PROCEEDINGS OF THE

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VORWORT

In der Biologie und allen mit ihr zusammenhängenden Fächern muss es wohl so sein, dass sich mit jedem Problem, das wir lösen, eine Vielzahl neuer Probleme auftut. Die Vielfalt müsste hoffnungslos, unbeherrschbar wachsen, würden nicht im gleichen Masse Probleme und Disziplinen, die gestern voneinander noch nichts wussten, zusammenfliessen und auf eine gemeinsame Lösung drängen, die heute wohl die molekulare Interpretation ist. Kaum anderswo zeichnet sich dieser Vorgang so eindrucksvoll ab, wie gerade bei den Nukleinsäuren, wo Erbgenetik, chemische Struktur und physikalische Morphologie des Moleküls aufs engste zusammenhängende Probleme geworden sind. Dieser Umstand und der Besitz der von Ochoa und Grunberg-Manago erforschten synthetischen Polynukleotide als Modellsubstanzen für die natürlichen Nukleinsäuren berechtigte wohl dazu, die Nukleinsäuren im Rahmen dieses Symposiums durch eine so grosse Zahl von Vorträgen herauszuheben. — Der Physiko-Chemiker sieht sich beim Studium der biologisch wichtigen Hochpolymeren Problemen gegenüber, deren ungeheure Vielgestaltigkeit sich nur zum Teil in der Vielfalt der im Vordergrund stehenden Untersuchungsmethoden wiederspiegelt. Lichtstreuung, Ultrazentrifuge, Diffusion, Viskosität und Elektronenmikroskopie, Verfahren, deren Anwendung auf die Nukleinsäure in den Vorträgen von Alexander und Stacey, Butler, Hall und Sadron als wichtig hervorgehoben werden, erschliessen uns die molekulare Morphologie und Heterogenität dieser Stoffe sowie ihrer Modelle, der Polynukleotide (Warner). Sie helfen mit, die Wirkung von ionisierenden Strahlen und alkylierenden Agenzien auf die Nukleinsäuren zu studieren und damit Grundlagen für ein Verständnis der Mutationsauslösung dieser Agenzien zu gewinnen (Alexander und Stacey). — Die Röntgen-Kristallstrukturanalyse in ihrer höchstgezüchteten Form gibt uns, wie wir aus dem Vortrag von Kendrew entnehmen, Angaben über die spezielle Faltungsform der Polypeptidkette im korpuskularen Molekül. Auch die Deutung der optischen Aktivität gewinnt an Hand der Messungen an Polypeptiden und Proteinen im Lichte der neueren strukturellen Erkenntnisse hohe Aktualität (Doty). Die Faserröntgenographie erlebt neue Triumphe in ihrer Anwendung auf die zu orientierten Fäden geformten synthetischen Polynukleotide, sie lehrt uns, die Helixstruktur dieser unübertrefflichen Modellsubstanzen zu deuten (Rich).

Bei der Cellulose dagegen macht uns das gleiche Röntgenverfahren mit grossräumigen Periodizitäten der micellaren Struktur bekannt (Porod). Die Elektronenmikroskopie, die täglich ihre Anwendungsgebiete erweitert, lehrt uns, diese Perioden, soweit sie in der Faserrichtung verlaufen,

als den regelmässigen Wechsel von dicht und locker gebauten — besser und schlechter geordneten — Bereichen zu erkennen, ein bemerkenswertes Phänomen, das, wie der Vergleich mit den synthetischen Hochpolymeren zeigt, zumindest *auch* als makromolekulare Eigenschaft gedeutet werden darf (Hess).

Auch andere synthetische Hochpolymere und solche, die wir künstlich mit natürlichen Fermenten herstellen, vermögen uns biologisch interessante Erkenntnisse zu bringen, wie Mark und Morawetz durch eine Sammlung derartiger Beispiele dartun. Ihr Aufruf zu einer engeren Zusammenarbeit zwischen den Biologen und jenen Forschern, die von Seite der Physik und Chemie her die Makromoleküle bearbeiten, ist nur zu berechtigt; man mag auch die Kuhn'schen Modellmuskel aus Polyacrylsäure und Polyvinylalkohol hierherzählen: wunderbare Maschinen, die chemische Energie verlustlos in mechanische Energie zu verwandeln vermögen, wenngleich die Frage der morphologischen Analogie mit dem natürlichen Muskel in vielen Punkten vorläufig ungeklärt bleibt.

