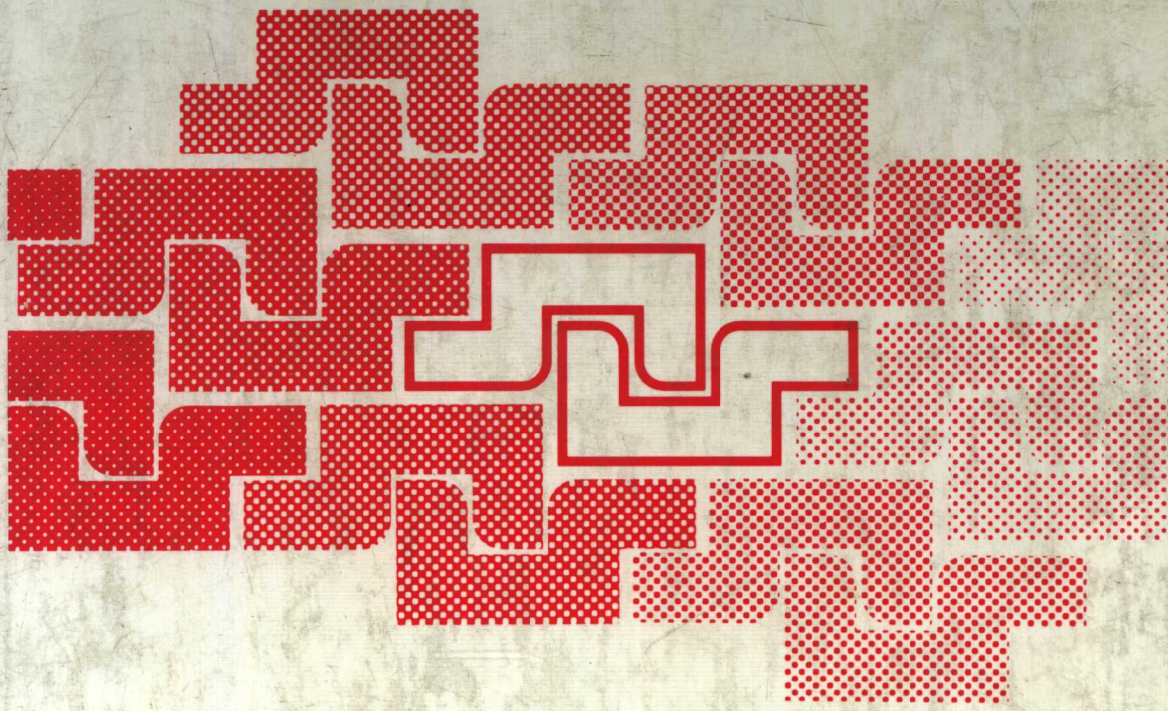

The Enzyme Catalysis Process

Energetics, Mechanism, and Dynamics

Edited by

Alan Cooper

Julien L. Houben and

Lisa C. Chien

NATO ASI Series

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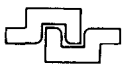

Series A: Life Sciences

PREFACE

This volume represents the proceedings of a NATO Advanced Studies Institute held near Barga (Italy), July 11-23, 1988, involving over 90 participants from more than twelve countries of Europe, North America and elsewhere. It was not our intention at this meeting to present a complete up-to-the-minute review of current research in enzyme catalysis but rather, in accord with the intended spirit of NATO ASIs, to give an opportunity for advanced students and researchers in a wide variety of disciplines to meet together and study the problem from different points of view. Hence the lectures cover topics ranging from the purely theoretical aspects of chemical reaction kinetics in condensed matter through practical experimental approaches to enzyme structure, dynamics and mechanism, including the new experimental opportunities arising from genetic engineering techniques. Our approach was unashamedly physical, both because the more biochemical aspects of enzymology are amply covered elsewhere and because progress in our understanding and application of the molecular basis of enzymic processes must ultimately come from advances in physical knowledge. We tried to cover as wide a spectrum as possible, and succeeded in gathering an expert and enthusiastic team of speakers, but there are some inevitable omissions. In particular, and with hindsight, our discussions might have been enriched by more detailed coverage of general aspects of chemical catalysis - but readers requiring this background should find adequate references herein.

