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Simulation of an oscillatory reaction of alcohol dehydrogenase in an oil/water system

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With numerical analysis of the enzyme-catalyzed reaction in the so-called 'ordered bi-bi' mechanism and the model presented by Theorell and Chance (Acta Chem. Scand. 5 (1951) 1127) for alcohol dehydrogenase, oscillations of the concentrations of coenzyme and intermediate complex were obtained on gradual entry of substrate. Neither autocatalysis nor allostery was included in the reaction mechanism. The features of the reaction were that the oscillations did not occur in a limit cycle and the interval became longer with time. In addition, it was found that the oscillations with an upwardly directed peak occurred at very low concentrations of enzyme in the model of Theorell and Chance. The results were consistent with the experimentally determined data reported previously.

1. Introduction

The oscillatory reaction of alcohol dehydrogenase in an oil/water system has been reported in the preceding paper [1]. It was shown that an increase in NADH concentration was accompanied by an increasing extent of oscillation, so that the oscillation was not considered to have a limit cycle. The causative factor of the oscillations appeared to differ from those presented previously, since the mechanism for alcohol dehydrogenase included neither allostery nor a process of autocatalysis. When alcohol exists in the bulk phase in the first place, oscillations do not occur naturally. Hence, the underlying cause of the oscillation was assumed to be the gradual migration of ethanol from the oil phase to the aqueous phase. Since such gradual entry might lead to a temporary shortage of ethanol in the aqueous phase, the contribution of the reverse reaction due to the increase in concentration of the products, acetoaldehyde and NADH, is probably dominant.

In order to confirm this assumption, in the present paper, a simulation of the oscillatory reaction has been performed by numerical analysis, through addition of the term for the gradual entry of substrate to the mechanism already presented for alcohol dehydrogenase and the other hydrogenase using NAD$^+$ as a coenzyme.

2. Model and calculation

Cleland [2] classified enzymes which react with two or more substrates and analyzed the enzyme reaction in the stationary state. Here, we treat two types, the ordered bi-bi reaction as termed by Cleland and the model presented by Theorell and Chance [3] for alcohol dehydrogenase.

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The mechanism for the ordered bi-bi enzyme reaction is as follows:

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA$$

$$EA + B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EAB \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EPQ \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} EQ + P$$

$$EQ \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} E + Q$$

where A and B denote substrates, P and Q products, E enzyme and EA, EAB, EPQ and EQ their intermediate complexes. In the case of dehydrogenase, NAD^+ is used as coenzyme and is denoted by A. The product NADH is represented by Q.

In contrast, the mechanism proposed by Theorell and Chance for alcohol dehydrogenase is as follows;

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA$$

$$EA + B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EQ + P$$

$$EQ \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + Q$$

Now, however, the ordered bi-bi mechanism is considered to be applicable for dehydrogenase, which includes alcohol dehydrogenase, using NAD^+ as coenzyme.

We assume that the substrate B enters stationarily the solution of enzyme E and coenzyme A at a rate k_0 and that the enzyme-catalyzed reaction occurs slowly. The rate equations for the concentration of each species in the ordered bi-bi reaction are as follows;

$$\frac{d[E]}{dt} = -k_1[E][A] + k_{-1}[EA] + k_5[EQ] - k_{-5}[E][P] \quad (1)$$

$$\frac{d[A]}{dt} = -k_1[E][A] - k_{-1}[EA] \quad (2)$$

$$\frac{d[EA]}{dt} = k_1[E][A] - k_{-1}[EA] - k_2[EA][B] + k_{-2}[EAB] \quad (3)$$

$$\frac{d[B]}{dt} = k_0 - k_2[EA][B] + k_{-2}[EAB] \quad (4)$$

$$\frac{d[EAB]}{dt} = k_2[EA][B] - k_{-2}[EAB] - k_3[EAB] + k_{-3}[EPQ] \quad (5)$$

$$\frac{d[EPQ]}{dt} = k_3[EAB] - (k_{-3} + k_4)[EAB] + k_{-4}[EQ][P] \quad (6)$$

$$\frac{d[EQ]}{dt} = k_4[EPQ] - k_{-4}[EQ][P] - k_5[EQ] + k_{-5}[E][Q] \quad (7)$$

$$\frac{d[P]}{dt} = k_4[EPQ] - k_{-4}[EQ][P] \quad (8)$$

$$\frac{d[Q]}{dt} = k_5[EQ] - k_{-5}[E][Q] \quad (9)$$

Using the method of Gear the rate equations for the concentration of each species were analyzed numerically.

Similarly, in the model of Theorell and Chance for alcohol dehydrogenase, rate equations for each species were derived and analyzed.

