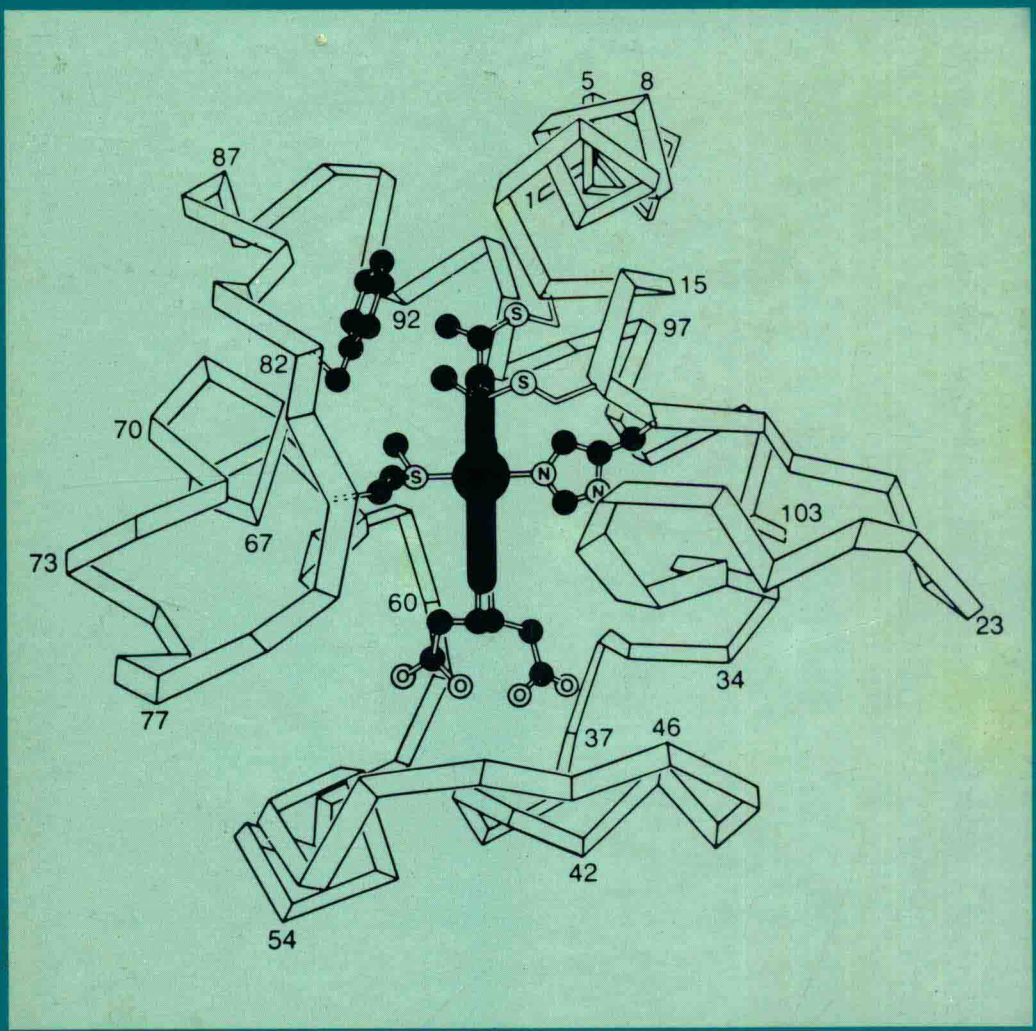
Ciba Foundation Symposium 93

Mobility and function in proteins and nucleic acids



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1983

Pitman
London

ISBN 0 272 79657 3

Published in February 1983 by Pitman Books Ltd, London.
Distributed in North America by CIBA Pharmaceutical Company (Medical Education Administration), Summit, NJ 07006, USA

Suggested series entry for library catalogues:
Ciba Foundation symposia

Ciba Foundation symposium
x + 357 pages, 100 figures, 20 tables

British Library cataloguing in publication data:
Mobility and function in proteins and nucleic acids.
(Ciba foundation symposium; 93)
1. Nucleic acids—Congresses
2. Proteins—Congresses
I. Porter, Ruth II. O'Connor, Maeve
III. Whelan, Julie IV. Series
574.87'328 QP620