It is difficult to judge the effectiveness of a meeting such as this, but perhaps one measure of success is that at least three international research collaborations were initiated here. Other benefits are less tangible, but we hope that a greater understanding of the capabilities, and failings, of various experimental and theoretical techniques will further progress in this important area. Perhaps most important of all, the opportunity for younger scientists to meet with established workers, warts and all, in a friendly and informal setting, shows the next generation that science is an intensely human activity, with all its attendant frailities, but is no less exhilarating for that.

We would like to thank the NATO Scientific Affairs Division for their financial and organizational support. Additional financial support and assistance with local organization, in most congenial surroundings, was generously provided by the Comitato Scienze Fisiche and the Gruppo Nazionale Cibernetica e Biofisica of the Consiglio Nazionale delle Recerche (CNR, Italy).

Alan Cooper
Julien L. Houben and
Lisa Chien

Glasgow and Pisa
1988

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(A) Introductory Lectures

"If a man will begin with certainties, he shall end in doubts; but if he will be content to begin with doubts, he shall end in certainties."

Francis Bacon

"First listen, my friend, and then you may shriek and bluster."

Aristophanes

ENZYME CATALYSIS: AN OVERVIEW FROM PHYSICS

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00185 Roma, Italy

INTRODUCTION

The organizers of this workshop asked me, as the first speaker, to present "a broad overview/introduction/ philosophy-like oriented lecture, as it appears that many of our collegues still have problems accepting the idea that a theoretical model can be of any use". I have accepted their invitation gladly, but let me say at the start that I share some of the perplexities of my colleagues, and that I am not so naive as to believe that "our ultimate goal must be to develop a general and practically useful model of the total enzyme catalysis process", as it is mentioned among the aims of this NATO-ASI. In the following, I will try to convince you that instead of a model we would do better to focus on a few general physical features of the process in question, each of these features coming into play with different emphasis in specific cases met in the laboratory.

Before entering into business, let me comment on the notion of "general physical features" for a process like enzyme action, which is only one part of the larger one called the living process. There is little doubt that some of these general physical features cannot be accounted for by physics alone, but require the wider viewpoint of biology. Let us take a look at a couple of examples:

1 - Why are enzymes such large and sophisticated macromolecules? An answer to this question can come only from biology: because the living process needs highly reliable kinetic units (e.g. the enzymes) to accomplish its operative tasks, leaving only DNA to introduce appropriate changes to its operative program.

2 - Why do enzymes work at the highest speed compatible with physical laws? Again an answer from physics is impossible, while biology suggests that an intrinsic property of the living process is the continuous urge to change itself and to control itself, and the quicker these operations are carried out the better for survival.

We may ask ourselves if there are general features of enzyme action that can be described from the viewpoint of physics only, even if the enzymes we deal with today result from a large evolution towards some biological goals. My opinion on this matter is optimistic, not because I am a physicist, but because I believe that what is going on in enzyme catalysis is similar to what happens in any physical process involving molecular media.

Therefore let me outline at least 3 physical features which seem

intrinsic to catalytic action, leaving to the following part of this lecture and even more to other lecturers, the task of expanding these statements:

a) While fully accepting the body of structural data offered by biochemistry, we must add that the enzyme macromolecule must have the capability to display some useful fluctuations in its space-charge distribution when coupled to a random medium.

b) In order to operate as a single system, the macromolecule must display some long-range connectivity among its subsystems.

c) Under stresses imposed by the environment or by other subsystems, each subsystem changes its space-charge distribution. Eventually, some of these induced changes can became cross-correlated in time.

SPONTANEOUS FLUCTUATIONS

Although globular proteins have long been considered "floppy bodies", meaning systems capable of displaying a large set of conformational fluctuations, the description of these fluctuations and of their biological significance is a comparatively recent acquistion. Because of the small size of a single macromolecule, transient fluctuations are inevitable, even at thermodynamic equilibrium. This is not a unique property of globular proteins, but here the occurrence of these large fluctuations can have pertinent implications upon their function, for instance as enzymes.

