Crystal Structure of IcaR, A Repressor of the TetR Family Implicated in Biofilm Formation in *Staphylococcus epidermidis*

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Expression of the gene cluster *icaADBC* is necessary for biofilm production in *Staphylococcus epidermidis*. The *ica* operon is negatively controlled by the repressor IcaR. Here, the crystal structure of IcaR was determined and the refined structure revealed a homodimer comprising entirely α-helices (Fig. 1A), typical of the tetracycline repressor protein family for gene regulations. The N-terminal domain contains a conserved helix-turn-helix DNA-binding motif with some conformational variations, indicating flexibility in this region (Fig 1B,D). The C-terminal domain shows a complementary surface charge distribution about the dyad axis, ideal for efficient and specific dimer formation (Fig 1C). The results of the electrophoretic mobility shift assay and isothermal titration calorimetry suggested that a 28-base-pairs core segment of the *ica* operator is implicated in the cooperative binding of two IcaR dimers on opposite sides of the duplex DNA. Computer modeling based on the known DNA-complex structure of QacR and site-specific mutagenesis experiments showed that direct protein-DNA interactions are mostly conserved, but with slight variations for recognizing the different sequences. By interfering with the binding of IcaR to DNA (Fig 2.), aminoglycoside gentamicin and other antibiotics may activate the *icaADBC* genes and elicit biofilm production in *S. epidermidis*, and likely *S. aureus*, as a defense mechanism.

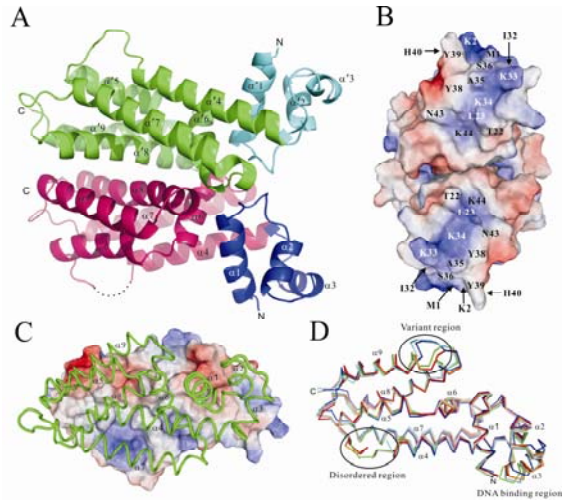

Figure 1. Structure of IcaR. (A) Overall structure of the IcaR homodimer. The protein structure is shown as a ribbon diagram with the N-terminal domains in blue and cyan, the C-terminal domains in magenta and green, and the disordered region as dotted line. (B) The DNA binding domains. The electrostatic surface of the dimer is viewed along the dyad axis, after a rotation of approximately 90° from (A). The electrostatic surfaces are drawn either blue for positive or red for negative. Possible residues involved in binding DNA are labeled. (C) The dimer interface. One subunit is shown as a surface representation, colored according to the electrostatic potentials as in (B), and another subunit is shown as a worm tracing in green. (D) Superposition of the IcaR models. The polypeptide backbones of six IcaR subunits are shown as stick models. Four subunits from the native crystal are colored in red, pink, orange and green, and two subunits from the SeMet derivative crystal are in cyan and blue. *Nucleic Acids Research*, 2008 (in press).

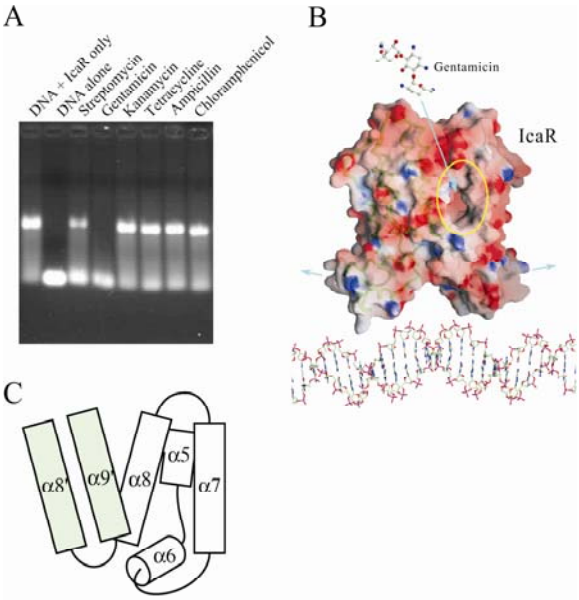

Figure 2. Gentamicin interrupts IcaR and DNA interaction. (A) The effects of antibiotics to the IcaR binding to *ica* operator in EMSA experiment. Only two aminoglycosides are capable of interfering the DNA binding, whereas gentamicin inhibited the binding to a significantly greater extent than streptomycin. The other four antibiotics kanamycin, tetracycline, ampicillin and chloramphenicol did not show significant effect on the IcaR-DNA binding. (B) Possible effect of gentamicin on the IcaR binding to *ica* operator. A negatively charged cavity was observed in each IcaR monomer, near the dimer interface. Binding of gentamicin to this cavity may result in conformation changes that lead to a larger separation of the N-terminal domains. (C) A schematic diagram of the putative antibiotics-binding cavity. The cavity is located at the dimer interface and surrounded by helices α5, α6, α7 and α8 of one monomer as well as α8' and α9' of the counter monomer. The α8-α9 loop has different conformations in the two crystal forms (see Figure 1D), suggesting some functional flexibility. *Nucleic Acids Research*, 2008 (in press).