COMMENT

PROTEIN CRYSTALLOGRAPHY AND THE STRUCTURE OF INSULIN*

THE origin of protein crystallography can be traced to the first recording of the diffraction pattern from crystals of the protein pepsin in 1934 by J. D. Bernal and Dorothy Crowfoot (Hodgkin)[1]. This was soon followed, in 1935, by the X-ray diffraction photography of crystals of the well-known protein hormone insulin by Dorothy Crowfoot Hodgkin[2]. Professor Hodgkin often describes the X-ray photography of insulin (which were later identified as those of 2Zn insulin with two zinc ions per insulin hexamer) as the most exciting event in her long and illustrious scientific career, matched only by the eventual solution of its structure at 2.8 Å resolution 34 years later[3].

I remember how, at the International Congress of Crystallography at Stony Brook, NY (in 1969), Dorothy Hodgkin stole the thunder when it was announced that the structure of insulin had been solved in her laboratory. It is also characteristic of her that the paper was presented by the youngest member of her group. It is a reflection of her steadfastness and singlemindedness in pursuing a difficult problem for long years that no one in the insulin group of her laboratory in 1969—Guy Dodson, Eleanor Dodson, Tom Blundell and M. Vijayan—who finally helped her accomplish the solution of the structure, was even born when she started her work on insulin in 1935! It must be a matter of great happiness to her that these 'young crystallographers' have established renowned schools, at York, London and Bangalore, respectively. Subsequent to the solution of the structure, Hodgkin and her colleagues concentrated on its refinement at high (1.5 Å) resolution. The refined structure has been presented at length in the 6 July 1988 issue of the *Philosophical Transactions of the Royal Society, London*[4]. The paper contains the most comprehensive and elaborate discussion to date on a protein structure and the associated water molecules.

Insulin has been involved in many landmarks in protein biochemistry. It was the first protein to be sequenced and also the first to be chemically synthesized. More recently, the insulin gene has been among the first to be cloned using genetic engineering techniques and the protein has been expressed in large quantities. Its role in protein crystallography has been no less important. As indicated earlier, crystals of insulin were among the first protein crystals to be X-ray-photographed. Among the early applications of the then recently developed Patterson method in the early thirties was the one on insulin crystals[5], a formidable undertaking indeed in the pre-computer days. Rotation and translation functions are the most important components of the now widely used molecular replacement method. The elucidation of the internal symmetry in insulin crystals was among the earliest applications of these functions[6]. The comparatively small size of insulin molecules and the tight packing of molecules in the crystals made the preparation of several independent heavy-atom derivatives rather difficult. The derivatives ultimately used in the structure solution were related to one another to varying degrees. This, as well as the total absence of centric reflections in the data, led to serious crystallographic problems. These were overcome largely through the extensive use of methods based on X-ray anomalous scattering[7,8]. The experience gained through the X-ray analysis of 2Z insulin in the effective use of anomalous scattering in protein crystallography has indeed been of considerable general interest. 2Zn Insulin has also been among the first protein crystal structures to be refined at high resolution. The work on insulin led to valuable contributions toward the development of protein refinement methodologies and the elucidation of the nature of problems encountered during refinement[9-11].

The detailed paper on 2Zn insulin in the *Philos. Trans.* indeed marks the culmination of an effort of epic proportions spread well over half a century. During this period, Professor Dorothy Hodgkin determined the structures of several important molecules, including those of cholesterol, penicillin and vitamin B_{12}, came to be recognized as perhaps the most distinguished X-ray crystallographer in the world, and received many honours, including the Nobel Prize. However, insulin has been the molecule

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closest to her heart and the work on it spanned her entire scientific career. In this process she trained generations of crystallographers in different continents and laid the foundations for the spread of protein crystallography to different parts of the world. The *Philos. Trans.* paper itself bears testimony to this truly international character of hers. The authors of the paper belong to five different countries, namely England, Australia, New Zealand, Japan and India.

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