X-ray Crystal Structure of Michaelis Complex of Aldoxime Dehydratase*\#1

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Aldoxime dehydratase (Oxd) catalyzes the dehydration of aldoximes (R–CH=N–OH) to their corresponding nitrile (R–C=≡N). Oxd is a heme-containing enzyme that catalyzes the dehydration reaction as its physiological function. We have determined the first two structures of Oxd: the substrate-free OxdRE at 1.8 Å resolution and the n-butyraldoxime- and propionaldoxime-bound OxdREs at 1.8 and 1.6 Å resolutions, respectively. Unlike other heme enzymes, the organic substrate is directly bound to the heme iron in OxdRE. We determined the structure of the Michaelis complex of OxdRE by using the unique substrate binding and activity regulation properties of Oxd. The Michaelis complex was prepared by x-ray cryoradiolytic reduction of the ferric dead-end complex in which Oxd contains a Fe3+ heme form. The crystal structures reveal the mechanism of substrate recognition and the catalysis of OxdRE.

Nitrile compounds are important intermediates in some industrial processes to produce nylon and acrylic fibers, insecticides, and pharmaceuticals. Although one of the most useful methods for nitrile production is dehydration of aldoxime, the chemical dehydration of aldoxime used in the industrial process requires harsh conditions. Therefore, a more environmentally benign process of aldoxime dehydration is needed, for which a biological dehydration of aldoxime is a possible candidate. In nature, some microbes have an “aldoxime-nitrile pathway” (supplemental Fig. 1), where aldoximes are metabolized to the corresponding carboxylic acids through nitriles formed by dehydration of aldoximes with aldoxime dehydratase (Oxd) EC 4.99.1.5. There are two pathways for the conversion of nitriles to carboxylic acids.

One is hydrolysis of nitriles by nitritase, and the other is the combination of the reactions catalyzed by nitrile hydratase and amidase (1–4). Nitriles are the important intermediate not only in some industrial processes but also in this biological system. The detailed characterization of such a biological process to produce nitriles will give some useful information to develop an environmentally benign process for the production of nitriles in the industrial field.

Oxd is a new heme-containing enzyme that works as a hydrolyase (2, 5, 6). The enzymatic activity of Oxd is dependent on the oxidation state of the heme iron, although the reaction catalyzed by Oxd is not a redox reaction. Ferrous Oxd containing a Fe2+ heme shows the enzymatic activity, but ferric Oxd containing a Fe3+ heme does not. Previous spectroscopic analyses reveal a novel mechanism, where the change in the coordination mode of the substrate plays a crucial role for the regulation of the enzymatic activity (7) (supplemental Fig. 1).

Although the oxygen atom of aldoxime is coordinated to the ferric heme, the nitrogen atom of aldoxime is coordinated to the ferrous heme (7). The dehydration reaction proceeds only via N-coordinated substrate in the ferrous heme. The organic substrate is directly coordinated to the heme iron in dehydration of aldoxime, which is a unique example among heme enzymes, although the coordination of O2 or H2O2 to the heme is well known in the heme-containing oxygenases, catalases, and peroxidases. It is proposed that the dehydration reaction of the heme-bound aldoxime proceeds in a general acid-base catalysis with a histidine working as a catalytic residue in the distal heme pocket (7–10). However, the detailed reaction mechanisms remain to be elucidated mainly because the structural information of Oxd was lacking.

In this study, we determined the crystal structures of Oxd from Rhodococcus sp. N-771 (OxdRE) in the substrate-free and substrate-bound forms. We could determine the crystal structure of the Michaelis complex of OxdRE by means of the unique property of OxdRE for the substrate binding. As the reaction spontaneously proceeds when mixing ferrous Oxd and the substrate, the crystallization of the substrate-bound OxdRE in the ferrous form (the Michaelis complex of OxdRE) is not possible by the usual methods. However, we could prepare the crystal of the Michaelis complex of OxdRE by the reduction of the crystal of the substrate-ferric Oxd complex using x-ray radiation under cryogenic temperature. The structures of the resting state and the Michaelis complex provide structural insights into the mechanisms of substrate recognition and the catalysis of OxdRE.
Crystal Structure of OxdRE

**Table 1**

Statistics of X-ray data collection and structure refinement of OxdRE

| PDB code     | Substrate-free | PrOx (soaking) | BOx (co-crystal) | BOx (soaking) |
|--------------|----------------|----------------|-----------------|---------------|
| PDB code     | PrOx (soaking) | BOx (co-crystal) | BOx (soaking) |
| PDB code     | PrOx (soaking) | BOx (co-crystal) | BOx (soaking) |
| PDB code     | PrOx (soaking) | BOx (co-crystal) | BOx (soaking) |

