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Oscillatory reaction of alcohol dehydrogenase in an oil / water system

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With the use of an oil/water system, oscillatory reactions of an enzyme have been demonstrated. This reaction system has been conceived as an example of the metabolic oscillations of living cells. When a substrate (ethanol) in the oil phase of toluene or chloroform slowly migrated into the aqueous phase containing alcohol dehydrogenase and NAD$^+$, oscillations were observed in the concentration of NADH produced. The gradual entry of substrate into the aqueous phase was essential for the oscillatory reactions to occur. A possible mechanism to account for the appearance of oscillatory reactions of enzymes is proposed, which differs from that presented previously.

1. Introduction

Glycolysis is a phenomenon of the greatest importance for the energetics of living cells. This process provides the first example in biology where the role of temporal dissipative structures has been unambiguously recognized. Many reconstituted glycolytic systems have been studied and these investigations have been reviewed by Hess and Boiteux [1]. Glycolytic oscillations are the best known example of metabolic oscillations. Nicolis and Prigogine [2] described the features of this type of oscillation as follows: (1) oscillation in the concentrations of all metabolites is observed in the case of slow injections of glycolytic substrate and (2) the period of such oscillation is of the order of a minute and depends on temperature. The concentrations of metabolites undergoing oscillation were found to vary periodically within the range 10^{-5}–10^{-3} M.

In the present investigation, a reaction system was constructed consisting of an oil/water phase as a model of a living cell and attempts were made to observe a dissipative structure in the enzyme-catalyzed reaction.

2. Materials and methods

The enzyme, coenzyme and substrate used in this study were alcohol dehydrogenase, β-nicotinamide adenine dinucleotide (NAD$^+$) and ethanol. Alcohol dehydrogenase from yeast (ADH-1) was purchased from Boehringer Mannheim GmbH, NADH$^+$ from Orient Yeast Co. and ethanol from Wako Pure Chemical Co. All reagents were used without further purification. The pH of aqueous solutions was adjusted to 7.6 with Tris-HCl.

The absorbance was determined in 1 × 1 × 4 cm quartz cuvettes mounted in a brass block which was thermally controlled by circulating water at constant temperature. When toluene was used as the oil solvent, 1 ml of toluene containing ethanol

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was layered onto an aqueous solution containing alcohol dehydrogenase and NAD$^+$ in the cuvette. In the case of chloroform as the oil solvent, 2 ml aqueous solution was layered onto the 1 ml chloroform solution containing ethanol. After masking the oil phase, the absorbance of the aqueous solution at a wavelength of 340 nm was recorded at intervals of 20 s using a Shimadzu UV-160 spectrophotometer.

All experiments were carried out at 37.0 ± 0.2° C.

3. Results

Fig. 1 shows the time dependence of the absorbance at 340 nm for an aqueous phase consisting of alcohol dehydrogenase and 1 mM NAD$^+$ onto which 1 ml toluene solution containing 0.2 ml ethanol had been layered. Evidently, at concentrations of alcohol dehydrogenase greater than 1.0 mg/ml, oscillation in the absorbance continued for about 20 min after layering the toluene solution onto the aqueous solution. The period of the oscillation was 2–3 min and the amplitude was of the order of 10^{-3} mM as the concentration of NADH. After this oscillation, the reaction proceeded monotonically in the direction of increasing NADH concentration. When the concentration of alcohol dehydrogenase was increased further at fixed concentrations of NAD$^+$ and ethanol, oscillation occurred up to 2 mg/ml alcohol dehydrogenase as shown in fig. 2. In contrast, oscillation, being reduced in extent by decreasing enzyme concentration, was observed even in 0.5 mg/ml alcohol dehydrogenase.

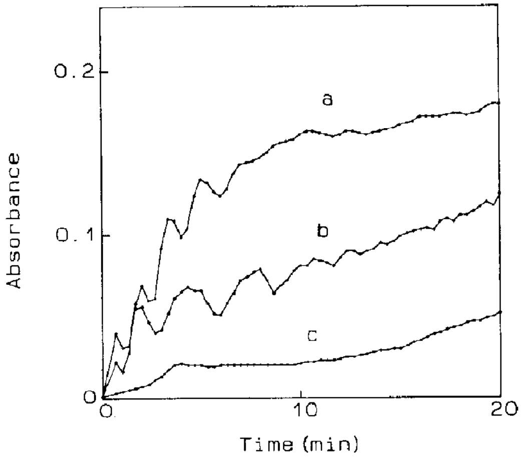

Fig. 1. Change in absorbance at 340 nm as a function of time for [NAD$^+$] = 1 mM and [ethanol] = 0.2 ml/ml in the toluene/water system. Concentration of alcohol dehydrogenase: 1.1 mg/ml (a), 1.0 mg/ml (b) and 0.9 mg/ml (c).

