Nitric Oxide 46 (2015) 14–24

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Review

On the chemical biology of the nitrite/sulfide interaction

Miriam M. Cortese-Krott [a], Bernadette O. Fernandez [b], Malte Kelm [a], Anthony R. Butler [c], Martin Feelisch [b,*]

[a] Cardiovascular Research Laboratory, Department of Cardiology, Pneumology and Angiology, Medical Faculty, Heinrich Heine University of Düsseldorf, Düsseldorf, Germany
[b] Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, UK
[c] Medical School, University of St-Andrews, St-Andrews, Fife, Scotland

ARTICLE INFO

Article history:
Available online 23 December 2014

Keywords:
Sulfide
Nitrite
Nitroxyl
$SSNO^-$
Sulfane sulfur
Hypoxia

ABSTRACT

Sulfide (H_2S/HS^-) has been demonstrated to exert an astounding breadth of biological effects, some of which resemble those of nitric oxide (NO). While the chemistry, biochemistry and potential pathophysiology of the cross-talk between sulfide and NO have received considerable attention lately, a comparable assessment of the potential biological implications of an interaction between nitrite and sulfide is lacking. This is surprising inasmuch as nitrite is not only a known bioactive oxidation product of NO, but also efficiently converted to S-nitrosothiols in vivo; the latter have been shown to rapidly react with sulfide in vitro, leading to formation of S/N-hybrid species including thionitrite (SNO^-) and nitrosopersulfide ($SSNO^-$). Moreover, nitrite is used as a potent remedy against sulfide poisoning in the clinic. The chemistry of interaction between nitrite and sulfide or related bioactive metabolites including polysulfides and elemental sulfur has been extensively studied in the past, yet much of this information appears to have been forgotten. In this review, we focus on the potential chemical biology of the interaction between nitrite and sulfide or sulfane sulfur molecules, calling attention to the fundamental chemical properties and reactivities of either species and discuss their possible contribution to the biology, pharmacology and toxicology of both nitrite and sulfide.

Contents

* Corresponding author. Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton General Hospital, South Academic Block, Level F, Mailpoint 810, Tremona Road, Southampton, SO16 6YD, UK. Fax: +44 (0)2380 511761.
E-mail address: m.feelisch@soton.ac.uk (M. Feelisch).

http://dx.doi.org/10.1016/j.niox.2014.12.009

1. Introduction

Hydrogen sulfide (H_2S), previously recognized as a noxious, malodorous gas and potent inhibitor of mitochondrial respiration, has been shown to exert a dazzling variety of beneficial biological effects some of which resemble those of nitric oxide (NO) and may hold potential for therapeutic exploitation [1–3]. Several enzymatic and non-enzymatic sources have been identified to contribute to the existing pool of sulfide in circulation and tissues [1–3], with a purportedly essential role in blood pressure regulation [4]. Indeed, it has been suggested that H_2S should be recognized as the third "gasotransmitter", alongside NO and carbon monoxide (CO) [5]. Although this recognition lacks universal agreement [6,7], and the three molecules differ in potency and appear to mediate their biological action via different signaling pathways, their action spectrum shows remarkable overlap.

As a weak diprotic acid (pk_{a1} 6.9–7.1, pk_{a2} >12) H_2S ionizes as,

$$H_2S(g) \rightleftharpoons H_2S(aq) \rightleftharpoons HS^- + H^+ \rightleftharpoons S^{2-} + 2H^+ \quad (1)$$

Therefore, at physiological pH H_2S exists largely (75–80%) in the form of the hydrosulfide anion (HS^-), with negligible amounts of S^{2-} [8,9], and very little of it is in the form of the dissolved uncharged gas [9]. However, in the following discussion, we will refer to the sum of H_2S+HS^-+S^{2-} in solution as "sulfide".

The striking similarities between some of the effects of NO and sulfide, along with intriguing co-operative effects, raise the possibility of a cross-talk between these species [10–16], involving the interaction with different proteins belonging to the same pathway (e.g. activation of soluble guanylate cyclase by NO and inhibition of phosphodiesterase by sulfide [17]) together with the possibility of direct chemical interaction [15,18,19].

Alongside the discovery of sulfide as a physiological transmitter, the past decade has witnessed the recognition that nitrite (NO_2^-) can serve as an alternative source of NO and is a molecular entity with intrinsic signaling properties [20,21]. Nitrite can be reduced to NO in an oxygen-dependent manner by a variety of enzymatic and non-enzymatic pathways [22]. Even nitrate (NO_3^-) can be reduced to nitrite by mammalian tissues [23,24] demonstrating that these anions are not mere end products of oxidative NO metabolism but subject to partial recycling. This nitrate–nitrite–NO cycle offers a conceptually attractive explanation for the blood pressure lowering and cardioprotective effects of diets rich in nitrate and may provide the molecular basis for past and future therapeutic uses of nitrite/nitrate [20,25], although definitive chemical evidence for the significance of this pathway under physiological conditions is still lacking.

While the chemistry, biochemistry and potential pathophysiology of the cross-talk between sulfide and NO (as well as between sulfide and S-nitrosothiols) have received considerable attention in recent years and is discussed in detail elsewhere [9,26–29], a critical analysis of the chemistry and potential biological implications of the interaction between nitrite and sulfide is lacking. This is surprising inasmuch as nitrite is not only a known bioactive product of NO oxidation but also efficiently converted to S-nitrosothiols (and other NO metabolites) if administered in vivo [21]. There is little doubt that the nitrite/sulfide interaction is of some biological relevance as nitrite is the most potent remedy known against sulfide poisoning [25]. These observations appear to be at odds with the notion that in simple aqueous solutions nitrite does not appear to react directly with sulfide, at least not at any significant rate [30]. However, sulfide may facilitate nitrite reduction to NO via interaction with Fe^{3+} protoporphyrin [30], while earlier studies showed that nitrite can be reduced to NO following direct reaction with sulfide [31,32]. To reconcile adequately such controversial chemical and biological observations, a better understanding of the chemical properties of sulfide and nitrite, and their potential interaction is needed.

In this review we aim to focus on the chemistry of the interaction between sulfide and nitrite, calling attention to the fundamental chemical properties of both species and discussing their potential biological chemistry and the consequences of this interaction for their biological and pharmacological activities in vivo. While the exact chemical nature of the cross-talk between sulfide and NO remains ill defined at this stage, two possible reaction products have received particular attention in this context lately: thionitrous acid (HSNO) and nitrosopersulfide ($SSNO^-$) [15,18,19]. Might the same two species also be formed during chemical interaction between sulfide and nitrite?

2. Nitrite and sulfide chemistry

2.1. Chemical properties and reactivities

Nitrite had been long considered as a biologically inert product of spontaneous [33] or metalloenzyme-catalyzed oxidation of NO [34]. NO is a poor one-electron oxidant, a property that allows this molecule, even in the presence of millimolar concentrations of thiols, to diffuse from endothelial cells to the underlying smooth muscle cells and reach the iron atom in the heme group of soluble guanylate cyclase [35]. However, NO can be readily oxidized to more reactive species in a process known as autoxidation [33]:

$$2\,NO + O_2 \rightarrow 2\,NO_2 \quad (2)$$

$$NO_2 + NO \rightleftharpoons N_2O_3 \quad (3)$$

Although it has been argued that in biological environments N_2O_3 is formed efficiently only in lipid membranes because of the reactivity of the NO_2 radical toward cellular antioxidants [36–38], it is fairly certain that some of the NO produced by NOS will follow this fate. N_2O_3 is, of course, the anhydride of nitrous acid:

$$N_2O_3 + H_2O \rightleftharpoons 2HNO_2 \rightleftharpoons 2H^+ + NO_2^- \quad (4)$$

The K value for the equilibrium formation of N_2O_3 is $3.0 \times 10^{-3}\,M^{-1}$ [39]. Nitrous acid, once formed, ionizes with a pK_a of 3.15 and so there is very little of the undissociated HNO_2 present around physiological pH.

Nitrite is a weak nucleophile [40], and in aqueous conditions and at biological pH, it will not easily react with electrophiles, also because of the solvation effects of water molecules. Nitrite can be used to nitrosate thiols but the exact mechanism depends on pH. At very low pH (<3) protonation of HNO_2 results in the formation of NO^+, a particularly powerful electrophile able to rapidly react with thiolate according to:

$$HNO_2 + H_3O^+ \rightleftharpoons [H_2NO_2]^+ + H_2O \quad (5)$$

$$[H_2NO_2]^+ \rightarrow NO^+ + H_2O \quad (6)$$

$$NO^+ + RS^- \rightarrow RSNO \quad (7)$$

The K value for equilibrium (Eq. 5) is $1.2 \times 10^{-8}\,M^{-1}$ [41]. Although there is little anion present in an acidic solution of, say cysteine ($pK_a = 8.3$), it is a sufficiently strong nucleophile to react on encounter with NO^+ and thus produce S-nitrosocysteine. It is worth pointing out that the release of NO^+ in its "free" form does not occur in biological environments other than perhaps the stomach; rather the [NO^+] moiety is formally transferred from a bound form to another acceptor in a so-called transnitrosation reaction.

At higher pH values (pH 3–7) the reaction pathway is rather different. Undissociated nitrous acid exists in equilibrium with N_2O_3

Box 1. Reactivity of nitrite.

Nitrite is a product of spontaneous or metalloenzyme-catalyzed oxidation of NO. In aqueous solution nitrite is a weak nucleophile and a poor one-electron oxidant, and therefore not expected to react with thiolates, except at acidic pH when nitrite is converted into the nitrosating species N_2O_3 or, at pH < 3, to [NO^+]. This might suggest that there is no interaction of nitrite with sulfide and other thiolates under conditions of biological relevance. However, there is experimental evidence to suggest otherwise.

(Eq. 4), which is also a nitrosating species. Water is a weak nucleophile and if other, more powerful, nucleophiles are present N_2O_3 will react preferentially with these, instead of forming HNO_2. While the anion of glutathione is present at only, say 10^{-4} M at physiological pH, compared with water at 55.5 M (the concentration of water in water), its far greater nucleophilicity means it will be nitrosated preferentially. According to the Ritchie's N^+ nucleophilicity scale [42] thiolate is 10^{11} times more nucleophilic than water (Box 1).

