The Elucidation of the Structure of Ribonuclease by Stanford Moore and William H. Stein


A previous Journal of Biological Chemistry (JBC) Classic (1) on Stanford Moore (1913–1982) and William H. Stein (1911–1980) offered a look into their early careers, including their development of chromatographic methods for determining protein composition, their invention of the photoelectric drop-counting fraction collector, and their creation of the first amino acid analyzer. This Classic focuses on their elucidation of the amino acid sequence of pancreatic ribonuclease, its disulfide bonds and active site residues.

In the early 1950s, Moore and Stein started using their ion-exchange chromatography methods to separate peptides and proteins. Encouraged by their success, they decided to embark on the structural analysis of an entire protein. They chose ribonuclease, an enzyme that is more than twice the size of insulin, which was the first protein to be fully sequenced as a result of the pioneering studies of Fred Sanger in Cambridge, England. At the time that these studies were begun, Christian B. Anfinsen and his associates at the National Institutes of Health also started studying the chemical structure of ribonuclease. Anfinsen’s work on ribonuclease will be the subject of a future JBC Classic.

To determine the sequence of ribonuclease, Moore, Stein, and their first postdoctoral associate, Werner Hirs, hydrolyzed the protein with trypsin and then separated the peptide mixture by ion-exchange chromatography. The peptide sequences were then analyzed by Edman degradation. By repeating this experiment with chymotrypsin and pepsin they were able to deduce the sequence of the entire protein, which is published in the first JBC Classic reprinted here. Their sequence, shown in Fig. 1, was almost entirely in agreement with the partial sequence of ribonuclease reported by Redfield and Anfinsen (2, 3), except for a disagreement on the nature of the amino acid residue at position 11. According to Anfinsen, the residue was glutamic acid, whereas Moore and Stein believed it was serine. Eventually, after using improved conditions for Edman degradation, Moore and Stein revised their published sequence (4) and stated that the amino acid was, indeed, glutamic acid.

In the Discussion section of the Classic, Moore and Stein also describe some modifications to their approach that they suggest adopting in future elucidations of the chemical structure of a protein to make the task easier and improve the results. These modifications include performing the peptide separations on larger columns, using highly purified proteolytic enzymes to cleave the protein, and using additional enzymes such as carboxypeptidase B.

While elucidating the sequence of ribonuclease, Moore and Stein also attempted to determine the positions of the four disulfide bonds in the molecule. This is the subject of the second JBC Classic reprinted here. Moore, Stein, and Darrel Spackman hydrolyzed ribonuclease with...
pepsin, trypsin, and chymotrypsin and then used an ion-exchange column to locate the cystine-containing peptides. These peptides were oxidized, separated chromatographically, and then subjected to amino acid analysis. From these experiments, they determined that the following cystine pairings occurred: I-VI, II-VII, III-VIII, and IV-V. Again, their results differed from those obtained by Anfinsen (5). His group had determined that II-VIII and III-VII pairings occurred. It was eventually shown that Moore and Stein were correct in their disulfide bond predictions.

Not content to stop at the sequence of ribonuclease, Moore and Stein also studied the inactivation of the enzyme by iodoacetic acid, which is the subject of the final JBC Classic reprinted here. They, along with Arthur Crestfield, discovered that two monocarboxymethyl derivatives were formed when iodoacetic acid was added. When they subjected these products to amino acid analysis they determined that the alkylation had occurred at the histidine residues occupying positions 119 and 12. From this they concluded that the imidazole rings of histidine 119 and histidine 12 were at the active center of ribonuclease. Using similar methods, they identified additional residues in and around the active site of the enzyme.

Moore and Stein’s work on ribonuclease was recognized in 1972 when they were awarded the Nobel Prize in Chemistry, which they shared with Anfinsen “for their contribution to the understanding of the connection between chemical structure and catalytic activity of the active centre of the ribonuclease molecule.”

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REFERENCES
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