Conformation of Biopolymers

PAPERS READ AT AN INTERNATIONAL SYMPOSIUM HELD AT THE UNIVERSITY OF MADRAS 16–24 JANUARY 1967

Volume 1

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Volume 1

Edited by

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Centre of Advanced Study in Biophysics
University of Madras, India

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Preface

An International Symposium on Conformation of Biopolymers was organized at the University of Madras by the Centre of Advanced Study in Biophysics of the University, from 18 to 21 January 1967. The Symposium, which had the sponsorship of the International Union of Pure and Applied Biophysics, was presided over by Professor Linus Pauling and was in the hands of an Organizing Committee consisting of Dr C. B. Anfinsen, Bethesda, U.S.A., Dr W. F. Harrington, Baltimore, U.S.A., Dr F. M. Richards, New Haven, U.S.A., Dr H. A. Scheraga, Ithaca, U.S.A., Dr G. N. Ramachandran, Madras (Convener), Dr C. Ramakrishnan, Madras (Secretary), Dr S. Tyagaraja Rao, Madras (Secretary) and Dr V. S. R. Rao, Madras (Treasurer).

The possibility of holding a Workshop on Protein Conformation, attended by some of the leading workers in the field, was the stimulus for the organization of the Symposium on the wider subject on Conformation of Biopolymers at Madras. It is gratifying to note that forty-eight papers were offered for the Symposium by scientists from different countries. Most of them (thirty-nine) deal with the conformation of proteins and polypeptides, five with nucleic acids and nucleotides and four with polysaccharides. The papers cover a wide variety of techniques used for the study of conformational aspects of these macromolecules, such as X-ray, chemical, optical and theoretical methods.

These volumes will be found to be of interest by all biochemists who are working on the study of proteins, nucleic acids and polysaccharides. In particular, they will appeal to those who are interested in the conformational aspects of these materials and the relation between conformation and biological activity. Those dealing with physical and chemical techniques for the study of conformation would find the papers particularly useful, as they deal with the latest developments in these fields.

The organizers would like to thank the Commission on Molecular Biophysics of the IUPAB, particularly Dr R. C. Williams, the Chairman, and Dr J. A. V. Butler, the Secretary, for their sponsorship of the Symposium, which enabled many of the scientists to attend the Symposium. Financial support for the Symposium came from various sources in India—in particular the University Grants Commission, the Council of Scientific and Industrial Research and the Atomic Energy Commission and we would like to thank all these for their generous assistance. The Organizers would also

like to record the continuous support and encouragement given to them by Dr D. S. Kothari, Chairman, University Grants Commission and Dr A. L. Mudaliar, Vice-Chancellor, University of Madras. They are grateful to all the members of the Centre of Advanced Study in Physics for their assistance in the actual conduct of the conference—in particular Dr R. Srinivasan for the organization of the Symposium and Dr V. Sasisekharan for that of the Workshop.

The Editor wishes to thank Academic Press for providing preprints from galleys for the Symposium and for their assistance in various ways for the speedy publication of the volumes. He would like to thank in particular Dr C. Ramakrishnan and his other colleagues for assistance in editing and in correcting the proofs.

March 1967
Madras

G. N. RAMACHANDRAN

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Proteins and Polypeptides

PRESIDENTIAL ADDRESS

Molecular Structure of Proteins

LINUS PAULING

Research Professor of the Physical and Biological Sciences, Center for the Study of Democratic Institutions, Santa Barbara, California, U.S.A.

addressed The Symposium as follows

I begin by expressing to Professor Ramachandran and his associates in the University of Madras my deep appreciation of the honor of having been chosen as President of this Symposium on Conformation of Biopolymers, and as Raman Visiting Professor of Physics in the University of Madras.

The problem of the structure of proteins is one that has interested me for 33 years. So far as I can remember, I had not developed any significant interest in this field until 1934, when I began work on hemoglobin, as I shall describe later on.

It was the discovery of the phenomenon of X-ray diffraction by Max von Laue, in 1912, and its successful application to the determination of the structure of crystals by W. H. Bragg and W. L. Bragg, in 1913, that was ultimately responsible for the great progress in the field of protein structure made during the last two decades. The first X-ray diffraction photographs of proteins were made in 1913 by S. Nishikawa and S. Ono, in Japan. In 1914, Nishikawa continued this task, but the diffraction patterns were so diffuse as to discourage further work. Better diffraction photographs were then made by Herzog and Jancke in 1920. They studied muscle, nerve, sinew, hair and silk, and a year later Brill made the suggestion that in silk the polypeptide chains are essentially in the extended form, in which each amino acid residue has a length along the fiber axis of about 3·5 Å. Then, in 1931, Astbury and Street pointed out that hair, wool and related fibers exist in both a contracted form, called α-keratin, and an extended form, called β-keratin. They suggested that in β-keratin the polypeptide chains are in the extended conformation, and that a chemical change involving the formation of rings of atoms held together by covalent bonds takes place on contraction to α-keratin. Astbury and Woods in 1933 described β-keratin as involving sheets of extended polypeptide chains, the chains being held together in the

