Structure, Dynamics and Function of Biomolecules

Edited by
A. Ehrenberg R. Rigler A. Gräslund L. Nilsson

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The First EBSA Workshop

A Marcus Wallenberg Symposium

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Preface

This is a collection of papers presented and discussed at the first EBSA workshop held at Saltsjöbaden outside Stockholm in Sweden, July 6-10, 1986. The common theme of these papers is dynamics of biomolecules, and how the dynamics depends on the molecular structure and organization, and connects to and determines the biological function. This is a rapidly expanding field of research which combines many different aspects of molecular biophysics. Much material is new and presented for the first time. Even if the work so far has been of the kind that is usually called basic research, practical applications are clearly indicated in some articles, and are waiting around the corner in several other cases.

At the workshop only one third of the time was used for the formal presentations and two thirds for discussion. To this should also be added discussions during the poster sessions. During these lively and unrecorded discussions fresh viewpoints emerged and new ideas were created. Admittedly, our knowledge at present is only fragmentary but when pieces of the puzzle are brought together at a workshop or in a publication of this kind more extended and sometimes unexpected contours and shapes become visible. It is our hope that this rapid publication of camera-ready manuscripts will transfer some of the spirit at the workshop to the reader, and in his or her institute or laboratory initiate further discussions, bring forward more ideas and start new experimental aproaches.

Without the very substantial support from the Marcus Wallenberg Foundation for International Scientific Collaboration it had not been possible to organize the workshop at such a short notice as seven months. Further financial support was obtained from Skandigen AB, the Swedish Natural Science Research Council, Pharmacia AB, LKB Bioteknik AB, Nuclear Data AB and Kabi Vitrum AB. Our work with the organization has depended very much on the patient and reliable assistance of Mrs Haidi Astlind and Mrs Karin Nilson. The staff of Grand Hotel Saltsjöbaden created a friendly atmosphere for the workshop. We gratefully acknowledge the provided financial support and all personal efforts that helped us to organize the workshop.

Stockholm, August 1986

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Introduction

EBSA (European Biophysical Societies Association) was initiated December 9, 1981, when representatives of the biophysical societies of nine European countries, following a proposal of the British Biophysical Society and an invitation from Professor A. Müller-Broich and the German Biophysical Society, met in Frankfurt a.M. to discuss forms of cooperation between the societies. An executive committee was established with A. Ehrenberg, Sweden, president, W. Kreutz, Germany, and P. Bayley, England, vice presidents, and J. Clauwaert, Belgium, secretary. This committee had to work out a proposal for statutes and activities of EBSA. The proposals of statutes of EBSA were circulated among the societies and finally adopted at the first meeting of the general assembly of EBSA, July 30, 1984, in Bristol. For the next three year period the following executive committee was elected: A. Ehrenberg (Stockholm), president, J. Engel (Basel), vice president, P. Bayley (London), member, and J. Clauwaert (Antwerp), secretary-treasurer. At present EBSA has eleven member societies.

It was stated at the general assembly that one important task for the executive committee would be to initiate the organisation of discussion meetings on specialized topics in the forefront of biophysical research, if possible combined with rapid publication of collected short papers on the theme of the meeting. The first EBSA workshop took place this summer. The hope is that many will follow.

Stockholm, August 1986 Anders Ehrenberg

List of Contributors

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The Nature of Mechanical Devices in Biological Systems

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A mechanical device must have moving parts. Even the simplest lever turns about a fulcrum. A biological system clearly directs stress and strain so that action and information are transmitted in a deliberate spatial pattern. The question arises as to how this can be done at the molecular level. The available motions which must be built into a molecular machine are known to be side-chain motions (vibrations or rotations), segment motions (vibrations, rotations or lateral translations) domain motions (vibrations, rotations or lateral translations) and whole molecule movements. We know that the polymers involved are proteins, polysaccharides and polynucleotides. I have been concerned with the first two only. I shall describe some observations and views.

Lysozyme

NMR studies of lysozyme against the background of the crystal structure led to clear demonstration of the four types of motion (i) Rotational flipping of phenylalanine and tyrosine rings and of valine and leucine asymetric tops. Similar motions of many side-chains are possible. (ii) Oscillation of tryptophan side chains. (iii) General motion of side-chains on the surfaces of the protein. (iv) Changes in structure during inhibitor binding. The last of these had been recognised earlier in the X-ray studies. The observations led to a reconsideration of the possible definition of the X-ray structure itself and generated an analysis of thermal motions (B-factors) in protein crystals. Similar observations using NMR studies of other proteins e.g. the trypsin inhibitor and cytochromes were made at about the same time by ourselves and

others. As a consequence of this work we can now see that proteins (and equally DNA, RNA and polysaccharides) are dynamic cooperative structures. Changes of local structure generally give changes sometimes small, sometimes large, in global structure. Simple consequences of the development of the ideas are that the matchings of biopolymer surfaces (protein-substrate interaction or polymer-polymer interaction) are not die/mould fittings nor are they simple induced fittings of the R → T type. They are hand in glove energy minimisation fittings in which dynamics and structure are both modified. The great advantage of such fittings is that rates of coming together are controlled as well as binding energies. Action in biology can be associated with transients. Of course the very mobility of the system in all states causes a loss of structural definition. This means that to some degree ultimate specificity (die in mould) is sacrificed for rate control. Selectivity is retained.