3. Results

3.1. The ordered bi-bi mechanism

We assumed the following values for each rate constant: $k_1 = 3.0 \times 10^3\ M^{-1}\ s^{-1}$; $k_{-1} = 25.0\ s^{-1}$; $k_2 = 2.0 \times 10^3\ M^{-1}\ s^{-1}$; $k_{-2} = 10.0\ s^{-1}$; $k_3 = 200.0\ s^{-1}$; $k_{-3} = 1.0 \times 10^4\ s^{-1}$; $k_4 = 10.0\ s^{-1}$; $k_{-4} = 1.0 \times 10^6\ M^{-1}\ s^{-1}$; $k_5 = 20.0\ s^{-1}$; $k_{-5} = 5.0 \times 10^5\ M^{-1}\ s^{-1}$.

Under the present conditions, a stable solution is obtained in the order of 10^3–10^5 k_1. Fig. 1 shows the results. Evidently, oscillations were clearly obtained for [Q] as well as [EQ]. It was also found that [A], [E], [EA], [EAB] and [EPQ] underwent oscillation. A characteristic feature of the solution is that increasing [Q] is accompanied by oscillation and that the period of oscillation becomes longer with time. Furthermore, the effect of influx of B (alcohol) was investigated. Fig. 2 shows the time dependence of [Q] with respect to variations in influx of B. At this time, the enzyme

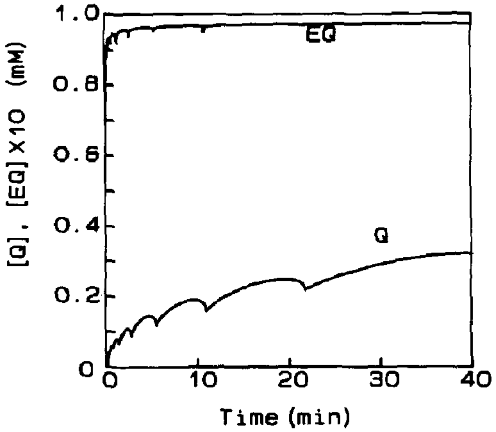

Fig. 1. Time dependence of [Q] for the ordered bi-bi mechanism. $k_0 = 0.1$ M s^{-1}; $[E]_i = 1.0 \times 10^{-4}$ M; $[A]_i = 1.0 \times 10^{-3}$ M.

concentration, $[E]_i$, and initial concentration of coenzyme, $[A]_i$, were fixed at 5.0×10^{-5} and 1×10^{-3} M, respectively. It was found that oscillation decreased in extent with increasing influx of B and ceased at very large influx of B. On the other hand, very small influx led to the disappearance of oscillations or random oscillation. It was found that, in order to obtain clear oscillation, a proper rate of entry of substrate was necessary.

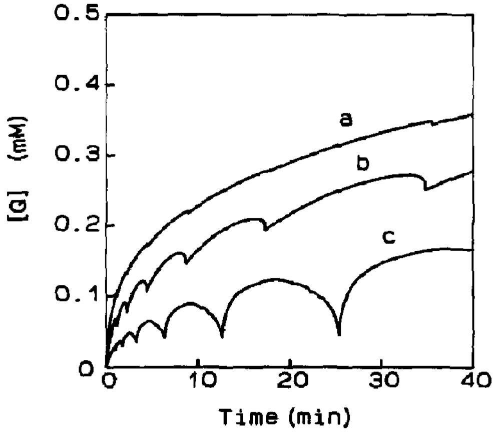

Fig. 2. Time course of [Q] for the ordered bi-bi mechanism with variation in influx of B. k_0: 1.0 (a), 0.1 (b), 1.0×10^{-2} (c) M s^{-1}. $[E]_i = 5.0 \times 10^{-5}$ M; $[A]_i = 1.0 \times 10^{-3}$ M.

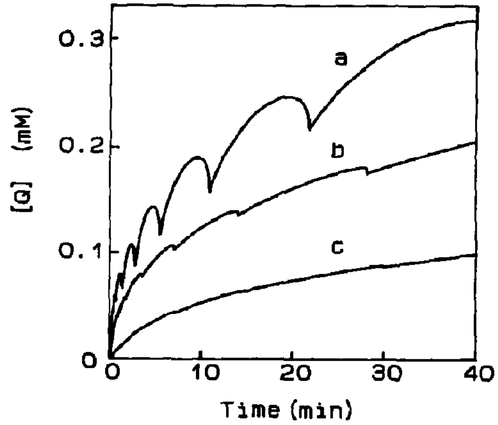

Fig. 3. Time course of [Q] with variation in the concentration of enzyme (ordered bi-bi reaction). $[E]_i$: 1.0×10^{-4} (a), 1.0×10^{-5} (b) and 1.0×10^{-6} (c) M. $[A]_i = 1.0 \times 10^{-3}$ M; $k_0 = 0.1$ M s^{-1}.

