Dynamics of Biochemical Systems

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Plenum Press
New York and London
Published in cooperation with NATO Scientific Affairs Division

Proceedings of a NATO Advanced Research Workshop on
Dynamics of Biochemical Systems,
held September 19–23, 1983,
in Marseille, France

Library of Congress Cataloging in Publication Data

NATO Advanced Research Workshop on Dynamics of Biochemical Systems (1983: Marseille, France)
Dynamics of biochemical systems.

(NATO ASI series. Series A, Life sciences; v. 81)
"Proceedings of a NATO Advanced Research Workshop on Dynamics of Biochemical Systems, held September 19–23, 1983, in Marseille, France"—T.p. verso.
"Published in cooperation with NATO Scientific Affairs Division."
Includes bibliographical references and index.
1. Enzymes—Congresses. 2. Biological chemistry—Congresses. I. Ricard, Jacques, 1929– . II. Cornish-Bowden, Athel. III. Title. IV. Series.
QP601.N29 1983 574.19'25 84-17857
ISBN 0-306-41830-4

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

PREFACE

This book collects together a series of communications given at a NATO Advanced Research Workshop held in Marseilles, France, from September 19 to September 23, 1983. Its aim is to describe the principles that govern the dynamics of biochemical systems. An obvious question that arises at this point is the need to define what a biochemical system is : in this book it is defined operationally, as a set of co-ordinated chemical reactions conditioned either by a single enzyme existing in different conformations, or by different enzymes, or by enzymes in association with membranes. The book thus describes how to tackle the dynamics of biochemical systems with different levels of complexity. The simplest level of complexity is represented by different conformational states of the same enzyme that can interact to control an enzyme reaction. An already more complex level arises if the polypeptide chains bearing the active site are packed together as an oligomeric enzyme. A third degree of complexity occurs if different enzymes are packed together as a multi-enzyme complex, with added potential for channelling effects. Many enzymes in the living cell are associated with membranes or cell envelopes, and one may wonder how such association may modify an enzyme's behavior. Finally, the enzyme reactions that occur in the living cell are interconnected, and constitute a very complex integrated network that represents the highest degree of complexity.

The methods, both theoretical and experimental, for studying biochemical dynamics originate from the kinetic study of isolated enzymes in solution. The unifying concept in the study of biochemical systems of all levels of complexity is a physico-chemical and quantitative study of their temporal evolution. Indeed, the concept of evolution occupies a central position in biology, and any kind of biological problem has to be viewed with an evolutionary perspective if one is to make sense of it. The properties of enzymes and enzyme systems today are the result of long evolution. The last part of the book is therefore devoted to understanding how fine tuning of the behavior of enzymes and enzyme systems may have occurred in the course of evolution.

In line with these ideas, the book comprises six sections, of which the first is entitled *"Slow" conformation changes of enzymes and their relevance to the regulation of biochemical systems.* Its aim is to show that "slow" conformation changes of an enzyme occurring far from pseudo-equilibrium conditions may generate apparent kinetic co-operativity. This behavior may occur even with monomeric one-site enzymes, and implies interaction of different conformational states of the enzyme in the overall enzyme reaction. The concepts of enzyme hysteresis and enzyme memory are related to this view. Two papers, one by K.E. Neet, G.V. Ohning and N.R. Woodruff (Cleveland, Ohio), and the other by A. Cornish-Bowden, M. Gregoriou and D. Pollard-Knight (Birmingham, England) give strong experimental support to these ideas.

The second section is entitled *Dynamics of subunit interactions in multimeric enzymes and the control of catalysis.* It describes how identical subunits interact to modulate the rate of product appearance. E.P. Whitehead (Rome, Italy) presents a linkage approach to the analysis of steady-state rates, whereas K. Dalziel (Oxford, England) offers a pre-steady-state kinetic study of polymeric oxidative decarboxylases.

