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Crystallization and preliminary X-ray study of two crystal forms of *Klebsiella oxytoca* diol dehydratase–cyanocobalamin complex

Jun Masuda,[a] Tetsuya Yamaguchi,[a] Takamasa Tobimatsu,[b] Tetsuo Toraya,[b] Kyoko Suto,[a] Naoki Shibata,[a] Yukio Morimoto,[a] Yoshiki Higuchi[a]† and Noritake Yasuoka[a]*

[a]Department of Life Science, Himeji Institute of Technology, 1475-2 Kanaji, Kamigori, Ako-gun, Hyogo 678-1297, Japan, and [b]Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, Tsushima-Naka, Okayama 700-6082, Japan

† Present address: Division of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.

Correspondence e-mail: yasuoka@sci.himeji-tech.ac.jp

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Two crystal forms of *Klebsiella oxytoca* diol dehydratase complexed with cyanocobalamin have been obtained and preliminary crystallographic experiments have been performed. The crystals belong to two different space groups, depending on the crystallization conditions. One crystal (form I) belongs to space group $P2_12_12_1$ with unit-cell parameters a = 76.2, b = 122.3, c = 209.6 Å, and diffracts to 2.2 Å resolution using an X-ray beam from a synchrotron radiation source. The other crystal (form II) belongs to space group $P2_1$ with unit-cell parameters a = 75.4, b = 132.7, c = 298.8 Å, β = 91.9°, and diffracts to 3.0 Å resolution. For the purpose of structure determination, a heavy-atom derivative search was carried out and some mercuric derivatives were found to be promising. Structure analysis by the multiple isomorphous replacement method is now under way.

1. Introduction

Adenosylcobalamin or coenzyme B_{12} is one of the most fascinating molecules in nature because it contains a unique naturally occurring Co—C bond. Recently, two structural studies have been reported in this area. One was of the cobalamin-binding domain of *Escherichia coli* methionine synthase (Drennan *et al.*, 1994) and the other was methylmalonyl-coenzyme A mutase from *Propionibacterium shermanii* (Mancia *et al.*, 1996). The imidazole N atom of the histidine residue of these enzymes is coordinated to the Co atom in cobalamin from the lower axial position ('base-off' mode).

Diol dehydratase is considered to be another type of adenosylcobalamin-dependent enzyme family, since it binds cobalamin in the 'base-on' mode (Yamanishi *et al.*, 1998; Abend *et al.*, 1998). The knowledge of the molecular structure of this enzyme is essential for the elucidation of the function of adenosylcobalamin. Diol dehydratase (1,2-propanediol hydrolyase, E.C. 4.2.1.28) is an enzyme which catalyzes the adenosylcobalamin-dependent conversion of 1,2-diols to the corresponding deoxyaldehydes. Toraya and coworkers (Tobimatsu *et al.*, 1995, 1997) have long been studying the molecular cloning, sequencing and expression of the genes encoding the enzyme and have finally established its molecular properties. The functional diol dehydratase consists of subunits of M_r = 60348 (α), 24113 (β) and 19173 (γ). The composition of the molecular assembly is found to be $(\alpha\beta\gamma)_2$ and the molecular weight observed is 220 kDa. A central issue is how the reactivity of adenosylcobalamin is controlled so as to favour homolytic cleavage of the Co—C bond in the enzyme. In this investigation we replaced adenosylcobalamin by cyanocobalamin, as the diol hydratase–adenosylcobalamin complex is unstable to light; the binding of cyanocobalamin to the apoenzyme is very similar to the binding of adenosylcobalamin. Therefore, we have initiated the crystallographic study of *Klebsiella oxytoca* diol-dehydratase–cyanocobalamin complex.