Fig. 3 shows the time course of the enzyme concentration dependence. In this calculation, the initial concentration of coenzyme and the influx of substrate were fixed at 1.0×10^{-3} M and 0.1 M s^{-1}, respectively. Both the frequency and the amplitude were found to decrease with decreasing enzyme concentration.

Fig. 4 demonstrates the time course of the coenzyme concentration dependence. The initial

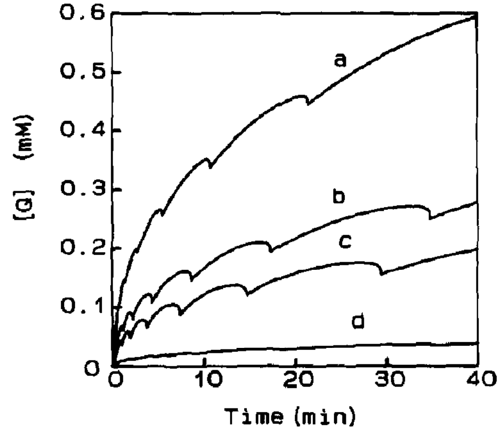

Fig. 4. Time course of [Q] with variation in concentration of A (ordered bi-bi reaction). $[A]_i$: 5.0×10^{-3} (a), 1.0×10^{-3} (b), 1.0×10^{-4} (c) and 2.0×10^{-4} (d) M. $[E]_i = 5.0 \times 10^{-5}$ M; $k_0 = 0.1$ M s^{-1}.

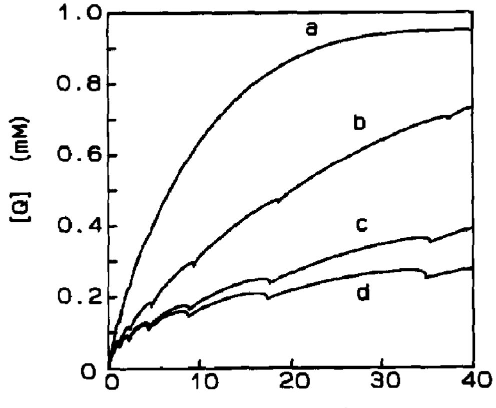

Fig. 5. Time course of [Q] vs removal of P (ordered bi-bi reaction). k_p: 0.1 (a), 0.01 (b) and 1.0×10^{-3} (c) and 0 M s^{-1} (d). $[E]_i = 5.0 \times 10^{-5}$ M; $[A]_i = 1.0 \times 10^{-3}$ M; $k_0 = 0.1$ M s^{-1}.

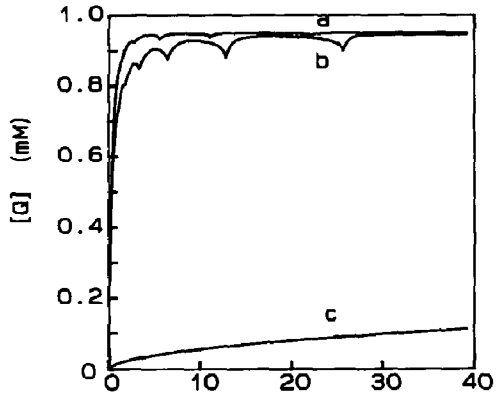

Fig. 6. Time course of [Q] with variation in k_0 (model of Theorell and Chance). k_0: 1.0 (a), 0.1 (b) and 1×10^{-5} M s^{-1}. $[E]_i = 5.0 \times 10^{-4}$ M; $[A]_i = 5.0 \times 10^{-4}$ M.

concentration of enzyme and influx were maintained constant at 5.0×10^{-5} M and 0.1 M/s, respectively. The highest frequency occurred in the order of 10^{-4} M $[A]_i$. This indicates that a proper concentration of A is necessary to bring about oscillations of high frequency.

So far the effect on the efflux of product has not been considered. Naturally, this factor should be taken into consideration. The product P was assumed to be removed in proportion to the concentration of P. Consequently, on the right-hand side of eq. 8, the term $-k_p[P]$ has been added (k_p, rate constant). Fig. 5 shows the dependence of the efflux of product P. The parameters used were the same as for trace b of fig. 2 except for the value of k_p. A high degree of efflux was found to lead to the disappearance of oscillations. This demonstrates that the removal of product P contributed to the disappearance of oscillation.