The reductive and nucleophilic properties of sulfide are likely the most predominant aspects of its chemistry that contribute to its biochemical and physiological actions [9,29]. While H_2S itself is relatively unreactive, the thiolate ion (HS^-) is a powerful nucleophile [36], exceeded only by hydrazine ($H_2N—NH_2$), which is a 'super nucleophile' because of the α-effect [39]. HS^- (where S is in the lowest formal oxidation state of –2) can react with oxidants to form sulfur compounds with higher oxidation states such as 0 (defined as S^0 as in sulfur-bound sulfur or 'sulfane sulfur', following the definition of Toohey [43,44]) as in polysulfides (S_x^{2-}) polythionates ($^-O_3S—S_n—SO_3^-$), elemental sulfur (S_8); +2 in thiosulfate ($S_2O_3^{2-}$); +4 in sulfite (SO_3^{2-}); and +6 as in sulfate (SO_4^{2-}). Like all thiols, sulfide can undergo both one-electron and two-electron oxidation, leading to formation of a radical (i.e. HS• [7]) or the disulfide (formally S^0), respectively. Therefore sulfide is considered as a mild reducing agent: its oxidized free radical form HS• is a good oxidant [7] and its two-electron reduction potential (S^0/H_2S) is comparable with that of cystine/2 cysteine and glutathione disulfide/2 glutathione [9]. As a two-electron reductant H_2S may also allow the conversion of higher oxidation state nitrogen oxides to lower-oxidation-state nitrogen oxides including NO, HNO, and NH_3 [28].

Although the reaction with oxygen itself is very slow and rate limiting, any aqueous solution of hydrogen sulfide in the presence of air will soon contain all sulfur compounds mentioned above (including polysulfide, sulfite and sulfate) [8,45] and related radical species (like R-SS•, HSS•) [7,26,46]. In an aqueous system, these compounds form complex equilibria, which themselves are strongly dependent on pH and at the same time affect the pH of the system [8,47,48]. Excellent reviews about the redox properties of sulfur-centered radicals were published recently [7,26].

Similar to nitrite, which at neutral pH has a tendency to form coordination complexes with metal centers (e.g. "nitro" (i.e. Fe—NO_2) or "nitrito" (i.e. Fe—O—NO) complexes in heme-protein [26]), sulfide forms complexes with divalent metals and with metal centers in proteins as reviewed in detail elsewhere [7–9,26] (Box 2).

2.2. Nitrite and sulfide interaction at acidic pH

The most cited work describing the interaction between nitrite and sulfide in the recent literature is an EPR analysis of the sulfide-mediated nitrite reduction to NO under acidic conditions by Grossi [31]; a limitation of this work is that the chemistry of the trapping can be changed by the reactants. By incubating equimolar concentrations of nitrite (NO_2^-) and NaHS in water or in buffer at

Box 2. Reactivity of sulfide.

In a biological environment at neutral pH, sulfide is mainly present in the form of the anion HS^-, which is a powerful nucleophile, reacts readily with metals/metal centers, or with oxidants leading to formation of inorganic and organic sulfane sulfur species including hydropersulfides (RSSH, HSS^-) and related radical species (like R-SS•, HSS•).

pH 3 or 6 under N_2 and trapping of the released NO with ferrous diethyldithiocarbamate ($Fe(DETC)_2$) in $CHCl_3$ a clear EPR signal, due to the NO—$Fe(DETC)_2$ radical (a_N = 1.28 mT and g = 2.039), was detected, and a yellow precipitate of elemental sulfur (S_8) was recovered from the aqueous solution 48 h later. At pH 6, nitrite alone showed a signal only after a longer incubation time (t > 40 min) indicating that the reaction was more efficient in the presence of sulfide, and not necessarily dependent on the presence of strong acid. Although the spectrum itself was not shown, a small signal was recorded even at pH 7, which the author attributed to "impurities", likely from transition metals [31].

There is a considerable amount of lesser-known older work on the reaction of sulfide with nitrite demonstrating that under acidic conditions the reaction leads to a range of products. Its age does not diminish the value of the results obtained. The earliest work appears to be that of Divers and Haga [49], who found that a solution of H_2S with silver nitrite gave S_8, NO, hydroxylamine and ammonia on acidification. A more comprehensive study was undertaken by Bagster [50], who noted that HNO_2, obtained by the acidification of $NaNO_2$ or KNO_2 solutions, reacts with sulfide leading to the formation of different combinations of nitrogen-containing (e.g. HNO, NO, NH_2OH or NH_4) and sulfur-containing compounds (e.g. SO_3^{2-}, polysulfides or sulfur), depending on the concentration ratio of the reactants, or NH_2OH and NH_3 (or their protonated form at acidic pH) when an overwhelming concentration of sulfide was used. By contrast, NO or HNO (nitroxyl) were the main products if the HNO_2 concentration was greater than that of sulfide. Bagster, in the same study [50], noted that if H_2S was passed continuously into a concentrated nitrite solution ($AgNO_2/NH_3Cl$) nitrite was slowly consumed, and that consumption was faster if conducted with excess sulfide, as soon as sulfur was spontaneously formed, or if conducted in the presence of sulfide and polysulfides. The reaction was attributed to the formation of low amounts of HNO_2 stemming from nitrite protonation even at neutral pH, which was reacting with sulfide to form sulfur according to the reactions described above. Reports about the reactivity of nitrite with sulfide in water at higher temperature (60 °C) leading to oxidation of sulfide to S_8 can be found elsewhere [32]. From the insights offered by the analysis of the products described in the literature, it is possible to propose a unified reaction scheme that satisfactorily explains formation of all these products and involves the intermediacy of thionitrous acid (HSNO) as summarized in Fig. 2.

It is not difficult to see how HNO_2 can react with sulfide under strongly acid conditions (pH < 3) to yield thionitrous acid, HSNO (Eq. 8). At higher pH, HNO_2 is in equilibrium with its anhydride N_2O_3 (Eq. 4), which will nitrosate HS^- as described for thiolates in Eq. 7, also leading to formation of HSNO.

$$HS^- + [H_2NO_2]^+ \rightarrow HSNO + H_2O \quad (8)$$

$$2\,HS^- + N_2O_3 \rightarrow 2\,HSNO + NO_2^- \quad (9)$$

2.3. HSNO – a highly reactive intermediate

Long before the recent interest in the biology of NO, nitrite and H_2S, HSNO was suggested by Williams as a likely instable