2. Crystallization

The *Klebsiella oxytoca* diol dehydratase overexpressed in *Escherichia coli* was purified following the method of Toraya and coworkers (Tobimatsu *et al.*, 1995, 1997). The cultured *E. coli* was disrupted by sonication in a phosphate buffer solution containing 2% 1,2-propanediol (pH 8.0) and was centrifuged. The crude membrane fraction was washed three times by repeating sonication of the suspension in a buffer solution containing 2% 1,2-propanediol (pH 8.0) and 2 m*M* EDTA and centrifugation. The crude membrane fraction was further washed three times in a buffer solution containing 0.2% $C_{12}H_{25}(OCH_2CH_2)_{23}OH$ (Brij 35) and then suspended in a buffer containing 1.0% Brij 35. The suspension was sonicated to extract the enzyme effectively. After centrifugation, the extracted enzyme was loaded on to a DEAE–cellulose column, which was then eluted with a buffer containing 20 m*M* sucrose monocaprate. About 50 ml of elute was collected. About 300 mg of the purified protein was obtained from about 15 g of wet *E. coli*

Table 1
Data-collection conditions.

Crystal type	Form I		Form II		
Crystal number	1	2	1	2	3
Method	Oscillation	Oscillation	Oscillation	Oscillation	Weissenberg
Beamline	BL-18B	BL-18B	BL-18B	BL-6B	BL-6B
Wavelength (Å)	1.00	1.00	1.00	1.00	1.00
Total oscillation range (°)	82.0	103.5	47.5	45.0	45.0
Overlap (°)	0	0	0	0	0
Coupling constant (° mm^{-1})	—	—	—	—	1.2
Oscillation speed (° s^{-1})	1.0	1.0	1.0	1.0	1.0
Oscillation time	10	30	40	35	20

cells. After purification, 0.10% LDAO (lauryldimethylamine oxide) was added to the protein solution, which was then concentrated and substituted by ultrafiltration. Excess cyanocobalamin was added to the protein solution, and the mixture was incubated for 30 min at 303 K in order to form a diol dehydratase–cyanocobalamin complex. The complex formed was dialyzed against a buffer containing 0.10% LDAO, which was used for the crystallization experiment. Crystals of diol dehydratase–cyanocobalamin complex were obtained by the sandwich-drop vapour-diffusion method. A single crystal of diol dehydratase–cyanocobalamin complex (form I) with dimensions 1.5 × 1.0 × 1.0 mm was grown under the conditions 20 m*M* Tris–HCl (pH 8.0), 0.24 *M* ammonium sulfate, 15% PEG (polyethylene glycol) 6000 and 0.20% LDAO. Crystals of form II with dimensions 2.5 × 0.5 × 0.5 mm were grown using a buffer containing 0.45 *M* potassium chloride, 15% PEG 6000 and 0.20% LDAO.

3. Diffraction experiments

Native data were collected from a single crystal using the Weissenberg camera for macromolecular crystallography (Sakabe, 1983, 1991) at the Photon Factory (KEK).

The form I data set was collected at the BL-18B beamline from two crystals using the oscillation method. The form II data were obtained from three diffraction data sets from three different crystals. Two of these data sets were collected using the oscillation method, while the third was collected using the Weissenberg method. The conditions and statistics for data collection for crystal forms I and II are listed in Table 1. All data sets were collected at 280 K. The radius of the Weissenberg camera cassette was 430 mm at BL-18B and 573 mm at BL-6B and the beam path from the beam collimator to the camera cassette was filled with helium gas to reduce the diffraction-image background during data collection. The wavelength of the X-ray beam was adjusted to 1.0 Å. The data were processed with the programs *DENZO* and *SCALEPACK* (Otwinowski, 1993).

4. Results and discussion

The form I crystals were found to have space-group symmetry $P2_12_12_1$ with unit-cell parameters a = 76.2, b = 122.3, c = 209.6 Å. Combined data from two crystals included 449078 indexed reflections and merged into 76970 unique reflections to 2.2 Å resolution, with an $R_{\rm merge}$ of 7.7%. The overall completeness was 87.7%. Assuming one dimer per asymmetric unit, the volume per unit molecular weight (V_m) is 2.21 $Å^3$ Da^{-1}. This value is in the range found for proteins (Matthews, 1968). A typical diffraction pattern from crystal form I is shown in Fig. 1.

