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Published in:
European Journal of Biochemistry

DOI:
10.1111/j.1432-1033.1989.tb15188.x

1989

Document Version:
Publisher's PDF, also known as Version of record

Eur. J. Biochem. *186*, 145–148 (1989)

A theoretical treatment of damped oscillations in the transient state kinetics of single-enzyme reactions

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(Received May 16/July 5, 1989) – EJB 89 0600

An extension of the available kinetic theory for reactions in the transient state is presented which establishes that single-enzyme reactions may exhibit damped oscillations under the conditions of standard kinetic experiments performed by stopped-flow techniques. Such oscillations may occur for reasonable magnitudes of rate constants in the enzymic reaction mechanism and at physiological concentrations of enzyme and substrate. In the simplest reaction systems, the oscillations will be strongly damped and lead to progress curves resembling those of a reaction governed by standard exponential transients; statistical regression methods may then have to be applied for their detection and characterization. The observation that single-enzyme reactions may exhibit oscillatory behaviour points to a previously unrecognized possible source of the damped oscillations observed in metabolic systems such as the pathways of glycolysis or photosynthesis.

Present mechanistic knowledge about enzymic catalysis is based to a large extent on results obtained by kinetic methodology. The corresponding theory has been the subject of extensive investigations and several generalized treatments are now available to show how experimentally applicable rate equations can be derived for enzyme reactions in steady as well as transient states [1–5]. Since the time dependence of enzymic reactions is governed by non-linear differential equations lacking a general analytical solution, the applicability of analytically derived rate equations has to be justified by a proper choice of (linearizing) experimental conditions. In the case of steady-state kinetic studies, one examines initial reaction velocities using substrate in large excess to enzyme. Similar precautions have to be taken in transient state kinetic experiments performed by stopped-flow techniques to ensure that reactions proceed under approximately linear (pseudo first-order) conditions.

Assuming that linearizing pseudo first-order conditions do occur, the initial time course of an enzymic reaction will be governed by one or several exponential transients [4]. Previous detailed theoretical treatments have considered only the case that rate parameters for the exponential transients are real. The possibility cannot be excluded, however, that the transient rate parameters under certain conditions may be complex, such that the time course exhibits the characteristics of a damped oscillation rather than conforming to an exponential first-order process in the standard kinetic sense.

The occurrence of damped oscillations in biological reaction systems has received much attention during the last two decades [6–8]. Considering the attempts to interpret these oscillations in terms of a kinetic coupling between distinct enzymes in metabolic pathways, it is of obvious interest to examine if a single-enzyme reaction may exhibit an oscillatory behaviour. The theoretical analysis now presented establishes that such may well be the case. Generalized and specific relationships are derived to illustrate the possible behaviour of a single-enzyme system in that respect. The implications of the results are discussed with regard to the practical evaluation and interpretation of kinetic data relating to the transient phase of enzymic reactions performed under linearizing experimental conditions.

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THEORY

General behaviour of reactions of King-Altman type

Previous generalized treatments of the kinetics of a single-enzyme reaction [4] have been based on the mechanism discussed by King and Altman [1], according to which the reaction involves $n + 1$ enzymic species $EX_0, EX_1, \ldots, EX_n$ (including free enzyme). Reaction steps in the mechanism are assumed to be either monomolecular isomerizations

$$EX_r \xrightarrow{k_{rs}} EX_s; \qquad \kappa_{rs} = k_{rs} \tag{1}$$

or bimolecular reactions between an enzyme-containing species and a non-enzymic reactant R_q:

$$EX_r + R_q \xrightarrow{k_{rs}} EX_s; \qquad \kappa_{rs} = k_{rs}[R_q]. \tag{2}$$

κ_{rs} in Eqns (1 and 2) represents the κ values defined by Wong and Hanes [2].

