

PROTEIN STABILITY FOR SINGLE SUBSTITUTION MUTANTS AND LOCAL COMPACTNESS IN THE DENATURED STATE

Sanzo Miyazawa

Gunma University, Faculty of Technology, Kiryu, Gunma 376, Japan

The stability changes caused by single amino acid substitutions are studied by a simple, empirical method which takes account of the free energy change in the compact denatured state as well as in the native state. The estimation of conformational free energy is based upon effective inter-residue contact energies evaluated in our previous study for proteins in water that are based on frequencies of non-bonded residue pairs in a set of protein crystal structures. When this method is applied, with a simple assumption about the compactness of the denatured state, for single amino acid replacements at Glu-49 of the tryptophan synthase α subunit (Yutani et al. (1987) *Proc. Natl. Acad. Sci. USA* 84, 4441-4444) and at Ile-3 of bacteriophage T4 lysozyme (Matsumura et al. (1988) *Nature* 334, 406-410), the estimates of the unfolding Gibbs free energy changes correlate well with observed values not only for hydrophobic amino acids but also for the aromatic and polar, charged residues. In the case of tryptophan synthase α subunit, the changes of hydrophobic energy estimated by the original authors were not large enough in magnitude to explain the changes of unfolding Gibbs free energy. The present method yields the same magnitudes of energies as the observed values for both proteins. When this method is also applied for amino acid replacements at various residue positions to estimate the average number of contacts at each position in the denatured state from the observed value of unfolding free energy change, those values for Gly and Ala replacements at the same residue position in staphylococcal nuclease (Shortle et al. (1990) *Biochemistry*, 29, 8033-8041) correlate well with each other. Those estimated values indicate that the compact denatured state has a native-like topology, supporting the molten globule model, because there is a correlation between the estimated average number of contacts at each residue position in the denatured state and the number of contacts in the native structure. These results provide further evidence that the inter-residue contact energies as applied here properly reflect actual inter-residue interactions, including hydrophobic energy.