MILESTONES IN BIOLOGICAL RESEARCH

From Protein Coagulation and Reversible Denaturation to the Protein Folding Problem: Chris Anfinsen Defining the Transition

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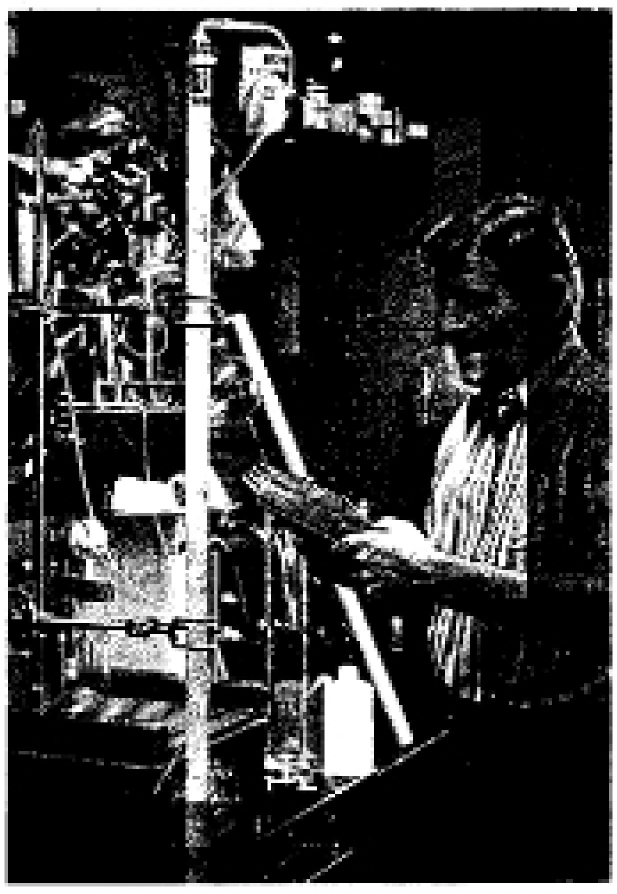
The name Christian B. Anfinsen is intricately interwoven with the concept of protein folding, the theme of this issue. I feel both honored and humbled to have been asked to write an appreciation of Chris's contributions to this field of scientific endeavor. In addition to providing the usual vital statistics, I have decided to paint a personal portrait of the originator of the "thermodynamic hypothesis" of protein folding. In this way, I hope that it will become easier to appreciate the elegance with which Chris combined his sensitivity, intuition, and sense of order and beauty with a persistent drive, an adaptable imagination, and a quest for rigorous answers to fundamental problems. It was this rare combination of sensitivity and rigor, self-assurance and humility, fearlessness and vulnerability, that instilled feelings of respect, admiration, and love in those who were fortunate to have met and worked with him.

Several articles have been written about Chris, some of which are more complete than what I can hope to present here. Some were written by persons who were closer to Chris at the peak of his laboratory achievements. I will not attempt to duplicate them, but I would like to direct the reader to a recent article by Alan Schechter (1) that I believe delivers both a complete and a sensitive account of Chris as a scientist and as a humanitarian. Here I will summarize only those points that can help one appreciate the scientific world of Chris.

Chris (as he was known to his colleagues, his students, and the laboratory custodians) was the son of a Norwegian family from Bergen that settled in Pennsylvania. He received his undergraduate degree from Swarthmore and his M.S. in organic chemistry from the University of Pennsylvania. In 1939, he went on an American-Scandinavian Foundation Fellowship to the Carlsberg Laboratories in Copenhagen to study enzyme chemistry, but World War II interrupted this visit. After returning to the States, he began graduate studies and received his Ph.D. in biological chemistry from Harvard Medical School in 1943. In 1946 he joined the faculty of the Department of Biological Chemistry at Harvard, where he stayed until 1950. During that period he spent a year (1947–1948) in the laboratory of Hugo Theorell at the Medical Nobel Institute in Stockholm developing his interests in enzyme purification and protein chemistry.

In 1950 Chris left Harvard to become chief of the Laboratory of Cellular Physiology in the National Heart Institute at NIH. In the years that followed, Chris's laboratory published findings along two distinct lines of research: one more applied and dealing with the metabolism of plasma lipoproteins, and the other more basic and addressing fundamental issues of protein structure, which ultimately became the area Chris's most recognized contributions, and for which he was awarded the Nobel Prize in Chemistry in 1972 (shared with Stanford Moore and William H. Stein of Rockefeller University).

Chris's stay at NIH was interrupted twice, once in 1954 for a second visit to the Carlsberg Laboratories to work on the physical biochemistry of RNAase with Kai Linderstrom-Lang, and again in 1962–1963 as Professor of Biological Chemistry at Harvard.



