SUMMARY

DafA is a protein encoded by the *dnaK* operon of *Thermus thermophilus* and mediates the formation of a highly stable complex between the molecular chaperone $DnaK_{Tth}$ and its co-chaperone $DnaJ_{Tth}$. This 87 amino acid residues protein is the only member of the $DnaK_{Tth}$ chaperone system for which no corresponding protein has yet been identified in other organisms and whose particular function has remained elusive.

The main aim of this work was to characterize $DafA_{Tth}$ using the stably expressed $DafA(L2V)_{Tth}$ variant. The studies presented here involved the application of various biochemical and biophysical methods offering new insights into structural and functional features of $DafA_{Tth}$.

Although not complete, the structural analysis of free $DafA(L2V)_{Tth}$ provided evidence for a well-folded protein comprising defined secondary structure elements including alpha-helical, beta-sheet and coiled coil regions. From these studies and from spectroscopical analyses of engineered $DafA(L2V)_{Tth}$ point mutants it became also evident that the two tryptophane residues of $DafA(L2V)_{Tth}$ have different localization with respect to solvent accessibility.

Studies on the dynamics of the $DnaK_{Tth}$-$DnaJ_{Tth}$-$DafA(L2V)_{Tth}$ complex formation and dissociation using a fluorescently labeled $DafA(L2V)_{Tth}$ cysteine mutant show that, at 25°C, both events occur at low rates. The rate constant characterizing complex assembly (k_{on}) is $9.3 \cdot 10^3\ M^{-1}s^{-1}$, whereas the complex dissociation rate (k_{off}) is $1.3 \cdot 10^{-3} s^{-1}$. As anticipated from previous studies, the affinity existing between the complex components is very high. The equilibrium dissociation constant K_D is in the submicromolar range (~140 nM). Also, these studies suggest once more that the complex formation is a highly synergic process occurring only in the presence of all three protein species. Fluorescent complexes comprising only two protein species could not be observed.

Except for its essential role in mediating in $DnaK_{Tth}$-$DnaJ_{Tth}$ association, no other specific function could be assigned to $DafA_{Tth}$. Studies with peptides showed that $DafA(L2V)_{Tth}$ competes with peptides binding to $DnaK_{Tth}$ in the presence of $DnaJ_{Tth}$. The same inhibitory effect was observed here in a luciferase refolding assay. $DafA(L2V)_{Tth}$ inhibits $DnaK_{Tth}$-assisted protein refolding thus confirming the model for $DnaK_{Tth}$ chaperone cycle proposed by Klostermeier et al. Since $DafA_{Tth}$ must be released from $DnaK_{Tth}$-$DnaJ_{Tth}$-$DafA_{Tth}$ complex before substrate proteins can bind, free $DafA_{Tth}$ might have regulatory function connected to the heat shock response. Here, the 70 S ribosomal particle was identified as the new binding target of $DafA(L2V)_{Tth}$ thus supporting this hypothesis. $DafA(L2V)_{Tth}$ is targeted *in vitro* to the translational machinery without recruiting $DnaK_{Tth}$ and $DnaJ_{Tth}$. Competition experiments show that $DafA(L2V)_{Tth}$ is shuttled between the two complexes. These findings strongly suggest the involvement of

$DafA_{Tth}$ in regulatory processes occurring at a translational level, which could represent a new mechanism of heat shock response as an adaptation to elevated temperature.

The second part of this study involved the cooperation between $DnaK_{Tth}$ and $ClpB_{Tth}$ chaperone systems in reactivation of protein aggregates. The aim of this work was to establish *in vitro* conditions that will allow for protein-aggregate reactivation using the co-expressed $DnaK_{Tth}$-$ClpB_{Tth}$ systems. The *dnaK*$_{Tth}$ operon containing both DnaK and ClpB chaperone systems was cloned into an expression vector and the corresponding proteins were produced in *E. coli*. Importantly, the *dafA* gene was successfully removed from the operon, a prerequisite for a $DnaK_{Tth}$ system functionally active in protein refolding. During this study it became evident that *E. coli* lysates containing the thermophilic proteins are not able to reactivate protein aggregates. Moreover, the lysates have a strong inhibitory effect on $DnaK_{Tth}$-$ClpB_{Tth}$-assisted refolding in an assay using purified chaperones. This observation raises the question of a DafA-like protein produced also by *E. coli* cells.