Naturwissenschaften (2000) 87:360–362

SHORT COMMUNICATION

Klaus-Peter Zauner · Michael Conrad

Enzymatic pattern processing

Received: 3 April 2000 / Accepted in revised form: 16 June 2000

Abstract A table-top prototype has been constructed that uses the enzyme malate dehydrogenase to recognize input signal patterns. The device is controlled by the enzyme in response to injection of Mg^{2+} used as a signaling substance. Output is monitored spectroscopically. If Mg^{2+} is injected along either of two signal lines (i.e., if the input signal pattern is 10 or 01) the device emits an output of 1. Injection along neither or both lines results in an output of 0. The enzyme in effect is used as a transform that converts the linearly inseparable exclusive-or problem into a linearly separable problem.

Enzymes are molecular pattern recognizers that respond to specific features of a complex chemical milieu. The simplest example of a recognition task that cannot be performed by a single layer network of summing devices, such as threshold neurons, is the exclusive-or (XOR) operation (Minsky and Papert 1969; Ellacott and Bose 1996). This requires that the device give a 1 output in response to binary signal inputs 01 and 10 and a 0 output to 00 and 11 input patterns. Whether actual neurons can perform tasks of this type, as opposed to responding to an average field, is an open question (Koch 1997). Simulation studies of abstract reaction schemes have shown that the XOR could conceivably be accomplished by a single enzyme (Arkin and Ross 1994). The issue is pertinent to the potential role of enzymes in computational devices (Conrad 1985, 1990) and of possible relevance to their role in cellular information processing (Bray 1995). Here we describe a table-top signal processing module that utilizes mitochondrial malate dehydrogenase (mMDH) for two variable pattern grouping tasks and show that this prototype can perform the XOR operation in response to Mg^{2+} used as a signal carrier.

The XOR operation is linearly inseparable, corresponding to the fact that input patterns cannot be separated into the output categories by a single threshold. This is in contrast to summing operations such as AND and OR. An element whose response is linear apart from a single threshold cannot perform the XOR, since it would be necessary for it to fire when the strength of the combined inputs exceeds a lower threshold and not fire when it exceeds a higher threshold. If the response of the element is nonmonotonic then it is possible to eliminate the need for the higher threshold and therefore to convert the linearly inseparable pattern recognition problem to a linearly separable problem. For an enzyme to perform this transformation it must exhibit nonmonotonic behavior.

Malate deyhydrogenase catalyzes the reaction of L-malate and NAD^+ to oxalacetate, NADH, and a proton. A nonmonotonic response of mMDH to phosphate ions has been demonstrated for the reverse reaction (Kun et al. 1967). Mg^{2+} has been reported to have an activating effect on the forward reaction in blood serum (Wong and Smith 1976; Smith 1983). High ionic strength is reported to have a suppressive effect on mMDH (Bracht and de P. Campello 1979). We worked with the forward reaction, favored by high pH. The reaction was monitored by spectroscopic detection of NADH at 339 nm. MDH has been one of the most intensively studied enzymes so far as response to milieu conditions and kinetic mechanisms are concerned (Wolfe and Raval 1970; DuVal et al. 1985; Gelpí et al. 1992). However, our purpose here is not to investigate the mechanism but rather to utilize the phenomenology. A response surface approach is therefore appropriate (Box and Draper 1987).

Figure 1 shows the absorbance at 120 s after the start of the reaction for various concentrations of Mg^{2+} and Ca^{2+}. The surface is convex along both the Mg^{2+} and Ca^{2+} axes. Thus a nonmonotonic response occurs when either of these ions is used as a signaling sub-

K.-P. Zauner · M. Conrad (✉)
Department of Computer Science, Wayne State University, Detroit, MI 48202, USA
e-mail: biocomputing@cs.wayne.edu

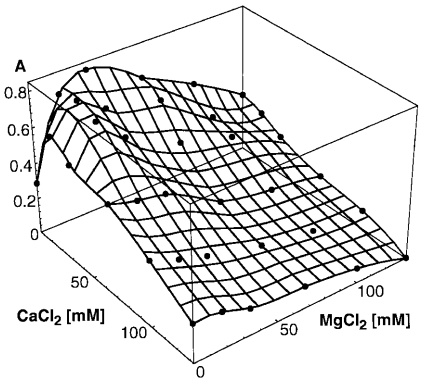

Fig. 1 Mg^{2+}/Ca^{2+} response surface of porcine heart mMDH. The convex character of the surface is due to the nonmonotonic dependence of reaction speed on ion concentrations. *Dots* represent absorbance (*A*) at 339 nm for a time 120 s after start of the reaction. The surface is obtained by interpolation. The reaction medium contained 4.8 mM L-malic acid, 1.8 mM NAD^+, and 13.2 mM MOPS (3-[*N*-morpholino]propanesulfonic acid, from the enzyme/NAD solution) and was buffered by 92 mM glycine adjusted to pH 10 with NaOH

stance. This response was observed only for malate concentrations that were low in comparison with reported assay protocols (Englard and Siegel 1969; Mullinax et al. 1982). Figure 2 shows the logic operations that can be obtained at different ion concentrations. One ion, say Mg^{2+}, can be used as the input signal and the second can be used as a modulator that changes the logic operation performed. The Mg^{2+} concentration of