O. KRATKY

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A THREE-DIMENSIONAL REPRESENTATION OF THE MYOGLOBLIN MOLECULE AT A RESOLUTION OF 6 Å

J. C. KENDREW

Medical Research Council Unit, Cavendish Laboratory, Cambridge, England

One of the most decisive advances in our understanding of protein structure was the elucidation by Sanger of the complete amino-acid sequence of insulin; his work gave us the first real hope of obtaining a full understanding of the behaviour of a protein molecule in terms of its chemical structure. The amino-acid sequence alone does not, however, tell the whole story, for it reveals nothing of the spatial relationships between the component parts of the molecule. Myoglobin, for example, consists of a single polypeptide chain of 153 residues; this chain must be extensively folded and re-folded to produce a molecule of the near-spherical shape characteristic of globular proteins. It is conventional to distinguish a *secondary* folding—very likely the α-helix of Pauling and Corey—and, superposed on this, a *tertiary* fold which brings the helical chain into a compact shape. The only technique which is available for obtaining detailed information about the secondary and tertiary folding is the use of X-ray methods to determine the structure of a protein crystal.

A crystal can be thought of as an indefinitely repeating three-dimensional periodic distribution of electron density. It can be shown that it bears the same relation to its X-ray diffraction pattern as does a musical note to the set of harmonics into which it can be analysed. Solving the structure of a crystal is essentially the reverse process, a harmonic synthesis or recombination of the elements of the diffraction pattern to give the electron-density of the repeating unit (or unit cell) of the crystal. To carry out this process one must know not only the amplitudes of all the reflexions in the pattern, which are directly measurable, but also their relative phases, that is to say the amount by which the various wave-trains are out of step with one another. Phases are not directly determinable, and herein lies the difficulty of X-ray analysis. In simple structures one can often proceed by trial and error, starting with an initial guess about the structure of the molecule; but in very complicated molecules such a method is out of the question. Indeed even a small protein is at least ten times larger than the

biggest molecule whose structure has so far been completely determined by X-ray methods (vitamin B_{12}, studied by Dorothy Hodgkin), so at the best one cannot at present hope for more than the discovery of the general outlines of the arrangement of the polypeptide chain within a protein molecule.

The studies of myoglobin here described depend not on trial and error, but on the application of the so-called *method of isomorphous replacement*, which has been applied in studies of simpler structures for a number of years, but which no one had used in protein work until Perutz first successfully did so in his studies of haemoglobin in 1953. The essence of the method is to replace some small group in the molecule by a very heavy atom or group. This replacement causes a slight change in the diffraction pattern; and provided that the positions of the heavy groups in the unit cell are known, and that the replacement causes no distortion of the crystal structure, it is possible to determine phases by comparing diffraction patterns before and after replacement. Perutz attached mercury atoms to the free sulphydryl groups of haemoglobin and observed appreciable changes in the diffraction pattern. Hence he was able to calculate some phases and proceeded to derive a two dimensional Fourier projection of the unit cell. Such a projection gives one as it were a silhouette of the molecule; but although it could be rigorously proved to be correct his projection unfortunately revealed very little of the structure of the molecule, for in it one is looking through as many as forty atoms at each point, and the resulting overlapping confuses the picture so much that little or nothing of the three-dimensional arrangement can be discerned. Nevertheless this projection represents a landmark in the history of subject, being the first unequivocal picture of a protein molecule obtained by X-ray methods. Perutz's work made it clear that if further progress was to be made the method had to be extended to three dimensions: only in this way could one hope to obtain a picture of the molecule which would be interpretable in structural terms.

In myoglobin we also began by making a two-dimensional analysis. We were unable to use the methods developed by Perutz for attaching mercury atoms to the molecule, because no known species of myoglobin contains free sulphydryl groups. Our approach was to crystallize myoglobin in the presence of various heavy ions, and then by X-ray methods to determine whether attachment of these ions had taken place (simply by observing whether or not the diffraction pattern had been significantly changed), and if so whether or not they were located at specific sites in the unit cell. We found, for example, that myoglobin forms useful isomorphous derivatives with mercuri-iodide, with auri-chloride, with silver, with *p*-chloromercuribenzene sulphonate, and with mercuri-diammine, most of these ions attaching themselves at different sites (Fig. 1). In most cases we under-

