Crystal structure analysis of DsrD in Desulfovibrio vulgaris

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Introduction

DsrD (dissimilatory sulfite reductase D) encoded by the *dsr* operon[1] is a small protein consisting of only 78 amiono acid residues. The *dsr* operon also contains the dsrAB genes for dissimilatory sulfite reductase (DsrAB)[2], of the anaerobic sulfate-reducing eubacterium, *Desulfovibrio vulgaris* Hildenborough. A function of DsrD was suggested as an anion-binding protein (especially sulfite-binding protein) for DsrAB because of its content of many positively charged lysine residues[1]. However, a recent spectroscopic study of purified DsrD failed to indicate that the either sulfite or sulfide bind of the DsrD protein with high affinity[3]. We indicated no strong similarity to protein sequences except for those of DsrD homologs in other sulfate-reducing bacteria from searches with the amino acid sequence of DsrD using the programs of BLAST and FASTA. Therefore, the real function of the DsrD protein in these bacteria is not still clearly identified. However, DsrD should have an important role such as a transcription factor for DsrAB. In order to determine its function with the three-dimensional crystal structure, we have started the X-ray structural study of DsrD.

Experiments, Results and Discussions

A diffraction data set was collected from the native DsrD crystal at 100K up to 1.4 Å resolution by using x-ray beam of a 1.0 Å wavelength at beam line BL-6A with CCD camera (ADSC Quantum 4R). The crystal was soaked into the 80 % saturated ammonium sulfate solution with 10 % glycerol for 48 hours, and soaked into the mineral oil for several seconds just before X-ray diffraction experiment of the DsrD crystal. The crystal belongs to space group $P2_12_12_1$. The unit cell parameters are a = 60.03 Å, b = 64.56 Å, c = 45.22 Å. The data were scaled and merged by using DPS Mosflm and CCP4. The data consist of 197,902 measured reflections and 34,837 unique reflections. Overall R_{merge} and average I/σ were 0.047 and 9.0, respectively. The structure of DsrD has been refined with crystallographic R=22.7 at 1.5 Å resolution by using CNS. The electron density map clearly shows that DsrD had dimeric structure in crystal (Fig.1) with five sulfate anions. These anions were suggested the capability of the binding of sulfite to this molecule. The structure of DsrD is similar with the structure of Zα domain, Z-DNA binding motif, of ADAR1 protein. Further refinement using with the SHELX program is now in progress.

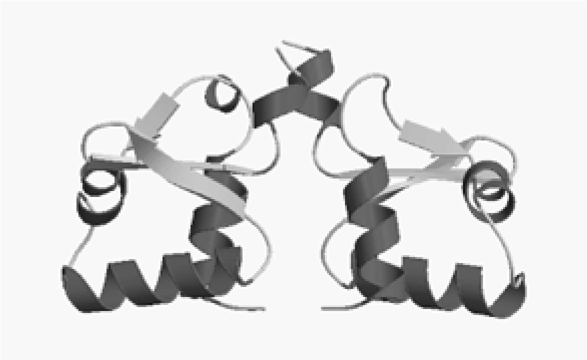

Fig.1 Dimeric structure of DsrD

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