The third section is devoted to the analysis of a more complex situation and is entitled *Enzyme interactions and dynamics of enzyme complexes.* Enzyme aggregation and compartmentation represent a functional advantage and may improve the performance of enzymes in the intracellular milieu. Three communications illustrate these views. G.R. Welch (New-Orleans, Louisiana) presents a rather general picture of enzyme dynamics in organized states. T. Keleti (Budapest, Hungary) describes how channelling may occur within a multi-enzyme complex, and A.H. Lane, C.H. Paul and K. Kirschner (Basel, Switzerland) present a detailed kinetic study of the conformation changes that occur in the assembly of the $\alpha_2\beta_2$ complex of tryptophan synthase.

A step further in the complexity of dynamic biochemical systems is taken in the fourth section devoted to the *Dynamics of enzyme reactions in heterogeneous media.* Under non-equilibrium conditions, diffusion of substrates and products as well as repulsion of these ligands by the fixed charges of a membrane, coupled to an enzyme reaction, may generate surprising effects. Among these are the recognition of "signals" from the external milieu (short-term memory) and the conduction of these "signals" at the surface of the membrane. Two contributions illustrate these ideas, one by J. Ricard, G. Noat and M. Crasnier (Marseilles, France) and the other by J.F. Hervagault, J. Breton, J.P. Kernevez, J. Rajani and D. Thomas (Compiègne France).

The last step in the complexity of biochemical systems is offered by the analysis of metabolic systems. This matter is aptly discussed in the fifth section of the book entitled *Dynamics of metabolic pathways: Self-organization and chaotic behavior.* The dynamic behavior

of a metabolic pathway considered as a whole is quite different from that of any enzyme acting as an element of the same pathway. Small random perturbations of an external parameter may create temporal organization of the whole system. The kinds of temporal organization possible may include sustained oscillations, birhythmicity and chaos as well. Three contributions illustrate the development in this area. The first is by A. Goldbeter, J.L. Martiel and O. Decroly (Brussels, Belgium) and discusses the rhythmicity displayed by metabolic processes; the second, by B. Hess, D. Kuschmitz and M. Markus (Dortmund, Federal Republic of Germany), is specifically dedicated to the study of glycolysis; and the last , by J. Stucki (Zurich, Switzerland) discusses biological energy conversion from the point of view of non-equilibrium thermodynamics.

In the last section of the book these ideas are placed in an evolutionary context. This section, entitled *Evolutionary considerations*, is specifically concerned with three important problems : theevolution and mutation of the genetic code; the progressive transformation of binding proteins into enzymes; the kinetics of complex self-replicating molecular systems and the role played by mutation and selection in this process. The first problem is examined by J.T. Wong (Toronto, Canada), the second by B. Gutte (Zurich, Switzerland) and the last by P. Schuster (Vienna , Austria).

The content of the book is obviously interdisciplinary in character and describes research carried out at the borderline between theory and experiment. We hope that these contributions shed some light on the physical bases of the complex dynamics of biological processes.

We are glad to thank Paule Cassa, Marie-Thérèse Nicolas and Jacques Victor for their help in the preparation of the manuscript. We are especially grateful to Brigitte Videau who typedmost of the contributions in camera-ready form and took a major part in the practical organization of the meeting.

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CONTENTS

SECTION I - "SLOW" CONFORMATION CHANGES OF ENZYMES AND THEIR RELEVANCE TO THE REGULATION OF BIOCHEMICAL SYSTEMS

HYSTERETIC ENZYMES, SLOW INHIBITION, SLOW ACTIVATION, AND SLOW MEMBRANE BINDING

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INTRODUCTION

Enzymes are generally considered to utilize rapid responses since they , indeed, catalyze reactions. In fact, many early and current approaches attempt to study the rapid catalytic steps by decreasing the rate of reactions, e.g. cryoenzymology, or by increasing the instrumental methods, e.g. rapid kinetic techniques. However, it has become apparent over the past fifteen years that slower responses in enzymes may be beneficial for catalysis or regulation of the activity. Numerous enzymes have been described that undergo relatively slow changes that are manifested in non-linear progress curves, cooperativity in their steady state kinetics, or otherwise demonstrable slow structural changes in response to ligands. Early suggestions by Rabin (1967), Keleti (1967), and Witzel (1968) of the observation of slow changes during assay resulted in 1970 in the coining of the term 'hysteresis' by Frieden (1970) to describe this class of enzymes. Frieden (1970) suggested that a hysteretic enzyme with a response time of minutes might serve to dampen or buffer cellular responses to changing metabolite concentrations. Shortly threafter, we (Ainslie, et al, 1972) provided the theoretical basis that described the potential and the limitations of Ligand Induced Slow Transitions in enzymes that may contribute to the kinetic cooperativity of the steady state. Whitehead (1970) suggested that this kinetic cooperativity is through 'time' rather than 'space' as in site-site interactions. The notion that this property represented a memory of the enzyme for a previous conformation was discussed by Ricard et al, (1974) and extensively analyzed in terms of the 'mnemonic' enzyme to

