Anfinsen’s experiment

1. The Observation

Ribonuclease A (RNaseA) is an extracellular enzyme of 124 residues with four disulfide bonds. In the first phase of the experiment, the S-S bonds were reduced to eight -SH groups (using mercaptoethanol, $HS\text{-}CH_2\text{-}CH_2\text{-}OH$); the protein was then denatured with 8 M urea. Under these conditions, the enzyme is inactive and becomes a flexible random polymer. In the second phase, the urea was slowly removed (dialysis); then the -SH groups were oxidized back to S-S bonds. If the protein was able to regain its native structure spontaneously after removal of the urea, we expect that it would also regain its activity. In fact, the activity was >90% of the untreated enzyme. Moreover, sequence analysis showed that nearly all of the correct S-S bonds had been formed.

2. The Control

A reasonable objection can be raised to the above result by suggesting that perhaps RNaseA was not completely unfolded in 8 M urea. To address this class of objections, RNaseA was first reduced and denatured as above. But in the second phase, the enzyme was first oxidized to form S-S bonds, and then the urea was removed, i.e. the order of steps in the second phase of the experiment was reversed. The resulting activity was only about 1-2% of the untreated enzyme. Sequence analysis showed a random assortment of S-S bonds.

Anfinsen's work showed convincingly that proteins can indeed adopt their native information spontaneously, i.e. sequence determines structure. His demonstration of this fundamental property of proteins opened the problem to a massive amount of experimental and theoretical effort. His summary of the experiments was presented as a Nobel Prize Lecture and published in:

Anfinsen, C.B. (1973) "Principles that govern the folding of protein chains." Science 181 223-230.

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