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BIOLOGICAL STRUCTURE
AND
FUNCTION AT THE MOLECULAR LEVEL

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CONTENTS

THE STRUCTURE OF GLOBULAR PROTEINS

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THE study of the structure of globular proteins by X-ray methods began in the late 1930's when the first X-ray photographs of protein crystals were obtained by Bernal, Crowfoot, Fankuchen and Perutz. Proteins are so much more complex than the molecules which were within the scope of X-ray methods at that time and for many years afterwards, that little progress was made in terms of results, though much in terms of techniques, for about 15 years. The decisive advance came in 1953, when Perutz showed[1] that it was possible to prepare heavy-atom derivatives of haemoglobin which crystallized isomorphously with the unsubstituted protein and whose crystals gave appreciably different diffraction patterns; he was then able to use the so-called method of isomorphous replacement which in principle enables an unambiguous structure determination to be carried out. During more recent years a number of crystalline proteins has been studied intensively with the object of applying this method, especially haemoglobin, myoglobin, insulin, ribonuclease and lysozyme. Always the difficulty has been to obtain suitable isomorphous heavy-atom derivatives; only one chemically rational method—the attachment of mercury or silver atoms to free sulphydryl groups, as originally used by Perutz—is known even today, and this is more often than not inapplicable since most of the proteins suitable for analysis on other grounds (e.g. low molecular weight, ease of crystallization) do not contain free sulphydryl groups. For the most part it has been necessary to search *ad hoc* for methods of attaching heavy atoms, those used successfully for one crystalline protein generally not being applicable to others. Only in haemoglobin and myoglobin has the isomorphous replacement method so far been applied with complete success. Perutz's early results with haemoglobin took the form of a projection of a row of haemoglobin molecules onto a plane—they were the first pictures of a protein molecule which were demonstrably correct, but the amount of structural information which could be derived from what was in effect a silhouette of a very complicated molecule was

small. It was clear that if the method was to be exploited it was necessary to extend it into the third dimension, and this was first achieved in 1957 in the analysis of the simpler protein myoglobin.[2]

The first stage of the X-ray analysis of myoglobin resulted in a three-dimensional representation of the molecule at a resolution of 6Å, insufficient to reveal the side-chains or even the configuration of the polypeptide chain itself, but which did give a picture of the general arrangement of the polypeptide chain and of the haem group—in other words of the tertiary structure of the molecule. This tertiary structure proved to be unexpectedly irregular and complex: clearly the simple geometrical arrangements proposed in many earlier theories of protein structure could not be correct. More recently the resolution of the analysis has been increased to 2Å.[3] Although neighbouring covalently-bonded atoms are still not distinctly resolved, it is now possible to separate atoms which are hydrogen-bonded or in Van der Waals contact, with the result that the atomic arrangement of most of the molecule can be inferred. At this resolution the appearance of the haem group with its central iron atom corresponds closely with theoretical expectation, thus providing a valuable check on the correctness of the analysis, and it can be seen that the iron atom is attached to a neighbouring polypeptide chain by means of a group which is almost certainly the imidazole ring of a histidine residue. Most of the polypeptide chain consists of a series of segments of right-handed α-helix, with parameters closely corresponding to those proposed by Pauling and Corey in 1951.[4] This was the first time that the α-helix had been directly seen in a protein molecule; the results confirmed earlier plausible evidence that this configuration was important in the structure of globular proteins as well as in those of many fibrous proteins. It turns out that the myoglobin molecule consists of 8 segments of α-helix joined by irregular regions of varying length: the helical segments comprise 75 per cent of the amino acid residues, in agreement with estimates made on the basis of optical rotation and deuterium exchange studies.

One reason for the choice of 2Å as the resolution of the second stage of the myoglobin analysis was that the secondary structure (or polypeptide chain configuration) should be clearly revealed at this resolution, as indeed proved to be the case; it was not anticipated that side-chains would be identifiable, so that progress could be made towards a direct determination of the amino acid sequence by X-ray methods alone. In the event expectation was much exceeded, for it was possible to see the individual side-chains as dense regions emerging from the helical main chain at intervals corresponding to the parameters of the α-helix, and a close ex-

amination of these regions often made it possible to identify side-chains with certainty; in other cases some ambiguity remained but the choice of side-chain could be reduced to two or three. At the present resolution about one-third of the side-chains can be identified with certainty, and another third with fairly high probability.[5] It now becomes possible to correlate these X-ray results with the preliminary data obtained by Dr. A. Edmundson who is engaged in working out the amino acid sequence of myoglobin by chemical methods.[4] He has broken down the molecule into short peptides by means of tryptic digestion, and he has determined the composition, and in a few cases the internal sequence, of these peptides. By comparing his results with our own it has proved possible to place almost all the tryptic peptides along the polypeptide chain, and the order of peptides thus ascribed corresponds with the order suggested in a few cases by Edmundson on the basis of preliminary work using chymotryptic digestion. Although some discrepancies remain and although the amino acid sequence has not yet been completely determined, its main features are now beyond doubt. We are at present engaged in an attempt to increase the resolution of the X-ray results still further, and we hope that the remaining ambiguities will then be removed.