**Experimental Procedures**

Protein Expression, Purification, and Enzymatic Activity Assay—The expression, purification, and enzymatic activity assay of OxdRE were performed according to reported methods (7), with a slight modification as described below. The OxdRE used in this work has an additional 20 N-terminal residues encoded in the plasmid, including a His6 tag. Site-directed mutagenesis was performed using the QuikChange site-directed mutagenesis kit (Stratagene). The deoxyoligonucleotide primers used in this work has an additional 20 N-terminal residues (7), with a slight modification as described below. The OxdRE expression was induced by adding isopropyl-β-D-thiogalactopyranoside to a final concentration of 1 mM after 5 h of growth. The cells were incubated for 1 h and then with DNase mixture (10 μg ml⁻¹ DNase 1, 5 mM MgCl₂, and 0.5 mM MnCl₂) for 30 min. The resulting solution was centrifuged at 100,000 × g for 1 h, and the cellular debris was discarded. The supernatant was loaded onto a nickel-ni-triltriacetic acid agarose (Qiagen) column. The column was washed with 3 bed volumes of lysis buffer followed by 10 bed volumes of lysis buffer containing 50 mM imidazole. The adsorbed proteins were eluted with lysis buffer containing 250 mM imidazole. The OxdRE-containing fractions were combined and dialyzed against 40 mM MOPS buffer (pH 6.8). The dialyzed sample was loaded on a RESOURCE Q column (GE Healthcare), and the adsorbed proteins were eluted under a
linear gradient of NaCl from 0 to 0.75 M. The OxdRE-containing fractions were combined, concentrated, and subsequently purified using a HiLoad 26/60 Superdex 200 pg column (GE Healthcare). OxdRE was concentrated by centrifugation with Amicon Ultra (Millipore).

OxdRE was oxidized to the ferric form by adding excess potassium ferricyanide. Potassium ferricyanide was removed by a prepacked PD-10 column (GE Healthcare), which was previously equilibrated with 20 mM HEPES buffer (pH 7.4) (Hampton Research). The purity of the sample used in the crystallization experiments was ~98%, as estimated by SDS-PAGE.

The OxdRE activity was assayed at 30 °C under anaerobic conditions by measuring the amount of phenylacetonitrile formed from Z-phenylacetaldoxime. The assay reaction was started by the addition of Z-phenylacetaldoxime (5 mM in the final concentration) dissolved in N,N-dimethylformamide into a 500 mM phosphate buffer (pH 7.0) solution containing 5 mM sodium dithionite and 1 mM purified OxdRE, in a final volume of 0.5 ml. High pressure liquid chromatography analyses were performed as described previously (7). The activity was measured at least three times for each sample. The average values are shown in Table 2.

Crystallization—Proteins were crystallized with hanging drops of 3 µl of protein mixed with an equal volume of precipitant solution in a 24-well crystallization VDX MtM plate (Hampton Research) at 20 °C. OxdRE was concentrated to 30 mg ml⁻¹ in 10 mM HEPES buffer (pH 7.4). Tris-(2-carboxyethyl)phosphine was added to the concentrated sample to a final concentration of 2 mM, and the mixture was centrifuged at 50,000 × g for 20 min before crystallization. Initial screening using Natrix screening kit (Hampton Research) revealed substrate-free OxdRE crystals. The final crystallization condition of the substrate-free OxdRE was 18% (w/v) polyethylene glycol 4000, 75 mM sodium cacodylate (pH 7.4), and 100 mM magnesium acetate. For cryoprotection, the crystal was soaked in a
reservoir solution containing 16% (w/v) sucrose and 12% (w/v) trehalose.

To prepare substrate-bound crystals by the soaking method, substrate-free crystal was soaked for a few minutes in a reservoir solution supplemented with 16% (w/v) sucrose, 12% (w/v) trehalose, and 2% (v/v) substrate (BOx or PrOx). For co-crystallization of OxdRE and BOx, the concentrated OxdRE solution (10 mg ml$^{-1}$) was mixed with BOx at a final concentration of 2% (v/v) and incubated for 1 h at 20 °C before starting crystallization. The final crystallization condition was 17% (w/v) polyethylene glycol 2000 monomethyl ether, 100 mM Trizma (Tris base)/HCl (pH 7.0), and 200 mM calcium acetate. The crystal was soaked in reservoir solution containing 22% (w/v) xylitol and 2% (v/v) BOx for cryoprotection.