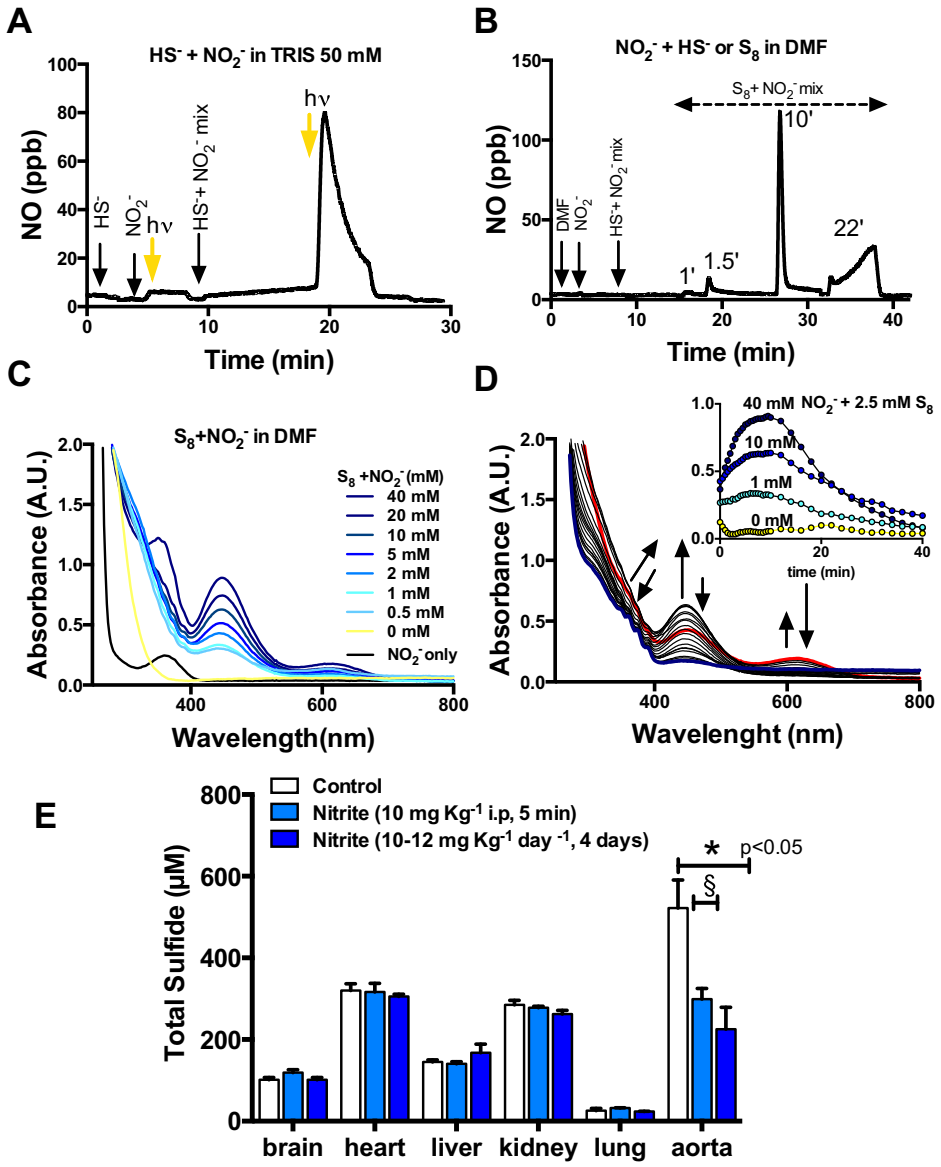


Fig. 1. (A) Pre-incubation of equimolar nitrite and hydrosulfide (50 mM each in 50 mM Tris buffer at pH 7.4) generates a photolabile compound that releases NO as detected by gas phase chemiluminescence (hv–light). (B) Original chemiluminescence tracing of NO release from nitrite and sulfide alone (controls) as compared to aliquots of a nitrite/S_8 mixture (1:1) pre-incubated for the indicated time points. (C) Concentration-dependent accumulation of $SSNO^-$; selected spectra taken 8 min after start of incubation. (D) Reaction of 2.5 mM S_8 with 1 mM nitrite. Attributions: λ_{max} 448 nm $SSNO^-$, λ_{max} 358 nm NO_2^-, λ_{max} 608 nm S_3^-; spectra taken every 2 min for 40 min. Red line: spectrum taken 30 s after addition of nitrite; Blue line: Spectrum taken at 40 min. Inset: time course of absorbance changes at 448 nm (formation of $SSNO^-$). (E) Total (free and acid-labile) sulfide pool in various organs of the rat as assessed by methylene blue assay (see Appendix: Supplementary materials for details). Acute and chronic nitrite applications of $NaNO_2$ do not affect total tissue sulfide content in most organs, with exception of the aorta. Data are plotted as means ± SEM. Group size: Control $n = 4$; acute nitrite (5 min after intraperitoneal, i.p. application), $n = 2$; chronic nitrite (4 days administered with the drinking water), $n = 2$; *t*-test: * chronic nitrite vs. control $p = 0.049$; § $p = 0.068$ acute nitrite vs. control, non significant.

intermediate of sulfide nitrosation [51]. It had been prepared and characterized by Goehring in 1950 [52,53] as a compound stable at –60 °C obtained by the reaction of $HN(SONH_2)_2$ (from SO_2 or $SOCl_2$ and NH_3 according to Schenk [54]) with HCl [53]. Later, Müller and Nonella prepared HSNO using the same method and characterized it, along with its isomers (see Eq. 13), at 12K in an argon matrix using modern methods and instrumentation [55,56]. As pointed out by all these authors, the extreme conditions used for its characterization were needed because of the known instability of HSNO and its isomers in solution, its high reactivity and a tendency for polymerization and formation of an insoluble brown material [53]. It has been claimed recently that it can also be prepared by reaction of sulfide and nitrite under acidic conditions and detected by ESI-TOF mass spectrometry [18]. The same authors also claimed that HSNO (obtained by the reaction of GSNO with sulfide) was "stable for less than 1 h at pH 7.4 and 21 °C", as assessed by ^{15}N-NMR [18].

The instability of HSNO can be understood by considering its chemical and structural properties. Similarly to other nitrosothiols, HSNO can undergo homolysis to give NO and the thiyl radical (Eq. 10), followed by dimerization leading to formation of disulfane (HSSH), which deprotonates to form hydrodisulfide or persulfide (HSS^-).

$$HSNO \rightarrow HS^{\cdot} + {}^{\cdot}NO \quad (10)$$

$$2\,HS^{\cdot} \rightarrow HSSH \quad (11)$$

It is generally assumed that nitrosothiol instability is due to the facile fission of the S—N bond. However, calculations suggest that the S—N

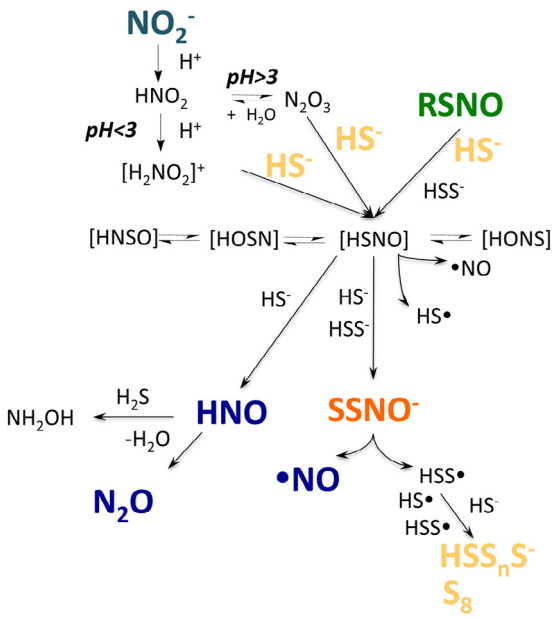
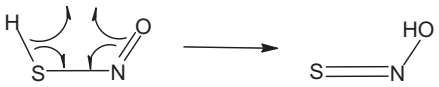


Fig. 2. Summary of possible reactions leading to formation of stable intermediates with potential signaling properties. At acidic pH, nitrite can be converted to the nitrosating species $[H_2NO_2]^+$ (at pH < 3) and N_2O_3 (at pH 3–7), leading to nitrosation of HS^- to form the reactive intermediate HSNO. The same intermediate is formed via transnitrosation from S-nitrosothiols (RSNOs). HSNO is highly reactive and can isomerize to other similarly unstable species; in the presence of sulfide it can generate either nitroxyl (HNO), which dimerizes under water loss to nitrous oxide or is reduced by sulfide to hydroxylamine. HSNO can be attacked by HS^- or HSS^-, which are good nucleophiles in a S_N2 reaction forming the more stable $SSNO^-$. $SSNO^-$ can accumulate in the presence of excess sulfide. This molecule belongs to the class of perthionitrites; in contrast to polysulfides, it is stable in the presence of high concentration of reduced thiols, but can generate both NO and polysulfides at pH 7.4, thus serving as a dual-purpose signaling molecules carrying both sulfane sulfur and NO bioactivity. Each of the intermediates and reaction products, including NO, HNO, H(S)S$^-$, H(S)S•, $SSNO^-$, can further react with one another, all in all feeding the reaction forward and increasing the complexity of the reaction equilibria.

in bond in nitrosothiols is quite strong, and it is its lengthening by complexation with cuprous ion that dramatically weakens it (M. Buehl, unpublished observation). Further calculations suggest that in HSNO the strength of the S—N bond is not substantially different from that in other nitrosothiols [55,56].

Rather than its smallness, a reason for its notability may be its possession of a mobile hydrogen, allowing isomerization by a facile cyclic 1,3 hydrogen shift.



(12)

There are four isomers with the same formula (HNSO, HOSN, HSNO, HONS and their corresponding anions, respectively; Eq. 13) [56]), and all have been characterized.

$$HNSO \rightleftharpoons HOSN \rightleftharpoons HSNO \rightleftharpoons HONS \quad (13)$$

According to recent calculations by Lai et al. [57] the most stable of these is *trans*-thionitrous acid, but Nonella et al. [56], like Goehring et al. before [53], concludes it must be thionyl imide (HNSO), which can be synthesized by the reaction of thionyl chloride with ammonia. Whatever the truth of the matter, all the energy barriers to isomerization are small because of the mobile hydrogen, and all isomers appear to degrade thermally to unknown products or rapidly polymerize [53,55]. In view of this reactivity the recent "characterization" of HSNO at physiological pH and room temperature [18] is rather surprising.

2.4. $SSNO^-$ – a likely biological mediator

The reaction of HSNO in the presence of an excess of sulfide may explain some of the unexpected products. Then, nucleophilic attack of HS^- on HSNO becomes dominant (Eq. 14):

$$HS^- + HSNO \rightarrow HSSH + NO^- \quad (14)$$

$$HSSH \rightleftharpoons H^+ + HSS^- \quad (15)$$

This is a second order reaction (rate = k[HSNO][HS^-]) and so, while negligible at low concentrations of HS^-, at higher hydrosulfide concentrations it dominates. The persulfide (hydrodisulfide anion; HSS^-) formed is also a strong nucleophile; while persulfides are unstable under acidic conditions (generating H_2S and elemental sulfur), at pH > 7 HSS^- can undergo nitrosation with N_2O_3 (Eq. 16) or transnitrosation with HSNO (Eq. 17) in a fashion similar to HS^-.

$$HSS^- + N_2O_3 \rightarrow HSSNO + NO_2^- \quad (16)$$

$$HSS^- + HSNO \rightarrow SH^- + HSSNO \quad (17)$$

The resulting nitroso compound, nitrosopersulfide (perthionitrite; $SSNO^-$) appears, from our recent work [19] and that of Seel et al. [58,59], to be considerably more stable than HSNO – even in the presence of high concentrations of thiols – and, therefore, a potentially better signaling molecule. The formation of sulfur chains (a chain of two in this instance) is so common in sulfur chemistry that to find evidence for the long-term presence of HSNO under conditions of excess sulfide would be highly surprising. Longer sulfur chains are common at higher sulfide concentrations, and elemental sulfur exists, of course, as S_8. It is not unreasonable to postulate that, because of its enhanced stability, for example in the presence of high concentrations of thiols (like GSH, Cys and DTT) [19], HSSNO/$ONSS^-$ is better able to provide NO and/or polysulfide in a biological environment than HSNO, as discussed in the section "Potential Biological Chemistry of Nitrite and Sulfide". Indeed, we found that $SSNO^-$ was able to release NO and induce sGC-dependent cGMP production in cells [19], but its detection in cells/tissues necessitates development of specific and sufficiently sensitive analytical methods, a task that has not yet been accomplished.

Since both HS^- and HSS^- are strong nucleophiles, their reaction with an electrophilic site in HSNO is also to be expected. If HSNO is attacked by persulfide instead of sulfide, the product is trisulfane (HSSSH) rather than disulfane (HSSH). The leaving group (nitroxyl anion, NO^-) is a very weak acid and will thus be protonated to give HNO, and dimerization of HNO gives rise to N_2O.

$$HS^- + HSNO \rightarrow HSSH + NO^- \quad (18)$$

$$2\,HNO \rightarrow\rightarrow H_2O + N_2O \quad (19)$$

The reduction of HNO by a thiol explains the formation of hydroxylamine and nitrogen (Eqs. 20 and 21). In other words, HNO is first reduced to NH_2OH, then further to N_2 by H_2S/HS^- or rather H_2S_2/HSS^- forming S^0.

$$HNO + 2\,H_2S \rightarrow NH_2OH + HSSH \rightarrow N_2 \quad (20)$$

$$2\,HNO \rightarrow N_2O + H_2O \quad (21)$$

Assuming homolytic cleavage of $ONSS^-$, the most likely initial product formed, in addition to NO, is the disulfide radical, $SS^{\bullet-}$ (Eq. 22).

