Dissertation abstract

Recent research indicates that protein stability and folding are key features for understanding a number of diseases. The importance of understanding the principles behind protein stability is also realised from many industrial applications for proteins with an improved stability. The present study focuses on structure, function and stability of two model proteins: azurin from *Pseudomonas aeruginosa* and proton-translocating transhydrogenase from *Escherichia coli.* These proteins represent two interesting cases since they constitute a soluble protein and a membrane bound protein, respectively. Azurin is a small copper protein composed of a β barrel and an α helix. Transhydrogenase is arranged as a membrane bound domain and two soluble domains, binding NAD(H) and NADP(H), respectively. The study is based on recombinant and affinity-purified proteins, and includes the development of a new one step purification protocol for transhydrogenase.

Azurin was investigated in order to identify stabilising interactions in the protein structure. Four azurin mutants were designed to increase the stability. Three mutants were selected to improve electrostatic interactions in the C-terminus. These modifications had a minor effect on the stability. The forth mutant introduced a novel disulfide bond, connecting a flexible loop with a small α helix, which improved the stability as compared to wild-type. Crystal structures of the mutants with increased stability supported the selected design.

The stability and folding properties of the soluble, NADP(H) binding domain of transhydrogenase, ecIII, were investigated in the presence of NADPH or $NADP^+$. It is concluded that ecIII unfolds reversibly in a two state transition manner. The results indicate that folding of ecIII and substrate binding occur simultaneously. EcIII has a higher affinity for NADPH and this is reflected as an increased stability compared to the $NADP^+$-bound form of the protein. Stabilising interactions of the membrane spanning domain of transhydrogenase have also been investigated. Linker peptides of different length were introduced to connect the alpha and beta subunits of the enzyme. It was demonstrated that a proper interface between the two subunits is essential for correct folding of the enzyme. Furthermore, a split in a loop connecting two helices of the beta subunit resulted in a functional enzyme and illustrated the importance of helix-helix interactions for a correct assembly.

A novel one-step purification protocol for transhydrogenase was developed based on a calmodulin-binding peptide. This efficient protocol produced a pure and stable protein with an excess of α subunits. The results suggest that, depending on the construct, the calmodulin binding peptide may be a suitable affinity purification tag for membrane proteins in general.

Keywords: protein stability, azurin, proton-translocating transhydrogenase, membrane protein purification

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