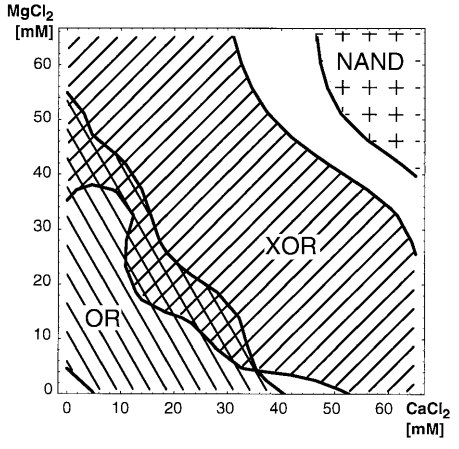

Fig. 2 Two variable groupings (logic functions) implicit in the mMDH response surface (Fig. 1). Every point on the plane corresponds to a combination of Mg^{2+} and Ca^{2+} used to represent a 1-signal; 0-signals are represented by the absence of these ions. The response of the enzyme groups the different possible input signal patterns. Zones corresponding to common logic functions were computed from the response surface by assuming that a minimum separation in absorbance values of 0.17 is required to distinguish two output states. If a smaller separation were allowed, the size of the zones would increase. Where zones overlap the choice of threshold determines which function is performed. NAND is the negation of an AND gate, OR is inclusive-or, and XOR is exclusive-or

interest is two orders of magnitude higher than typical physiological concentrations (Heaton 1993). The high pH conditions under which the reaction was run are also nonphysiological. For our purposes it has the advantage that the signal solutions are more stable (Ochoa 1955).

The response surface described above shows that it is possible to use mMDH to perform an XOR. We developed a table-top signal processing system to implement this operation in a device-like context and more generally to explore the possibilities for enzymatic pattern grouping from a computational point of view. Five glass capillaries were sealed into a spectrophotometer cuvette (1 cm light path). Two of the capillaries were used for injecting the signals and one for injecting enzyme/NAD solution. Three small pumps, each comprising a syringe and two valves, were used for injection. The remaining two capillaries (connected to a peristaltic pump) served to clear and wash the curvette. Signals were injected in two 0.8 ml portions, taken in any of four possible combinations from solutions representing 0 and 1. Both solutions contained 7.1 mM L-malate (in 114 mM glycine-NaOH at pH 10.5). The 1-solution in addition contained $MgCl_2$ (190 mM) used as signal. This quantity was chosen so that the absorbance resulting from 01 or 10 inputs is maximally separated from the absorbances produced by 00 and 11.

The reaction is initiated by injecting 0.5 ml enzyme/NAD buffered in 0.1 M MOPS at pH 7.4. The reaction medium thus contained 5.4 mM L-malate, 1.3 mM NAD^+, 24 mM MOPS, and 87 mM glycine. The input signals were represented in the reaction medium by the concentration of $MgCl_2$. The 00 input was represented by the absence of $MgCl_2$; the 01 and 10 signal patterns both corresponded to a $MgCl_2$ concentration of 72 mM; the 11 input was represented by 144 mM $MgCl_2$. Note that this encoding scheme associates a substance with a state of a signal, but does not differentiate between different signal lines. This is sufficient for implementing the commonly used logic gates, since these are all commutative. To implement noncommutative functions, such as implication, it is necessary to associate different substances with different signal lines to represent the order of the operands.

Switches connected to the syringe used for injection of enzyme/NAD solution send a start signal to the computer that controls the spectrophotometer. An absorbance measurement at 339 nm is taken at a fixed time after the start of the reaction. The accumulation of NADH serves as the output signal.

The performance of the device is exemplified by the classification of a series of 135 consecutive signal patterns arriving in random order (45 corresponding to 00, 46 to 01/10, and 44 to 11). The time from the start of the reaction to measurement of output was 10 s. All patterns were classified correctly. The mean absorbance for the 00 signal was 1.02, with a sample standard deviation (SD) of 0.01; for the 01/10 case the mean absorbance was 1.40 (SD = 0.06); and for 11 the mean was

1.07 (SD = 0.06). Extensive experimentation with variations of the apparatus confirm this level of performance. The 10-s response is close to what can be achieved with our instrumentation and is not an inherent limitation. The setup provides a convenient means for exploring the sensitivity of enzymes to chemical context. The methodology can be used to investigate the signal processing capabilities of multi-enzyme systems.

The above result shows that enzymes can be employed to perform pattern recognition tasks that cannot be realized with linear components. The enzyme is, in effect, a network that integrates milieu signals through conformational interactions. Networks of electronic elements would be required to duplicate this signal processing capability. Of great practical relevance is the fact that it is easy to replicate enzymes and therefore to precisely replicate their nonlinear characteristics. Nonlinear conformational interactions are expensive to simulate on a digital computer. For the purpose of utilizing enzymes as pattern processing elements they constitute a computational resource.

Acknowledgements This material is based upon work supported by the US National Science Foundation under Grant Nos. ECS-9704190 and CCR-9610054. The authors thank F. Hong, H. Martin, and C. Prothmann for helpful comments.

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