Box 3. HSNO and $SSNO^-$.

HSNO is a nitrosothiol, which was first characterized in the 1950s as being highly unstable, and rapidly undergoing isomerization, homolysis and polymerization. It was considered by Williams [51] as a likely unstable intermediate in sulfide nitrosation. HSNO reacts with excess sulfide to form the more stable nitrosopersulfide, ($SSNO^-$), various nitrogen oxide species (including NO, HNO, N_2O) and higher oxidized sulfur species (including polysulfides, thiosulfate, polythionate, and elemental sulfur).

$SSNO^-$ is a colored compound containing a sulfane sulfur and a nitrosonium (NO^+) moiety attached to opposite ends of a sulfur atom. $SSNO^-$ was first characterized by Seel et al. in the 1980s by ^{15}N-NMR, IR, ESR spectrometry, and X-ray crystallography. In contrast to polysulfides, $SSNO^-$ is stable in the presence of millimolar concentrations of thiols. $SSNO^-$ generates both NO and sulfane sulfur on decomposition, making it an attractive candidate signaling molecule, in particular in the context of the cross-talk between the NO/NO_2^- and H_2S/HS^- pathways *in vivo*.

$$ONSS^- \rightarrow {}^{\bullet}NO + SS^{\bullet -} \quad (22)$$

$$SS^{\bullet -} \rightarrow S_4^{2-} \rightarrow\rightarrow \left(S_x^{2-}\right) \xrightarrow{H_2O} S_{(x-1)}^{2-} + H_2S \quad (23)$$

$$2\,HS_6^- \rightarrow 2\,HS_2^- + S_8 \downarrow \quad (24)$$

Dimerization and reaction with excess thiolate may give rise to formation of higher polysulfides (S_x^{2-}/HS_x^-). In neutral aqueous solutions, and particularly at acidic pH, this is followed by hydrolysis and disproportionation reactions, yielding S_8 in addition to free H_2S [48] (Eqs. 23, 24).

To summarize, the reaction of sulfide with nitrite under acidic conditions leads to formation of nitrogen compounds and sulfur compounds, which may be explained by the formation and decomposition of HSNO (See Fig. 2). A similar range of products was noted by Tannenbaum et al. [60] in the reaction of GSH with GSNO; the explanation given is rather different from that proposed in this review. The second order nature of the nucleophilic substitution reaction was established by Dicks et al. [61], and for the reaction of L-Cys with S-nitrosocysteine by Komiyama and Fujimori [62] (Box 3).

2.5. Nitrite and sulfide interaction at neutral pH

At pH 7.4 vanishingly low concentrations of $[H_2NO_2]^+$ are expected in nitrite-containing aqueous solutions and according to our calculations (see Appendix: Supplementary materials), the reaction of nitrite with HS^- becomes encounter controlled and impossibly slow. This is consistent with the lack of changes in UV/Vis absorbance characteristics of nitrite/sulfide containing solutions under these conditions. Nevertheless, in our hands, mixtures of buffered aqueous solutions of sulfide/nitrite always contain small amounts of a photolabile NO-containing species (Fig. 1; unpublished data). When equimolar mixtures of nitrite and sulfide (50 mM each in Tris-buffer at pH 7.4 with diethylenetriaminepentaacetic acid (DTPA) at room temperature) are pre-incubated for more than a couple of minutes[1], injected into the reaction chamber, and then exposed to high-intensity white light (Fig. 1) or copper (not shown) a clear NO signal can be detected by gas phase chemiluminescence, whereas nitrite or sulfide alone produce no signal at all under these conditions (Fig. 1A). These observations are consistent with the formation of trace amount of a nitrosothiol (or another species sensitive to light and transition metals), but we were unable to further characterize the reaction product.

One possibility to explain the oxidation of sulfide by nitrite to S_8 [32], the formation of NO-Fe-DETC complexes [31], or the formation of the photolabile NO releasing compound as observed by us (Fig. 1A), is by invoking the formation of intermediates by radical mechanisms as initiated by sulfide oxidation, for example. Indeed, over the range of physiological pH between 6.5 and 8.0 the rate of HS^- oxidation increases approximately 10-fold [63]. Oxidation of HS^- will lead to formation of a potent oxidant radical anion $S^{\bullet -}$ giving rise to formation of SO_2, polysulfides, thiosulfate and polythionate [64], that changes the acid/base and redox equlibria of the solution allowing the reaction to occur. Quantitative and qualitative product analyses with sensitive spectroscopic and analytical techniques are needed to confirm or refute these speculations.

2.6. Nitrite and sulfide or sulfur in polar aprotic solvents

Nitrite is nucleophilic by virtue of its negative charge, a charge that is delocalized. Thus, its reactivity as nucleophile is decreased by solvation in neutral aqueous solutions. In polar aprotic organic solvents like dimetlyformamide (DMF) and dimetylsulfoxide (DMSO), anions like nitrite (and sulfide) are not solvated and exist essentially in their "naked" form (unlike in water where solvation is via formation of hydrogen bonds) [65]. Thus in these solvents, the nucleophilicity of nitrite is markedly enhanced because of the lower activation energy needed due to the absence of hydrogen bonding and can react with electrophilic compounds. Sulfide is a powerful nucleophile, and therefore, is not expected to react with nitrite. Indeed, Schmidt and Wägerle [66,67] and Seel et al. [68] did not observe any reaction between nitrite and sulfide dissolved in DMF or DMSO in the absence of air, even after boiling the solutions. This is consistent with the lack of changes in UV–visible spectral characteristics and the lack of NO release on incubation of nitrite/sulfide mixtures in DMSO under aerated conditions (Fig. 1B; arrow $HS^- + NO_2^-$; unpublished data). To be able to react with each other, either nitrite or sulfide has to be converted into an electrophilc compound. As mentioned before, nitrite can be protonated to give NO^+ or 'dehydrate' to give N_2O_3 where H_2O is the departing nucleophile. What is the electrophilic counterpart of sulfide?

Sulfane sulfur, i.e. sulfur atoms bound to other sulfur atoms, as present in polysulfides and colloidal sulfur (S_8), are good electrophiles because in S—S bonds sulfur has the tendency to delocalize the octet and form double bonds [66,67]. Indeed nitrite reacts very efficiently with elemental sulfur in polar aprotic solvents (albeit not in water [66–68]) under deaerated conditions [66,67], and according to Seel et al. [68], the reaction leads to formation of NO and N_2O. A similar reaction of nitrite with polysulfides was described, but no results were shown [68]. The reaction of nitrite with polysulfide or sulfur in aqueous solution, although predicted to be thermodynamically favorable and highly exothermic [68], does not occur. Using UV–visible spectrometry we found that nitrite reacts with S_8 (and under some conditions also with polysulfides) to form an NO-containing yellow compound with λ_{max} 448 nm (Fig. 1B–D; unpublished data). Based on Seel's earlier findings [58,68,69] we assign this product to be nitrosopersulfide, $SSNO^-$ [19]. So, how can $SSNO^-$ be formed under these conditions?

According to Schmidt and Wägerle [66,67], the reaction of nitrite with inorganic sulfur takes place via a nucleophilic attack of nitrite on the S_8 ring, leading to ring opening and formation of a linear polysulfide carrying a nitrite grouping at one end; the species formed belongs to the class of nitrosulfides.

[1] Please refer to Appendix: Supplementary materials for detailed description of the reaction conditions.

$$NO_2^- + S_8 \rightarrow {}^-S(S)_6\text{-}S\text{-}NO_2 \quad (25)$$

S_8 is easily attacked by a nucleophile via a S_N2 mechanism because in S_8 sulfur atoms are bound covalently to one another and have the tendency to distribute the electronic shell beyond the octet forming double bonds, where the sulfur atoms can act as electrophiles. These sulfur atoms with valence 0 or –1 are called sulfane sulfur and are present not only in S_8 but also in polysulfide, polythionate and other sulfur compounds [43,45]. This step is rate limiting, and, as pointed out before, probably is favored in non-aqueous 'electron pair donor' solvents for two reasons: first, because of the better solubility of inorganic sulfur in these solvents as compared with water, and second because the anions are not solvated and "masked" by water molecules via hydrogen bonds, as indicated by the typical absorption spectrum of nitrite in hexamethylphosphoramide displaying five different absorption bands at 340, 351, 363, 377, 393 nm similar to nitrous acid in water [68] and as also observed in the spectrum of isopentyl nitrite (see for example [19]).

The thionitrate anion ${}^-S(S)_6—S—NO_2$ may be further attacked at the sulfane sulfur by a second nitrite anion leading to formation of an unstable SNO_2^-, thionitrate anion (with absorption at 350 nm).

$$ {}^-S(S)_6\,SNO_2 + NO_2^- \rightarrow {}^-S(S)_5\,SNO_2 + {}^-SNO_2 \quad (26)$$

The monothionitrate may react further either with another thionitrate anion, with nitrite or with a polythionitrate leading to a mixture of thionitrates with different numbers of sulfur atoms. Alternatively, thionitrate can decompose into polysulfide and NO_2, which can act as potent oxidant leading to the formation of nitrate and NO (from nitrite) and oxidized sulfur metabolites (from sulfide und disulfide) including sulfite, sulfate, thiosulfate and polythionate (from polysulfide).

$$ {}^-S_xNO_2 \rightarrow NO_2 + S_x^{\bullet -} \quad (27)$$

$$2\,NO_2 + S_x^{\bullet -} \rightarrow 2\,NO + S_xO_2^{\bullet -} \quad (28)$$

$$2\ NO_2 \rightleftharpoons N_2O_4 \rightleftharpoons NO^+ + NO_3^- \quad (29)$$

Taken together, nitrite reacts with S_8 and, seemingly less efficiently, with polysulfides in non-aqueous polar solvents leading to the formation of similar products as observed previously in the reaction of NO with sulfide [69], and nitrosothiols with thiols [70], and with sulfide [19,71] including $SSNO^-$. Although polysulfides, and perhaps even S_8, are likely to co-exist in certain cells the significance of these reactions for the biological environment is unclear at present. Nevertheless, it appears reasonable to assume that some reactions of nitrite with sulfur species occur in regions of the cell that are non-aqueous (Box 4).