Crystals of form II belong to space group $P2_1$, with unit-cell parameters a = 75.4, b = 132.7, c = 298.8 Å, β = 91.9°. Data to 3.0 Å resolution were obtained from 368718 indexed reflections, which were reduced to 69072 unique structure factors with an $R_{\rm merge}$ of 9.6% and a completeness of 59.4%. Assuming three dimers per asymmetric unit, V_m = 2.24 $Å^3$ Da^{-1}.

Trials for the preparation of heavy-atom derivatives have been carried out with form

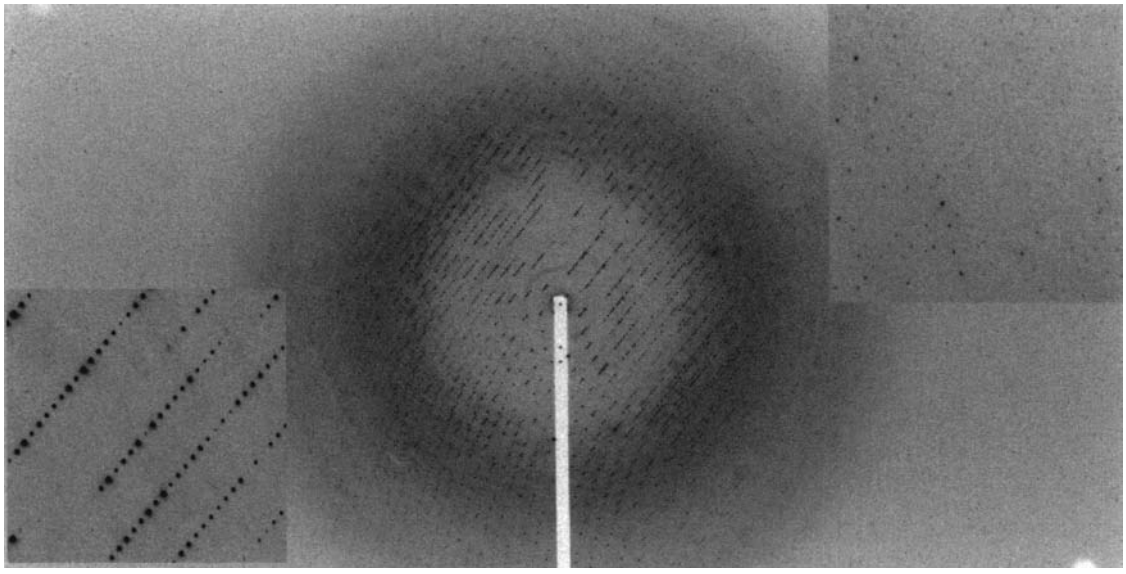

Figure 1
X-ray diffraction pattern of a single crystal of diol-dehydratase–cyanocobalamin complex (form I). The oscillation photograph was taken at an oscillation angle of 4°. The images were recorded using the X-ray beam from the synchrotron radiation on a Weissenberg camera for macromolecular crystallography with a Fuji imaging plate as a detector system. The image in the centre of the figure shows the oscillation photograph, while the image on the upper right shows an enlarged portion of the higher angle reflections and the image on the lower left shows an enlarged portion of the lower angle reflections.

I crystals. Methylmercuric compounds and ethylmercurithiosalicylate (EMTS) have been introduced to the enzyme crystals and significant changes in the diffraction pattern were observed. The preparation of heavy-atom derivatives, the collection of intensity data from them and the detailed description of the structure analysis will be reported in the near future. Structure analysis using the multiple isomorphous replacement method with the anomalous dispersion effect is being carried out.

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