Dr. Anfinsen in his laboratory at NIH, circa 1978.

In 1963 he returned to NIH (The Laboratory of Chemical Biology of the National Institute of Arthritis and Metabolic Diseases), where he remained until his retirement in 1981. He then spent a year at the Weizmann Institute, and in 1982 he joined the faculty of the Biology Department of Johns Hopkins Univer-

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sity as Professor of Biophysical Chemistry, a position he held until his death on May 14, 1995.

Chris approached the study of proteins with a perspective best captured in the following lines from his 1959 book (2): "To construct a final picture of the covalent structure of the protein requires, in addition to the complete sequence of each peptide fragment, a study of the disulfide bridges. . . . [However,] knowledge of the complete covalent structure . . . is, unfortunately, not a magic key to the understanding of physicochemical and catalytic behavior in itself. A real appreciation of Nature's intent with respect to protein molecules must be sought through additional considerations of structure in three-dimensional terms." This theme, i.e., the need for correlation of the beauty of the architecture of the macromolecule to its "organochemical details [and] physicochemical behavior in solution," is, in my opinion, the hallmark of Chris's research approach, the origins of which can be traced to his organic chemistry training and to the influence of his father, a construction engineer.

For Chris, all the experimentally obtainable parameters were but partial glimpses or snapshots of the whole life cycle of the molecule, the understanding of which was his real objective. Thus, "The study of the internal structure of proteins in the crystalline form . . . indicates a well defined arrangement of atoms, more or less 'frozen' into a definite pattern which is the same for each molecule in the crystal. . . . From the purely chemical point of view such a picture is completely satisfactory. In living cells, however, proteins are not in solid state but are dissolved in the intracellular fluid and can be shown to be in a constant state of minor rearrangements in response to shifting hydrogen ion concentrations and salt levels and to reversible adsorption to intracellular surfaces" (2).

To better appreciate the uniqueness of Chris's contributions to the field of protein organization and folding, we need to look at the state of that field from the beginning of this century to the early 1960s. Others have written on this issue extensively, in particular John Edsall in the Annals of the New York Academy of Sciences (3).

At the turn of the century, protein solutions were considered ill-defined, polydisperse systems. Fractionation and purification methods were rudimentary and rarely yielded pure preparations. The available criteria for homogeneity would today be considered unconvincing. The methods for molecular weight determination that had served the organic chemists well were of limited value for the study of large protein molecules. Indeed, the very idea that proteins could exist as unique structural entities, endowed with definite size, was in sharp contrast to the views of the colloid chemists. To them, drawing from the properties of inorganic colloids, the particles in such preparations were heterogeneous in size; fractionation of the preparation only narrowed the width of the size distribution of the system. Several early workers in the field of protein chemistry were practicing colloid chemists or were at least strongly influenced by them. Contemporary developments in physics had lent strength to the quantitative treatments and predictions of the colloid chemists (as cited on p. 59 of ref 3).

From 1917 (Sorensen and his associate Linderstrom-Lang) to 1926 (Adair in England and Svedberg in Sweden), with the application of new methodologies, evidence began to accumulate that protein solutions could be obtained exhibiting a single (albeit large) molecular weight for the molecules in the preparation. Svedberg, who started as a colloid chemist and earlier believed that protein solutions were also heterogeneous mixtures, was, by 1939, converted to the view that "proteins are . . . [entities] possessing the hallmark of individuality, and are in reality giant molecules . . . built up according to a plan which makes every atom indispensable for the completion of the structure" (4). Remember that between 1939 and 1948 Chris, on separate occasions, had visited the Carlsberg Laboratories in Copenhagen and the Medical Nobel Institute in Stockholm, and thus had been directly exposed to the ideas and the leaders of this transition period.

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Perhaps the most profound influence on Chris's research, however, was the accomplishment of Fred Sanger, i.e., the determination of the complete amino acid sequence of insulin. As Chris recalled in a conversation, "this meant that one could now determine the order of the building blocks of any enzyme and hope to unravel its functional fluctuations. Even better, there was now hope that some day one could make synthetic enzymes in the laboratory; what a crazy but exciting idea!"

At about the same time another great discovery had an immense impact on Chris's thinking—the discovery of the double helix by Watson and Crick in 1953. The impression of this discovery on Chris has perhaps been less well appreciated by those who knew him primarily for his work with proteins (more on this later). But in The Molecular Basis of Evolution, it becomes obvious, indeed impressive, that a remarkable synthesis had occurred in his mind by that time (2). Chris writes an elegant and awe-inspiring account of the development of ideas from Mendel and Darwin through the founding years of microbial genetics and the one gene–one-enzyme concept of Beadl and Tatum (5), through the experiments on the mode of DNA replication and chromosome replication of Meselson and Stahl (6) and H. Taylor (7), respectively. Soon after Chris's book came Dintzis's experiments (1961–1962) demonstrating the ordered and sequential progression of the synthesis on the protein chain from the amino to the carboxyl end (8). In 1962 Canfield and Anfinsen reproduced this pattern of synthesis with chicken oviduct lysozyme (9).