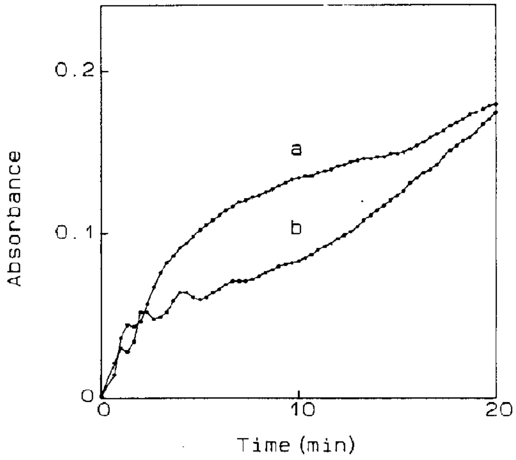

Fig. 2. Change in absorbance at 340 nm in the toluene/water system. The initial concentrations of NAD$^+$ and ethanol were the same as those given in fig. 1. Concentration of alcohol dehydrogenase: 2.0 mg/ml (a) and 1.5 mg/ml (b).

One of the characteristic features of such oscillatory reactions is that the period of oscillation increases in duration with time.

When the concentrations of both alcohol dehydrogenase and NAD$^+$ were lowered whilst maintaining the same ratio of [alcohol dehydrogenase] to [NAD$^+$] as that for trace b in fig. 1, oscillation was also found to occur. However, the concentration of alcohol dehydrogenase required to induce the oscillation was found to be within a narrow range in the case of lower [NAD$^+$]. For example, in the case where the concentrations of NAD$^+$ and ethanol were fixed at 0.7 mM and 0.2 ml/ml, respectively, the range of concentrations of alcohol dehydrogenase for which oscillation was observed covered the region from 0.5 to 0.7 mg/ml. In the case of [NAD$^+$] = 0.4 mM and [ethanol] = 0.2 ml/ml, oscillation was clearly observed at concentrations of alcohol dehydrogenase between 0.2 and 0.4 mg/ml. For the region of [alcohol

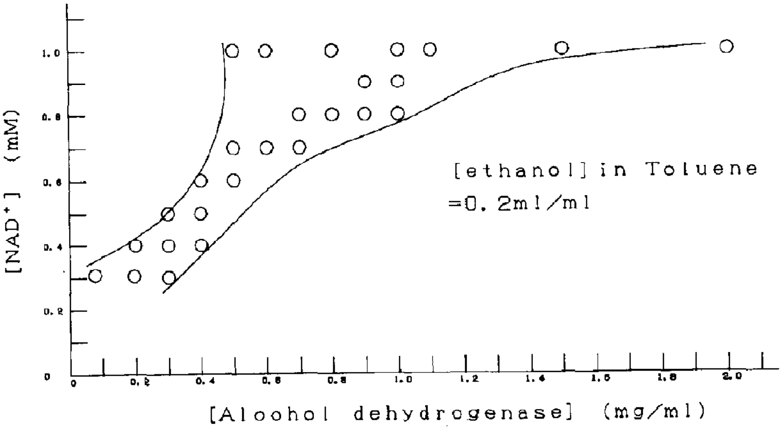

Fig. 3. Phase diagrams in the toluene/water system.

dehydrogenase] = 0.007–0.2 mg/ml, oscillation occurred in [NAD$^+$] = 0.3 mM.

Summarizing these results, we can construct a phase diagram for the oscillatory reaction as shown in fig. 3.

When chloroform was used as the oil solvent, the monotonic increase in NADH concentration was observed at the onset of the reaction, the oscillation appearing later with a period longer than that observed in the case of toluene and continuing for a greater length of time. In the case shown in fig. 4, oscillation took place for more than 3 h. Such regular oscillation could be observed even for a lower concentration of enzyme compared with that in toluene solution when equal concentrations of NAD$^+$ were present in both solutions. In chloroform solutions, oscillation occurred over a wide range of concentrations of enzyme and NAD$^+$, and displayed a longer oscillatory period (about 10 min in the case shown in fig. 5) with amplitudes of the order of 10^{-2} mM NADH. On lowering the concentration of alcohol dehydrogenase, oscillation with a long period and demonstrating a sharp peak was found to result.

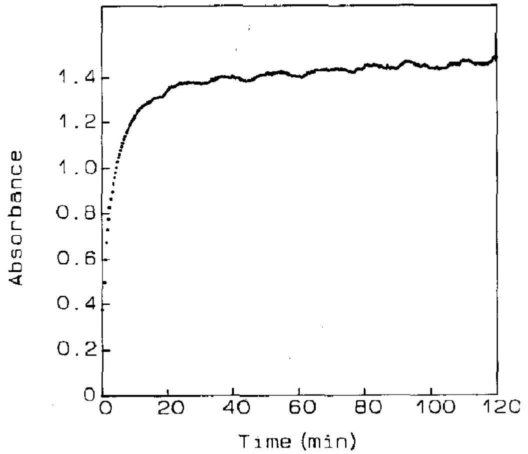

Fig. 4. Change in absorbance at 340 nm in the water/chloroform system: [alcohol dehydrogenase] = 0.1 mg/ml, [NAD$^+$] = 0.3 mM and [ethanol] = 0.2 ml/ml.

