Abstract

Type II toxin-antitoxin (TA) modules are bacterial genetic systems which encode a stable toxin and an unstable antitoxin. The unbound toxin causes bacteriostasis or cell death by binding to its cellular target. Its action is inhibited by binding of the antitoxin. TA modules are involved in general cell stress management or contribute to plasmid maintenance, which makes them interesting for studying the molecular bases of their functioning in bacteria. In addition, they represent interesting systems for biotechnological applications and development of new antimicrobial agents.

In the first part of this work we have used global analysis of urea denaturation curves to determine the thermodynamic stability of the $CcdB_{Vfi}$ toxin encoded on the *V. fischeri* chromosome. We compared the obtained thermodynamic parameters with those for the $CcdB_F$ toxin encoded on the *E. coli* plasmid F. We found that differences in stability are mainly due to differences in enthalpies and that driving forces of $CcdB_{Vfi}$ and $CcdB_F$ unfolding are substantially different. We have shown that these differences can be explained by differences in structures of native $CcdB_{Vfi}$ and $CcdB_F$.

In the second part we have studied the association of disordered C-terminal part of the antitoxin, $CcdA_F$, encoded on the *E. coli* plasmid F with the toxins $CcdB_F$ from *E. coli* and $CcdB_{Vfi}$ from *V. fischeri*. The C-terminal part folds into alpha helical structure upon binding, which makes it very interesting for studying general characteristics of an important group of proteins, called intrinsically disordered proteins. Analysis of isothermal titration calorimetry data, CD spectra and molecular modeling was used to explain, how do changes in amino acid sequence of the C-terminal fragment of $CcdA_F$ affect its binding-coupled folding. We have designed two types of mutant fragments, the first type exhibits the same binding surface as the wild-type fragment, while the second one does not. We have shown that mutations of the first type affect only folding properties of the fragment, which enabled us to dissect the differences in thermodynamic parameters, caused by mutations, into the contributions of folding and binding. We show that mutations of the second type lower the binding affinity of the fragment. Moreover, we explain the accompanying changes in enthalpy and entropy at the level of side chain interactions. We have also identified how changes in amino acid sequence influence the binding of the C-terminal fragment of $CcdA_F$ to a different recognition surface ($CcdB_F \rightarrow CcdB_{Vfi}$) and demonstrated that the effects are thermodynamically favorable, accompanied by extensive enthalpy-entropy compensation. A general conclusion of this part is also, that the secondary structure C-terminal fragment of $CcdA_F$ assumes on binding is to a large extent encoded in its amino acid sequence.

In the last part we used global analysis of isothermal titration calorimetry data to determine the thermodynamics of protein-protein and protein-DNA interactions driving the functionality of the *E. coli maz* module. We have shown that binding sites for MazE on MazF are different, mainly due to the fact that the binding of the first MazE molecule disables binding of a small part of the second MazE molecule. We have shown that the promotor DNA sequence contains two high and one low affinity binding site for MazE. Analysis of competitive calorimetric titrations suggests, that the expression of *maz* module could be regulated by various MazE:MazF complexes, and shows that the most probable repressor complex is the one with MazE:MazF molar ratio 1:1. Simulation of the *maz* system behavior suggests that fast response to changes in MazE and MazF concentrations is possible only if the total concentrations of MazE and MazF are about 100- to 1000-times higher than the DNA concentration, and that MazE successfully inhibits the toxic action of MazF up to 40 °C.

Keywords: toxin-antitoxin modules, thermodynamics, *ccd*, *maz*, structure