11 Φ-Value analysis

In Chap. 1 some inter-residue contact maps of protein transition states were presented. Here, the method of Φ-value analysis underlying such maps and some of its high-resolution applications are presented in more detail: the correlation of inter-residue contacts with Φ-values (see Fig. 1.9) is the currently available method with the highest resolution for protein folding transition states (Nölting, 1998, 1999a, b; Nölting and Andert, 2000).

The transition state corresponds to the state with the highest free energy in the course of the reaction. Since it is only extremely short-living, at present its structural resolution can not be carried out with NMR or X-ray crystallographic analysis. Φ-Value analysis uses mutants as structural reporters and a combination of equilibrium thermodynamics and kinetics methods (Nölting, 2005).

11.1 The method

Fig. 11.1 shows the free energy changes in the folding reaction for a wild-type protein and a mutant of this protein. One can see that in the course of the reaction

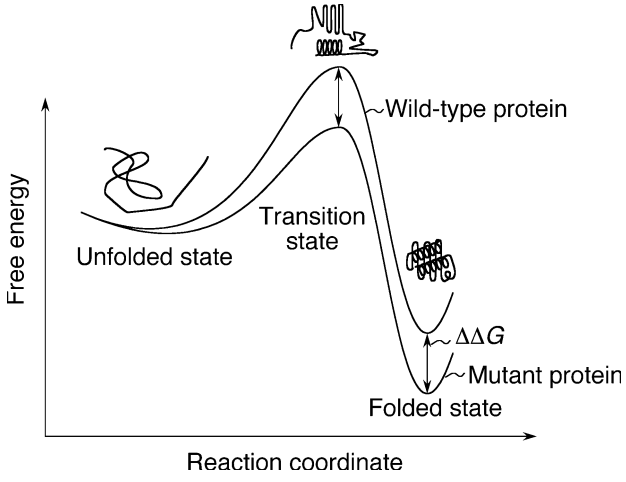

Fig. 11.1 Energy landscape along the reaction coordinate for the folding reaction of a wild-type protein (top curve) and a mutant of this protein (bottom curve)

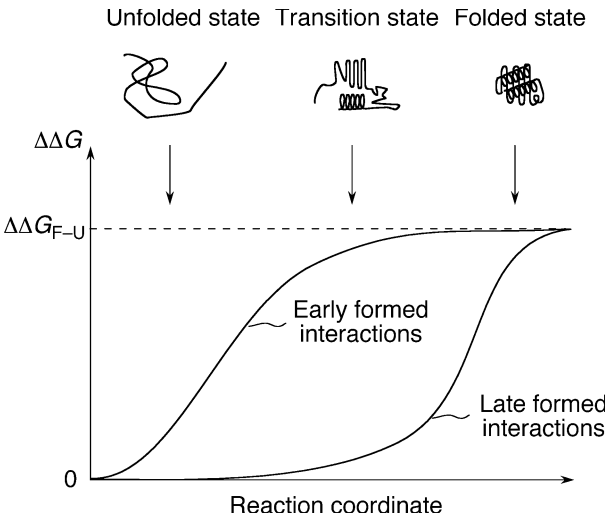

Fig. 11.2 Built-up of an energy difference, $\Delta\Delta G$, between wild-type protein and a mutant in the course of the folding reaction. $\Delta\Delta G_{F-U}$ is the energy difference between wild-type and mutant protein in the folded state

an energy difference, $\Delta\Delta G$, builds up between wild-type and mutant protein. This build-up of $\Delta\Delta G$ corresponds to the build-up of structure in the molecule. In particular, the time point in the reaction at which $\Delta\Delta G$ becomes significant depends on the time at which the interactions probed by the mutation build up in the molecule: If the interactions altered by the mutagenesis form early in the folding reaction (left curve in Fig. 11.2), one usually observes an early increase of $|\Delta\Delta G|$. In contrast, if the interactions probed by mutagenesis are formed late in the folding reaction, there is usually no significant $\Delta\Delta G$ till late in the reaction (right curve in Fig. 11.2). So, by measuring $\Delta\Delta G$ at the different stages of the folding reaction one can find out when certain interactions in the molecule are becoming formed. For the methods of measurement of $\Delta\Delta G$ see Nölting (2005).

The formation of stable interactions in the molecule is usually expressed by the Φ-value which is a measure of the structure consolidation at the position of the mutation on a scale from 0 to 1. Φ is defined as $\Phi = \Delta\Delta G / \Delta\Delta G_{F-U}$, where $\Delta\Delta G_{F-U}$ is the $\Delta\Delta G$ in the folded state (Nölting, 2005). A Φ of 0 at a certain stage of the folding reaction suggest the absence of stable structure at the position of the mutation at this time. If structure is completely formed at the position of the mutation at this stage of the reaction, one would expect a Φ-value of 1. Possible sources of error in this analysis, e.g., the effect of non-native interactions, can be decreased by using several mutants for the same part of the molecule.

So, in order to obtain information on the structure of a transition state one simply needs to measure Φ of the transition state for many mutants and correlate the data with the inter-residue contacts in the molecule (Nölting, 1998, 1999a, b).