Text set in 10/12pt Linotron 202 Times, printed and bound
in Great Britain at The Pitman Press, Bath

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Symposium on Mobility and function in proteins and nucleic acids, held at the Ciba Foundation, London, 2–4 March 1982
This symposium was held at the suggestion of G. C. K. Roberts
Editors: Ruth Porter (organizer), Maeve O'Connor and Julie Whelan

Introduction

F.M. RICHARDS

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May I first record our gratitude to Gordon Roberts, who initiated this meeting and has contributed immensely to the organization of the programme. From my own experience of previous symposia at the Ciba Foundation, and with this topic, I anticipate considerable differences of opinion during this meeting. I hope that we can resolve differences where possible, and clearly spell out both the questions and opposing views when agreement is not possible.

The symposium is centred on certain properties of macromolecules of biological origin. You will have noted that the words 'mobility' and 'function' both occur in the title. The phrasing strongly implies, although it does not so state, that there is a connection between the two. Some major questions which we should attempt to answer at this meeting are:

(1) Is mobility correlated with function?
(2) If such a correlation exists, is the observed mobility essential for function or incidental to it?
(3) If the answer to (2) is unclear, in what directions should we look for definitive experimental or theoretical answers?

The range of times that we shall be considering is enormous—12 to 15 decades on a logarithmic scale. At the fast end are atomic vibrations in the sub-picosecond region. Towards the slow end are structural fluctuations, measurable by leisurely physical or chemical techniques, in the range of 1 to 1000 seconds. The motions that most of us go through in our daily lives as macroorganisms occur in the latter time range. If we extrapolate another 15 decades along the logarithmic time-scale we come roughly to the age of the universe. From the point of view of two vibrating atoms, the fine detail of a nerve action potential would appear to unfold at a rate comparable, for us, to the movement of the tectonic plates. These comparisons serve to emphasize the care that we should exercise in comparing and correlating molecular motions in the various time ranges to which the different techniques give us access.

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I would hope that by the end of the symposium we shall have a better idea of the meaning of the commonly used words and phrases. For example, mobility may refer to small fluctuations about a single mean structure or to the ability to encompass a major transition between two quite different mean structures. In either case, the same system may appear either mobile or rigid, depending on the time-scale of the observation. The transition may be gradual on the time axis with many intermediate states and a low degree of correlation in local motions, or it may be abrupt, implying highly concerted movement.

(4) What qualifications need to be added in each example (and many others) for these terms to be meaningful and unambiguous?

The correlation of the experimental results with each other and with theoretical predictions will require the assumption of certain mechanical models for the presumed motions. The validity, or lack of it, of the various models requires our most careful consideration. A model successfully describing motion may or may not include any direct or implied connection with function. Models related to rapid small-scale motion are easily related to chemical theory, but are difficult to correlate with much slower biological phenomena. On the other hand, large-scale concerted motions of easily imagined functional interest present formidable theoretical problems. A general model will specify an amplitude and time function for each defined component of the system, and will include the time range over which the model is presumed to apply.

(5) Are models for small fluctuations inherently different from those attempting to describe large-scale motions?

(6) How many models are required to cover usefully the entire time range for the motion of a macromolecule?

Much of our discussion will centre on the detailed application of certain physical and chemical techniques and on the analysis of data obtained from their application to specific systems. We shall consider X-ray diffraction, optical and magnetic resonance spectroscopy, and certain chemical procedures, including hydrogen exchange. We shall discuss the theories of what molecular structure and motion might look like. X-ray diffraction data, in general, offer no time base for fast processes, and no information on correlations between movements, but they do contain information on the mean amplitude of motion.

(7) What is the proper method for extracting amplitude data, given that the standard Debye-Waller formalism is demonstrably inappropriate?

For spectroscopic procedures, time-based parameters can frequently be measured quite accurately, but estimates of amplitude are absent or can be inferred only indirectly.

