PSEUDO-MICHAELIAN KINETICS AND FLIP-FLOP TYPE MECHANISMS

Christian GACHE
Centre de Biochimie – Faculté des Sciences, 06034 Nice, France

Received 19 July 1974
Revised version received 16 September 1974

1. Introduction

Analysis of reaction rate dependence upon substrate concentration is one of the primary approaches in enzyme kinetics. Deviations from classical Michaelian kinetics are often taken to reflect the multiplicity of sites and either their dependence [1–5] or the existence of a conformational pre-equilibrium between states with different affinities for the substrate [3,4,6,7]. Nevertheless some authors have shown that non-Michaelian v_0 versus S responses may occur with monomers [3,8–13]. On the other hand if uniqueness or independence of the active sites is always associated with Michaelian behaviour, the reciprocal is not always true as pointed out by Lazdunski et al. [14]. Their studies with the dimeric *E. coli* alkaline phosphatase have shown the tight dependence of the two subunits in catalysis although this enzyme exhibits Michaelis-Menten kinetics. The flip-flop mechanism that they propose has also been established for calf intestine alkaline phosphatase [15]. In fact literature analysis shows that a growing number of enzyme seem to conform to flip-flop type mechanisms. Their main properties have been recently reviewed [16,17].

The particular flip-flop scheme which has been demonstrated for alkaline phosphatase and alcohol dehydrogenase is purely Michaelian under steady-state and initial velocity assumptions [14,18–21]. However in a general case, similar patterns do not always give a pure Michaelian form. Velocity as a function of substrate concentration shows hyperbolic dependence for particular values of some rate constants only.

Several authors [22–28] have emphasized the usefulness of a phenomenological systematic kinetic analysis of enzyme systems. An approach of this kind is used here for a minimum system which includes the flip-flop mechanism of Lazdunski et al. [14,16] as a particular case. Conditions for Michaelian approximation are established for typical mechanisms, irrespective of the number of sites and ligands. This paper is complementary to other phenomenological approaches from this laboratory concerning functional polydimers [17,29,30].

2. Description of the system

The enzyme is an oligomer involving identical protomers, equivalent within the free enzyme. For each class of ligands there exists a unique site on each subunit. Free enzyme exists only under one conformation. Among the possible ligands, one considers only one substrate S and its corresponding product P. Each subunit may appear under three liganded states: free, S-bound, P-bound. Elementary steps are binding, transformation or desorption steps of one ligand at a time.

The general scheme which takes into account all macroscopic species and elementary steps is given in fig. 1. Only the simplest case in which n =2 will be investigated here. It is sophisticated enough to permit occurrence of some characteristic mechanisms specific to polymeric enzymes. The corresponding scheme is given in fig. 2A.

All possible interactions are included within this

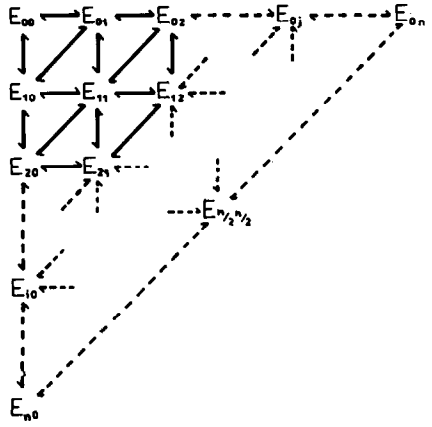

Fig. 1. General scheme for a two-ligand system. E_{ij} stands for any enzymatic species. Subscripts i and j refer to the number of S molecules and P molecules bound to the enzyme. Vertical arrows represent S binding steps, the horizontal ones P binding steps, and diagonal arrows catalytic transformation steps.

