7. Summary

7.1. Crystal structure analysis of site directed mutated Cold Shock Proteins

Crystal structures of site directed mutated cold shock proteins from *Bacillus caldolyticus* were solved to elucidate the structural reason for differences in the thermostabillity on atomic level. Five mutants represent the transition from the thermophile protein to the mesophile homolog. Here, the rest are mutated whose responsible for the differences in the thermostabillity. The both rest Arg3 and Leu66 have a exceptional interest. Both residues contribute to the difference in the free energy of unfolding of 15.8 $kJ \cdot mol^{-1}$ essentially. The other ten residues have no remarkable influence on the thermostabillity. Both proteins have the same structure in principle and differ in detail only. The small and compact proteins contain no cysteins, *cis*-prolines and strong bonded cofactors.

From the structures of *Bc*-Csp and *Bs*-CspB is known, that the rest Arg3 and Leu66 are neighboring in the structure very closely. The structure analysis are enclosed neighboring residues, which can interact with Arg3 and Leu66. The crystal structure of the mutants *Bc*-Csp R3E and *Bc*-Csp L66E were solved with nearly atomic resolution (1.4 Å and 1.27Å) and refined to R-values of R/R_{free} 13.9%/20.2% and 15.8%/18,9%. Both structure have the same global fold. Remarkable is, that they show varying patterns of hydrogen bonds and salt bridges between the both molecules in the asymmetric unit. Under consideration of the adjacent Glu46 and triple mutants *Bc*-Csp R3E/E46A/L66E and *Bc*-Csp V64T/L66E/67A could be examined the distribution on surface charges around positions 3 and 66.The mutants *Bc*-Csp E46A, *Bc*-Csp R3E/E46A/L66E and *Bc*-Csp V64T/L66E/67A were crystallized, the structure were solved up to a resolution of1.8 Å, 1.32 Å respectively 1.8 Å and refined to R-values of R/R_{free} 18.5%/23.4%, 13.9%/18.1% and 19.3%/24.6%.
A systematic analysis of all mutants shows very similar structures of all mutants. But in every structure are found different patterns of hydrogen bonds, electrostatic and hydrophobic interactions around position 3 and 66 and adjacent residues. It seems, that the thermal destabilization appears to correlate with extent of an acidic patch near the C-terminal carboxylate group on the surface of the cold shock mutants. But from the thermodynamic studies is no hint known for an existence of attractive electrostatic interaction between the mutated residues. That is not in contrast to the crystal structure. It could be concluded, that the cold shock proteins are stabilized by removing of repulsive electrostatic interactions.

7.2. Crystallographic investigations on KorB

KorB is a repressor protein from *E. coli* and regulate the expression of RP4 genes.
For that, KorB binds to a pseudo symmetric 13 bp operator (O_B) sequence. The 60kb plasmid contain twelve copies of this O_B. The binding of dimeric KorB to DNA is influenced by other proteins encoded by RP4.
A C-terminal fragment (residues 297 to 356) was obtained after an *in situ* proteolysis in a crystallization droplet. Surprisingly, the C-terminal fragment crystallized in the same droplet. Further domain analysis and DNA binding studies of KorB revealed, that the N-terminal fragment contain the DNA bindings side. With limited proteolysis, with and without DNA, KorB-N could be restrict to a shorter fragment, that contain the DNA binding region. The fragment is localized in the central region of KorB.
Such a fragment could be crystallized in the presence of 17 bp DNA fragment, containing the O_B sequence. It was shown, that the crystals contain DNA and protein. This crystals diffract to a maximum of 1.7Å resolution.

The crystal structure of C-terminal domain of the repressor protein KorB ,KorB-C, were solved in two different space group with a maximum resolution of 1.7Å. The C-terminal domain contains the rests 297 to 356. KorB-C is folded into a five stranded antiparallel β sheet. The strands 1 to 4 form a up and down sheet. Strand 5 closed the sheet on the open side from strand 1. Strand 4 and 5 are connected by a short 3_{10}-Helix. Quite unexpectedly a structure comparison shows a fold similar to the Src homology 3 domain (SH3-domain) for KorB-C. SH3 domains are well known from the eukaryotic signal transduktion pathway and bind to proline rich sequences.
From the arrangement of the molecules in the structures was concluded, it is concluded that two molecules form a functional relevant dimer. The detailed examination of the dimer interface and chemical cross-link studies suggest that KorB-C forms a dimerisation domain. The dimer interface is dominated by hydrophobic residues and the size is big enough to stabilize the dimeric form of intact KorB. In this way, KorB-C stabilized the dimeric form of KorB in solution to facilitate binding to the palindromic operator sequence..
The crystal structure of KorB-C is a further example for a structure with a SH3 similar fold with prokaryotic origin. The observed structure extend the range of protein-protein interactions known to be promoted by SH3 and SH3-like domains. The SH3-SH3 interaction is connected with a biologic function clearly. This form and arrangement of interaction is described the first time for SH3-like domain here.