

CHAPTER 1

ANFINSSEN: AMINO ACID SEQUENCE DETERMINES PROTEIN SHAPE

In 1973, Christian B. Anfinsen and his colleagues performed the definitive experiment showing that a protein takes its specific shape based on the “directions” encoded in the sequence of amino acids.

ANFINSSEN'S EXPERIMENT

The hypothesis that “protein amino acid sequence determines the final shape a protein assumes in a water solution” was proven to be correct when Christian B. Anfinsen showed that if the enzyme ribonuclease was opened out into a linear chain and then allowed to reform, it reassumed the correct catalytic shape. This experiment is a critical one in the understanding of the nature of gene expression, because it establishes the ultimate translation of the genetic information into functional difference. It is in determining the *shape* of proteins that genes express the information necessary to carry out and govern metabolism.

UNFOLDING RIBONUCLEASE

In order to test the hypothesis that a protein's amino acid sequence determines its shape, Anfinsen needed to measure or otherwise assess protein shape and to find some way of watching the folding process. Anfinsen solved the first problem by the simple expedient of working with an enzyme, ribonuclease. Ribonuclease catalyzes the hydrolysis of RNA, and its enzymatic activity depends entirely upon the protein being in a particular shape; thus, the level of enzyme activity could be used to monitor the degree to which ribonuclease protein successfully achieved the proper catalytic shape.

To watch the folding process, one might start with nascent proteins, newly made and not yet folded, or one might choose to unfold mature active ribonuclease and then watch it refold. Anfinsen chose the latter course. Ribonuclease is particularly suitable for this latter approach because it is a small protein of simple construction: it has a single polypeptide chain of 124 amino acids, and it is organized into its final shape by the formation of four *disulfide* (Cys-Cys) *bonds*. These bonds form cross-links between particular portions of the polypeptide, and thus are the major factor that determines what shape the ribonuclease protein assumes. Anfinsen found that the bonds can be *reduced* (electrons removed) with high concentrations of the sulfhydryl reagent β -mercaptoethanol (known to generations of students by its rich aroma of rotten eggs), so that $-S-S-$ becomes $-SHHS-$. If one then imposes a stress on the reduced protein, such as altering the polar nature of the solvent by adding urea, the reduced ribonuclease, lacking the disulfide bonds to resist the stress, open up (*denatures*) into a *random coil* that has no enzyme activity.

REFOLDING RIBONUCLEASE

Having succeeded in obtaining unfolded protein, Anfinsen was now in a position to study the process of refolding. Analysis of the physical properties of the reduced ribonuclease clearly indicated a random coil shape, so that looking at refolding (rather than the initial folding that occurs at synthesis) was a fair test of the hypothesis: because all eight cysteine residues were reduced and the polypeptide was free to assume random shape, there could be no residual information on folding left over from the protein's previous life as an active enzyme.

There are 105 different ways to pair eight cysteine residues two at a time to form four disulfide bonds, and only one combination corresponds to a ribonuclease protein that is active. If the information determining protein shape is inherent in the amino acid sequence, then that one form should always be produced—the inevitable thermodynamic consequence of repeatedly trying all alternative bond configurations until the

absence of urea, lacked the means of overcoming the thermodynamic barrier between it and the catalytic form. This deficiency could be remedied by adding *trace* amounts of the reducing agent - mercaptoethanol back to the solution and thus promoting the rearrangement of disulfide bonds. The result is a fully active enzyme, and the transition is driven entirely by the reduction in free energy that occurs in going from the “scrambled” to the catalytic form of the enzyme. This demonstrated that the shape that a protein realizes in solution is dictated by amino acid sequence information, which is expressed in terms of thermodynamic stability (figure 1.1).