Box 4. Does nitrite react with sulfide in biological environments?

Nitrite and sulfide are both nucleophiles and anions, and as such do not chemically react with each other. However, both can be converted with relative ease into metabolites with distinct chemical properties (e.g. molecules carrying respectively NO^+ or sulfane sulfur moieties such as N_2O_3 or DNIC, and polysulfides) the (indirect) chemical interaction of which gives rise to formation of other bioactive metabolites, including $SSNO^-$. In a biological environment, nitrite and sulfide are readily converted into more reactive metabolites by a variety of enzymatic and non-enzymatic reactions. Experimental evidence is needed to confirm the relevance of these reactions for the biology of either species *in vivo*.

3. Potential biological chemistry of nitrite and sulfide

It is now well appreciated that in biological systems "free" sulfide levels ($HS^- > H_2S > S_2^-$) are relative low (nM), and the majority of sulfide is indeed bound to biomolecules via different chemical bonds of various strengths [72]. Chemical and/or enzymatic reactions might contribute to releasing sulfide from these storage forms, and therefore the equilibria between these "labile" sulfide stores and "free" sulfide may play a central role in controlling the bioactivity of sulfide [72]. In analogy with the pool of nitric oxide metabolites [73–76], sulfide stores appear to be characterized by more complex chemistry and equilibria [72]. The existence of such stores was proposed some time ago following critical revision and comparison of the different concentrations of sulfide in blood, plasma and tissues obtained using different detection methods. Based on the chemical conditions (i.e. acidification, alkalization or reduction) required for sample preparation to release sulfide, these were defined as "acid labile", "alkaline labile" and "persulfide" pools [72,77]. The nature of the chemical interaction between different species belonging to either the sulfide or the NO stores is far from fully characterized at present.

As with nitrite [78], sulfide concentrations in rodents are higher in tissues than in blood, and the highest concentrations are observed in the vasculature [79], rendering the aorta a relevant target for the interaction of nitrite with sulfide. In preliminary experiments, both acute and chronic administration of sodium nitrite markedly reduced aortic sulfide levels, while no changes whatsoever were seen in any other tissues (Fig. 1D; unpublished data). The speed with which nitrite affects the total vascular sulfide pool suggests a direct interaction rather than an effect of nitrite on H_2S production as systemic NOS inhibition is not known to lead to comparable drops in nitrite that quickly [78]. The nature and relevance of this interaction is currently unclear, although our observation is unlikely the result of a direct chemical reaction between nitrite and sulfide. Why this interaction is only observed in vascular tissue is somewhat of a mystery and warrants further investigation.

3.1. May sulfide participate in nitrite-derived NO formation in hypoxic tissue?

It is thought that nitrite is the source of NO for vasodilation in hypoxic tissue, but the means whereby NO is released on demand is not known. Nitrite may form NO either via deoxyhemoglobin (deoxyHb, $Fe^{II}Hb$) [80,81], deoxymyoglobin [82] or xanthine oxidoreductase (XOR)-mediated reduction [83], or via spontaneous [84] and carbonic anhydrase-facilitated disproportionation [85]. Most of these processes show a clear oxygen-dependence, and several are favored by low oxygen tension. The relative contribution of either mechanism to NO formation is likely to vary with oxygen partial pressure along the vascular tree [86,87].

Hypoxic tissue is not acidic enough to release NO from nitrite spontaneously [88], but it could be argued that reaction of N_2O_3 with H_2S/HS^- (hypoxia may shift the equilibrium from HS^- into the direction of H_2S) would lead to the formation of HSNO or the more stable $SSNO^-$ with subsequent release of NO via the route depicted in Fig. 2. However, there is what appears to be a serious flaw in this suggestion. If we assume that the pH is 6, the concentrations of both nitrite and H_2S are 10^{-5} M, and that N_2O_3 and HS^- react on encounter, it is possible to calculate the rate of reaction leading to the formation of HSNO. The concentration of HS^- at this pH is 1.3×10^{-6} M, while the concentration of N_2O_3 at this pH is only 3×10^{-19}M. The very low value of the latter is due to the fact that nitrous acid is still largely dissociated at this pH and, in the equilibrium for the formation of N_2O_3, nitrous acid formation is second order. The second order rate constant for a reaction occurring on encounter is generally taken to be 4×10^9 $M^{-1}s^{-1}$ [89] and so the rate of reaction is:

$$\text{Rate} = k[HS^-][N_2O_3] = 4\times10^9 \times 1.3\times10^{-6} \times 3\times10^{-19} = 1.6\times10^{-15}\,M\,s^{-1}$$

This is far too low for the reaction to occur on a meaningful time scale.

The reaction might become significant if N_2O_3 is formed from nitrite by other means, as for example in the reaction between deoxyhemoglobin and nitrite [90]. Considering the affinity of sulfide for metals, and metals centers and the fact that the biochemistry of these interactions is still unclear [26], these considerations are purely speculative and need experimental confirmation.

Taken together, sulfide may indirectly participate to release NO from nitrite in biological tissues only if nitrite is transformed into a potent nitrosating intermediate like N_2O_3, leading to nitrosation of H_2S/HS^- and formation of HSNO, as depicted in Figs. 2 and 3.

3.2. Sulfide releases NO from nitrosothiols via formation of $SSNO^-$

A number of S-nitrosothiols (S-nitrosoglutathione, S-nitrosoalbumin and s-nitrosohemoglobin) are found in blood and tissues and believed to represent storage forms capable of releasing NO on demand [73,74,78,91–93], but the means of release have never been clearly established. Stamler [94] proposed the involvement of a specific enzyme, GSNO-reductase, for the release of NO from S-nitrosoglutathione but its substrate specificity is thought to prevent interaction with other nitrosated species, and the mechanistic details of NO release from the nitrosothiol at Cys-93 on hemoglobin likewise remain a bit of a mystery. However, the presence of HS^- may provide a route for such release assuming formation of HSNO via transnitrosation. The general equation for transnitrosation is:

$$R'SH + RSNO \rightleftharpoons RS^- + R'SNO \qquad (30)$$

The first reported observation of such a reaction was by a Japanese group [95] and the process was investigated fully by Williams and co-workers [96,97]. They found the reaction to be very fast, requiring stopped-flow techniques for investigating the kinetics. Their data showed that the nitrosation favored thiols with a lower pK_a.

It is possible that a naturally occurring S-nitrosothiol on encountering tissue rich in HS^- like the vasculature will immediately transnitrosate to form HSNO first and then the more stable $SSNO^-$ (see Figs. 2 and 3), restoring the thiol group to its reduced state. The low pK_{a1} of sulfide favors this process. If sulfide is in excess over the nitrosothiol (which are found in tissues typically at nM concentrations), $SSNO^-$ is likely to accumulate and may more easily transport and release NO than GSNO or S-nitrosoalbumin [19].

$SSNO^-$ is a colored species containing a sulfane sulfur and a nitrosonium (NO^+) moiety attached to opposite ends of a sulfur atom. It formally belongs to the group of polysulfides (S_x^{2-}); however, contrary to polysulfides and other nitrosothiols, it is stable in the presence of millimolar concentrations of reduced thiols, including cysteine and glutathione (even dithiotheritol, DTT) [19]. Contrary to nitrosothiols, $SSNO^-$ like polysulfides is unstable under strong acidic conditions, leading to rapid decomposition and excision of S_8 [19]. Such a compound would seem to be of particular interest in the context of the cross-talk between the NO and H_2S signaling pathways. In fact, considering its structural characteristics, $SSNO^-$ has the potential to generate both NO and sulfane sulfur on decomposition. Evidence of pharmacological activity of $SSNO^-$ leading to sGC activation with concomitant cGMP formation in cells was presented by us recently [19]. However, evidence for $SSNO^-$ generation *in vivo* awaits experimental confirmation.

Of note, under hypoxic conditions it has been demonstrated that nitrite administration increases the level of nitrosothiols and nitrosylheme in the vasculature and in erythroctes [21,98]. Since H_2S is oxidatively inactivated by the action of sulfide-quinone-oxidoreductase in mitochondria, while its formation is essentially oxygen independent [99,100], hypoxic conditions are expected to increase the availability of H_2S/HS^-. It is tempting to speculate that increased sulfide and nitrosothiol concentrations (due to enhanced nitrite reduction) could lead to selective accumulation of $SSNO^-$ (and potentially other downstream reaction products), which is sufficiently stable in the reducing environment of cells and at the same time, a better NO releaser compared to the originating nitrosothiol (Fig. 3).

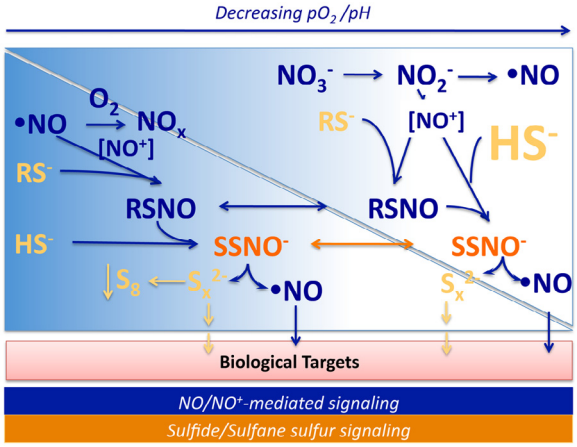
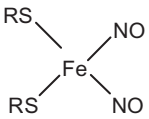


Fig. 3. Hypothetical scheme depicting the possible interactions between constituents of the sulfide pool and the NO pool along the physiological oxygen and pH gradient. Release of NO^+ in its "free" form does not occur in biological environments, rather the [NO^+] moiety is formally transferred from a bound form to another acceptor via a transnitrosation reaction. The reaction of nitrosothiols with HS^- will lead to formation of SSNO-. Hypoxic conditions are expected to lower the prevailing pH and increase the availability of H_2S/HS^- (due to reduced oxidative clearance) and that of nitrosothiols (due to enhanced nitrite reduction and/or metabolic conversion). These conditions could potentially lead to an enhanced accumulation of $SSNO^-$ (and other downstream reaction products). $SSNO^-$ should be sufficiently stable in the reducing environment of the cell; at the same time, it is a better NO releaser than the parent nitrosothiol [19].