The non-linear differential equations describing the kinetics of this reaction can be linearized with the assumption that non-enzymic reactants are present in large excess to enzyme and that there is no kinetically significant accumulation of reaction products. Under such conditions, concentrations of all non-enzymic reactants may be treated as constants and one obtains [4] the solution

$$[EX_r] = [EX_r]_\infty + \sum_{s=1}^{n} A_{rs} e^{-\lambda_s t}; \qquad r = 0,1,\ldots,n \tag{3}$$

where $[EX_r]_\infty$ denotes the steady-state concentration of species EX_r. A_{rs} and λ_s represent amplitudes and rate parameters, respectively, for the exponential transients. The amplitudes are functions of the transient rate parameters and of the initial state of the system. The transient rate parameters, λ_s, are the n roots of the characteristic equation [4]

$$\lambda^n - p_{n-1}\lambda^{n-1} + p_{n-2}\lambda^{n-2} \ldots (-1)^{n-1} p_1 \lambda + (-1)^n p_0 = 0 \tag{4}$$

where the coefficients p_r are sums of products of the true or pseudo first-order rate constants κ_{rs}. Since all κ_{rs} are real, the rate parameters λ_s are either real or complex conjugated pairs

$$\lambda_s = a_s + b_s i, \qquad \lambda_{s+1} = a_s - b_s i \tag{5}$$

where a_s, b_s are real, $b_s > 0$ and $i = \sqrt{-1}$. Assuming that m is the number of complex-conjugated pairs of transient rate parameters, Eqn (3) may be rewritten (after renumbering the rate parameters) as

$$[EX_r] = [EX_r]_\infty + \sum_{s=1}^{m} e^{-a_s t}[C_{rs}\cos(b_s t) + D_{rs}\sin(b_s t)] + \sum_{s=2m+1}^{n} A_{rs} e^{-\lambda_s t} \tag{6}$$

where

$$C_{rs} = A_{rs} + A_{rs+1} \tag{7}$$

$$D_{rs} = i(A_{rs+1} - A_{rs}) \tag{8}$$

Each term in the first sum of Eqn (6) represents an oscillatory transient that can be characterized by its period time $T = 2\pi/b_s$ and its damping factor a_s. The period time is determined by the imaginary part and the damping by the real part of the corresponding complex-conjugated pair of transient rate parameters.

Existence of oscillations

Pettersson, in his treatment of the King-Altman mechanism [4], considered only the case where all transient rate parameters are real. To show that such must not necessarily be the case, we may examine the kinetic behaviour of the one-substrate enzyme reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P \tag{9}$$

where E, S and P denote, respectively, free enzyme, substrate and product. With the linearizing approximations described above, we have

$$[S] = c_S \gg c_E \tag{10}$$

$$[P] = 0 \tag{11}$$

where c_S and c_E denotes the total concentration of, respectively, the substrate and enzyme. The time dependence of the concentrations of enzymic species in Eqn (9) then becomes of the form indicated by Eqn (3) with the transient rate parameters defined by

$$\lambda = \frac{1}{2}(k_1 c_S + k_{-1} + k_2 + k_{-2} + k_3) \pm \frac{1}{2}\sqrt{(k_1 c_S + k_{-1} + k_2 - k_{-2} - k_3)^2 - 4k_2(k_1 c_S - k_{-2})}. \tag{12}$$

The transient rate parameters will be complex if

$$(k_1 c_S + k_{-1} + k_2 - k_{-2} - k_3)^2 - 4k_2(k_1 c_S - k_{-2}) < 0. \tag{13}$$

Since c_S and all k_r are positive, necessary (but not sufficient) conditions for oscillations are

$$k_1 c_S > k_{-2} \tag{14}$$

$$k_3 > k_{-1}. \tag{15}$$

When $k_1 c_S = k_2 = k_3 = k > 0$ and $k_{-1} = k_{-2} = 0$ we have

$$\lambda = \frac{k}{2}(-3 \pm i\sqrt{3}). \tag{16}$$

Eqn (16) establishes that oscillations indeed may occur in a single-enzyme system.

Characteristics of the oscillations

In the general solution given by Eqn (6), the quotient b_s/a_s provides a measure of how much oscillation s is damped every period. For the particular reaction defined by Eqn (9) it follows from Eqn (12) that

$$\frac{b_1}{a_1} = \frac{\sqrt{4k_2(k_1 c_S - k_{-2}) - (k_1 c_S + k_{-1} + k_2 - k_{-2} - k_3)^2}}{k_1 c_S + k_{-1} + k_2 + k_{-2} + k_3}. \tag{17}$$

Analysis of Eqn (17) shows that

$$\frac{b_1}{a_1} \leqslant \frac{1}{\sqrt{3}} \tag{18}$$

where the equality holds true if, and only if,

$$k_1 c_S = k_2 = k_3 \text{ and } k_{-1} = k_{-2} = 0. \tag{19}$$

Eqn (18) implies that the oscillation is strongly damped, such that its amplitude declines to less than 1/5000 of its initial value within one period. The oscillation, therefore, would be expected to be of experimentally detectable magnitude only for about one-fourth of a full period and, when observed, may not be immediately recognized as an oscillation.

