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Commentary by

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on 'Studies on the structural basis of ribonuclease activity'
by C.B. Anfinsen, W.F. Harrington, Aa. Hvidt, K. Linderstrøm-Lang, M. Ottesen and J. Schellman
Biochim. Biophys. Acta 17 (1955) 141–142

The short communication selected for inclusion in the 1000th volume of Biochimica et Biophysica Acta was the result of experimental efforts and discussions involving six of us working at the Carlsberg Laboratory in 1955 under the guidance of Professor K. Linderstrøm-Lang. This communication is a beautiful

example of how an entirely acceptable conclusion can be reached that is entirely wrong because of the paucity of knowledge at that particular time. Indeed, I spent the following 15 years or so completely disproving the conclusions reached in this communication, namely, that an ordered secondary structure in a protein is quite unnecessary for its properties as a catalyst. These were the days when, except for Sanger's work on insulin, no sequence information of any significance was available for protein molecules, and three-dimensional structure derived from crystallographic work was still a product of the future.

In the 3 or 4 years that followed, I was fortunate to be joined in my laboratory at the National Institutes of Health by Michael Sela, William Harrington, Fred White, and a number of others. In a relatively short time we discovered that the ribonuclease molecule, which is indeed highly disoriented in strong urea solutions, is held together and stabilized in its native conformation by its substrate and, indeed, by a number of other polyvalent cations such as polymetaphosphate, poly(aspartic acid), and even orthophosphate itself. We have frequently referred to this kind of stabilization of structure in denaturing solvents as 'rigidification', and it seems to be a common phenomenon with many enzymes whose activities are preserved by substrate molecules or substrate analogs. When I now look at the original BBA communication, I find myself thinking in terms of the current witch-hunting that is now so popular in which published material based on inadequate or incomplete data is occassionally referred to as "fraud". In think this term is frequently probably correctly applied, although I do believe that in many instances such premature incorrect conclusions may simply reflect the fact that the advance of science and the deeper understanding of nature are under continual modification. Ongoing refinement of data frequently requires considerable reinterpretation of formerly held 'truths'.

As the result of a number of studies with many colleagues on the refolding of denatured molecules, accumulated even after conversion to extended polypeptide chains by disulfide bond reduction, it became clear that a generality could be proposed that seemed to be applicable to essentially all proteins that were examined. This generality stated that the details of the three-dimensional structure of a protein molecule were determined entirely by the amino-acid sequence of the molecule and that no other outside information was required. The air oxidation of a reduced protein frequently led to 'scrambled' molecules with incorrect pairing of half-cystine residues, particularly when the concentration was too high during the reoxidation. In dilute solutions, however, such mispairing, or intermolecular bonding, was generally avoided. I remember, as one of my more exciting moments, an experiment which Dr. Edgar Haber and I carried out on ribonuclease refolding from the reduced form. After stirring in air overnight, almost no activity had been regenerated, but the addition of a small amount of mercaptoethanol led to a rapid reshuffling of the scrambled SS bridges with full recovery of activity in a relatively short time. The path of our research on formation of three-dimensional structure thereafter was fairly easy sailing.

In recent years, with the acceptance of the generality of the process of spontaneous refolding based on sequence alone, a very large effort has been progressing in many laboratories to elucidate the nature of the interacting forces that lead to the correct structure and to deduce the three-dimensional structure of proteins from the sequence alone, employing a large number of different thermodynamic and stereochemical parameters. One of these days, our thermodynamic and computer experts will solve this problem of prediction, and the so-called 'folding problem' can be put on the shelf along with other solutions to Nature's secrets.

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