emphasize this point. These several related concepts represent different viewpoints of the same basic process rather than any real conflict (Neet and Ainslie, 1980; Frieden, 1979). Several other contributions to our understanding or application of these ideas have been made by several investigators (Jarabak and Westley, 1974; Whitehead , 1976; Kurganov, 1977; Storer and Cornish-Bowden, 1977).

As discussed in the published literature (Frieden, 1979; Neet and Ainslie, 1980), the following definitions will be used. Hysteresis or hysteretic enzyme applies to any observable, slow process affecting enzymatic activity, particularly with respect to its potential for physiological function. Transient refers directly to the observation of the early, nonlinear stages of an assay progress curve, before the true steady state is attained. Slow transition refers to the molecular change that the enzyme undergoes in its hysteretic response, which may be a conformational change, an association-dissociation, or a ligand displacement (Frieden, 1970; Ainslie, et al, 1972). We are concerned with reversible, noncovalent interactions, thus eliminating the area of enzymology dealing with regulation of activity by covalent modification, e.g. phosphorylation. 'Slow' also needs to be defined since it is a term relative to the scale of the observer. 'Slow' may simply mean 'in the range of minutes' and thereby allow easy observations of a transient in standard laboratory assays and potentially influence rapid metabolic changes through its hysteretic response. Alternatively, 'slow' may refer to particular molecular steps and have certain relationships among different steps in a mechanism; e.g. , for the Ligand Induced Slow Transition mechanism to generate cooperativity, the rate of the isomerization step must be on the same order of magnitude as the other unimolecular steps (dissociation, catalysis) in the mechanism (Ainslie, et al, 1972).

The general form of the slow transition (isomerization) is given (Fig. 1) for the two substrate, ordered mechanism for a monomeric enzyme in which substrate is capable of binding to two forms of an enzyme that are slowly interconvertible. For a single substrate or conditions of saturating levels of one substrate, the mechanism would simplify to one involving only one side of the figure. Strictly concerted mechanisms, in which the second conformer can only occur with both substrates (or products) bound, and rapid equilibrium mechanisms, in which the binding of substrates is at equilibrium (Ainslie, et al, 1972), can only give rise to hysteretic transients and not to kinetic cooperativity in the steady state. The mnemonic mechanism (Fig. 2), in which there is one EA form and two free enzyme forms (no E'A), is shown for comparison and is extensively discussed for a particular enzyme , glucokinase, in the next paper. This simpler mechanism can account for kinetic cooperativity in a monomeric enzyme and produce transients. The observable assay, transient, and its physiological

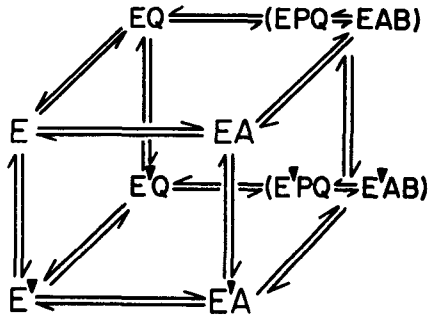

Fig.1. The Ligand Induced Slow Transition Mechanism for a hysteretic, monomeric enzyme catalyzing a two substrate, ordered reaction. The slow transition shown is an isomerization (vertical steps) between different enzyme conformations, E and E', with different catalytic properties. A and B are substrates and P and Q are products. Vertical steps are slow steps. (Reprinted by permission from Neet and Ainslie, 1980).