The latter point can be illustrated by examination of the kinetics predicted by Eqn (9) when rate constants in the mechanism are of the realistic magnitudes indicated in the legend to Fig. 1. In this specific case, reactions performed under standard initial conditions ([E] = c_E, and [ES] = [EP] = 0 at $t = 0$) will exhibit an oscillatory behaviour for substrate concentrations within the range 0.1 – 40 μM, i.e. around the K_m value of 5 μM for the enzymic reaction. Progress curves obtaining for enzymic species when c_S = 30 μM are given in Fig. 1 and show that the concentrations of free enzyme and species EP appear to vary monotonously with time, despite the fact that they are governed by a transient corresponding to a damped oscillation. The variable [ES], however, exhibits a distinct overshoot before it attains its steady-state value. Fig. 2 shows that the overshoot exhibited by [ES] becomes increasingly pronounced when c_S is increased, but also illustrates that there is no immediately obvious difference between the progress curves obtained in the oscillatory region and those obtained for c_S values exceeding 40 μM (i.e. giving rise to two exponential transients with real rate parameters). This indicates that the detection and characterization of an oscilla-

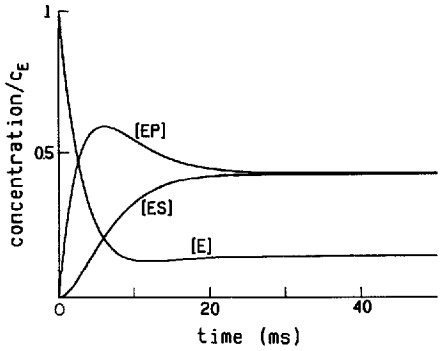

Fig. 1. *Oscillatory transients in a single-enzyme reaction.* Progress curves calculated by numerical integration of the differential equations governing the reaction system in Eqn (9) using $k_1 = k_{-3} = 10^7\ M^{-1}\ s^{-1}$, $k_{-1} = k_{-2} = 1\ s^{-1}$, $k_2 = k_3 = 100\ s^{-1}$, $c_E = 0.01\ \mu M$ and $c_S = 30\ \mu M$

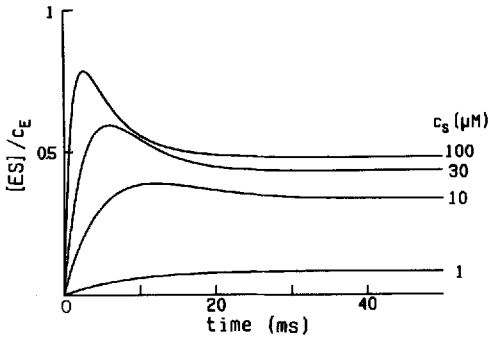

Fig. 2. *Effect of substrate concentration on the progress curve for [ES] in Eqn (9).* Conditions as in Fig. 1 except that c_S was varied

tory progress curve requires careful data processing by application of, for instance, non-linear regression methods [9, 10].

Oscillations in more complex reaction systems

Results in the preceding two sections were obtained by analysis of the kinetic scheme in Eqn (9). This scheme was chosen because it represents the simplest realistic case of a one-substrate enzyme reaction [11]. Similar analysis (with the linearizing assumptions $c_S \gg c_E$ and $[P] \approx 0$) of the extended reaction system in Eqn (20),

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EX_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EX_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \ldots\ldots \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} EX_n \overset{k_{n+1}}{\longrightarrow} E + P \qquad (20)$$

establishes that the kinetic characteristics of the latter system are largely analogous to those of the system in Eqn (9). In particular, the reaction in Eqn (20) may exhibit damped oscillations for certain values of rate constants and substrate concentrations in the system. The quotient of the imaginary part and the real part for each of the complex-conjugated pairs of transient rate parameters $[\mathrm{Im}(\lambda_s)/\mathrm{Re}(\lambda_s)]$ is always bounded, attaining its maximum value when

$$k_1 c_S = k_2 = \ldots = k_{n+1} = k \qquad (21)$$

$$k_{-1} = k_{-2} = \ldots = k_{-n} = 0. \qquad (22)$$

Assuming that Eqns (21) and (22) hold true, all transient rate parameters will be complex (except one if n is odd) and evenly distributed around a displaced unit circle such that