> ***"...We had to learn what questions to ask, along with the search for the answers."****—J. Edsall*

Between 1962 and 1963 in Chris's mental framework, the issue of reversible protein denaturation had been transformed to the protein folding question.

Earlier work by such pioneers as Mona Spiegel-Adolph (1925), Hsien Wu (1931), Mirsky and Pauling (1936), Anson and Mirsky (1931–1936), Neurath and Bull (1936), and several others in the 1940s and early 1950s, had addressed ingeniously the problems of "reversible denaturation" of proteins. At those early times, however, the problem of protein folding could not be defined with sufficient rigor, nor could it be approached with the necessary incisiveness, because the critical threshold of knowledge had not yet been crossed. John Edsall, describing the activities and thoughts in the Department of Physical Chemistry at Harvard between 1930 and 1940, draws a sobering picture of the state of the field of peptides and proteins: ". . . at that time we did not know enough to ask such coherent questions. We had to learn what questions to ask, along with the search for the answers" (10).

Chris emerged at that critical moment to provide the clear and simple answer. "In the fields of observation, chance favors only the mind that is prepared," Pasteur had said some years earlier. And Chris's mind was prepared. He had been schooled and trained within the emerging field and had lived and experienced the explosive growth in what later became known as molecular biology. All he had to do was to allow his fertile mind and creative imagination, his genius, to synthesize on the keyboard of discovery with virtuosity and sensitivity, and synthesize he did.

In a 1963 article, Chris wrote: "To discuss the topic intelligently, I shall also have to consider . . . the chemical structure of the chains and the beauty and precision of the genetic information that resides along their amino acid sequence" (11). This sentence reveals Chris's unique outlook on the problem, i.e., considering it both an object of hard chemistry as well as one of architectural beauty. Throughout the article he used this agile and balanced blend of rigorous experimental facts and concepts with anthropomorphic metaphors and humanly meaningful examples. This article is worth reading even today, since it also reviews some important experiments by several key figures (up to that time) of the Anfinsen laboratory: R. Canfield, C. Epstein, R. Goldberger, E. Haber, M. Sela, and F. White.

This paper was presented at a symposium at Rutgers University sponsored by the National Science Foundation. In the discussion that followed Chris's presentation, which was chaired by his good friend W. F. Harrington, one finds many telling comments by P. Berg, H. Fraenkel-Conrat, J. Fresco, W. Kauzmann, and V. Luzzati, among others. Fraenkel-Conrat asked the last question of the session. It addressed Chris's use of the term molecular "hovering" to describe some intermediate, metastable states in the protein folding process as applied to bovine pancreatic ribonuclease. Such a loose use of language by Chris appears to have alarmed the formal chemist within the discussant (famous for his work on tobacco mosaic virus), who asked for clarification of "hovering." Chris provided the following simple but exquisitely correct reply: "I think it is just a question of how 'hovering' you want to get; tobacco leaves live in cold fields . . . whereas bovine pancreatic ribonuclease lives at 37°. . . . Don't you think that it is selection under given environmental conditions that determines structure?" Thinking a bit about the depth of this answer one cannot help but see the foundation of what would become the main focus of Chris's research during his years at Johns Hopkins University, namely, protein thermostability.

The NIH Years

The work of the Anfinsen group at NIH between 1955 and 1962, primarily with bovine pancreatic ribonuclease, led to the enunciation of the "thermodynamic hypothesis" of protein folding. The hypothesis stated that " . . . the native conformation [of a protein] is determined by the totality of interatomic interactions, and hence the amino acid sequence, in a given environment" (12). But as we have already seen, this concept of the protein molecule as a harmonious constellation of atoms in tune with their environment had surfaced several times earlier in Chris's presentations. After the discovery of molecular chaperones, there were some short periods of confusion concerning the general applicability of the thermodynamic hypothesis. However, such concerns, which Chris considered carefully, missed the significance of the contributions of the molecular microenvironment to the folding process, as had been repeatedly emphasized by Chris. A nascent protein chain, being folded on the ribosomal surface, experiences a constellation of environmental contributions very different from those that the mature, unfolded molecule will experience upon refolding either in some corner of the cytoplasm or in the test tube. When the proper distinctions are made between kinetic and thermodynamic consid-