Data Collection, Structure Determination, and Refinement—The optical absorption spectra of the crystals were measured by a single-crystal microspectrophotometry system (11) on the beam line BL44B2 (SPtmg-8, Hyogo, Japan). Diffraction data were collected on beam lines BL41XU and BL44B2 (SPtmg-8). All image data were collected at 90 K. The HKL2000 (12) or XDS (13) program was used for integration of diffraction intensity and scaling. Initial phases of substrate-free crystal were calculated by the SHELX-97 (14) and HKL2MAP (15) programs from multil wavelength anomalous dispersion (16) data measured at the absorption edge of the iron atom (supplemental Table S2). Phases were further improved and extended to 1.8 Å resolution data. Most parts of the atomic model of OxdRE were built automatically using the ARP/warp (17) program. The heme and remaining residues were manually built using the graphics program Coot (18). The model was refined using REFMAC5 (19). Non-crystallographic symmetry restraints were applied to the core region of the protein molecule throughout all refinement cycles. The model geometry was checked using the program WHAT IF (20). Crystal structures of the substrate-bound form were determined by the molecular replacement method using the MOLREP program (21). The substrate-free OxdRE structure was used as a search model.

The open and closed states were defined based on the conformations of the A- and C-chains in PrOx-soaked crystal and of the co-crystallized BOx-bound OxdRE, respectively. The α10 and following 3$_{10}$ helix region of B- and D-chains of PrOx-soaked crystal shows two conformations of the open and close states. When the model was refined with only a single conformation of the open state, the calculated difference Fourier map ($F_o - F_c$ map) showed the positive density for the close state. The occupancies of alternative conformations were not refined by the program but estimated from manual adjustment in 0.05 steps, which is followed by the refinement and the check of $B$-factor of each atom and $F_o - F_c$ map. The additional N-terminal 20 residues derived from the vector, including the His tag, were disordered in density for most of the polypeptide chains.

Coordinates—The coordinates for OxdRE and BOx-bound OxdRE prepared by co-crystallization and for BOx-bound OxdRE and PrOx-bound OxdRE prepared by the soaking method were deposited in the Protein Data Bank.
The barrel was composed of eight
prepared by the soaking method.
formed a homodimer with non-crystallographic two-fold symmetry (Fig. 1
refined at a 1.8 Å resolution (supplemental Table 2). OxidRE was solved by multiwavelength anomalous dispersion (16) and
Overall crystal structure of substrate-free OxidRE was
results (22). Each monomer contained one heme molecule. The
hydrophobic pocket composed of non-polar side chains of Trp-
insertion of a short turn or loop, which deformed the β-barrel. The interior of the β-barrel primarily consisted of hydro-
(PDB) with accession codes of 3A15, 3A17, 3A18, and 3A16, respectively.

RESULTS AND DISCUSSION

Overall Structure and Protein Fold—Statistics of the data collection and refinement of OxidRE are summarized in Table 1. The overall crystal structure of substrate-free OxidRE was solved by multiwavelength anomalous dispersion (16) and refined at a 1.8 Å resolution (supplemental Table 2). OxidRE formed a homodimer with non-crystallographic two-fold symmetry (Fig. 1A), consistent with previous gel filtration analysis results (22). Each monomer contained one heme molecule. The α10 helix of one monomer interacted with the α10 helix of the other to create the dimer interface, which was stabilized by hydrogen bonds and electrostatic interactions.

Each monomer of OxidRE has a α + β structure consisting of an elliptic β-barrel flanked on both sides by α-helices (Fig. 1B). The barrel was composed of eight β-strands, β3  β4  β2  β5-5'  β11-11'  β8-8'  β9  β10 , with all strands exhibiting an antiparallel pattern. Some regions of β-strands failed to form hydrogen bonds with the neighboring strand because of the insertion of a short turn or loop, which deformed the β-barrel. The interior of the β-barrel primarily consisted of hydro-

Crystal Structure of OxidRE

Environment of the Heme in the Substrate-free OxidRE—The crystal of the substrate-free OxidRE was obtained in ferric form, in which the heme iron was in the Fe3⁺ state. However, the heme was reduced by x-ray radiation, as confirmed from the visible crystal spectra obtained with the single-crystal microspectrophotometry system. As data collections for the structural analyses were begun after heme reduction was complete, the structure of the substrate-free OxidRE reported here is the same as that of ferrous OxidRE.

