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Protein Crystallography

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PROTEIN CRYSTALLOGRAPHY

（内部交流）

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PROTEIN CRYSTALLOGRAPHY

MOLECULAR BIOLOGY

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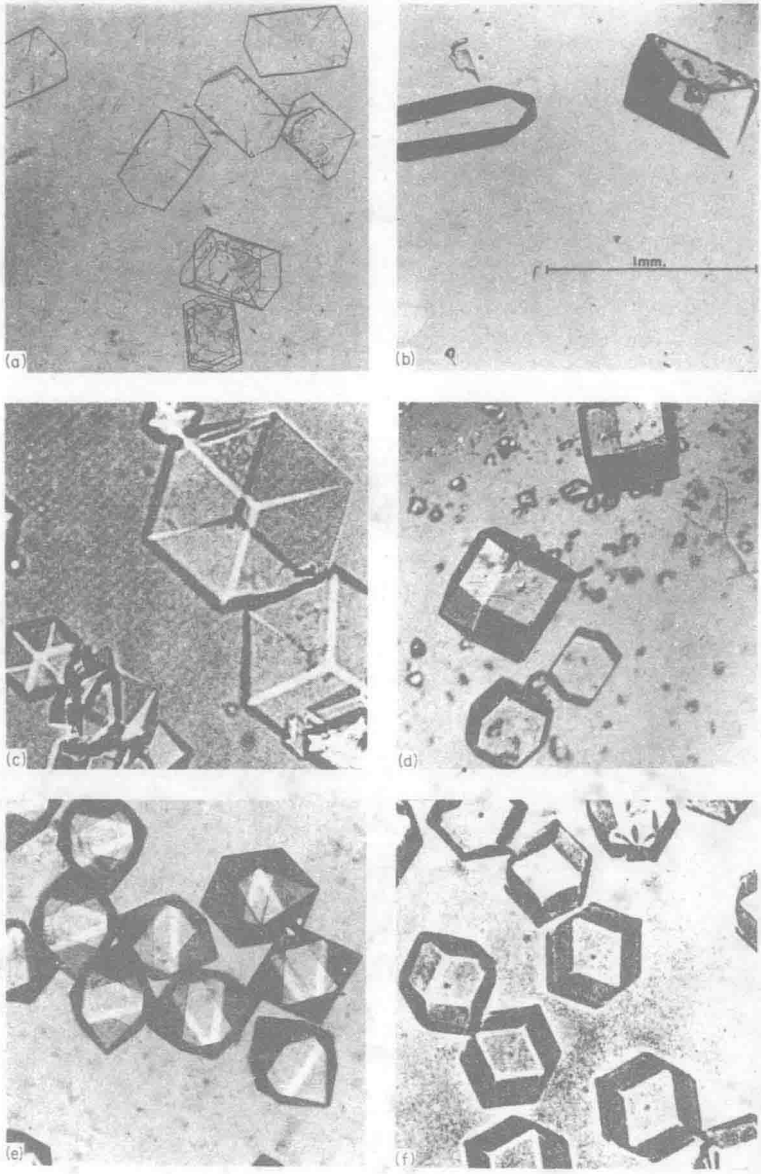
Protein Crystals. a) monoclinic aldolase. b) orthorhombic triosephosphate isomerase. c) trigonal insulin. d) tetragonal lysozyme. e) hexagonal aldolase. f) cubic glucagon.



PREFACE

The scope of this book is simply defined: we have tried to cover all aspects of the X-ray diffraction analysis of protein crystals, with special emphasis on the differences between the analysis of protein crystals and the analysis of crystals of small molecules. In several cases we have had a difficult task in selection of material. In general we have limited our detailed descriptions to those procedures which have contributed to successful structure determinations. We also include chapters on neutron diffraction, γ-ray resonance and electron microscopy. The information obtained from the application of these techniques to protein crystals is complementary to that obtained by X-ray analysis and in recent years there have been considerable advances in these fields.

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April, 1976

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INTRODUCTION

We ought to be neither like spiders which spin things out of their own insides nor like ants which merely collect but like bees which both collect and arrange.

Sir Francis Bacon

1.1 INTRODUCTION

Protein Crystallography is the application of the techniques of X-ray diffraction (and to a lesser extent, those of neutron, γ ray and electron diffraction) to crystals of one of the most important classes of biological molecules, the proteins. Proteins are ubiquitous molecules. They include the enzymes, the biological catalysts which control all chemical processes in living systems; the storage and carrier molecules such as haemoglobin which is responsible for the transport of oxygen in the blood; the hormones, which act as messengers between different parts of the organism; and the antibodies which provide immunity against infection. It is known that the diverse biological functions of these complex molecules are determined by and are dependent upon their three-dimensional structure and upon the ability of these structures to respond to other molecules by changes in shape. At the present time X-ray analysis of protein crystals forms the only method by which detailed structural information (in terms of the spatial co-ordinates of the atoms) may be obtained. The results of these analyses have provided firm structural evidence which, together with biochemical and chemical studies, immediately suggests proposals concerning the molecular basis of biological activity.

