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X-ray crystallographic analysis of P-450cam operon repressor CamR

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Introduction

Pseudomonas putida cam repressor (CamR) is a homodimeric protein that binds to the operator DNA (camO) of cytochrome P450cam operon (camDCAB) to inhibit its transcription. The inducer D-camphor binds toward the CamR protein to repress the binding of CamR protein toward the camO, resulting in the activation of the transcription. Based on biochemical studies, there are some interesting binding properties of CamR toward the operator DNA camO and the D-camphor; (1) CamR protein has two domain structure, the N-terminal DNA binding domain which is proposed to have a typical helix-loop-helix motif and to recognize the camO, and the C-terminal regulatory domain which binds the D-camphor to induce the inhibition of CamR binding to camO, (2) two D-camphor molecules can bind to one molecule of homodimeric CamR in a negative cooperative manner, (3) similar amino acid sequence responsible for camphor binding exist in three proteins of the camDCAB; CamR, cytochrome P450cam and purtidaredoxin reductase. In order to clarify the molecular mechanism of the CamR/camO/D-camphor recognition, we are trying to determine the crystal structures of CamR and its complexes with camO and D-camphor.

Result

The recombinant wild-type (wt-camR) and selenomethionyl derivative CamR proteins (SeMet-CamR) have been overproduced in *Escherichia coli* and purified. The wt-CamR was crystallized in two conditions of (1) 12-14% PEG4K,0.1M KCl, 1% Glycerol, 50mM Na-Pipes (pH7.3), with and without 2mM camphor, 15°C, and (2) 1.6M Pi, Na-Pipes(pH6.7), 2mM camphor, 5°C. On the other hand, the SeMet - CamR protein was not crystallized in the above conditions, but was successfully crystallized in two other conditions of (1) 10% MPD, 25mM Na-cacodylate, 20mM $MgCl_2$, pH 6 at 15°C, and (2) 12.5% PEGMME 550, 25mM Na-Pipes, 2.5mM $MgCl_2$, pH 7.3 at 25°C.

The crystals of SeMet-CamR in the condition (1) have large cell dimensions and large mosaicity, it was difficult to solve the structure. On the other hand, the crystals from condition (2) have the P222 space group with the cell dimension, a= 47 Å, b= 87 Å, c= 105 Å, a=b=c=90° (one molecule per asymmetric unit). The crystals of SeMet-CamR were suitable for cryo-experiment by increasing the concentration of PEGMME 550 to 30%. MAD data using the SeMet-CamR crystal were collected at several wavelengths including, 0.9793 Å (peak), 0.9795 Å (edge) and 0.9879 Å (remote) at the beam line BL18B. For all these data sets, 90 image data with an oscillation range of 2 degree were collected utilizing the ADSC CCD detector system.

All diffraction data were autoindexed, integrated and corrected for Lorentz and polarization effects with the program MOSFLM. Scaling and merging of each data in SCALA indicated a p222 space group (Rmerge~12% on data to 3 Å resolution, data completeness= more than 98%). The data collections for the crystals of the wt-CamR with or without the inducer CamR were also done. A full structure determination is in progress.

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