Abstract

The ability to form capsules is a widespread feature between various bacterial species. The capsules consist of long, modular polysaccharides and represent the interface for the interaction of the bacteria with their environment. Furthermore, the exopolysaccharide (EPS) of pathogenic organisms serves as a protection against host-defense mechanisms and is also an important virulence determinant.

The enterobacteria *Escherichia coli*, *Erwinia amylovora*, and *Pantoea stewartii* subsp. *stewartii* synthesize the EPS colanic acid, amylovoran, and stewartan, respectively. All these capsules belong to the group IA of bacterial polysaccharides. Their biosynthetic genes are organized in operon-like clusters (the *wca*-, *ams*-, and *cps*-cluster) and are regulated by the Rcs (regulation of capsule synthesis) system, consisting of the three proteins RcsA, RcsB, whose activity can be modulated by phosphorylation, and RcsC, a membrane located sensor kinase. Activation of transcription is achieved by binding of an RcsA/RcsB heterodimer (RcsAB) to suitable promoter sequences.

In this work, the RcsAB/DNA interaction was further characterized. The apparent equilibrium constant K_D = 100 nM of the RcsAB/*ams*-promoter complex has been determined using the bandshift technique, while the K_D = 77 nM of the RcsAB/*wza*-promoter complex was calculated using the surface plasmon resonance (SPR) technique. An *in vitro* selection of the *ams*-promoter made it possible to formulate a first consensus motif for RcsAB binding (Wehland *et al*., .1999), which allowed to find 13 more RcsAB binding sites in the EPS- and *rcsA*- promoters from *E. coli, Ew. amylovora, P. stewartii, Salmonella typhi*, and *Klebsiella pneumoniae* as well as in the *bvgA*- and *fha*-promoters from *Bordetella pertussis* and *B. parapertussis* by data bank search. A compilation of these sequences resulted in the finding of a common RcsAB binding motif, the RcsAB box : TaAGaatatTCctA (Wehland *et al*., 2000). The essential role of the RcsAB box in EPS regulation was additionally confirmed by *in vivo* experiments

Besides the RcsAB binding sites in the EPS main promoters, internal, weaker binding sites inside the *ams*- and *wca*-operons were identified, but their biological function remained unclear.

The RcsAB binding to various *rcsA* promoters has been shown for the first time. *In vitro* and *in vivo* experiments confirmed the central role of the RcsAB box for RcsAB/DNA interaction.

Expression of an RcsB protein bearing a mutation in its phophorylation motif ($RcsB_{(11D-A)}$) led to constitutive, RcsA independent EPS synthesis in *E. coli*. SPR analyses showed a tenfold increased DNA affinity of $RcsB_{(11D-A)}$ compared to the wildtype protein, which cannot be suppressed by phosphorylation. Furthermore, SPR and *in vivo* studies provided evidence for the involvement of the RcsAB box in RcsB/DNA interaction as a deletion of the RcsAB box abolished RcsB binding completely.

Additionally, the first experimental proof together with the kinetic data for an RcsA/RcsB interaction in solution is presented.