The success of these physical techniques in describing biological structure and function does not mean that biology has become a branch of physics. As Bernal (1969) has written: "The use of physical or chemical knowledge to explain mechanical, electrical or chemical aspects of living organisms has brought into greater relief their biological aspects. These phenomena however well they could be described in physical terms do not occur in mechanisms made by some divine craftsman from ideal models laid down from all eternity, but in self-regulating

and self-producing entities whose present form is the result of an evolution stretching back over billions of years".

Protein Crystallography has advanced extremely rapidly in the last decade and in certain cases protein structures may now be determined relatively easily using standard methods. (Previously such analyses had proved difficult and time consuming.) Many of the techniques which were being tentatively tried out a few years ago are now well established; other approaches have still to prove fruitful and in certain areas there is a need for new ideas. This recent knowledge is scattered in many different journals and doctoral theses or is simply passed by word of mouth from one generation of research students to the next. It seemed to us that the subject has now reached a stage of maturity when a detailed review in the form of a book is needed. Our aim has been to draw together in a systematic and comprehensible manner those methods that are now well established and to indicate those areas where further expansion is needed.

The subject attracts workers from many different disciplines, such as chemistry, biochemistry, biophysics, zoology, physics and mathematics, and the different insights and skills contributed by these workers are vitally important in this multi-disciplinary subject. The book is primarily intended for those who wish to do research in the field. However, we have attempted to write it in a style which will be comprehensible to workers from these various disciplines.

The principles of protein structure and the implications of the X-ray analyses of biological molecules form important parts of many undergraduate courses in both biological and physical sciences. We hope that this book will also prove useful to undergraduates especially where a deeper insight into the scope and limitations of the technique than is usually provided in review articles is required.

In this introductory chapter we first survey the biochemistry of the proteins which have been and are being studied by protein crystallographers and which recur frequently in our discussions of techniques. We then review the history of our subject and provide a synopsis of the special problems encountered in protein crystallography and the means by which they have been overcome. Thus this chapter to some extent forms a resumé of the book.

1.2 THE NATURE AND FUNCTION OF GLOBULAR PROTEINS

We have already seen that globular proteins may have many roles in organisms including biological catalysis and regulation of cellular processes. It was in fact their wide range of biological functions and their consequent importance in living systems which led to the name "proteins" which is derived from a Greek word meaning first. We shall see in Chapter 2 that proteins are characterized by their hydrolysis to amino acids in dilute solutions of mineral acids at boiling point. In fact all proteins are built up from linear polymers of amino acids called

polypeptide chains. They may be divided into two classes, fibrous proteins and globular proteins, on the basis of their structure and function.

Fibrous proteins are relatively insoluble in aqueous solutions. These include the keratins of wool, hair and horn, the myosin of muscle, the fibroin of silk and the collagen of tendon. These fibrous proteins have a structural role. They are either protective materials or they are nature's ropes and girders. The fibrous nature and the insolubility of these materials make crystallization with good three dimensional order almost impossible. Consequently the information we can gain about their structure is much less precise and the techniques of their structure analysis are quite different from that of globular proteins. The techniques are reviewed by Holmes and Blow (1966); we do not intend to discuss them in this volume.

In globular proteins the polypeptide chain has a complex folded structure. Most globular proteins are soluble in aqueous solutions, but others which are normally located in or on membrane structures will be more soluble in nonpolar solvents. The difficulty of purifying and stabilizing the membrane proteins has meant that most proteins studied by X-ray analysis at high resolution are water soluble proteins and so our present knowledge of structure is really rather unrepresentative of globular proteins as a whole. Further, the crystallographer has tended to study the more stable proteins and these have often been extracellular proteins.

Let us consider in more detail the globular proteins which have been the subject of X-ray analysis. For the reader who has no biochemical training we recommend one of the standard texts such as "Biological Chemistry" by Mahler and Cordes (1968) or "Biochemistry" by Lehninger (1975). Here we will try to outline the biological roles of some of the most intensely studied proteins. We hope that this will give the reader some impression of the purpose of the structural study when we are discussing the techniques of protein crystallography.

Almost all of the proteins studied by X-ray analysis can be classified in one of the five following groups:

1. Enzymes
2. Redox proteins
3. Carrier and storage proteins
4. Hormones
5. Antibodies

Let us consider enzymes first.

Enzymes are catalysts; they alter the rate of biochemical reactions without altering the position of equilibrium. They are important in all biological processes. They are classified according to the kind of reactions catalysed; the classification is illustrated in Table 1.I with the enzymes which are the subject of discussion in this book.

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