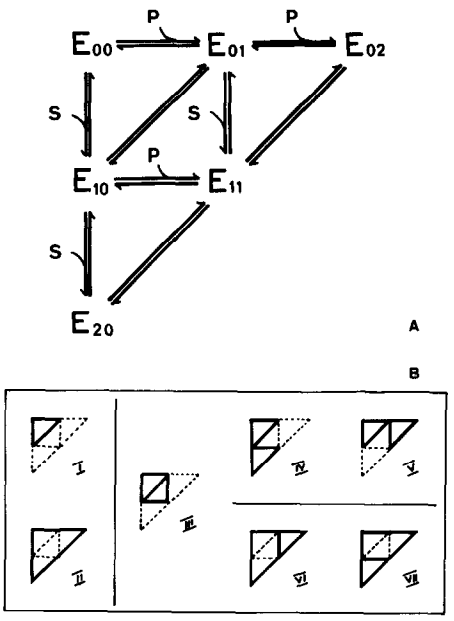

Fig. 2A). The two-ligand, two-subunit system. Conventions are the same as for fig. 1. (B) Basic mechanisms derived from the complete system. By deleting systematically all steps minus the first S and P binding steps from the complete scheme of fig. 2A one find 125 possible systems. Among these, one rules out nonsense combinations and systems which exhibit dead-end pathways, or which need product for activity in the S→P direction or which cannot reduce to a previous case or to a basic system. The 7 basic systems which remain are drawn here. In each case, deleted steps are in dotted line.

scheme. Each of the three types of steps occurring on one subunit appears three times, corresponding to each of the three possible states presented by the other subunit. Homotropic and heterotropic interactions for the binding of the two ligands can be easily introduced. The scheme in fig. 2A takes into account interactions at the catalytic level. Transformations on one subunit depend upon the state of the other subunit(s). Homo- and heterotropic effects are defined for the transformation of a ligand according to the occupancy or the opposite subunit by the same or the other ligand.

3. Discussion

The analysis of this system according to King and Altman [31] and Wong and Hanes [25], shows that under steady-state and initial velocity assumptions the solution is of the non-Michaelian form given by equation (1), with x=3.

$$v_0/E_T = \frac{\sum_{1}^{x} a_i S^i}{\sum_{0}^{x} b_i S^i} \qquad (1)$$

The coefficients of S in equation (1) are complex combinations of rate constants which cannot be easily discussed. In consequence only simpler partial systems will be considered here. The 7 systems given in fig. 2B are of particular interest and will be briefly surveyed.

It should be noted that systems (I) and (III) are symmetrical, so their behaviour is identical with respect to S and P. Systems (V) and (VII) have the same properties, with interchanged S and P, as systems (IV) and (VI) respectively. All these systems except (I) are described by a rate equation simpler than but similar to equation (1) with x equal to 2 or 3. In each case approximations necessary to obtain a typical Michaelian behaviour of flip-flop enzymes will be discussed.

3.1. *Linear systems (I and II)*

Case (I)

This case is formally identical to the minimum system for a monomer. The introduction of only one substrate molecule leads to a rate expression of the Michaelian form. The system describes a dimer with Michaelian kinetics and half-site reactivity. It derives from the whole system by introducing homotropic and heterotropic absolute antico-operativity at the binding level.

Case (II)

This mechanism is obtained by introduction in the whole system (fig. 2A) of an absolute negative heterotropic interaction for the binding steps and two positive effects, homo- and heterotropic, for the catalytic steps. The rate equation is of the following form:

$$v_0/E_T = \frac{2a_2 S^2}{b_2 S^2 + b_1 S + b_0} \qquad (2)$$

$b_0 \simeq o$ will give the Michaelian character. Such a condition is fulfilled if the second binding step is quasi-irreversible.