equivalent, is seen (Fig. 1) to be due to a time dependent change in distribution between the primed and unprimed cycles altered by the presence or change in concentration of the substrate. Cooperativity, if it exists in the monomeric enzyme, is dependent upon both a slow step as well as a shift in distribution between cycles in the steady state in a non-Michaelis-Menten fashion. Cooperativity can be positive or negative with apparent bursts or lags (or , indeed, no easily observed transient) in the transient; conditions for generating these have been discussed (Neet and Ainslie, 1980). These mechanisms have been drawn for a conformational change in a monomeric enzyme but could also be due to a slow association/dissociation (Frieden, 1970; Ainslie et al, 1972; Klinov and Kurganov, 1982), to a slow ligand dissociation (Frieden, 1970; Ainslie et al, 1972; Williams and Morrison, 1979), or occur

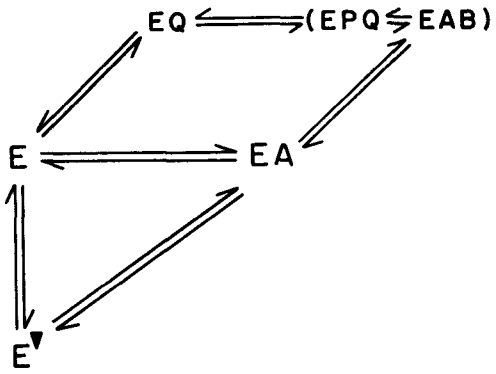

Fig. 2. The Mnemonic Mechanism for a hysteretic, monomeric enzyme catalyzing a two substrate reaction. E and E' are two different enzyme conformations that convert to the same catalytic form upon binding substrate, A. An ordered reaction is shown. A, B, P and Q have the same meaning as Fig. 1.

in an oligomeric enzyme that might also display cooperativity due to site-site interactions (Kurganov, 1982). Sorting out the contribution, significance, and interaction of the hysteretic response in many enzymesis the subject of important, current investigations.

CONCEPTS RELATED TO HYSTERESIS

Slow inhibition

Slow-binding inhibitors are those in which the establishment of equilibrium between E, I and EI complexes does not occur instantaneously but occurs in the time range of minutes (Cha, 1975; Williams and Morrison, 1979). Such properties were implicit in the observation and formulation of early hysteretic and slow transition concepts (Frieden, 1970; Ainslie, et al, 1972). The observable , slow step could either be the initial encounter complex formation or a slow transition to an altered EI' complex after

a rapid initial binding step. In the latter case the overall equilibrium constant would be the resultant of both steps and could lead to a quite high affinity. If the affinity is high enough, then significant depletion of the free inhibitor concentration could also result and the effector would then be operationally classified as a tight binding inhibitor (Williams and Morrison, 1979; Morrison 1982), i.e. one with which stoichiometric ratios of inhibitor and enzyme are used during experimental analysis. A slow binding inhibitor can be one type of tight binding inhibitor if its affinity is high enough (or the conditions of analysis are appropriate) or it may be a relatively weak inhibitor but with a slow onset of the full inhibitory state. Progress transients may be 'bursts' or 'lags' depending upon the preincubation and assay conditions. Analysis of the non-linear transient curves and consideration of possible artifacts (Williams and Morrison, 1979; Morrison, 1982) are similar to those discussed for substrate-induced, hysteretic enzymes (Frieden, 1979; Neet and Ainslie, 1980).

Mechanistically, the simple, competitive case or dead-end inhibition (Fig.3A) is the most straightforward and can be directly

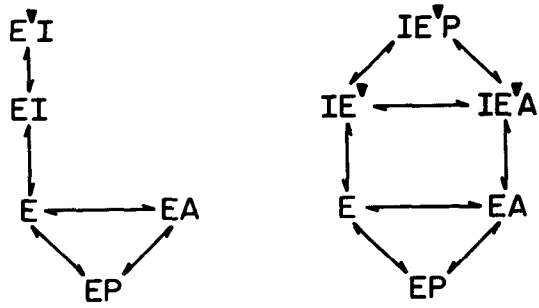

Fig. 3. (a) A slow, competitive inhibition mechanism for the conversion of A to P by the enzyme E. The inhibitor (I) binds only to the free enzyme form. (b) A more general slow inhibition scheme which can give rise to non-competitive or mixed inhibition. The binding of inhibitor, I, and the putative subsequent isomerization are shown as one step for simplicity.

