Structure-Function and De novo Design of Protein Disulfide Isomerases

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Protein disulfide isomerases are ubiquitous proteins that catalyze the formation of disulfide bonds during oxidative protein folding. Even though these enzymes were first discovered by Christian Anfinsen over 40 years ago, their mechanism of action is still not understood. The bacterial protein disulfide isomerase DsbC is a homodimeric V-shaped enzyme that consists of a dimerization domain, a α-helical linker and a thioredoxin fold catalytic domain. By analyzing the properties of swapped domain variants of DsbC, where the catalytic domain had been replaced with structural homologous domains from other thioredoxin proteins, we determined that the key features for catalysis of the rearrangement of disulfide bonds are: (a) the availability of a peptide binding site to mediate interaction with substrate proteins; (b) the existence of two thioredoxin domains; (c) optimal positioning of the thioredoxin catalytic sites such that they face each other in the final molecule structure (Segatori *et al*., 2004. *PNAS*). On the basis of this information we designed a completely artificial disulfide isomerase enzyme comprising of the peptide binding domain of a chaperone fused to two thioredoxin domains derived from the bacterial oxidase DsbA. This protein, which has no amino acid homology to DsbC, exhibited high catalytic activity for disulfide bond isomerization and could support the folding of multi disulfide proteins expressed in *E. coli*. Our ability to *de novo* design a disulfide isomerase reveals that we now understand the salient features of these enzymes, and have opened the way for the engineering of improved catalysts for protein expression and metabolic engineering applications.