3.2. *Branched systems (III to VII)*

3.2.1. Conditions for Michaelian behaviour.

The scheme homologous to the flip-flop mechanism discussed by Lazdunski et al. for *E. coli* alkaline phosphatase [14,18], belong to this category. The schemes of this class, as most of those which can be drawn for higher degree polymers, present more than one introduction of S and the rate equation is not of degree 1 for S. For the specific class under study, which is probably of common occurrence, conditions can be found which lead to a Michaelian form whatever *the number of active sites, substrates, products, and conformational change steps* may be. These conditions are related to the three following points (see fig. 3):

a) The general pattern of the system: this branched system, with any number of steps, presents one loop (steady-state loop) which does not contain the free enzyme. Between the free enzyme and the loop two linear pathways (α and β) are drawn.

b) The introduction of substrate molecules: if

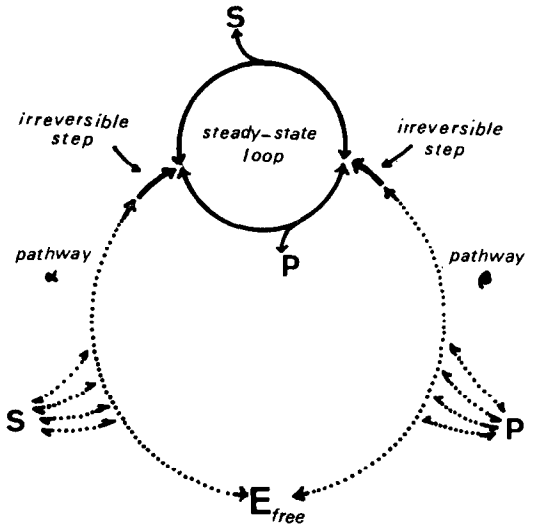

Fig. 3. General pattern for Michaelian branched mechanisms. The steady-state loop is in heavy line; forward and reverse (α and β) presteady state pathways are in dotted line.

there are x substrate binding steps in the system, $x - 1$ are on pathway α. There is at least one P desorption step on pathway β. The loop contains only one introduction of S and at least one exit of P and one catalytic step.

c) The irreversibility of some steps: the two steps of pathways α and β contiguous to the loop are quasi- irreversible as indicated in fig. 3.

The use of King and Altman rules [31] easily shows that when the above conditions are fulfilled, and in absence of the product, Michaelian kinetics are always observed. (see appendix). As previously noted a characteristic property of the flip-flop mechanism is that in the steady-state there is not regeneration of free enzyme but only regeneration of one of the active sites.

Mechanisms involving considerable co-operativity (binding co-operativity and kinetic co-operativity), Michaelian kinetics, and any fractional stoichiometry at equilibrium (equilibrium dialysis with one of the substrates or an analogue) and/or during steady-state (by rapid kinetics or quenching of the complexes which accumulates in the steady-state) are perfectly compatible; see for example half-sites enzymes [32,17].

Before treating the dimer case it is important to underline that polymeric enzymes with flip-flop

type mechanisms and Michaelian kinetics are not necessarily polydimers.

3.2.2. The dimer case

Application of the previous discussion to the particular case of the dimer can be readily carried out. Patterns for analogue binding, for saturation during steady-state and of presteady-state events may be easily predicted.

Even in the limited case under study it becomes evident that despite their classical Michaelian responses flip-flop type mechanisms can display rather uncommon properties: Different presteady-state pathways can lead to a same steady-state. Mechanisms (IV) and (VII) in fig. 2B show the same steady-state where the enzyme acts with one subunit occupied by S, but presteady-states differ for the P→S direction. In both schemes (V) and (VI) the enzyme acts with one subunit always occupied by P in the steady-state but presteady-state pathways differ in the forward (S → P) direction. Mechanisms (VI) and (VII) involve unsaturated forms for one of the ligands in the steady-state loop, whereas the enzyme is saturated during presteady-state. Stoichiometry as evaluated by equilibrium binding or presteady-state measurements will be higher than those determined from steady-state experiments. The reciprocal case may also occur. Mechanism (V), under Michaelian conditions, displays on one hand, half-site reactivity towards binding, on the other hand a full reactivity during presteady-state (two-sites). For schemes (IV), (VI) and (VII) approximations for Michaelis-Menten kinetics are consistent with any binding properties. Antico-operativity is not a necessary property of flip-flop mechanisms. Evidently scheme (III) shows similar properties as the mechanism described by Lazdunski et al. for alkaline phosphatase. It arises from the overall scheme if antico-operativity operates for both binding and catalysis of both S and P. One can see that this mechanism is the only one which involves uniquely disymmetric species, so microscopic forms follow one another in a strictly ordered manner.