analyzed for the rate constants in the pre-steady state (Cha, 1975). The more complex situation in which the EIA' complex is catalytically competent (Fig.3B)can lead to a non-competitive or mixed inhibition and a more complex relationship between the relaxation time for the transient and the inhibitor or substrate concentration (Cha, 1975). Both of these models can also produce inhibition of the apparent initial velocity if the formation of the first EI complex is rapid. Note that the noncompetitive model (Fig. 3B) predicts the possibility of cooperativity for either inhibitor or substrate in the steady state velocity (but not the initial) just as the conformational form of the Ligand Induced Slow Transition model (Fig. 1) does. The situation for threonine inhibition of homoserine dehydrogenase (E. coli) that we described several years ago (Bearer and Neet, 1978) is essentially this noncompetitive model, except that it is complicated by a tetrameric structure and site-site interactions of binding; the slow inhibition is on the order of milliseconds and the kinetic contribution is in addition to the equilibrium cooperativity.

Slow activation

Slow activation is an analogous molecular process to that described for inhibition, except that the modifier in Fig. 3B would produce an activation, i.e. the primed cycle would be more active than the unprimed (bottom) cycle. Laidler (Hijazi and Laidler, 1973) has provided the steady state and pre-steady state equations for at least one case of this mechanism. Numerous allosteric enzymes have been described in which the activator produces its effects slowly, e.g. the response of liver Acetyl CoA carboxylase to citrate activation has been reported to require several minutes (Greenspan and Lowenstein, 1968) and AMP (activator) affects the slow association and degree of activity of threonine dehydrase of E. coli (Dunne and Wood, 1975).

Hysteretic, allosteric activators are well known, but a less well understood situation is one which involves hysteretic, isosteric activators. The simplest (monomeric, one substrate enzyme) case of an essential, slow activator (Fig. 4C) could refer either to an allosteric activator or to one required for the catalytic mechanism. Of particular interest are those essential activators that are cofactors of the reaction. The vitamin derived cofactor, thiamin pyrophosphate, TPP, appears to play this role in at least four different enzymes. A slow transient lag occurs in the activation by TPP of alpha-ketoglutarate dehydrogenase of cauliflower (Craig and Wedding, 1980), of pyruvate dehydrogenase of E. coli (Graupe et al, 1982; Horn and Biswanger, 1983), of yeast pyruvate decarboxylase (Hubner et al, 1978), and yeast transketolase (Egan and Sable, 1981). The cofactor also promotes a dimerization of the latter enzyme that does not appear to be at equilibrium (Egan and Sable , 1981). In collaboration with Shreve and Sable