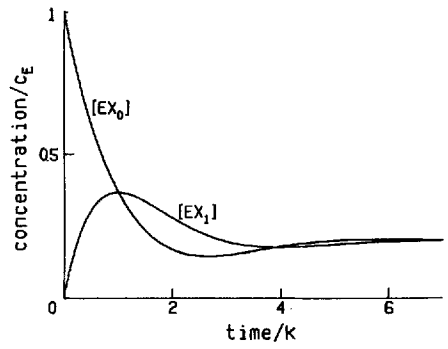

Fig. 3. *Progress curves for representative enzymic species in the reaction in Eqn (20).* Curves calculated by numerical integration, using rate constants in Eqns (21) and (22)

$$\lambda_s = k[-1 + \cos(\phi_s) + i\sin(\phi_s)]; \qquad s = 1,2,\ldots,n \quad (23)$$

where

$$\phi_s = \frac{2\pi s}{n+1}. \qquad (24)$$

The oscillatory transient corresponding to $s = 1$ will exhibit the largest $\mathrm{Im}(\lambda_s)/\mathrm{Re}(\lambda_s)$ value as well as the smallest $\mathrm{Re}(\lambda_s)$ value. Consequently, this transient will be the main one governing the time-course of the reaction system near the steady state. Hence it follows that the oscillatory behaviour of the reaction system will become increasingly conspicuous with increasing magnitude of n. This is illustrated in Fig. 3 for the case that Eqns (20) and (22) occur with $n = 4$. Under such conditions, the oscillation will remain of kinetic significance for almost a full period and concentrations of all enzymic species in the reaction mechanism will exhibit a pronounced overshoot in the approach to the steady state.

Approximate relationships between transient rate parameters and rate constants for the mechanism

The transient rate parameters, $\lambda_1, \lambda_2, \ldots, \lambda_n$, for an arbitrary reaction of the King-Altman type are solutions of the characteristic Eqn (4). Pettersson [4] described a method for the derivation of approximate relationships between transient rate parameters and rate constants in the case that the rate parameters are real and differ considerably in magnitude. This method can be generalized to include also the case of complex rate parameters. The assumption that rate parameters 'differ considerably in magnitude' then has to be specified as

$$\mathrm{Re}(\lambda_1) \ll \mathrm{Re}(\lambda_2) \ll \ldots \ll \mathrm{Re}(\lambda_n) \qquad (25)$$

and

$$|\lambda_1| \ll |\lambda_2| \ll \ldots \ll |\lambda_n| \qquad (26)$$

where

$$|\lambda_s| = \sqrt{\mathrm{Re}(\lambda_s)^2 + \mathrm{Im}(\lambda_s)^2} \qquad (27)$$

and where the rate parameters λ_s have been numbered according to their size as defined by Eqn (25). If two roots are complex-conjugated, the symbol $\ll$ describing their interrelationship in Eqns (26) and (27) should be substituted by the symbol $=$.

Provided that Eqns (26) and (27) apply, the coefficients p_r in Eqn (4) will be given approximately by

$$p_r = 2\mathrm{Re}(\lambda_{r+1}) \prod_{s=r+2}^{n} \lambda_s \qquad (28)$$

if λ_r and λ_{r+1} are a conjugated pair of complex roots, and otherwise by

$$p_r = \prod_{s=r+1}^{n} \lambda_s . \qquad (29)$$

Since the coefficients p_r are known functions of κ values in mechanisms of the King-Altman type, Eqns (28) and (29) provide generalized relationships for the interpretation of experimentally observed transient rate parameters, whether real or not.

DISCUSSION

The present investigation establishes that the transient approach of a single-enzyme reaction towards the steady state may exhibit the characteristics of a damped oscillation under standard conditions of kinetic experiments performed by stopped-flow techniques. Such techniques have been extensively applied for several decades, but there have been no reports of the experimental detection of oscillations in single-enzyme systems. (Oscillations have been observed in the reaction catalysed by peroxidase, but these oscillations origin from a complicated system of partly non-enzymic reactions [11] and are therefore of less general interest.) A possible reason for this is indicated by the present observation that oscillations will arise only for certain combinations of values of rate constants in the mechanism and over certain ranges of substrate concentrations. Many transient-state kinetic studies may well have been directed towards reaction systems, or performed over concentration ranges, where no oscillations may occur.