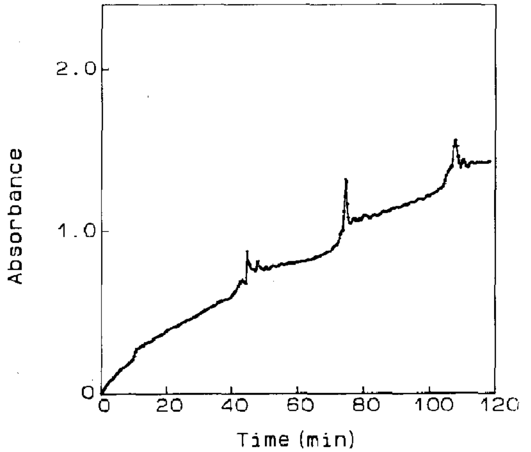

Fig. 5. Change in absorbance at 340 nm in the water/chloroform system: [alcohol dehydrogenase] ≈ 0.003 mg/ml, [NAD$^+$] = 0.5 mM and [ethanol] = 0.3 ml/ml.

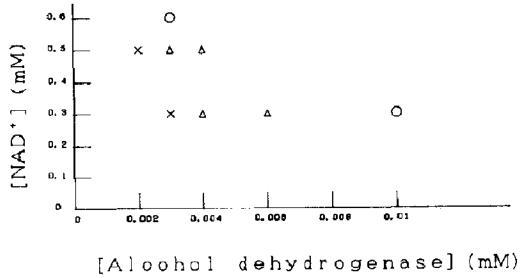

Fig. 6. Phase diagrams in the chloroform/water system [ethanol] in chloroform = 0.2 ml/ml (○), 0.3 ml/ml (△) and 0.4 ml/ml (×).

In order to induce such oscillation, an appropriate concentration of ethanol should be chosen. For initial concentrations of enzyme and NAD$^+$ of 0.003 mg/ml and 0.3 mM, respectively, regular oscillation was displayed at 0.3 ml/ml ethanol, decreasing enzyme concentration leading to the cessation of oscillation at fixed ethanol concentration. In the case of 0.001 mg/ml enzyme and 0.5 mM NAD$^+$, 0.4 ml/ml ethanol was suitable for bringing about oscillation. Fig. 6 shows the phase diagram for the system of chloroform.

4. Discussion

When the concentration of alcohol dehydrogenase in the aqueous solution was measured using the Biorad method, the concentration of enzyme in the bulk remained unchanged both before and after layering the toluene solution. Since the enzyme does not aggregate at the interface, the reaction observed in the present study is considered to take place in the bulk.

Using suspensions of yeast cells, Betz and Chance [3] were successful in demonstrating glycolytic oscillations using the well-known allosteric model which has been frequently employed in the interpretation of metabolic oscillations.

The mechanism of enzyme-catalyzed reaction for alcohol dehydrogenase was proposed and elucidated by Theorell and Chance [4] as follows:

$$\mathrm{E} + \mathrm{A} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{EA} \tag{1}$$

$$\mathrm{EA} + \mathrm{B} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \mathrm{EQ} + \mathrm{P} \tag{2}$$

$$\mathrm{EQ} \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} \mathrm{E} + \mathrm{Q} \tag{3}$$

Here, E, A, B, P and Q represent enzyme, NAD$^+$, ethanol, acetaldehyde and NADH, respectively, and the k_j denote the rate constants for each reaction. Cleland [5] discussed the possibility that the mechanism for process 2 consisted of the following steps:

$$\mathrm{EA} + \mathrm{B} \rightleftarrows \mathrm{EAB} \rightleftarrows \mathrm{EPQ}$$

$$\mathrm{EPQ} \rightarrow \mathrm{EQ} + \mathrm{P}$$

The number of enzyme species participating in the oscillatory reaction investigated in the present paper was only one. Hence, oscillation does not occur in this mechanism, since the process of allosterism or autocatalysis does not exist. These results suggest that the mechanism differs from those presented previously for the oscillatory reaction.

The underlying cause of oscillation is considered to be the gradual migration of ethanol from the oil phase to the aqueous phase. Since the gradual entry of ethanol leads to a shortage of ethanol in the aqueous phase with time, the contribution of the reverse reaction due to the increase in the concentrations of the products, [P] and [Q], probably is predominant. Numerical analysis on the basis of this assumption will be carried out in the subsequent paper.

References

1 B. Hess and A. Boituex, Annu. Rev. Biochem. 40 (1971) 237.
2 G. Nicolis and I. Prigogine, Self-organization in nonequilibrium Systems (Wiley, New York, 1977) p. 354.
3 A. Betz and B. Chance, Arch. Biochem. Biophys. 109 (1964) 585.
4 H. Theorell and B. Chance, Acta Chim. Scand. 5 (1951) 1127.
5 W.W. Cleland, Biochim. Biophys. Acta 67 (1963) 104.