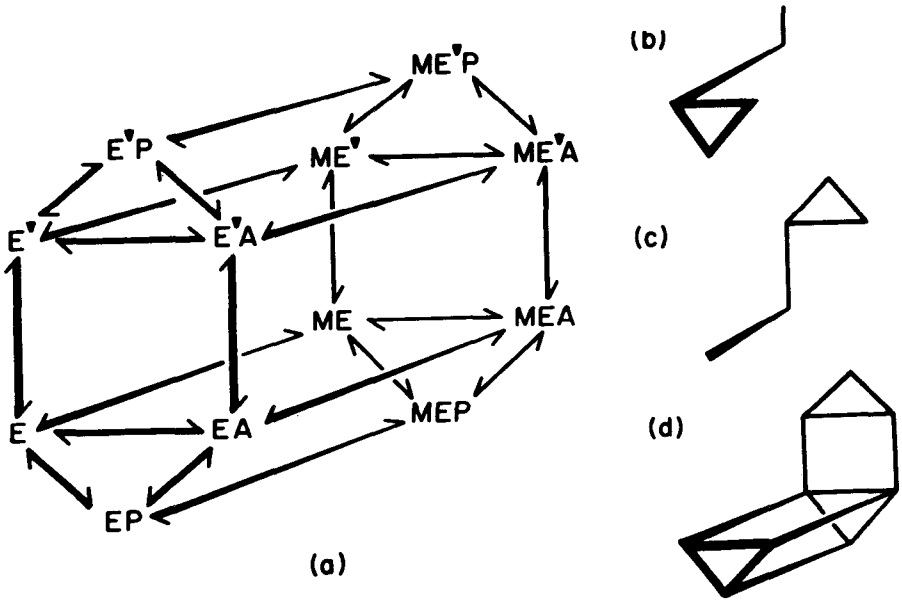

Fig. 4. (a) The general modifier effect on the Ligand Induced Slow Transition (isomerization) Mechanism for a monomeric, single substrate enzyme. The letters have the same meaning as Fig.1 with M representing an allosteric modifier. Enzyme forms with altered kinetic properties and altered distributions are demonstrated by the back plane (with M bound) of the mechanism.(b) A diagrammatic portion of the general modifier mechanism of (a) showing modifier binding only to free enzyme, E. In this case only competitive inhibition can occur; compare Fig. 3a.(c) A diagrammatic portion of the general modifier mechanism of (a) showing an essential modifier binding to free enzyme, E, to induce the active conformation, E', through a slow isomerization.(d) A diagrammatic portion of the general modifier mechanism of (a) showing modifier bound to E, EA and EP but with the second, primed conformation only present after induction by the modifier through a slow isomerization.

we have shown that the activation of transketolase is a complex process that is not simply a slow binding of the ligand, as it appears to be with α-ketoglutarate dehydrogenase (Craig and Wedding, 1980), since the dependence of the reciprocal half-time on TPP concentration is nonlinear for transketolase (Shreve et al, 1984). Furthermore, the slow stepcan not simply be attributed

to the dimerization step itself, since this also gives the wrong dependency of the rate on the TPP concentration. Whether this transition is related to the negative cooperativity observed in the kinetic activation of the enzyme is as yet unclear. The fact that four enzymes show slow activation by the TPP cofactor suggests that this behavior must be important for its functioning or that it is a necessary consequence of the reaction. It would be highly unlikely that the slow process would be involved in a hysteretic damping of the response to cellular concentrations of TPP, since the latter are not known to change rapidly under different physiological conditions. A relationship to the cooperativity, as suggested for two of the enzymes (Egan and Sable, 1981; Shreve et al, 1984; Horn and Bisswanger, 1983), would seem to be the most likely possibility.

The general form of modifier effects on the slow transition mechanism (Fig. 4A) is rather complex and, as usual, can account for nearly any observation. The special case in Fig. 4B is identical to the simple inhibition case (Fig. 3A) whereas the limiting scheme in Fig. 4D is essentially the same as the inhibition of Fig. 3B. The latter (Fig. 4D) can nicely accommodate slow effects seen in partial competitive inhibition, noncompetitive inhibition, V or K type activators, and effects of either type of modifier on apparent initial velocities as well as steady state rates. The complete modifier (Fig. 4A), or the partial (Fig. 4D), slow transition mechanism (monomer) can give rise to terms with powers greater than two in the rate equation for either substrate (A) or modifier (M) if the binding of modifier itself (front to back steps) is not at rapid equilibrium. The full mechanism (Fig.4A) is necessary to explain cases in which inhibition (or activation) does not cause changes in cooperativity in the absence of modifier and hence represents an entire new set of rate constants with similar relationships, i.e. EA forms are not pulled. We have interpreted the partial competitive inhibition of glucokinase by palmitoyl-CoA that does not change the positive cooperativity with glucose in this fashion (Tippett and Neet, 1982).

Slow membrane processes

We will now diverge for a moment from the main thrust of this book, namely enzymes themselves, and briefly consider a related topic, slow transition in membrane systems. In some cases these may be enzymatic activities that are part of an integral membrane protein, involved in transport or signalling. In other cases, the slow processes may not have yet been associated with a classical enzymatic activity but simply be manifested in binding equilibria. Membranes may well be the archetypal systems for such slow responses since more restrictive forces on the diffusion, aggregation, and/or conformational mobility may be operative (Ricard et al, 1984; Hervagault et al, 1984).