The first evidence that rapid structural fluctuations are present in a large number of globular proteins and enzymes comes from experiments on fluorescence quenching performed in 1973 by Lakowicz ad Weber [1]. Almost contemporaneously I suggested [2] that the ability to time-correlate these fluctuations could be an essential kinetic property of the macromolecule if it is to work as an enzyme. Since then, the notion of the enzyme as a fluctuating unit has gained credit; its experimental and theoretical basis has been progressively strengthened [3) (4].

In order to proceed in our physical description of biomacromolecules near equilibrium in their thermal bath, we shall first take a closer look at the interconnection between three major physical concepts, namely "events" "statistical macro-variables", and "statistical correlations". Let us begin with a proper definition of a statistical macrovariable Y. By this we mean a macroscopic statistical variable that is necessary for describing function at a molecular level. Examples of these macro-variables are the average charge on specific residues at the active site of an enzyme; the concentration of a particular ligand; and so on. Clearly, Y quantities are themselves functions of several microscopic quantities: for instance, the average charge on an active-site residue is a function of the charges and distances of nearby atoms, and so forth. Note that these Y macro-variables are of a truly statistical nature, because the small size of the system necessarily implies a certain number of fluctuations.

Events can now be defined as the measurable change dY of the Y macrovariables. Of course such a measurement is in practice possible only if amplitude and duration can be calculated using available experimental techniques; this means that the energy must be well above average thermal energy. One such typical event is the transition over a free-energy barrier, the measurable quantity being the rate of chemical species produced during the barrier transition. As a rule, only rare large amplitude fluctuations can be considered as measurable events.

Once a set of relevant macro-variables is identified, their cross-correlation can be mathematically expressed as the non-vanishing average of these quantities over a duration time. It stands to reason that in order to be cross-correlated, macrovariables must display close values of self-correlation time, so that some form of coupling can become effective

among them. The notion of "correlated events" then emerges in a simple and direct way. As a matter of fact, large amplitude fluctuation of the macro-variables can be described as relevant spontaneous events, that can become cross-correlated if their duration is similar and if their coupling is sufficiently strong. Thus it becomes quite evident that when too many such events become cross-correlated, the complexity limit within this description level may be reached.

Some aspects of enzyme action are truly general; they will be the focus of our concern hereafter. For instance physics aims to describe how free energy is exchanged in order to overcome free energy barriers, and since such barriers operate in a time sequence, physics hopes to understand how this planned sequence of internal events can be achieved in a thermal bath. This implies that an enzyme should be described not only by its spatial but also by its temporal structure, the two merging together in the spacetime fluctuation spectrum of the macromolecule. This ambitious programme has not been achieved as yet. Since the structural aspects of enzymes have been widely considered in the literature, only some of the temporal aspects will be considered below.

Let us start by trying to increase the precision of our notion regarding the statistical time event relevant for catalysis. According to the picture of the fluctuating enzyme, the reaction coordinate ought to be a function of only a few statistical macrovariables, which by high-amplitude spontaneous fluctuations create biological function. Therefore, we must first consider the relationship between the time scale of the spontaneous flutuations of the macrovariables involved and accordingly we must identify the main classes of these relevant macrovariables as a function of the time-data considered above. If this class is relevant for catalysis it must provide the active fluctuation; namely, the high-amplitude fluctuation that occurs rarely and well away from the mean value of the Gaussian curve. In our picture, this active fluctuation is the bottleneck for catalysis. We believe that a free-energy increase of one order of magnitude above the average thermal energy (say, up to 5 Kcal/mole) should be considered sufficient, because this increase must be lower than the net free energy of structure stabilization of a globular protein, which is the order of 10 Kcal/mole. Therefore, active fluctuations towards catalysis must occur with a probability factor around 10 exp -5, and if we take 10 exp -3 as a representative value for the enzyme turnover, we anticipate that some active fluctuations must originate from that part of the frequency spectrum which is centered around 10 exp -8[5].

As this stage we should combine the two main points: on the one hand, the free-energy transducing property of the enzyme between the thermal bath and the active site and, on the other, the value of the common-time constant of the spontaneous fluctuations involved in the transducing processes. This will help us identify the specific classes of fluctuating macrovariables which are relevant for catalysis. Expressed in different and more concrete terms, we should identify some specific process occurring on the macromolecule surface and others occurring at the active site. There is reason to assume these processes can couple, because both have a time constant close to 10 exp-8 sec. This search was performed some time ago [5], and among suitable candidates which could account for processes occurring at the protein surface, we have already pointed out the proton-transfer reaction of bound water, the charge fluctuations of the ionic medium and the side-chain motions.