4. Conclusions

As it can be seen in the previous examples, a wide diversity of behaviours can be expected for polymeric enzymes which all exhibit general properties defined by Lazdunski [15,17] for flip-flop type mechanisms, namely Michaelian kinetics associated with dependent sites.

The essential features of this model are as follows: Taking into account dependent sites for binding and catalysis on a polymer gives rise to a complex potential network. The reactivity on one site is dependent in a general case on the state of the other site. Strong interactions between sites may impose to the enzyme a particular pathway. However the other pathways remain potentially functional, and may be triggered by specific signals. The Michaelian feature in introduced by simple approximations for a given group of reaction patterns which are not purely Michaelian. The required irreversibility of characteristic steps leads for this type of mechanisms to a partial splitting between steps in the steady-state and some of the presteady-state steps. Specific active site titration properties arise from this feature.

Conformational changes have not been involved in a formal way here, their introduction being straightforward. Nevertheless they are of fundamental importance in flip-flop mechanisms since they mediate interactions between protomers in oligomeric structures.

Finally it could be pointed out that the Michaelian feature of flip-flop mechanisms which is often observed [16,17] can be lost without alteration of the fundamental property of these mechanisms, namely kinetic co-operativity. Alteration of the Michaelian behaviour of a flip-flop type enzyme may have an important regulatory function [16,17, 29,30].

Acknowledgements

I am greatly indebted to Professor M. Lazdunski, in whose laboratory this work was done, for introducing me to flip-flop mechanisms and for critical reading of this work. I thank R. Manfredini and D. Chappelet for very fruitful discussions and constant interest. Thanks are also due to Dr A. di Franco and to all my colleagues for carefully reading the manuscript.

Appendix

King and Altman [31] gave rules for deriving rate laws for enzyme catalyzed reactions.

They stated: 'The concentration of each EX_i relative to the concentration of enzyme, $[EX_i]/[E_o]$, is a quotient of two summations of terms, each term being the product of n−1 different rate constants and the appropriate concentrations. Each term in the numerator of the expression for $[EX_m]/[E_o]$ involves the rate constants (and appropriate concentrations) associated with reactions steps which individually or in sequence lead to EX_m, the enzyme-containing species in question'.

Because of the general form of the scheme defined in fig. 3 and in (a), if there are n enzymatic species, there are n+1 steps. In consequence non cyclic patterns of n−1 steps are obtained by deleting two steps, either both in the steady-state loop or one in this loop and the other in α or β pathways. The condition of irreversibility (c) implies that no King-Altman pattern can lead to any of the complexes in pathway α and β. The steady-state concentration of these complexes is zero. They only appear as transient intermediates. The steady-state rate equation contains only terms relative to enzymatic species of the steady-state loop. Condition (b) which limits to one the number of substrate introduction on the steady-state loop implies a Michaelian behavior.

Non-cyclic patterns (denominators terms in equation (1); King and Altman rules) can only be of x or x−1 degree for S whereas cyclic patterns (numerator terms) are all of degree x for S when conditions (a), (b), (c) are fulfilled. The rate equation will appear in the simple form of the following equation, which is a Michaelian one:

