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Crystal structure analysis of transcription regulator, Dnr from *Alcaligenes faecalis* S-6

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Alcaligenes faecalis S-6 has a dissimilatory nitrate reductin system called denitrification, in which nitrate is sequentially converted to nitrite, nitric oxide, nitrous oxide and finally to molecular nitrogen[1]. *A. faecalis* S-6 grows by means of aerobic respiration in the presence of oxygen, but the bacterium grows by means of denitrification. The activation of the gene encoding the denitrification enzymes is restricted in the anaerobic condition. In *Escherichia coli*, Fnr (fumarate nitrate reduction) activates the transcription of genes encoding the anaerobic respiration enzymes. Fnr forms 4Fe-4S cluster with the four cysteine residues in the N-terminal region, and the state of oxidation of the 4Fe-4S cluster serves as a switch between the activated form and inactivated form. In the upstream region of the genes which expression is regulated by Fnr, there is the nucleotide motif called Fnr box. Fnr in the activated form binds to Fnr box and activates the expression of the gene downstream of the motif. In *A. faecalis* S-6, Fnr boxes presents in the upstream region of the genes encoding the denitrification enzymes, it is thought that Fnr regulates the expression of these genes. On the other hand, the gene encoding the protein which belongs to Dnr (Denitrification regulation) subfamily was present in the cluster which contains denitrification enzymes. Unlike Fnr, the protein which belongs to Dnr subfamily have no conserved cysteine residues for sensing the redox state, suggesting that Dnr regulates the genes encoding the denitrification enzymes not by sensing redox state directly but through sensing other signals. Recently performed biochemical experiment using other denitrifying bacterium indicates that the proteins which belong to Dnr subfamily sense nitric oxide and activates the transcription of the genes encoding denitrificaiton enzymes. However, the mechanism for sensing nitric oxide by Dnr is unknown, and the direct evidence to proof that Dnr binds to Fnr box is not obtained. On the other hand, molecular size calibration and SDS-PAGE of Dnr indicated that Dnr from *A.faecalis* S-6 is present as a monomeric form. Gel mobility shift assay indicated Dnr is in a inactive form. To elucidate the mechanisms for nitric oxide sensing and binding to Fnr box by Dnr, we tried to determine the crystal structure of Dnr.

Crystals of apo-EX7 were grown at 20 ºC by vapor diffusion in 0.4~0.8 M Ammonium Sulfate, 0.1 M Tris-HCl pH7.0~8.0. X-ray data were collected from a crystal that was flash cooled to 95 K. The resolution of obtained data was 6~7Å, and data set did not provide the data for structural analysis. The space group for the crystal was C2. The unit cell parameters for the crystal was a=154Å, b=107 Å, C=73 Å, b=106º.

Reference

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