Meanwhile Perutz and his collaborators have obtained a three-dimensional representation of the haemoglobin molecule at a resolution of 5·5Å.[7] Haemoglobin is known to be made up of four sub-units, each consisting of a single polypeptide chain and an associated haem group. Larger protein molecules generally consist of several sub-units, whose spatial arrangement is termed the quaternary structure. This remarkable finding indicates that the irregular arrangement of the polypeptide chain now found in two distinct proteins,derived from very different species, namely horse and sperm whale, has some general significance which we cannot at present understand. A further extension of the range of this structure has been provided by the work of Scouloudi,[8] who has shown that the molecule of seal myoglobin very closely resembles that of horse myoglobin.

The structures of virus particles lie outside the scope of the present paper; it is, however, relevant to note that in these still more complex molecules protein sub-units are also arranged in a highly regular manner. For example in tobacco mosaic virus the 2000 identical protein sub-units each as large as a myoglobin molecule, are arranged in the form of a helix; in the spherical viruses the sub-units are disposed symmetrically on the surface of regular or semi-regular polyhedra.

Thus we are now beginning to get a first glimpse at the general nature of protein structure at all levels of complexity. Hitherto the chief difficulty in applying to biological problems the results of the exhaustive physico-chemical and chemical studies of proteins carried out in many laboratories during the last half-century has been our lack of knowledge of the three-dimensional arrangement of the atoms of which protein molecules are composed. Such knowledge is an essential complement to an understanding of the chemical interconnections, because it is the spatial relations between the side-chains which determine the chemical behaviour and biological specificity of the protein molecule as a whole, and these relations cannot be determined, except in a fragmentary manner, by purely chemical techniques. The rate of progress on all these fronts is now so great that we are undoubtedly on the eve of a major advance in our understanding of one of the central problems of biology—the relation between the structure of the molecules making up living cells and their biological functioning.

REFERENCES

1. D. W. GREEN, V. M. INGRAM and M. F. PERUTZ; *Proc. Roy. Soc.* A, **225**, 315 (1954).
2. G. BODO, H. M. DINTZIS, J. C. KENDREW and H. W. WYCKOFF; *Proc. Roy. Soc.* A, **253**, 70 (1959).
3. J. C. KENDREW, R. E. DICKERSON, B. E. STRANDBERG, R. G. HART, D. R. DAVIES D. C. PHILLIPS and V. C. SHORE; *Nature* **185**, 422 (1960).
4. L. PAULING, R. B. COREY and H. R. BRANSON; *Proc. Nat. Acad. Sci., Wash.* **37**, 205 (1951).
5. J. C. KENDREW, H. C. WATSON, *et al*;, (in preparation).
6. A. B. EDMUNDSON and C. H. W. HIRS; (in preparation).
7. M. F. PERUTZ, M. G. ROSSMANN, A. F. CULLIS, H. MUIRHEAD, G. WILL and A. C. T. NORTH; *Nature* **185**, 416 (1960).
8. H. SCOULOUDI; *Proc. Roy. Soc.* A, **258**, 181 (1960).

ADDENDUM

Elucidation of the quaternary structure of haemoglobin was facilitated by the results of study of the tertiary structure of myoglobin which is extremely close to the tertiary structure of each of the four sub-units of haemoglobin. The spatial structure of myoglobin is represented in Figs. 1 and 2. Figure 1 depicts the atomic model of the myoglobin molecule where each small offshoot of the basic skeleton corresponds to the position of the individual atoms in space. The thicker winding white line denotes the general course of the polypeptide chain.

In Fig. 2 the three-dimensional configuration of this polypeptide chain is represented diagrammatically.

The investigations of Perutz have shown that the quaternary structure of haemoglobin is very simple and symmetrical; four sub-units are located at the apices of an almost regular tetrahedron.

Figure 3 depicts the two α- and two β-chains of the haemoglobin molecule.

Figure 4 shows how these four sub-units are joined into the quaternary structure of the haemoglobin molecule.

It may be supposed from the similarity detected in the structure of the sub-units of haemoglobin to that for myoglobin that the secondary structure of these molecules must also be very similar.