sheets by interaction of carbonyl and imino groups of the amide groups. In 1936 Alfred E. Mirsky and I discussed the general problem of the structure of proteins and the phenomenon of denaturation, and suggested that the interaction between the chains in silk is the formation of hydrogen bonds between the carbonyl oxygen atom and the hydrogen atom attached to the amide nitrogen atom. The same suggestion about hydrogen bonding in silk was made at nearly the same time by Maurice L. Huggins. Several possible configurations for the coiled polypeptide chain in α-keratin were suggested by Astbury, Huggins, Bragg, Kendrew and Perutz, and finally, in 1949, the discovery of the α-helix was reported by Professor Robert B. Corey, Dr Herman R. Branson and me.

I have already mentioned that I became interested in the structure of proteins by way of hemoglobin. Between 1922 and 1934 I was engaged in the determination of the structure of many crystals by the X-ray diffraction method and of some gas molecules by the electron-diffraction method, as well as in theoretical work dealing with the nature of the chemical bond and the structure of molecules and crystals. The matter of the magnetic properties of substances also engaged my interest, and one day in 1934, when I happened to run across a discussion of the question of the nature of the attachment of oxygen molecules to hemoglobin in the red cells of the blood, the idea occurred to me that some significant information could be obtained by measuring the magnetic susceptibility of hemoglobin. The oxygen molecule is almost unique among gas molecules in having a permanent magnetic moment, corresponding to two electrons with parallel spin. I argued that if the oxygen molecules were attached to the hemoglobin molecule by physical interactions they would retain their magnetic moment, whereas if they formed chemical bonds with the hemoglobin molecule, presumably with the iron atoms, the unpaired electrons would be involved in the formation of pairs and the permanent magnetic moment would be lost. Charles Coryell and I carried out this experiment. We found that oxyhemoglobin is completely diamagnetic, with no magnetic moment; and in this way we proved that the oxygen molecules have entered into chemical combination with the hemoglobin molecule. A striking observation made in the course of these studies was that the hemoglobin molecule with oxygen removed from it has a large magnetic moment, which is the result of a deep-seated change in the electronic structure of the iron atoms that takes place on the removal of the oxygen molecules.

Before embarking, with Coryell, on these magnetic studies, I had carried out a theoretical discussion of the oxygen equilibrium curve of hemoglobin, in relation to the change in interaction energy of the four heme groups with one another that accompanies their oxygenation. Having decided that it would be well worth while to carry out some experimental investigations of

hemoglobin, I was faced with the fact that my background of experience did not include the handling of such delicate and complex chemical substances as hemoglobin, and that I would be wise to seek the help of an expert. Fortunately, I was successful in getting Dr Alfred E. Mirsky, of the Rockefeller Institute for Medical Research, to come to Pasadena for a year, and to give me (and Dr Coryell) instruction in the ways of preparing and handling hemoglobin solutions. In the course of this work Mirsky and I developed the idea that proteins in general are held in their native configurations through the formation of hydrogen bonds between one part of a polypeptide chain and another part.

The magnetic studies on hemoglobin were continued for several years. They led to the discovery of several previously unknown compounds of hemoglobin and to much information about equilibrium constants, rate of reactions and electronic structure of hemoglobin and hemoglobin derivatives. Moreover, this work on hemoglobin, which I described in a seminar at the Rockefeller Institute in 1936, caused Dr Karl Landsteiner, the discoverer of the blood groups, to ask me to discuss with him the question of the structure of antibodies and the nature of serological reactions, and this discussion led ultimately to a large amount of work on the subject in our Pasadena laboratories.

Moreover, Dr Robert B. Corey, who had been working at the Rockefeller Institute with Ralph W. G. Wyckoff, decided that he would spend a year of absence from the Rockefeller Institute working in our laboratories in Pasadena. When he arrived in Pasadena in the summer of 1937 he found me engrossed in the effort to formulate a structure for α-keratin. I had decided that the resonance of the double bond in the amide group would require this group of six atoms (including the two α-carbon atoms) to be planar, and that the acceptable ways of folding the polypeptide chain would be those in which the planarity of the amide groups was preserved, but that different orientations around the two single bonds to the α-carbon atoms were possible, and that in addition the hydrogen atom attached to nitrogen in the amide group should be directed toward the oxygen atom of a neighboring amide group, with a normal hydrogen-bond length, about 2·8 or 3·0 Å. I had constructed some ball-and-stick models, in the effort to get a repeating structure that would account for the strong 5·1 Å meridional reflection observed in the X-ray diffraction pattern of the α-keratin proteins. After several weeks of this effort, with no success in accounting for the 5·1 Å reflection, I gave up the search. I preserved some of the models for a year or two, and then dismantled them. It is my memory that my greatest effort was expended on ways of folding the polypeptide chain in which the chain lay in essentially one plane, as had been suggested by Astbury, and that I did not make a very serious effort to discuss three-dimensional models, especially helical ones, as

was done by Huggins and by Bragg, Kendrew and Perutz, as well as by Corey, Branson and me, a decade or two later.