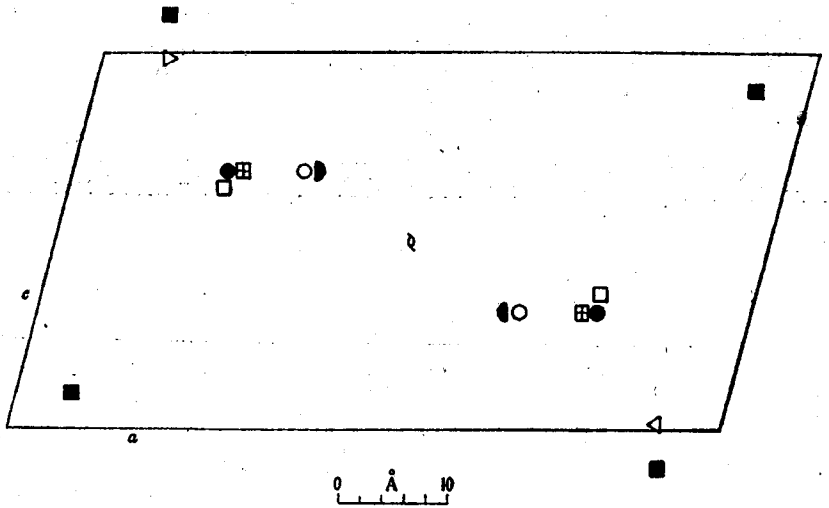

Fig. 1. Positions of heavy atoms in the unit cell of myoglobin. □ PCMBS; △ silver gold; ○ mercuri-iodide, auri-iodide; ■ mercury diammine, *p*-chloro-mercuri-aniline; ● *p*-iodo-phenyl hydroxylamine; ⊡ PCMBS derivative of *p*-mercapto-phenyl *iso*-cyanide; ◓ iodine. The unit cell contains two molecules of myoglobin, so each heavy atom is attached at two points per unit cell.

stand nothing of the chemistry of the process of attachment, but the X-ray evidence that it has taken place is unequivocal. In two-dimensional work it is not strictly necessary to have more than a single isomorphous replacement, nevertheless if several are available they serve as independent checks of the phase determination. By using them we were able to prepare a two-dimensional projection of the myoglobin unit cell, but it proved to be just as difficult to interpret as that of haemoglobin even though the myoglobin molecule is only a quarter as large as haemoglobin.

Our main objective, however, was to extend the analysis to three dimensions. Here it turns out to be essential to have several isomorphous derivatives with the heavy substituent at a different site in each; it is also necessary, for any given resolution, to study a great many more reflexions in the diffraction pattern than are needed for a two-dimensional analysis. Furthermore, the computations involved for each reflexion are now a good deal more complicated. In the event we decided to calculate a three-dimensional Fourier synthesis with a resolution of 6 Å, involving 400 reflexions (if we had chosen 2 Å, 10,000 reflexions would have been needed; atomic resolution would demand at least 20,000 reflexions); the figure of 6 Å was selected because at this resolution helical polypeptide chains should be revealed as rods of high density against a background of low-density side-chains. Space does not allow a discussion of the methods of computation adopted; the actual Fourier synthesis was carried out

on a high-speed computer, and was plotted as a series of electron density maps for sixteen parallel planes at different levels in the unit cell (Fig. 2).

The resulting three-dimensional representation of the density distribution, throughout the unit cell (which actually contains two myoglobin molecules) is readily seen to contain numerous dense rods, which often run nearly straight for distances of 30 or 40 Å (Fig. 3), and whose dimensions