As expected, by considering the chemistry of the reaction (Fig. 2), further products of this reaction might be nitroxyl as proposed previously [16,18,30], and nitrous oxide (N_2O). N_2O has potent biological activity when used at pharmacological concentrations, but is not thought to be produced by mammalian cells. Yet, N_2O has been detected in exhaled breath of humans [101]. Could nitrous oxide be another gaseous mediator produced by this reaction? Although unlikely, there was a time when NO seemed an unlikely biological mediator, too.

There is an increasing interest in dinitrosyl-iron-complexes (DNICs) as NO storage forms in cells and tissues, and Thomas et al. proposed these species to represent the most abundant NO-containing material in biological tissue [102]. It was proposed that DNICs are formed in the vasculature from nitrosothiols and that they are in equilibrium with the nitrosothiol pool [103,104].



(31)

That they can be prepared from S-nitrosothiols [105] suggests that NO^+ transfer from RSNO to iron occurs readily. In the presence of HS^-, the reverse process may occur with the formation of HSNO. The chemistry of DNICs is fairly complex [103], making it difficult to predict the exact course of any reaction involving them but, as most of their reactions are in equilibria; thus the reaction proposed is reasonable.

4. Summary and conclusions

Hydrogen sulfide, mainly existing as hydrosulfide anion in biological environments, has recently been recognized to exert an astonishing variety of biological and pharmacological effects, which resemble in part those of NO including regulation of vascular tone and blood pressure. Indeed, these two species were proposed to interact with each other both chemically and biochemically. Nitrite, once considered an inert byproduct of NO oxidation, is now considered an alternative source of NO by virtue of its metalloprotein-catalyzed reduction. Because of its chemical nature as a nucleophile and reducing agent, sulfide has been proposed to directly reduce nitrite to NO and/or to promote nitrite reduction by modulating enzymatic activities of xanthine oxidoreductase or porphyrins. Nitrite is used as a potent remedy against sulfide poisoning in the clinic. Not all authors agree that a direct chemical interaction between nitrite and sulfide occurs or is even necessary for nitrite to be effective in this setting. To adequately address these controversies, a better understanding of the chemical properties of sulfide and nitrite, and their potential interaction is required.

In this work we focused on the interaction of sulfide and nitrite calling attention to their fundamental chemical properties and discussing their biological chemistry. In an aqueous environment the reactivity of nitrite is limited by its weak nucleophilicity and by solvation with water molecules. On the other hand, the reductive and nucleophilic properties of H_2S, and its presence in solution mainly in the form of HS^- are likely the most predominant aspects of sulfide chemistry in a biological environment. Therefore, considering their basic chemical properties nitrite and sulfide as such are not expected to react with each other in cells and tissues. However, if nitrite is converted to more potent nitrosating species like $[H_2NO_3]^+$ or N_2O_3 (e.g. at acidic pH or in catalyzed reactions) these can react with sulfide leading to the formation of unstable and reactive intermediates such as HSNO. This intermediate is known to readily undergo isomerization, homolysis or polymerization, and to further react with sulfide to form the more stable nitrosopersulfide, $SSNO^-$ along with various nitrogen species (including NO, HNO, N_2O) and oxidized sulfur species (including polysulfide, thiosulfate, polythionate, and elemental sulfur, S_8). As HSSNO/$SSNO^-$ appears to be more stable than HSNO it could function more readily as a means of removing NO from very stable nitrosothiols, like S-nitrosoalbumin, and transporting it to wherever in tissues it is required and then releasing it. Although the reaction is thermodynamically favorable, nitrite does not seem to react with polysulfides and sulfur in aqueous solution. However, in non-aqueous polar aprotic solvents, nitrite reacts very efficiently already at room temperature with S_8 and, in the presence of sulfide, also with polysulfides leading to accumulation of $SSNO^-$, in turn releasing NO and polysulfides on decomposition. The biological relevance of this interaction is currently unclear, but the possibility that it may reflect their reactivity in hydrophobic cellular compartments cannot be excluded at present.

S-nitrosothiols appear to be more reactive toward sulfide compared to nitrite (and maybe to NO itself). In fact, nitrosothiols readily react with sulfide both in aqueous and non-aqueous media. Similarly to sulfide, nitrosothiols are found at nanomolar concentrations in most biological compartments; they are particular abundant in the vasculature indicating that an encounter of sulfide with nitrosothiols is plausible making the vasculature a particularly interesting compartment for these interactions to occur.

A further level of interaction could be due to nitrite-derived nitrosothiol formation occasioned by an increase in sulfide levels (due to increased stability and decreased enzymatic breakdown) under hypoxic conditions. Although it is important to point out that at present the interaction between NO species and sulfide stores in vivo is mere speculation this aspect warrants urgent experimental confirmation. It seems that sulfide provides a means of NO delivery from sources that might otherwise be too stable to become physiologically significant.

The chemistry of both sulfide and the nitrogen oxides, and their interactions had been extensively studied long before their biological actions were discovered. Rediscovering and analyzing the literature that describes the basic chemical principles governing these interactions is necessary not only for our understanding of the chemical biology and biochemistry, and the complex and sometimes controversial results obtained in the biological environment, but also for the development of therapeutic strategies that take the complex interactions between the pool of NO and sulfide metabolites, and that of the S/N hybrid molecules including $SSNO^-$ into account. Orthogonal to an improved understanding of the underlying chemistry is the ability to detect key reaction products and intermediates. This will require developing new analytical methods suitable to quantify these species at physiologically relevant concentrations in complex biological matrices, a challenging but potentially rewarding task.

Acknowledgments

We would like to thank Dr. Michael Buehl for computational analyses and helpful discussions, Annette Rauf for helping with UV–visible measurements as a part of her doctoral thesis, and Dr. Maria Francisca Garcia-Saura for expert assistance with the *in vivo* experiments. We are grateful to the Susanne-Bunnenberg-Stiftung of the Düsseldorf Heart Center (to MK), the COST action BM1005 (European Network on Gasotransmitters), and the Faculty of Medicine, University of Southampton (to MF) for financial support.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.niox.2014.12.009.

References

[1] L. Li, P. Rose, P.K. Moore, Hydrogen sulfide and cell signaling, Ann. Rev. Pharm. Toxicol. 51 (2011) 169–187. In: A.K. Cho, (Ed.).
[2] C. Szabo, Hydrogen sulphide and its therapeutic potential, Nat. Rev. Drug Discov. 6 (2007) 917–935.
[3] K.R. Olson, The therapeutic potential of hydrogen sulfide: separating hype from hope, Am. J. Physiol. Regul. Integr. Comp. Physiol. 301 (2011) R297–R312.
[4] G. Yang, L. Wu, B. Jiang, W. Yang, J. Qi, K. Cao, et al., H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase, Science 322 (2008) 587–590.
[5] R. Wang, Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter?, FASEB J. 16 (2002) 1792–1798.
[6] M. Feelisch, K.R. Olson, Embracing sulfide and CO to understand nitric oxide biology, Nitric Oxide 35 (2013) 2–4.
[7] K. Ono, T. Akaike, T. Sawa, Y. Kumagai, D.A. Wink, D.J. Tantillo, et al., Redox chemistry and chemical biology of HS^-, hydropersulfides, and derived species: implications of their possible biological activity and utility, Free Radic. Biol. Med. 77 (2014) 82–94.
[8] J.W. Morse, F.J. Millero, J.C. Cornwell, D. Rickard, The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters, Earth Sci. Rev. 24 (2002) 1–42.
[9] Q. Li, J.R. Lancaster Jr., Chemical foundations of hydrogen sulfide biology, Nitric Oxide 35 (2013) 21–34.
[10] K. Abe, H. Kimura, The possible role of hydrogen sulfide as an endogenous neuromodulator, J. Neurosci. 16 (1996) 1066–1071.
[11] M.Y. Ali, C.Y. Ping, Y.Y. Mok, L. Ling, M. Whiteman, M. Bhatia, et al., Regulation of vascular nitric oxide in vitro and in vivo; a new role for endogenous hydrogen sulphide?, Br. J. Pharmacol. 149 (2006) 625–634.
[12] M. Kajimura, R. Fukuda, R.M. Bateman, T. Yamamoto, M. Suematsu, Interactions of multiple gas-transducing systems: hallmarks and uncertainties of CO, NO, and H_2S gas biology, Antioxid. Redox Signal. 13 (2010) 157–192.
[13] P.J. Yoon, S.P. Parajuli, D.C. Zuo, P.K. Shahi, H.J. Oh, H.R. Shin, et al., Interplay of hydrogen sulfide and nitric oxide on the pacemaker activity of interstitial cells of cajal from mouse small intestine, Chonnam Med J 47 (2011) 72–79.
[14] K. Shatalin, E. Shatalina, A. Mironov, E. Nudler, H_2S: a universal defense against antibiotics in bacteria, Science 334 (2011) 986–990.
[15] M. Whiteman, L. Li, I. Kostetski, S.H. Chu, J.L. Siau, M. Bhatia, et al., Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric

oxide and hydrogen sulphide, Biochem. Biophys. Res. Commun. 343 (2006) 303–310.
[16] Q.C. Yong, J.L. Cheong, F. Hua, L.W. Deng, Y.M. Khoo, H.S. Lee, et al., Regulation of heart function by endogenous gaseous mediators-crosstalk between nitric oxide and hydrogen sulfide, Antioxid. Redox Signal. 14 (2011) 2081–2091.
[17] C. Coletta, A. Papapetropoulos, K. Erdelyi, G. Olah, K. Modis, P. Panopoulos, et al., Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 9161–9166.
[18] M.R. Filipovic, J. Miljkovic, T. Nauser, M. Royzen, K. Klos, T. Shubina, et al., Chemical characterization of the smallest S-nitrosothiol, HSNO; cellular cross-talk of H_2S and S-nitrosothiols, J. Am. Chem. Soc. 134 (2012) 12016–12027.
[19] M.M. Cortese-Krott, B.O. Fernandez, J.L. Santos, E. Mergia, M. Grman, P. Nagy, et al., Nitrosopersulfide ($SSNO^-$) accounts for sustained NO bioactivity of S-nitrosothiols following reaction with sulfide, Redox Biol. 2 (2014) 234–244.
[20] J.O. Lundberg, M.T. Gladwin, A. Ahluwalia, N. Benjamin, N.S. Bryan, A. Butler, et al., Nitrate and nitrite in biology, nutrition and therapeutics, Nat. Chem. Biol. 5 (2009) 865–869.
[21] N.S. Bryan, B.O. Fernandez, S.M. Bauer, M.F. Garcia-Saura, A.B. Milsom, T. Rassaf, et al., Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues, Nat. Chem. Biol. 1 (2005) 290–297.
[22] E.E. van Faassen, S. Bahrami, M. Feelisch, N. Hogg, M. Kelm, D.B. Kim-Shapiro, et al., Nitrite as regulator of hypoxic signaling in mammalian physiology, Med. Res. Rev. 29 (2009) 683–741.
[23] E.A. Jansson, L. Huang, R. Malkey, M. Govoni, C. Nihlen, A. Olsson, et al., A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis, Nat. Chem. Biol. 4 (2008) 411–417.
[24] F. Bernheim, M. Dixon, The reduction of nitrates in animal tissues, Biochem. J. 22 (1928) 125–134.
[25] A.R. Butler, M. Feelisch, Therapeutic uses of inorganic nitrite and nitrate: from the past to the future, Circulation 117 (2008) 2151–2159.
[26] J.M. Fukuto, S.J. Carrington, D.J. Tantillo, J.G. Harrison, L.J. Ignarro, B.A. Freeman, et al., Small molecule signaling agents: the integrated chemistry and biochemistry of nitrogen oxides, oxides of carbon, dioxygen, hydrogen sulfide, and their derived species, Chem. Res. Toxicol. 25 (2012) 769–793.
[27] G.K. Kolluru, X. Shen, C.G. Kevil, A tale of two gases: NO and H_2S, foes or friends for life?, Redox Biol. 1 (2013) 313–318.
[28] B.S. King, Potential biological chemistry of hydrogen sulfide (H_2S) with the nitrogen oxides, Free Radic. Biol. Med. 55 (2013) 1–7.
[29] M.L. Lo Faro, B. Fox, J.L. Whatmore, P.G. Winyard, M. Whiteman, Hydrogen sulfide and nitric oxide interactions in inflammation, Nitric Oxide 41 (2014) 38–47.
[30] J. Miljkovic, I. Kenkel, I. Ivanovic-Burmazovic, M.R. Filipovic, Generation of HNO and HSNO from nitrite by heme-iron-catalyzed metabolism with H_2S, Angew. Chem. Int. Ed Engl. 52 (2013) 12061–12064.
[31] L. Grossi, Hydrogen sulfide induces nitric oxide release from nitrite, Bioorg. Med. Chem. Lett. 19 (2009) 6092–6094.
[32] K.M. Kaster, A. Grigoriyan, G. Jenneman, G. Voordouw, Effect of nitrate and nitrite on sulfide production by two thermophilic, sulfate-reducing enrichments from an oil field in the North Sea, Appl. Microbiol. Biotechnol. 75 (2007) 195–203.
[33] H.H. Awad, D.M. Stanbury, Autoxidation of NO in aqueous solution, Int. J. Chem. Kin. 25 (1993) 375–381.
[34] S. Shiva, X. Wang, L.A. Ringwood, X. Xu, S. Yuditskaya, V. Annavajjhala, et al., Ceruloplasmin is a NO oxidase and nitrite synthase that determines endocrine NO homeostasis, Nat. Chem. Biol. 2 (2006) 486–493.
[35] S. Moncada, R.M. Palmer, E.A. Higgs, Nitric oxide: physiology, pathophysiology, and pharmacology, Pharmacol. Rev. 43 (1991) 109–142.
[36] X. Liu, M.J. Miller, M.S. Joshi, D.D. Thomas, J.R. Lancaster, Accelerated reaction of nitric oxide with O_2 within the hydrophobic interior of biological membranes, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 2175–2179.
[37] M.N. Moller, Q. Li, D.A. Vitturi, J.M. Robinson, J.R. Lancaster Jr., A. Denicola, Membrane "lens" effect: focusing the formation of reactive nitrogen oxides from the $^*NO/O_2$ reaction, Chem. Res. Toxicol. 20 (2007) 709–714.
[38] P. Wardman, Fluorescent and luminescent probes for measurement of oxidative and nitrosative species in cells and tissues: progress, pitfalls, and prospects, Free Radic. Biol. Med. 43 (2007) 995–1022.
[39] G.Y. Markovits, S.E. Schwartz, L. Newman, Hydrolysis equilibrium of dinitrogen trioxide in dilute acid solution, Inorg. Chem. 20 (1981) 445–450.
[40] R.G. Pearson, H.R. Sobel, J. Songstad, Nucleophilic reactivity constants toward methyl iodide and trans-dichlorodi(pyridine)platinum(II), J. Am. Chem. Soc. 90 (1968) 319–326.
[41] K.H. Becker, J. Kleffmann, R. Kurtenbach, P. Wiesen, Solubility of nitrous acid (HONO) in sulfuric acid solutions, J. Phys. Chem. 100 (1996) 14984–14990.
[42] C.D. Ritchie, Nucleophilic reactivities toward cations, Acc. Chem. Res. 5 (1972) 348–354.
[43] J.I. Toohey, Sulphane sulphur in biological systems: a possible regulatory role, Biochem. J. 264 (1989) 625–632.
[44] J.I. Toohey, Sulfur signaling: is the agent sulfide or sulfane?, Anal. Biochem. 413 (2011) 1–7.
[45] J.I. Toohey, A.J. Cooper, Thiosulfoxide (sulfane) sulfur: new chemistry and new regulatory roles in biology, Molecules 19 (2014) 12789–12813.
[46] J. Zhu, K. Petit, A. Colson, S. DeBolt, M. Sevilla, Reactions of sulfhydryl and sulfide radicals with oxygen, hydrogen sulfide, hydrosulfide, and sulfide: formation of $SO_2^{\bullet-}$, $HSSH^{\bullet-}$, $HSS^{\bullet 2-}$and $HSS^{\bullet}$, J. Phys. Chem. 95 (1991) 3676–3681.
[47] G. Schwarzenbach, A. Fischer, Die Acidität der Sulfane und die Zusammensetzung wässeriger Polysulfidlösungen, Helv. Chim. Acta 43 (1960) 1365–1390.
[48] A. Kamyshny Jr., J. Gun, D. Rizkov, T. Voitsekovski, O. Lev, Equilibrium distribution of polysulfide ions in aqueous solutions at different temperatures by rapid single phase derivatization, Environ. Sci. Technol. 41 (2007) 2395–2400.
[49] E. Divers, T. Haga, V. – Reduction of nitrites to hydroxylamine by hydrogen sulphide, J. Chem. Soc. Trans. 51 (1887) 48–51.
[50] L.S. Bagster, CCCXLVI. – The reaction between nitrous acid and hydrogen sulphide, J. Chem. Soc. (1928) 2631–2643.
[51] D.L.H. Williams, Nitrosation reactions and the chemistry of nitric oxide, Elsevier, Amsterdam, 2004.
[52] M. Goehring, H.W. Kaloumenos, J. Messner, Zur Kenntnis der schwefligen Säure. II. Über Das Amid der Imidodisulfinsäure, Z. Anorg. Allg. Chem. 264 (1951) 48–53.
[53] M. Goehring, J. Messner, Zur Kenntnis der schwefligen Säure. III Das Sulfinimid und seine Isomeren, Z. Anorg. Allg. Chem. 268 (1952) 47–56.
[54] P.W. Schenk, Über das Thionylimid SONH, Ber. Dtsch. Chem. Ges. 75 (1942) 94–99.
[55] R.P. Müller, M. Nonella, J.R. Huber, Spectroscopic investigation of HSNO in a low temperature matrix. UV, VIS, and IR-induced isomerisations, Chem. Phys. 87 (1984) 351–361.
[56] M. Nonella, R. Huber, T.-K. Ha, Photolytic preparation and isomerization of HNSO, HOSN, HSNO, and HONS in an Argon matrix. An experimental and theoretical study, J. Phys. Chem. 91 (1987) 5203–5209.
[57] C.-H. Lai, E.Y. Li, P.-T. Chou, Isomerization reactions of RSNO (R = H, C n H2 n+ 1 n ≤ 4), Theor. Chem. Acc. 117 (2007) 145–152.
[58] F. Seel, R. Kuhn, G. Simon, M. Wagner, B. Krebs, M. Dartmann, PNP-Perthionitrit und PNP-Monothionitrit, Z. Naturforsch. B 40 (1985) 1607–1617.
[59] F. Seel, M. Wagner, Reaction of sulfides with nitrogen monoxide in aqueous solution, Z. Anorg. Allg. Chem 558 (1988) 189–192.
[60] S. Singh, J. Wishnok, M. Keshive, W. Deen, S. Tannenbaum, The chemistry of the S-nitrosoglutathione/glutathione system, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 14428–14433.
[61] A.P. Dicks, E. Li, A.P. Munro, H.R. Swift, D.L.H. Williams, The reaction of S-nitrosothiols with thiols at high thiol concentration, Can. J. Chem. 76 (1998) 789–794.
[62] T. Komiyama, K. Fujimori, Kinetic studies of the reaction of S-nitroso-L-cysteine with L-cysteine, Bioorg. Med. Chem. Lett. 7 (1997) 175–180.
[63] K.R. Olson, A practical look at the chemistry and biology of hydrogen sulfide, Antioxid. Redox Signal 17 (2012) 32–44.
[64] R. Steudel, Mechanism for the formation of elemental sulfur from aqueous sulfide in chemical and microbiological desulfurization processes, Ind. Eng. Chem. Res 35 (1996) 1417–1423.
[65] A.R. Butler, Formation of Meisenheimer–Jackson complexes in a non-polar solvent, J. Chem. Soc. Perkin Trans 1 (1975) 1557–1558.
[66] M. Schmidt, R. Wägerle, Umsetzung von Schwefel mit Nitrit. Über Säuren des Schwefels, XIII, Angew. Chem 70 (1958) 594–595.
[67] M. Schmidt, R.R. Wägerle, Über die Reaktion von elementarem Schwefel mit Natriumarsenit (im Vergleich mit Nitrit und Phosphit), Z. Anorg. Allg. Chem 330 (1964) 48–58.
[68] F. Seel, G. Simon, J. Schuh, M. Wagner, B. Wolf, I. Ruppert, et al., Untersuchung der Umsetzung von Schwefel mit Natriumnitrit in DMF, DMSO und HMPT, Z. Anorg. Allg. Chem 538 (1986) 177–190.
[69] F. Seel, M. Wagner, The reaction of polysulfides with nitrogen monoxide in non-acqueous solvents- nitrosodisulfides, Z. Naturforsch 40 (1985) 762.
[70] S.P. Singh, J.S. Wishnok, M. Keshive, W.M. Deen, S.R. Tannenbaum, The chemistry of the S-nitrosoglutathione/glutathione system, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 14428–14433.
[71] A.P. Munro, D.L.H. Williams, Reactivity of sulfur nucleophiles towards S-nitrosothiols, J. Chem. Soc. Perkin Trans 2 (2000) 1794–1797.
[72] P. Nagy, Z. Palinkas, A. Nagy, B. Budai, I. Toth, A. Vasas, Chemical aspects of hydrogen sulfide measurements in physiological samples, Biochim. Biophys. Acta 2014 (1840) 876–891.
[73] M. Feelisch, T. Rassaf, S. Mnaimneh, N. Singh, N.S. Bryan, D. Jourd'Heuil, et al., Concomitant S-, N-, and heme-nitros(yl)ation in biological tissues and fluids: implications for the fate of NO in vivo, FASEB J. 16 (2002) 1775–1785.
[74] J. Rodriguez, R.E. Maloney, T. Rassaf, N.S. Bryan, M. Feelisch, Chemical nature of nitric oxide storage forms in rat vascular tissue, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 336–341.
[75] T. Rassaf, M. Preik, P. Kleinbongard, T. Lauer, C. Heiss, B.E. Strauer, et al., Evidence for in vivo transport of bioactive nitric oxide in human plasma, J. Clin. Invest 109 (2002) 1241–1248.
[76] T. Rassaf, M. Feelisch, M. Kelm, Circulating NO pool: assessment of nitrite and nitroso species in blood and tissues, Free Radic. Biol. Med 36 (2004) 413–422.
[77] X. Shen, E.A. Peter, S. Bir, R. Wang, C.G. Kevil, Analytical measurement of discrete hydrogen sulfide pools in biological specimens, Free Radic. Biol. Med 52 (2012) 2276–2283.
[78] N.S. Bryan, T. Rassaf, R.E. Maloney, C.M. Rodriguez, F. Saijo, J.R. Rodriguez, et al., Cellular targets and mechanisms of nitros(yl)ation: an insight into their nature and kinetics in vivo, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 4308–4313.