(8) Can one derive a convincing mechanical model of the motion to fit the spectroscopic data, and can it be shown to be unique?

Hydrogen exchange is an example of a chemical procedure that is capable of

providing kinetic data over an enormous time range.

(9) Can such measurements be correlated with X-ray or magnetic relaxation data, in the light of our present understanding of the details of the exchange process?

The theoretical approach to macromolecular dynamics invariably involves approximations: in the basic input such as potential functions, in the treatment of the solvent and other small molecule components, and in the calculation procedures. In general the motions will be anisotropic, anharmonic and frequently highly damped—all factors that make for difficulty in modelling. In view of the approximations and uncertainties on both the theoretical and the experimental sides:

(10) Is agreement between a model derived from theory and one derived from experiment to be greeted with joy or with suspicion?

Let us assume that these questions on experiments and their interpretation are satisfactorily answered in a particular system, not only for the macromolecule but for its complexes with appropriate ligands. Then,

(11) Is it possible to answer the first three questions even for this system?

(12) What criteria need to be met to establish convincingly a connection between motion and function?

As we consider motions and structural fluctuations in proteins and nucleic acids, ranging from localized vibrations and librations to motions involving the entire molecule, I hope that additional or alternative questions, either specific or general, will arise—the answers to which this symposium might reasonably be expected to provide. Each speaker will be concentrating on a particular macromolecule or macromolecular system. I hope that as a group we can try to extract from each case those elements or ideas that may be applicable to macromolecules in general and to focus especially on those structural fluctuations that may be characteristic of these systems as large structures.

The role of mobility in the substrate binding and catalytic machinery of enzymes

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Abstract Recent theoretical and experimental studies have demonstrated that proteins are fluctuating systems capable of large, seemingly random, excursions from the equilibrium conformation. Attention is now focusing on the functional consequences of these motions. X-ray diffraction is a powerful tool for mapping the spatial distribution of protein dynamics; studies on the temperature dependence of the apparent Debye-Waller factors of crystalline myoglobin demonstrate that proteins are flexible in the solid state. Crystallographic studies of a Michaelis complex of ribonuclease A show that a mobile lysine adapts its conformation to the changes in stereochemistry and charge distribution in the substrate during catalysis. The structure of the triose phosphate isomerase–substrate complex shows that a mobile region of 10 amino acids becomes ordered when ligand binds. These studies suggest several roles for protein mobility in enzymic catalysis: providing access to internal sites, allowing changes in substrate structure during the reaction, and reducing the observed binding constant of substrate and product to the enzyme by decreasing entropy. A flexible enzyme also does not need a communication system to signal binding or transformation, since a pre-existing equilibrium can be used. More speculative ideas, such as the guiding of thermal vibrations along the reaction coordinate, can only be tested when more detailed data are available.

Enzymes as machines

The earliest concepts of enzyme structure viewed these biological catalysts as colloids without a defined conformation. Since that time, our view of these molecules has oscillated from one of great flexibility to rigidity and back again. In the last five years, virtually the entire arsenal of biophysical methods has been trained on the problem of protein dynamics. We now understand

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that, in aqueous solution at ordinary temperatures, atoms in globular proteins can undergo a wide variety of motions, ranging in amplitudes from a few hundredths of an Ångstrom to several Ångstroms and in frequencies from less than one picosecond to seconds (Gurd & Rothgeb 1979). Attention is now beginning to focus on the functional consequences of the random fluctuations in protein structure, on the not unreasonable assumption that nature usually turns the inevitable to her advantage.

We are interested in the structural basis for enzymic catalysis. Some enzymes speed up the rate of their chemical reactions by factors of 10^{10} or more. Simple organic and inorganic catalysts cannot approach this efficiency. Since one characteristic these small molecule catalysts lack that enzymes possess is conformational flexibility, we have tried to identify what chemical advantages a dynamic structure might confer on a protein.