Calcium Proteins

The study of calcium proteins by many groups including my own has illustrated these principles extremely clearly. There are a series of proteins designed to respond differentially in rate, selectivity, and binding strength so as to allow the full value of calcium activations of cells and their recoveries to be expressed. In essence all the proteins are comprised of (i) a small two stranded β-sheet which has side-chains of considerable motion but a back-bone which is relatively constrained and which binds two calcium ions, (ii) a set of four helices which act as transmitting rods (levers) from the β-sheet to distant loops (iii) loops distant from the calcium site which undergo considerable restructuring easily (iv) a local fulcrum near, just behind, the β-sheet which is comprised of three rapidly flipping aromatic residues from two different helices and is a point of cross-over of the helical rods. Rotational/translational relative helix motion at the fulcrum propagates and enhances the changes generated by calcium binding (at the β-sheet) to the distant loops. This NMR picture has been fully confirmed by later X-ray structure studies. Apart from the demonstration of this general mechanical transmission device there are individual features in calmodulins and troponins (fast binding

and release but medium binding strength) and parvalbumins (slower binding and release but stronger binding) so that a series of rate controlled movements of the calcium ion can be managed in different devices for triggering and recovery. Possibly even calcium diffusion is controlled. It is likely that many other effectors work in this way. There is a further device, the calcium ion pump, and a possible model for this is given below.

Membrane Proteins

A second example of helical motion I would like to stress is helix movements in membranes. We have proposed following a study of glycophorin and of a variety of chemical structures such as alamethicin that membrane helices could have two types of motion. The first, the simple movement of a helix into or out of a membrane, would operate a push-pull signal from the extracellular to the intracellular matrix. Glycophorin was our example but the principle can be extended to treat the EGF-receptor for example. The link of the segmental protein motion to the side-chain motions of a space-inspecting polysaccharide joined to the protein is only one possibility for such a device. The second proposal is that relative motions like those of the helices of calmodulin could be used to create a gated ion channel. The F_0-peptides of the ATP-ase would be such a channel and as in calmodulin the driving force is ion (proton) binding to anionic side-chains but now on the surface of the helices. (We know that calmodulin-drugs bind to the surfaces of its helices and twist them). Perhaps F_0 is a proton-modulin in a membrane which drives ATP-formation.

Kinases

My final example concerns a joint study of kinases with two crystallographic groups - those of Blake at Oxford and Watson at Bristol. The study is of segmental motion controlling the release of such molecules as ATP from a cleft. The kinase we have analysed by NMR is phosphoglycerate kinase. We have shown that the protein

has a somewhat mobile region associated with the surfaces and the link between two domains. The domains are β-sheets (barrels) of high rigidity so that we see few highly resolved resonances in this protein of molecular weight 45,000. All the well resolved resonances appear to be related to the link region which is comprised in part of two crossing helices. The suggestion which has been made by Blake especially is that the two helices form an open/closed hinge. The change of state is detailed also by Watson. The NMR studies indicate that the structure is in fact constantly flipping between open and closed forms. We shall present a full description of this protein shortly. It is easy to see how calcium binding to a calmodulin bound to a kinase could be used as a mechanical switch for such a kinase.

We have taken over this model of a kinase together with the above calmodulin model, but now for a channel in a membrane, and given a provocative structure for ATP driven ion pumps or for ion (proton) driven ATP synthesis. In essence ATP release from a groove is driven by ion flow through a channel of helices which is gated by a conformational change of the helix rotation/translation type.

An overall reference to this work is
R.J.P. Williams, Europ. J. Biochem. 150, 231-248 (1985)

Physical Chemistry and Biological Strategy of Antigen Recognition

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In attempting to understand the molecular and cellular events involved in the recognition of antigen by the immune system, one encounters a fascinating mixture of physical chemistry, and biological strategy. Here we describe briefly how two classical techniques of physical chemistry, fluorescence spectroscopy, and NMR can be used to study certain aspects of some central processes in immunology: the recognition of antigen by antibodies on B cells, the MHC restricted recognition of processed antigen by T (helper) cells, and (T-cell)-(B-cell) collaboration.

One of a number of pathways involved in antigen recognition is sketched below:

$$B^d + Ag_o \rightarrow B^dAg_o \qquad (A)$$

$$B^dAg_0 \rightarrow B^dAg_1 + \ldots\ldots \qquad (B)$$

$$B^dAg_1 + T_H^d \rightarrow B^dAg_1T_H^d \qquad (C)$$

$$B^dAg_1T_H^d \rightarrow I\ell \cdot 2 + \ldots \qquad (D)$$

$$I\ell\text{-}2 + B^d \rightarrow IgG + \ldots \qquad (E)$$

$$IgG + Ag_o \rightarrow IgGAg_o \qquad (F)$$

$$IgGAg_o + M\phi \rightarrow 0 + \ldots \qquad (G)$$

In step (A) a protein antigen Ag_o binds to surface immunogloblin (sIgG) on the plasma membrane of a B lymphocyte. The protein antigen is internalized, degraded, and fragments (peptides), denoted Ag_1, appear on the surface of the B cell. For recent work, see ref. (1). If the peptide fragment Ag_1 is non-self, e.g., a peptide from a virus, then the B helper T-lymphocyte T_H^d with its receptor R_T can "recognize" this antigen and be stimulated to produce interleukin-2 (Iℓ-2) as shown in (D). The recognition event in (C) is said to be "MHC restricted",