[79] M.D. Levitt, M.S. Abdel-Rehim, J. Furne, Free and acid-labile hydrogen sulfide concentrations in mouse tissues: anomalously high free hydrogen sulfide in aortic tissue, Antioxid. Redox Signal 15 (2011) 373–378.
[80] K. Cosby, K.S. Partovi, J.H. Crawford, R.P. Patel, C.D. Reiter, S. Martyr, et al., Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation, Nat. Med 9 (2003) 1498–1505.
[81] E. Nagababu, S. Ramasamy, R. Albernethy, M. Rifkind, Active nitric oxide produced in the red cell under hypoxic conditions by deoxyhemoglobin-mediated nitrite reduction, J. Biol. Chem 278 (2003) 46349–46356.
[82] M. Totzeck, U.B. Hendgen-Cotta, P. Luedike, M. Berenbrink, J.P. Klare, H.J. Steinhoff, et al., Nitrite regulates hypoxic vasodilation via myoglobin-dependent nitric oxide generation, Circulation 126 (2012) 325–334.
[83] A. Webb, A. Milsom, K. Rathod, W. Chu, S. Qureshi, M. Lovell, et al., Mechanisms underlying erythrocyte and endothelial nitrite reduction to nitric oxide in hypoxia: role for xanthine oxidoreductase and endothelial nitric oxide synthase, Circ. Res 103 (2008) 957–964.
[84] J.L. Zweier, P. Wang, A. Samouilov, P. Kuppusamy, Enzyme-independent formation of nitric oxide in biological tissues, Nat. Med 1 (1995) 804–809.
[85] R. Aamand, T. Dalsgaard, F.B. Jensen, U. Simonsen, A. Roepstorff, A. Fago, Generation of nitric oxide from nitrite by carbonic anhydrase: a possible link between metabolic activity and vasodilation, Am. J. Physiol. Heart Circ. Physiol 297 (2009) H2068–H2074.
[86] R.P. Patel, N. Hogg, D.B. Kim-Shapiro, The potential role of the red blood cell in nitrite-dependent regulation of blood flow, Cardiovasc. Res 89 (2011) 507–515.
[87] B.Y. Owusu, R. Stapley, R.P. Patel, Nitric oxide formation versus scavenging: the red blood cell balancing act, J. Physiol 590 (2012) 4993–5000.
[88] A.R. Butler, J.H. Ridd, Formation of nitric oxide from nitrous acid in ischemic tissue and skin, Nitric Oxide 10 (2004) 20–24.
[89] A.J. Elliot, D.R. McCracken, G.V. Buxton, N.D. Wood, Estimation of rate constants for near-diffusion-controlled reactions in water at high temperatures, J. Chem. Soc. Faraday Trans 86 (1990) 1539–1547.
[90] S. Basu, R. Grubina, J. Huang, J. Conradie, Z. Huang, A. Jeffers, et al., Catalytic generation of N_2O_3 by the concerted nitrite reductase and anhydrase activity of hemoglobin, Nat. Chem. Biol 3 (2007) 785–794.
[91] J.S. Stamler, O. Jaraki, J. Osborne, D.I. Simon, J. Keaney, J. Vita, et al., Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 7674–7677.
[92] D.L.H. Williams, The chemistry of S-nitrosothiols, Acc. Chem. Res 32 (1999) 869–876.
[93] K.A. Broniowska, N. Hogg, The chemical biology of S-nitrosothiols, Antioxid. Redox Signal 17 (2012) 969–980.
[94] L. Liu, A. Hausladen, M. Zeng, L. Que, J. Heitman, J.S. Stamler, A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans, Nature 410 (2001) 490–494.
[95] S. Oae, K. Shinhama, Y.H. Kim, Direct conversion of arylamines to the halides by deamination with thionitrite or related compounds and anhydrous copper (II) halides, Bull. Chem. Soc. Jpn 53 (1980) 1065–1069.
[96] D.J. Barnett, J. McAninly, D.L.H. Williams, Transnitrosation between nitrosothiols and thiols, J. Chem. Soc. Perkin Trans 2 (1994) 1131–1133.
[97] D.J. Barnett, A. Rios, D.L.H. Williams, NO-group transfer (transnitrosation) between S-nitrosothiols and thiols. Part 2, J. Chem. Soc. Perkin Trans 2 (1995) 1279–1282.
[98] M. Feelisch, B.O. Fernandez, N.S. Bryan, M.F. Garcia-Saura, S. Bauer, D.R. Whitlock, et al., Tissue processing of nitrite in hypoxia an intricate interplay of nitric oxide-generating and-scavenging systems, J. Biol. Chem 283 (2008) 33927–33934.
[99] K.R. Olson, N.L. Whitfield, Hydrogen sulfide and oxygen sensing in the cardiovascular system, Antioxid. Redox Signal 12 (2010) 1219–1234.
[100] K.R. Olson, Hydrogen sulfide as an oxygen sensor, Clin. Chem. Lab. Med 51 (2013) 623–632.
[101] T. Mitsui, T. Kondo, Increased breath nitrous oxide after ingesting nitrate in patients with atrophic gastritis and partial gastrectomy, Clin. Chim. Acta 345 (2004) 129–133.
[102] J.R. Hickok, S. Sahni, H. Shen, A. Arvind, C. Antoniou, L.W. Fung, et al., Dinitrosyliron complexes are the most abundant nitric oxide-derived cellular adduct: biological parameters of assembly and disappearance, Free Radic. Biol. Med 51 (2011) 1558–1566.
[103] A.F. Vanin, Dinitrosyl iron complexes with thiolate ligands: physico-chemistry, biochemistry and physiology, Nitric Oxide 21 (2009) 1–13.
[104] C.A. Bosworth, J.C. Toledo Jr., J.W. Zmijewski, Q. Li Jr., J.R. Lancaster, Dinitrosyliron complexes and the mechanism(s) of cellular protein nitrosothiol formation from nitric oxide, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 4671–4676.
[105] M. Boese, P.I. Mordvintcev, A.F. Vanin, R. Busse, A. Mulsch, S-nitrosation of serum albumin by dinitrosyl-iron complex, J. Biol. Chem 270 (1995) 29244–29249.