Crystallography

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Crystal structure of *Staphylococcus epidermidis* TcaR

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Introduction

TcaR and IcaR are a weak and a strong negative regulator of transcription of the *ica* locus, respectively, and their presence prevents the poly-*N*-acetylglucosamine (PNAG) production and biofilm formation in *Staphylococcus epidermidis*. Although TcaR was shown to interact with the *ica* promoter, the precise binding region and the mechanism of interaction remained unclear. Here, in order to study how the *ica* operon is controlled, and to investigate the role of TcaR in antibiotic resistance, TcaR from *S. epidermidis* was over-expressed in *E. coli* with a His-tag to facilitate purification. The crystal structure of TcaR was solved by multi-wavelength anomalous dispersion (MAD) method using protein containing seleno-methionines.

Results and discussion

The crystal structures of SeMet TcaR derivative in the space group $P6_1$ were refined to 2.9 Å. The refined TcaR structure contains two TcaR molecules (denoted chain A and chain B) in the asymmetric unit, forming a dimer. The overall structure of TcaR belongs to the α/β family protein as observed in other MarR family proteins. The TcaR dimer adopts a triangular topology with each monomer consisted of secondary structure α1-α2-α3-α4-βA-βB-α5-α6 (Fig. 1*A*). The N and the C-terminus α-helices (α1, 5, 6) interdigitate with those of the other monomer to produce dimerization interaction. Strands βA and βB form a β-hairpin, which is slightly twisted and constitutes the typical winged motif. Between βA and βB is the flexible winged region (residues 84-96) which had poor electron density in the initial 2Fo-Fc map. In addition, using the program *CAVER*, we observed a highly porous structure with several cavities for potential ligand binding in the TcaR dimer. All cavities are located at the dimer interface, surrounded by helices α1, α2, α3, α5, and α6.

The wHTH DNA binding domain is composed of α2-α3-α4-βA-W1-βB which adopts the winged-helix- fold similar to the winged helix (wH) domain described in other MarR family protein. The DNA-binding site expected to be facing the DNA, is densely positively charged in a surface patch (Fig. 1*B*). Electrostatic interactions involving those positively-charged amino acids must play most important roles for DNA binding.

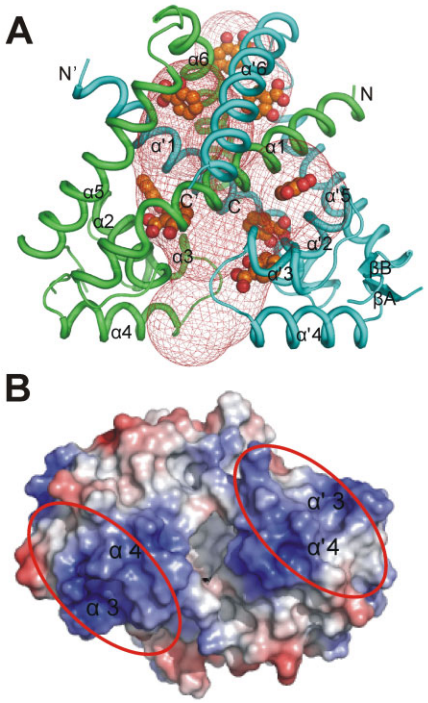

Fig. 1. Overall structure of apo TcaR. (*A*) The overall structure of the TcaR homodimer. The protein structure is shown as a ribbon diagram with chain A in green and chain B in cyan. In addition, we used the program to explore possible cavities in TcaR for ligand binding. The cavities identified by *CAVER* are shown in mesh representation and using a solvent probe of radius 2 Å. In addition, the binding positions of eight salicylate molecules in the TcaR-salicylate complex are shown in sphere, revealing that the porous structure of TcaR is able to interact with numerous small molecules. (*B*) The DNA-binding domains. The electrostatic surface of the dimer is viewed after a rotation of ~90° from Fig. 1*A*, with a horizontal axis in the plane of paper. The electrostatic surfaces are drawn either blue for positive or red for negative. Possible domains involved in binding DNA are labeled as red ovals.

Reference

[1] Y.-M. Chang et al., Proc. Natl. Acad. Sci. USA. 107, 8617 (2010).

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