$$v_o/E_T = \frac{a_x S^x}{b_x S^x + b_{x-1} S^{x-1}}$$

References

[1] Adair, G. S. (1925) J. Biol. Chem. 63, 529–545.
[2] Pauling, L. (1935) Proc. Natl. Acad. Sci. U.S. 21, 186–191.
[3] Weber, G. (1965) in: Molecular Biophysics (Pullman, B. and Weissbluth, M., eds.) pp. 369–396, Academic Press, New York.
[4] Weber, G. and Anderson, S. R. (1965) Biochemistry 4, 1942–1947.
[5] Koshland, D. E. Jr., Nemethy, G. and Filmer, D. (1966) 5, 365–385.
[6] Changeux, J. P. (1964) Brookhaven Symp. Biol. 17 (BNL 869 (C-40)) 232, 239.
[7] Monod, J., Wyman, J. and Changeux, J. P. (1965) J. Mol. Biol. 12, 88–118.
[8] Dalziel, K. (1957) Acta Chem. Scand. 11, 1706–1723.
[9] Atkinson, DK and Walton, G. M. (1965) J. Biol. Chem. 240, 757–763.
[10] Ferdinand, W. (1966) Biochem. J. 98, 278–283.
[11] Rabin, B. R. (1967) Biochem. J. 102, 22C–23C.
[12] Sweeny, J. R. and Fisher, J. R. (1968) Biochemistry 7, 561–565.
[13] Ainshie, R. E., Schill, J. R. and Neet, K. E. (1972) J. Biol. Chem. 247, 7088–7096.
[14] Lazdunski, M., Petitclerc, C., Chappelet, D. and Lazdunski, C. (1971) Eur. J. Biochem. 20, 124–139.
[15] Chappelet-Tordo, D., Fosset, M., Iwatsubo, M., Gache, C. and Lazdunski, M. (1974) Biochemistry 13, 1788–1795.
[16] Lazdunski, M. (1972) in: Curr. Top. Cell. Regul. (Horecker, B. L. and Stattman, E. R., eds.) Vol. 6, pp. 267–310, Academic Press, New York.
[17] Lazdunski, M. (1974) in: Progress in Bioorganic Chemistry (Kaizer, E. T. and Kezdy, F. J., eds.) Vol. 3, pp. 100–161, Wiley Interscience, New York.
[18] Chappelet-Tordo, D., Iwatsubo, M. and Lazdunski, M. (1974) Biochemistry (in press).
[19] Dunn, M. F. and Bernhard, S. A. (1971) Biochemistry 10, 4569–4575.
[20] Luisi, P. L. and Favilla, R. (1972) Biochemistry 11, 2303–2310.
[21] McFarland, J. T. and Bernhard, S. A. (1972) Biochemistry 11, 1486–1493.
[22] Cleland, W. W. (1963) Biochim. Biophys. Acta 67, 104–137.
[23] Cleland, W. W. (1963) Biochim. Biophys. Acta 67, 173–195.
[24] Dalziel, K. (1958) Trans. Faraday Soc. 54, 1247–1253.
[25] Wong, J. T. and Hanes, C. S. (1962) Can. J. Biochem. Physiol. 40, 763–804.
[26] Fisher, J. R. and Hoagland, V. D. Jr. (1968) Advan. Biol. Med. Phys. 12, 163–211.
[27] Ricard, J., Mouttet, C. and Nari, J. (1974) Eur. J. Biochem. 41, 479–497.
[28] Nari, J., Mouttet, C., Fouchier, F. and Ricard, J. (1974) Eur. J. Biochem. 41, 499–515.
[29] Lazdunski, M., Lazdunski, C., Petitclerc, C., Chappelet, D., Fosset, M., Gache, C. and Delaage, M. (1973) in: Dynamic Aspects of Conformation Changes in Biological Macromolecules (Sadron, C., ed.) pp. 285–299, Reidel Publishing Company, Dordrecht, Holland.
[30] Lazdunski, M. and Delaage, M. (in preparation)
[31] King, E. L. and Altman, C. (1956) J. Phys. Chem. 60, 1375–1378.
[32] Levitzki, A., Stallcup, W. B. and Koshland, D. E. Jr. (1971) Biochemistry 10, 3371–3378.