The concept of a changing structure for an enzyme is hardly revolutionary. Indeed, our conventional way of viewing biological catalysis is by analogy to machinery. Like the elaborate cause-and-effect contraptions created by the American cartoonist Rube Goldberg and his British counterpart Heath Robinson in the 1920s, enzymes are thought of as moving logically from one well-defined conformation to another in response to a set of signals. These signals originate with the binding of substrate to the enzyme or its conversion to an intermediate, and are propagated to distant parts of the protein by a linked series of small changes: a hydrogen bond breaks, leading to a shift in a helix–helix contact, which in turn, etc. (the pre-eminent example of this view is the transformation from the R to T state of haemoglobin).

The validity of this picture is long established. But this picture is not quite what we want to discuss here. The Rube Goldberg/Heath Robinson picture of conformational changes in a protein is a description of transitions from one equilibrium state to another. We are concerned, in this paper, with the fluctuations of the enzyme about any given equilibrium state, and the role of these thermal energy-driven motions in the catalytic process. Such movements are random and difficult to observe, but ubiquitous. And we have found, by examination of their traces in the crystal structures of two enzyme–substrate complexes, that they may be extremely important for efficient catalysis.

Proteins are flexible in the crystalline state

Since we shall rely on the results of X-ray diffraction to map the spatial distribution of protein fluctuations, it is essential to establish that these small random motions really occur in the crystalline state and can be measured. There is considerable indirect evidence that proteins maintain their flexibility

even on crystallization: protein crystals are typically 50% solvent by volume (Matthews 1968), so there is room in the lattice for substantial mobility; buried amide hydrogens in crystalline proteins exchange with solvent deuterons at rates comparable with their exchange rates in solution, implying that whatever motions admit water to the protein interior or expose the inner structure to solvent occur in the solid state (Tüchsen & Ottesen 1979), and there is considerable fall-off in the electron density distribution around each atom in a protein crystal structure, presumably from thermal motion of those atoms (Artymiuk et al 1979, Frauenfelder et al 1979). This last point is crucial to our use of diffraction as a tool to study protein motion, so we want to discuss it in some detail.

The fall-off of electron density around each atom can be fitted to a Gaussian curve whose width at half-height, the apparent mean-square displacement or $<x^2>$ of that atom, is related to the conventional Debye-Waller factor, B, of that atom by the expression $B = 8\pi^2 <x^2>$ (Willis & Pryor 1975). If high resolution X-ray data (i.e. 0.2 nm or better) have been collected for a structure, the apparent B for each atom can be refined in a restrained least-squares method, as described by J. H. Konnert and W. A. Hendrickson (Konnert 1976). Then the apparent $<x^2>$ for each atom can be calculated. This method requires two incorrect assumptions, the seriousness of which is unknown: first, that the motion is isotropic, and second, that it is harmonic. Interpretation of the $<x^2>$ values in terms of actual motion requires the additional assumption that the observed fall-off in electron density from the average position of each atom is really due largely to atomic motion. Unfortunately, it could be dominated by a build-up of errors in the structure determination and/or by static disorder in the crystal lattice. We do not believe this is the case, since the observed $<x^2>$ values correlate sensibly with structural features in the protein (Frauenfelder & Petsko 1980), but direct evidence is desirable.

Atomic motion should be temperature-dependent; lattice disorder should not be. Further, the magnitude of any temperature dependence should enable pure harmonic vibrations (small and linear dependence on T) to be differentiated from larger, more complex motions that require a potential energy barrier to be overcome (large and complex T dependence). In collaboration with Professor Fritz Parak, Drs W. Steigeman and H. Hartmann in Munich, and Professor H. Frauenfelder in Illinois, we have refined the crystal structure of sperm whale Met-myoglobin at 0.2 nm resolution at a temperature of 80 K (Hartmann et al 1982). Fig. 1 shows a comparison of the average $<x^2>$ of the backbone atoms of myoglobin as a function of residue number at 80 K with the values obtained earlier at room temperature (Frauenfelder et al 1979). The overall $<x^2>$ is reduced by more than a factor of two, and individual residues show reductions even larger than this. The apparent $<x^2>$