3.2. The model of Theorell and Chance

The parameters used in this calculation were as follows; $k_1 = 3.0 \times 10^3$ M^{-1} s^{-1}; $k_{-1} = 25.0$ s^{-1}; $k_2 = 4.0 \times 10^5$ s^{-1}, $k_{-2} = 1.0 \times 10^7$ s^{-1}; $k_3 = 20.0$ s^{-1}; $k_{-3} = 5.0 \times 10^5$ M^{-1} s^{-1}.

As shown in fig. 6, oscillations which were similar to those of the ordered bi-bi reaction were obtained. The oscillations were in the order of 10^3–10^5 k_1. It was also found that the results obtained with respect to the dependence of the influx of B, $[E]_i$, $[A]_i$ and the removal of P were similar to those in figs 3–5, respectively. However, at very low concentrations of enzyme, a different pattern of oscillation appeared. Here, k_1 was assumed to be 3.0×10^5. The results are depicted in fig. 7, in which the peak is directed upward, although the peaks shown above are all downwardly directed. The reason for the oscillation peak being

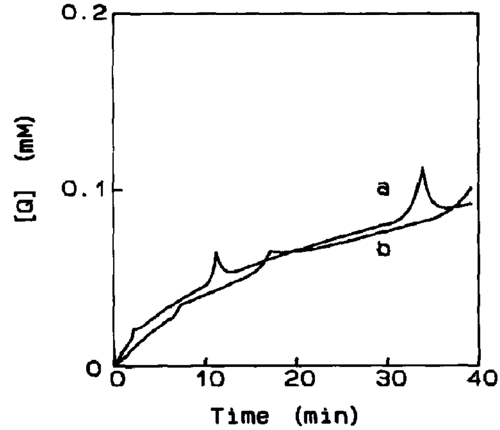

Fig. 7. Time course of [Q] (model of Theorell and Chance). $[E]_i = 5.0 \times 10^{-6}$ M (a) and 1.0×10^{-6} M (b). $[A]_i = 5.0 \times 10^{-4}$ M; $k_0 = 1.0 \times 10^{-6}$ M s^{-1}.

upwardly directed has not yet been verified. However, in the experiment such oscillations occurred at low concentrations of enzyme when chloroform was used as oil solvent. On the other hand, oscillations with upwardly directed peaks have not yet been demonstrated in the case of the ordered bi-bi reaction.

4. Discussion

Until now oscillations of enzyme-catalyzed reactions have been studied in detail in glycolytic processes and reactions of allosteric enzymes [5–7]. However, the mechanisms of enzyme reaction considered in the present paper do not include steps of autocatalysis or allostery. Thus, oscillations could not be evoked in both experiments and calculations when the substrate was present in the bulk phase from the outset. Oscillations were demonstrable on introducing the idea of gradual entry of substrate. Neither polysubstrate nor an allosteric effect was essential for oscillations in such a system. Further, the existence of product P should also contribute to the oscillation, since oscillations disappeared when the P was assumed to be removed rapidly.

In the experiment of the preceding paper [1], it was found that the gradual entry of the substrate (ethanol) from toluene or chloroform into an aqueous solution of alcohol dehydrogenase and NAD^+ caused oscillations in NADH concentration. The increase in [NADH] is accompanied by oscillations in enzyme concentration and increasing duration of the oscillatory period. Results of numerical analyses support these experimental data. In particular, the model of Theorell and Chance rather than the ordered bi-bi mechanism appears to be valid for interpreting the experimental results for alcohol dehydrogenase, since oscillations with a long period and an upward peak at lower concentrations of enzyme in the chloroform system as well as oscillations with a downward peak at higher enzyme concentrations in both toluene and chloroform systems could be reproduced. On the other hand, the result for the ordered bi-bi mechanism suggests that oscillations could occur in other enzyme systems using NAD^+ as coenzyme.

References

1 T. Hideshima, Biophys. Chem. 38 (1990) 265.
2 W.W. Cleland, Biochim. Biophys. Acta 67 (1963) 104.
3 H. Theorell and B. Chance, Acta. Chem. Scand. 5 (1951) 1127.
4 C.W. Gear, Numerical initial value problem in ordinary differential equations (Prentice-Hall, 1971); K. Hayashi and N. Sakamoto, Kosohanno no dynamics (Dynamics of enzyme reaction), Gakkai Shuppan Center 47 (1981).
5 B. Hess and A. Boiteux, Annu. Rev. Biochem. 40 (1971) 237.
6 G. Nicolis and I. Prigogine, Self-organization in nonequilibrium systems (Wiley, New York, 1977) p. 358.
7 J. Monod, J. Wyman and J.-P. Changeux, J. Mol. Biol. 12 (1965) 88.