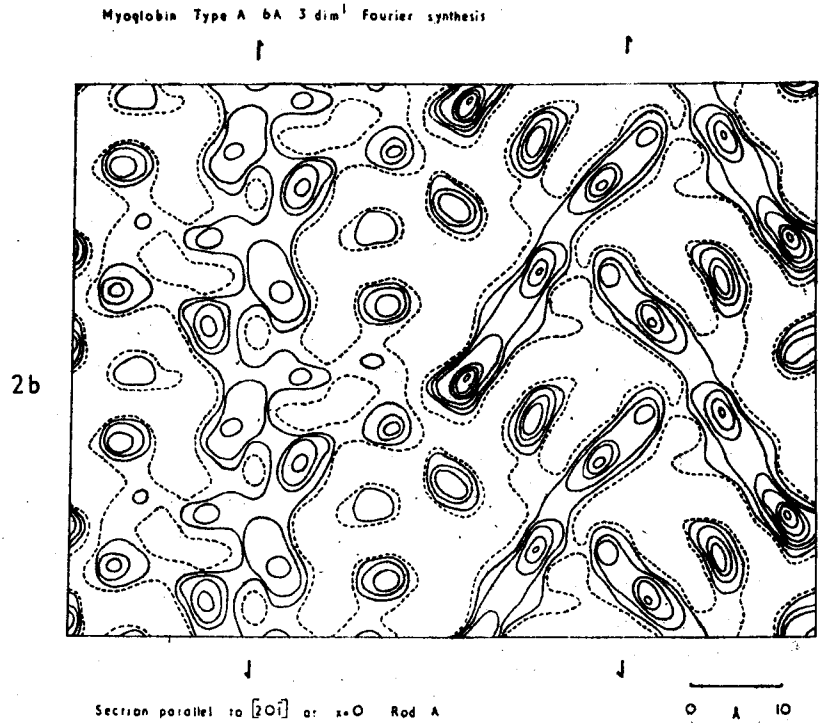

Fig. 3. Section through the three-dimensional Fourier synthesis, showing (on the right) straight rods of high electron density 40 Å long.

and separations and densities allow one to assert with confidence that they are indeed the polypeptide chains in some compact configuration (which may very well be an α-helix, though the exact nature of the configuration cannot at present be discerned). Indeed apart from a few ambiguities where the rods turn corners it is possible to trace a single rod throughout the molecules, presumably corresponding to the single polypeptide chain found by chemical methods. Ambiguities are to be expected at this resolution, since corner-turning necessarily involves a disruption of the assumed helical configuration, whose high density alone makes it distinguishable

from the matrix of sidechains in which it is embedded. The polypeptide chain is irregularly coiled to make a bundle measuring about 45 × 35 × 25 Å, and the main impression gained from a study of the model is of the complexity and apparently complete lack of symmetry of the tertiary folding of the chain (Fig. 4). Measurement of the total length of chain in the model suggests that some 70% of it is present in the form of a helix, a necessarily approximate estimate which does, however, agree with others based on techniques such as optical rotation and deuterium-exchange rates.

Myoglobin contains a single haem group, a planar porphyrin ring with an iron atom at its centre. This group can also be identified in the electron density map because of the iron atom, which is the heaviest atom in the molecule; it produces a peak of electron density 50% higher than any other in the Fourier synthesis. Though we cannot resolve the atoms of the porphyrin ring, the group appears flattened in a direction which corresponds well with what we already knew of the orientation of the haem group from optical and magnetic studies of myoglobin crystals. The identification of the haem group is confirmed by an isomorphous derivative prepared with *p*-iodo-phenylhydroxylamine, which is known to react directly with the haem group: in this the electron density was found to be raised at the appropriate place in the molecule.

The three-dimensional model of myoglobin gives us a first glance at the general layout of the molecule in three dimensions, but its resolution is too low to enable us to identify particular chemical groups, to establish definitely the configuration of the polypeptide backbone, or even to determine with certainty the complete course of that backbone. All these questions and many others of interest to biochemists should be answered if the resolution of the electron density map is to be improved. We are at present engaged in measuring reflexions to a resolution of 2 Å, and if we are successful in doing this the electron density map calculated from these data should contain very much more detail than does the present one; it will still, however, fall far short of revealing each atom in the molecule as a separate entity, and, indeed, it is still quite doubtful whether a further extension to atomic resolution will prove to be possible.

The studies described here have been carried out in the Medical Research Council's Unit for Molecular Biology in the Cavendish Laboratory at Cambridge and in the Davy–Faraday Laboratory of the Royal Institution in London, in collaboration with Drs. Bluhm, Bodo, Dintzis, Phillips and Wyckoff and Miss Mary Pinkerton. A full description of the two-dimensional analysis has been published (Bluhm, Bodo, Dintzis and Kendrew; *Proc. Roy. Soc.* **A246**, 369 (1958)), and further details of the three-dimensional work may be found in Kendrew, Bodo, Dintzis, Parrish, Wyckoff and Phillips; *Nature* (*Lond.*) **181**, 662 (1958).

Discussion

M. Perutz, *Cambridge, England:*

Kendrew's three-dimensional Fourier of myoglobin represents a great advance in protein chemistry. It was derived without any chemical assumptions about the nature of the myoglobin molecule. His results were arrived at by purely physical measurements, and their essential correctness has been rigorously proved.

As regards my own work on the crystal structure of haemoglobin, I have nothing to add to the paper I presented at the Protein Symposium in Paris last year. H. M. Dintzis, A. F. Collis and I are working on a three-dimensional Fourier at 6 Å resolution. We have been able to attach heavy atoms to two different sites on the haemoglobin molecule and we believe we have enough different heavy atom compounds to determine the phases of the majority of X-ray reflexions. However, until the three-dimensional Fourier is completed, this work yields no information whatever about the structure of the haemoglobin molecule, and I have therefore nothing of biochemical interest to report.

