

Protein Stability for Single Substitution Mutants and the Extent of Local Compactness in the Denatured State [1]

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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 conformational free energy is estimated from effective inter-residue contact energies, as 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 a 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 especially for hydrophobic amino acids. In the case of tryptophan synthase a 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 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 replacements with Gly and Ala at the same residue position in staphylococcal nuclease (Shortle et al. (1990) Biochemistry, 29, 8033-8041) correlate well with each other. Those estimated numbers of contacts indicate that the protein is not fully expanded in the denatured state, and also that the compact denatured state may have a substantially native-like topology, like the molten globule state, in the sense that there is a weak correlation between the estimated average numbers of contacts at each residue position between the denatured state and the native structure. These results provide some further evidence that the inter-residue contact energies as applied here properly reflect actual inter-residue interactions, and can be considered to be a pair-wise hydrophobicity scale. Also, the results indicate that characterization of the denatured state is critical to understanding the folding process.

References

[1] Miyazawa, S. and Jernigan, R. L. Prot. Engng., **7**, (1994), 1209-1220.

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