STABILITY OF A STRUCTURAL INTERMEDIATE WITH AN INCORRECT PROLINE ISOMER ON THE REFOLDING PATHWAY OF RIBONUCLEASE A

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Quantitative stability data for a structural intermediate on the refolding pathway of ribonuclease A (RNAase A) have been determined and compared to the stability of the fully native protein. The investigated folding intermediate I_N has previously been characterized as a native-like molecule, which contains at least one proline peptide bond in the incorrect configuration. The outline of the experiments is as follows. (i) The intermediate I_N is populated transiently by an interrupted folding experiment under "strongly native" conditions. (ii) The resulting reaction mixture is exposed to increasing concentrations of guanidine·HCl (GuHCl) to detect the stability of the I_N molecules towards unfolding induced by GuHCl. The experiments yield an unfolding transition of I_N as well as of native RNAase A (N). Employing these unfolding transition curves, the difference in stabilization energy between I_N and the native protein, N, $\delta\Delta G$ (N-I_N) is calculated to be 1.7 kcal/mol at 2.9 M GuHCl. In a theoretical study Levitt (1) provided a classification of proline residues on the basis of energy differences between the fully native protein and the folded structure containing the incorrect isomer of a particular proline. According to this model the proline (tentatively pro 93) which is still in the wrong *trans* configuration in the intermediate I_N is "type II". The energy difference of 1.7 kcal/mol is used to accelerate the rate of proline isomerization in the $I_N \longrightarrow N$ step about twentyfold.

1) Levitt, M., (1981) J. Mol. Biol., 145, 251.