On the other hand, it should be noted that the appearance of oscillations in reaction systems such as the one in Eqn (9) or (20) is favoured by conditions which imply that all forward rate constants in the mechanism (including the apparent first-order rate constant $k_1 c_S$ for substrate binding) are of similar magnitude and much greater than rate constants for the reaction steps in the reverse direction. Enzyme reactions showing such characteristics are by no means physiologically unreasonable. On the contrary, several investigations have been presented which indicate that an evolutionary pressure in the direction of higher reaction rates should tend to modify rate constants such that the condition expressed by Eqn (21) is approached [12, 13]. The condition in Eqn (22) depends on the reaction equilibria and will be approached for reactions in which the formation of products is thermodynamically favoured. Hence it may be concluded, firstly, that evolution would be expected to favour a functional design of enzymes that allows for the appearance of oscillatory transients. Secondly, it follows that the oscillations should obtain over a fairly wide range of substrate concentrations that may be considered as physiological in the sense that they are of the same order of magnitude as the K_m for the enzymic reaction.

Considering this, the absence of reports on an oscillatory transient behaviour of single-enzyme reactions seems unexpected and points to the possibility that such transients have not been recognized as oscillations when actually observed. This possibility is favoured by the results in Figs 1 and 2, which show that oscillatory transients are not necessarily conspicuous but may readily be mistaken for transients governed by real rate parameters. It seems reasonable to assume that the lack of precedence for an oscillatory behaviour of single-enzyme systems has led to neglect of the interpretational possibility that transients may be characterized by complex rate parameters. One may hope that the present results will stimulate the search for oscillatory events in single-enzyme reactions and lead to modified procedures for the evaluation of transient-state kinetic data. The inclusion (as a standard routine) of statistical tests to discriminate between transients exhibiting real and complex rate parameters would seem highly desirable and may be required for unambiguous interpretation of the observations made.

The present results also have an obvious bearing on the origin of the damped oscillations which have been observed in multi-enzyme systems [6–8] and which usually have been attributed to a kinetic coupling between different enzymes. If a single-enzyme reaction can oscillate, then the possibility exists that these single-enzyme oscillations may extend to the pathway as a whole through projection and amplification. This possibility will be considered in a future investigation.

This investigation was supported by grants from the Swedish Natural Science Research Council.

REFERENCES

1. King, E. L. & Altman, C. (1956) *J. Phys. Chem. 60*, 1375–1378.
2. Wong, J. T. F. & Hanes, C. S. (1962) *Can. J. Biochem. Physiol. 40*, 763–804.
3. Hammes, G. G. & Schimmel, P. R. (1970) in *The enzymes* (Boyer, P. D., ed.) vol. 2, pp. 67–115, Academic Press, London.
4. Pettersson, G. (1978) *Acta Chem. Scand. B32*, 437–446.
5. Cornish-Bowden, A. (1979) *Fundamentals of enzyme kinetics*, Butterworth, London.
6. Hess, B. & Boiteux, A. (1971) *Annu. Rev. Biochem. 40*, 237–258.
7. Noyes, R. M. & Field, R. J. (1974) *Annu. Rev. Phys. Chem. 25*, 95–119.
8. Goldbeter, A. & Caplan, S. R. (1976) *Annu. Rev. Biophys. Bioeng. 5*, 449–476.
9. Watts, D. G. (1981) in *Kinetic data analysis* (Endrenyi, L., ed.) pp. 1–24, Plenum Press, New York.
10. Bártfai, T. & Mannervik, B. (1972) in *Analysis and simulation of biochemical systems* (Hemker, H. C. & Hess, B., eds) pp. 197–209, North Holland, Amsterdam.
11. Degn, H. & Olsen, L. F. (1979) *Ann. N. Y. Acad. Sci. 316*, 623–637.
12. Brocklehurst, K. (1977) *Biochem. J. 163*, 111–116.
13. Albery, W. J. & Knowles, J. R. (1976) *Biochemistry 15*, 5631–5640.