Once this set of relevant macrovariables is identified, we should proceed to calculate their statistical cross-correlations, in order to derive the natural laws which describe the behaviour of this macromolecule. Such is the final aim of this description level, and it is obviously quite remote because of the non-linear coupling which probably exists among the macrovariables themselves. At this point different coupling regimes may occur, according to the degree of displacement of

the system from thermodynamic equilibrium. In practice, the enzyme will use the regime that fits best in the overall course of the functional order. There is no preconceived scheme according to which every enzyme must work; rather, in each instance, nature has evolved the best way to achieve its goal, based upon the laws of statistical physics.

LONG RANGE CONNECTIVITY

A general statistical-physical approach, called the percolation model, has been developed for a wide range of processes where spatially random events and topological disorder are of intrinsic importance. A typical physical application of the percolation theory [6] is to the electrical conductivity of a network of conducting and non-conducting elements. One of the most appealing aspects of the percolation process is the presence of a sharp transition, where long-range connectivity among the elements of a system suddenly appears at a critical concentration of the carriers. My aim here is to show that this model can successfully describe the emergence of catalytic function in a simple enzyme. This is because proteins are often disordered at the microscopic scale; then long-range connectivity between subunits must be established according to statistical laws, and the presence of a threshold should be expected according to the percolation model.

Recent work by our group has shown that powders of lysozyme at low hydration display protonic conductivity and that the conduction process follows the percolation model [7) (8]. In this picture, the conductivity reflects motion of protons along threads of hydrogen-bonded water molecules adsorbed on the surface of the macromolecule, with long-range proton movement developing along with the extended network at the percolation threshold.

For lysozyme-saccharide complexes a higher value of the percolation threshold has been found, suggesting that the presence of a "foreign body", where the water bridges may not be favorable for proton transfer, must affect the long-range connectivity on the protein surface. This hydration level, hc=0.25, is close to the critical level for the onset of enzymatic activity in Lysozyme catalysis. The value of the critical exponent found for the saccharide complex suggests that the protonic conduction remains a surface process. This observation is in agreement with the suggestion that preferred paths of proton movement pass through the active site; substrate would be expected to block these without changing the surface character of the percolation.

The percolation transition, although it exhibits some characteristics of a phase transition, differs importantly in that it reflects change in the connectivity within a system without there being also a change in the chemistry of the elements of the system. A percolative transition can be found even if there is no interaction between elements, as in the case of a mixture of conducting and non-conducting spheres. For this example, one can identify in principle two phases, one consisting of those conducting elements which are part of the infinite thread, the other of conducting elements not part of it; however, the chemistry of the conducting elements in the two putative phases is clearly indistinguishable. The percolative transition is detected as a discontinuity in a process, a property of the entire system, for example conduction, the onset of which reflects the onset of long-range connectivity. This is developed stochastically in a randomly-structured system of many elements, as a result of a change in system composition (in the example, change in the fraction of conducting elements). We note that one may develop an alternative description of the conductive regime of hydrated lysozyme powders, as a series of correlated single proton transfers along a random thread of water molecules. Since each single proton transfer can be considered a small-scale event due to a local fluctuation, the large-scale conductivity results from the

correlation of very many of these small-scale fluctuations.

We have extended the application of the experimental techniques developed through study of lysozyme to measurement of the dielectric properties of the purple membrane of Halobacterium halobium [9]. The data demonstrate a cooperative transition for proton movement, with a threshold hydration level close to the onset of photoresponse of this system.

