

Binding regions in the Skp chaperone for client membrane proteins identified by a site directed fluorescence study.

Meenakshi Sharma, University of Konstanz

In Gram-negative bacteria, outer membrane proteins (OMPs) are synthesized in the cytoplasm. The translocation of OMPs across the periplasm in unfolded state is assisted by periplasmic molecular chaperones. The Seventeen-Kilo-Dalton protein, Skp, is a homotrimeric periplasmic chaperone known to facilitate folding and insertion of various OMPs into the membrane. To gain a better insight into the mechanism, by which Skp binds its client proteins in the periplasm, we designed, expressed and isolated a new Skp construct, Sx3kp, from *E. coli*. In this construct, the three Skp monomers were linked together with two short and flexible linkers. The function of Sx3kp was confirmed by comparison with wild-type Skp₃ in membrane protein folding experiments with OmpA.

To examine the topology of Sx3kp·OmpA complexes, we have used site directed mutagenesis and fluorescence spectroscopy. Our first aim was to identify binding regions in the Sx3kp chaperone at the level of individual amino acid residues. Nine single-cysteine Sx3kp mutants were constructed. The cysteine residues in these mutants were spectroscopically labeled with the fluorescent probe IAEDANS. Fluorescence resonance energy transfer (FRET) was then used to probe the interaction between the Sx3kp mutants and a set of single-tryptophan mutants of OmpA. The transfer efficiency E , the Förster distance R_0 , and the average distance between the fluorescence donor tryptophan in the client membrane protein and the fluorescence acceptor IAEDANS in the Sx3kp chaperone, r , were calculated. For the Sx3kp-N115C mutant, the donor to acceptor distance was $r \sim 21\text{Å}$, which is less than the R_0 for the pair tryptophan/IAEDANS. This confirmed close proximity and binding of the donor and acceptor proteins to each other.

To examine conformation changes within Sx3kp, two Sx3kp double mutants were constructed, each containing a single cysteine and a single tryptophan located in two of the three tentacle tips. FRET experiments with IAEDANS-labeled mutants indicated no effective change for the donor to acceptor distance ($r \sim 26 \pm 2 \text{Å}$) value upon client binding, suggesting limited or no conformational change at the tip region of Skp trimer.