Corey had made some X-ray diffraction photographs of proteins while he was with Wyckoff at the Rockefeller Institute, and when he came to discuss with me his program of work for the year the question of the structure of proteins was brought up. I mentioned that I felt some doubt as to whether we were justified in assuming, as I had been doing, that the structure of the amide group in the polypeptide chains of proteins had the planarity, bond lengths and bond angles that I had predicted, and also whether the assumption that I had been making about the formation of hydrogen bonds was justified. The decision was made by Corey and me that he would attack the experimental problem of determining the structure of some amino acids and simple peptides. This decision led to his determination of the structure of diketopiperazine in 1938, of glycine (with Gustav Albrecht) in 1939, and of alanine (with Henry Levy) in 1941, and ultimately, through the efforts also of E. W. Hughes, Jerry Donohue, Werner Shomaker, David Shoemaker, Walter Moore, Kenneth Trueblood, R. A. Pasternak, Gene Carpenter, Harry Yakel, Jr., and others, to accurate structure determinations of many amino acids and simple peptides. Corey's year in Pasadena turned out to be 30 years, so far.

By 1948 it has become quite clear that the conclusions about the planarity of the amide group, values of bond lengths and bond angles, and formation and properties of hydrogen bonds that I had reached in 1937 were, in fact, valid. One day, in the spring of 1948, when I was in bed with a cold in Oxford, England, where I was serving as Eastman Visiting Professor, I decided to attack again the problem of the structure of the α-keratin proteins. Starting with the idea that the operation of a rotation and a translation, when repeated, gives rise to a helical structure of equivalent groups, I soon found, with the aid only of a sheet of paper which I could twist into cylindrical form, that helical configurations of polypeptide chains could be devised in which the structural parameters have the accepted values and hydrogen bonds are formed between amide groups and their neighbors in adjacent turns of the helix. During the next few years this idea led to a tremendous amount of work, in the prediction of many alternative helical structures, some with several strands of helices. Much of this work remained unpublished. In particular, our results about the effect of different amino acid residues in influencing the bending of the polypeptide chain from one α-helix segment to another has not been published in detail.

I shall not discuss, in this lecture, the recent history of the field of protein structure, except in relation to collagen. In 1951 Professor Corey and I proposed a structure for collagen, involving three polypeptide chains twisted about one another. In devising this structure we had found it necessary to assume that in each polypeptide chain there is an alternation of two amide

groups with the *cis*-configuration and one with the *trans*-configuration. It is well known that our structure turned out not to be right. In a lecture on the stochastic method and the structure of proteins that I gave in Stockholm in 1953, at the Thirteenth International Congress of Pure and Applied Chemistry, I pointed out that in applying the stochastic method the first step is to make a hypothesis, a guess. The second step is to test the hypothesis, by some comparison with experiment. In general the test cannot be sufficiently thorough to provide rigorous proof that the hypothesis is correct: it may happen that it can easily be shown that the hypothesis is incorrect, through the discovery of a significant disagreement with experiment, but agreement on a limited number of points cannot be accepted as verification of the hypothesis. In order for the stochastic method to be significant, the principles used in formulating the hypothesis must be restrictive enough to make the hypothesis itself essentially unique; in other words, an investigator who makes use of this method should, I contended, be allowed only one guess. If he were allowed many guesses he would sooner or later make one that was not in disagreement with the limited number of test points, but there would then be little justification for accepting that guess as correct. At that time (1953), however, I contended that Professor Corey and I together should be allowed two guesses on collagen, and I stated that we were determined that our second one would be right.

As you all know, it turned out that Professor Corey and I did not get to make our second guess. In 1955 Professor Ramachandran and his co-worker G. Kartha described the striking triple-helical structure of collagen that is now generally accepted as being essentially correct. Although I may have some feeling of regret that Professor Corey and I did not succeed in making our second guess (which I trust would have turned out to be the right one), I may point out that the problem was a very difficult one, and that Professor Ramachandran and his co-workers deserve great credit for their successful attack on it, and for their continuing vigorous effort in the solution of the many difficult problems in the field of protein structure and other aspects of structural chemistry to which they have devoted themselves for many years.