The percolation model bears on the transport of protons and small molecules across membranes. This movement is thought to involve, generally, a water channel, although for this discussion it is sufficient to imagine some collection of conducting elements. Several points can be made: (1) The channel or surface need be only partly filled with conducting elements. The percolation threshold, above which there is conduction, is 0.45 fraction filled for a surface, and 0.16 for a 3-dimensional region. (2) Gating might be associated directly with a simple chemical event, such as binding of a ligand or change in state of ionization of a protein group, without its being mediated by a change in protein conformation. If the system is poised near the critical percolation concentration, a small change in the fraction filled would serve to switch the system. A two percent decrease in water content shifts the lysozyme system from a proton conductive to a non-conductive mode. (3) The central focus of percolation theory is the randomness of the arrangement of conducting elements. A membrane channel or conducting surface that operates through a percolation mechanism would need no structure extending over the full thickness of the hydrocarbon core of the membrane. For example, in the case of the proton pump of the purple membrane there need be no proton wire of hydrogen-bonded groups leading from the membrane surface to the active site. Instead, it is possible that protons diffuse to and from the gate along the threads of a fluctuating random percolation network within the protein-lipid interface. The photoreaction at the active site would serve to give a vectorial kick to the proton movement. In this picture, the requirement for a special non-random structure would be limited to the gate, and the size of the active site would be that found to be typical for other biochemical processes such as enzyme catalysis.

INDUCED CHANGES

Broadly speaking, we can call "induced changes" all kinds of changes that one site of a macromolecule displays because of the action of the surroundings. Typically, this is the case of a change induced by binding of a substrate, or by the mutual interactions among nearby sidechains at the active site. All these effects are widely considered in the biochemical literature and will not be a matter of concern hereafter. Instead, what is worth mentioning here is the possibility that the change at one site of the macromolecule can result from the action of one which is far away. Although experimental evidence for this kind of long-range effect is still lacking, it seems quite possible that such effects can take place in some specific cases. The best-known example is the proton transfer along chains of water molecules adsorbed on the protein surface, along a pathway which may also include some sidechains. Of course, in this way some distant side chains relevant for acid-base catalysis can become time-correlated to develop concurrent factors for catalysis. A similar but much more sophisticated long range process can be assisted by such long living excitations as the so called Davydov solitons. However, in spite of the existence of vibrational trapped states in a model system for the amide group of proteins, there is no evidence as yet that this kind of process can be helpful in real enzymes [10].

It seems worthwhile to point out the great relevance of the hydrogen bond in most of the inductive effects considered here. As is well known, the active sites of several enzymes are just an embroidery of hydrogen

bonds connecting substrate to side chains, and even the protein surface is but a network of hydrogen bonds connecting absorbed water molecules to the amide backbone. It seems that the hydrogen bond frame which encompasses the whole protein is the best system occuring in nature to produce large amplitude fluctuations (because of the low energy of this bond), long-range connectivity paths (because of the nature of the water molecules and of the amide group), and strong inductive effects (because of the high polarizability of this bond). In other words, most of the general physical features occurring in enzymatic action are rooted in the properties of the hydrogen bond itself.

CONCLUDING REMARKS

i - In the previous sections I have used a dynamical picture of the enzyme which is still tailored to its structure. The design of the macromolecule was assumed to have been optimized by evolution in order to make good use of both thermal disorder and of strucutral order, the first playing the role of a chance carrying factor and the second of a selecting factor. And the flexibility of the enzyme structure we tacitly assumed to allow for the temporal series of space-charge changes. An irreducible complexity of the enzymes's structure thus appears, because the system is made of several interacting subsystems, each capable of spontaneous fluctuations and of mutual inductions assisted by random interconnecting links.

On the other hand, one can use a description grounded directly on the process itself, so that the functional complexity of the enzymatic action becomes expressed in terms of a series of cross-correlated temporal events [11]. Of course the two above description are complementary to each other, because an event is by definition a change occurring in a structure (or, better, a transition among two states of that structure). Yet the description stressing the process rather than the structural character of the enzymatic action is more abstract and general, and for these reasons should be better used when comparing the enzymatic process to other processes occurring in the living cell.

ii - Let me add something about the future work which seems more interesting from the personal viewpoint developed in this overview. In my opinion, there are at least three lines which are worth mentioning:
a - detecting cross-correlation among fluctuating macrovariables by non-destrctive probes.
b - using computer simulation to reach the fluctuation spectrum centered near 10 exp -8[5].
c - producing by bio-engineering simpler enzymes, to be used as model system ameneable to theoretical treatments.

I am glad to see that these topics will be a matter of concern for several of the next speakers. The progress we shall make here along these avenues will be a good indication of the success of this ASI.

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