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High Resolution Structure of FMN-binding Protein

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High resolution X-ray analysis of protein structure can detect hydrogen atoms, but it might be not same as neutron diffraction study can do.

KEYWORDS: FMN-binding protein, high resolution x-ray study, neutron diffraction study

§1. Introduction

Neutron diffraction studies play an important role in structural biology, because it can clearly detect the positions of hydrogen (deuterium) atoms which are essential to understand biological processes. High resolution synchrotron X-ray study can also contribute in this field. Sulfate-reducing bacteria possess many redox proteins including flavoproteins. FMN-binding protein (FMN-bp) from *Desulfovibrio vulgaris* Miyazaki F is composed of 122 amino acids and a FMN, which is the smallest in known proteins binding flavin.[1] The FMN-bp does not have any homology with other flavoproteins including flavodoxin. The function of FMN-bp *in vivo* is at present unclear, however it might take part in the electron-transfer pathway. The crystal structure was solved by X-ray crystallography with multiple isomorphous replacement method.[2,3]

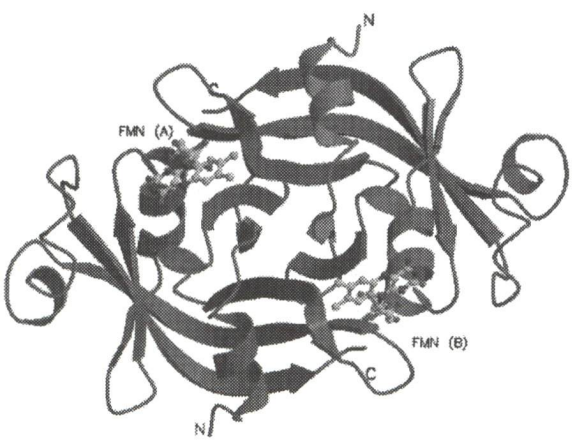

Fig. 1. Dimeric structure of FMN-bp. The protein chains were illustrated as ribbon models. The heterogen atoms illustrated as ball-and-stick models were FMNs.

§2. Experimental and Structure Refinement

The high resolution X-ray diffraction data were collected at the beamline BL44B2, SPring-8. The data were collected at a wavelength 0.7000 Å with an imaging plate detector, RIGAKU R-AXIS IV. The crystals diffract X-ray beyond 0.9 Å resolution at a temperature of 100 K. Several crystals were used for the experiments and X-ray diffraction data from the two good crystals out of them were used to merge. The cell dimensions were a=36.6, b=84.0, c=40.4 Å, β=93.4^o with the space group of $P2_1$. Summary of the data sets was shown in Table I. Since one dimer was contained in an asymmetric unit, the solvent content was assumed to 46 %. The preliminary

TableI. Data statistics.

Temperature	100 K
The number of crystals	2
The number of frames	157
Resolution range (Å)	20 - 0.90 (0.92 - 0.90)*
Total reflections	325290
Unique reflections	142777
Completeness (%)	79.3 (40.2)*
$R^{**}_{\rm merge}$ (%)	4.7 (12.7)*
$<I> / <\sigma(I)>$	26 (4.3)*
Redundancy	2.3

*) Values in parentheses are for the highest resolution shell.

**) $R_{\rm merge} = \frac{\sum_{hkl,i} |I_{hkl,i} - <I_{hkl}>|}{\sum_{hkl,i} I_{hkl,i}}$

phase were calculated from a model refined at 1.2 Å resolution data. After the model was refined using program CNS[4] up to 2.0 Å resolution, the structure was refined using program SHELX97[5] with reflections of 10. - 0.9 Å resolution. After isotropic temperature factors were refined, the R and $R_{\rm free}$ values were 0.158 and 0.179. Finally anisotropic temperature factors were introduced and refined, and the R and $R_{\rm free}$ values decreased to 0.109 and 0.135 (Table II). The figures were illustrated with MOLSCRIPT,[6] XTALVIEW[7] and RASTER3D.[8]

§3. Discussion

Dimeric structure of FMN-bp was illustrated in Fig.1. FMN-bp does not have any flexible loops, therefore the average temperature factor is very small (Table II). As the phases were improved along with the refinement of the structure, some hydrogen atoms were clearly observed on difference Fourier map above 2.5 σ level (Fig. 2). About 50 % of hydrogen atoms attached to protein

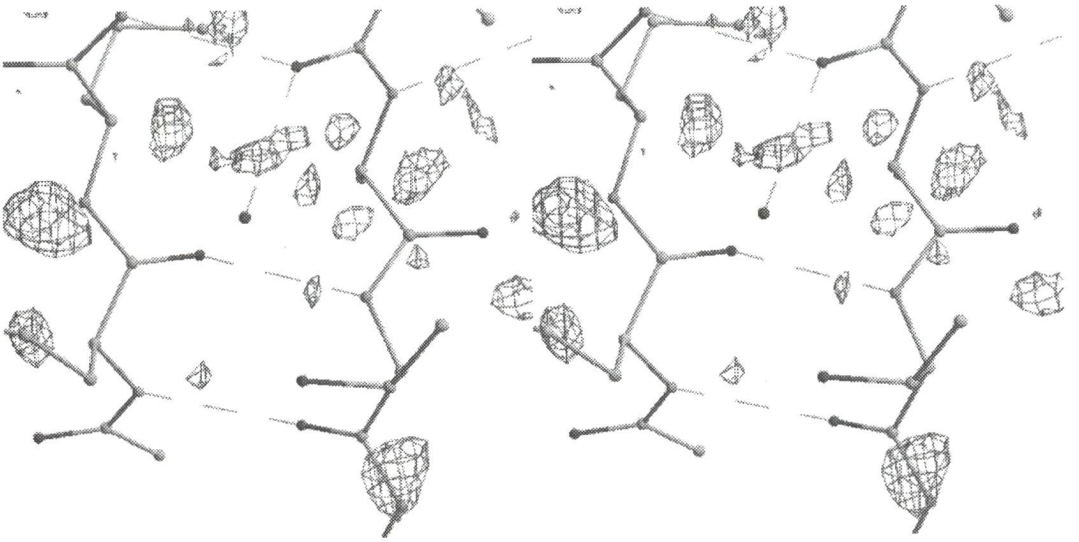

Fig. 2. Stereo view of sigma-weighted difference Fourier map. Many hydrogen atoms appeared in the map including atoms relative to hydrogen bonds.

Table II. Refinement statistics.

Resolution range (Å)	10 - 0.90
The number of reflections	135397
Water molecules	444 (402)*
R_{work} (%)	10.9 (15.7)*
R_{free} (%)	13.5 (17.8)*
Averaged B-factor** (Å^2)	8.8 (8.2)*
Parameters	21925 (9363)*

*) Values in parentheses are results of isotropic refinement.
**) Calculated with protein atoms.

Cα atoms could be located, and those attached to main chain N atom incorporating hydrogen bondings were also visible. On the contrary, any hydrogen atoms bonding to the side chain oxygen atoms could not be found, even if they are clearly included in hydrogen bonds. It may depend on the electoronegativity of the atoms bonding to hydrogen atoms whether hydrogen atoms could be found on electron density map or not. The crystals of FMN-binding protein can grow to the size larger than 1 mm, we are planning to carry out a neutron diffraction study of this crystal. Neutron diffraction study and high resolution synchrotron X-ray study will be complementary in the case of FMN-binding protein.

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