O. Kratky, *Graz, Österreich:*

Ich möchte auf die Möglichkeiten hinweisen, welche im Studium der diffusen Kleinwinkelstreuung von verdünnten Lösungen liegen. Das Verfahren ist zwar nicht geeignet, um Erkenntnisse ähnlicher Art zu liefern, wie sie durch eine solche Spitzenleistung der Röntgen-Feinstrukturanalyse, wie die eben dargelegte, zustandgebracht werden. Aber man kann doch recht wertvolle Angaben über die äussere Gestalt der Moleküle machen. Man darf nicht vergessen, dass bei der Patterson-Synthese (die Durchführbarkeit der dreidimensionalen Fourier-Synthese ist, wie wir gehört haben, bisher noch nicht generell möglich) trotz gewisser Angaben über die Massenverteilung häufig die Bestimmung der Molekülgestalt als Ganzes grösste Schwierigkeiten macht. Wenn wir nun mittels der diffusen Röntgen-Kleinwinkelstreuung der verdünnten Lösung zum Beispiel gefunden haben, dass das Hämoglobinmolekül einem Hohlzylinder mit angebbaren Dimensionen (r_1 = 25 bis 27 Å, r_2 = 15 Å, h = 64 bis 56 Å) jedenfalls sehr nahe kommt,* so sind das Angaben, die an sich Interesse beanspruchen dürfen und ausserdem bei der Deutung des Patterson-Diagrammes sowie der Durchführung der Fourier-Synthese weiterhelfen können. Das Gleiche ist natürlich für eine Reihe von sehr schönen Kleinwinkeluntersuchungen der Beeman-Gruppe in Wisconsin zu sagen.

* O. Kratky und W. Kreutz; *Angew. Chem.* **70**, 134 (1958); *Mh. Chem.* **83**, 169 (1958).

H. THEORELL, *Stockholm, Sweden:*

Since it is well known that myoglobin even in the best of crystals is inhomogeneous, and according to our experience consists of three well-defined, and one ill-defined components in horse myoglobin, I wonder whether this fact could have distorted Dr. Kendrew's pictures to any extent?

J. C. KENDREW:

Sperm-whale myoglobin as ordinarily prepared consists (even after recrystallization) of five components, which can be separated on a column. We normally take X-ray photographs of crystals prepared from the unseparated material; we have, however, compared these photographs with others taken of crystals derived from each of the two major components after separation. All three sets of photographs are so similar that it can be stated with confidence that their molecules must resemble one another extremely closely; nevertheless differences in a few individual amino-acid residues might well escape detection unless we studied the diffraction patterns more closely than we have yet done, and indeed such differences have been shown to exist by Mr. Edmundson at the Rockefeller Institute, using chemical methods. At our present resolution, however, it is clear that the results of an analysis of crystals from a purified component would be indistinguishable from those we have obtained using the unseparated material.

OPTICAL ROTATION AND THE STRUCTURE OF POLYPEPTIDES AND PROTEINS

PAUL DOTY

Department of Chemistry, Harvard University, Cambridge, Massachusetts

For many years it has been recognized that the specific rotation of a protein in a specified solvent is a reproducible and intrinsic property of the protein and that there is some kind of close relation between optical rotation and configuration of protein molecules. However, it is only within the three years since the last Congress that progress has been made in understanding this relation and in using it to gain some information on protein structure in solution. I should like to summarize some of these developments and to attempt to evaluate the present position of this problem.

It is an old observation that the specific rotation of proteins falls[1] upon denaturation.[2] During the last decade much specific support has been given to this generalization by quantitative studies, particularly by Kauzmann and his co-workers.[3] In 1951, shortly after the proposal of the α-helix by Pauling and Corey, Perutz[4] showed by means of X-ray diffraction that a few fibrous proteins in crystalline form did contain this configuration in unspecified amounts. However, it was not possible to extend this method to globular proteins.

POLYPEPTIDES

In 1953, Dr. E. R. Blout and I initiated a program of synthesis and characterization of synthetic polypeptides: this had as one of its aims the direct testing of whether or not polypeptides could take up unique configurations such as the α-helix in solution. In 1954 we found that poly-γ-benzyl-L-glutamate could exist in two configurations, the α-helix and the solvated, randomly coiled chain, depending on the solvent and, moreover, that the two forms showed a substantial difference in specific rotation similar in sign and magnitude to the difference between native and denatured proteins.[5] This fitted nicely with the suggestion made in 1955 by Cohen[6] that a specific main-chain configuration may be the cause of the difference in optical rotation between native and denatured states of proteins.