The heme exists between the β-barrel and the helix in the hydrophobic pocket composed of non-polar side chains of Trp-172, Ile-217, Ile-238, Leu-242, Leu-249, Leu-289, Trp-292, Ile-302, Phe-303, Phe-306, and Phe-307 (supplemental Fig. 5). His-299 in the α10 helix was the proximal ligand of the heme iron. The orientation of the His-299 imidazole ring was stabilized by a hydrogen bond between the N6 atom of His-299 and the O atom of the main chain at position 293 (Fig. 2). A water molecule (w1) coordinated to the distal coordination site of the heme in the substrate-free form (Fig. 2). The heme 6-propionate group was directed to the proximal side, and its conformation was stabilized by interactions with two waters (w2 and w3) and the side chain of Ser-174 (supplemental Fig. 5B). The
7-propionate was directed to the distal side and interacted with the side chain of His-169 and the main chain amide of Gly-170 (supplemental Fig. 5B). A water molecule (w4) was shared by two propionates for hydrogen bonding (supplemental Fig. 5B). One of the B strands in the barrel provided His-320, whose N1 atom is located within a distance capable of a hydrogen bonding with w1. The side chain of Ser-219 also formed a hydrogen bond to w1 (Fig. 2). Another water molecule (w5) interacted with the side chains of Ser-219 and Gln-221 near w1. In the distal heme pocket, a hydrogen-bond network existed among His-320, Glu-143, and Arg-178 (Fig. 2).

Preparation of the Crystal of the Michaelis Complex for OxdRE—The structure of substrate-free OxdRE was determined, as described above. Determination of the structures of the resting state and the Michaelis complex of OxdRE should provide useful information for the elucidation of the molecular mechanisms of OxdRE catalysis. In principle, crystallization of the Michaelis complex of a native enzyme is not possible because the complex is a transiently formed intermediate on enzymatic catalysis that is formed only at very low concentrations. We obtained the crystal of the Michaelis complex by using the unique properties of OxdRE. The substrate-bound ferric form of the OxdRE crystal was obtained by crystallization of ferric OxdRE in the presence of the substrate or by soaking the substrate-free crystal of ferric OxdRE in a substrate-containing solution. Ferric OxdRE is an inactive form that forms a dead-end complex with the substrate. The oxidation state of the heme iron and the coordination mode of the substrate were different between this dead-end complex and the Michaelis complex (supplemental Fig. 1). We postulated that the crystal of the OxdRE Michaelis complex could be obtained by reduction of the heme iron in the crystal of the dead-end complex using x-ray radiation under cryogenic temperature.

The optical absorption spectra of the substrate-free and substrate-bound OxdRE crystals were changed upon x-ray radiation (Fig. 3). The spectral change in the Q-band region indicates that the heme iron in the substrate-bound crystal was also reduced by x-ray radiation at 90 K, as was also the case for substrate-free OxdRE. The heme iron began to be reduced within seconds by the x-ray beam of SPring-8 BL44B2, and reduction was complete within 60 s (Fig. 3C). We collected x-ray diffraction images for structural analyses after confirming the reduction of the heme iron by microspectrophotometry.

The structures of the BOx-bound OxdRE and PrOx-bound OxdRE were determined at 1.8 and 1.6 Å resolutions, respectively (supplemental Fig. 6). The nitrogen atom of aldoxime was
coordinated to the heme in these complexes, indicating that reduction by x-ray radiation has successfully reconstructed the coordination structure of the aldoxime-heme complex from the inactive to the active form, even in the crystal. The overall structure of the substrate-bound OxRE was almost the same as that of the substrate-free OxRE, but there were some local conformational changes upon substrate binding, as discussed below.

**Environment of the Heme in the Michaelis Complex of OxRE**—The structures around the heme of PrOx- and Box-bound OxRE are shown in Figs. 4 and 5, which clearly reveal that PrOx and Box bound to the heme iron via the N atom. The structures around the heme were similar in PrOx- and Box-bound OxREs. The substrate alkyl group was surrounded by hydrophobic residues (Met-29, Leu-145, Phe-306, and Leu-318) in the distal side of the heme, but there was no specific arrangement of hydrophobic residues to discriminate the specific alkyl group (Fig. 5). A large cavity existed on the distal side of the heme (Fig. 6A). OxRE has a broad substrate specificity capable of converting various aryl- and alkyl-aldoximes to their corresponding nitriles (22). This is because the large cavity does not prevent accommodation of various substrates.

The OH group of the heme-bound substrate formed two hydrogen bonds with Ser-219 and His-320. In the distal heme pocket, the hydrogen-bond network was retained among Glu-143, Arg-178, and His-320 (Fig. 5), as was the case of the substrate-free form. Conformational differences between the substrate-free and substrate-bound forms were not observed in these distal residues.

Although no conformational differences were observed at the distal side of the heme among the determined structures, conformational variations were observed at the proximal side of the heme (Fig. 6B). The region 294–315, including the proximal α-helix (α10) and the following 3_{10} helix (γ4), displayed a conformational variation in each monomer in the asymmetric unit of the crystal. In the dimer of the substrate-free form, two conformations were present: one subunit with an open form and another with a closed form. In the open form, the substrate-binding cavity in the distal side of the heme was connected to the protein surface through a channel formed between the 3_{10} helix (309–312) and the loop (24–29). This cavity will serve as the substrate access and product release channel (Fig. 6A). However, the entrance of the channel on the protein surface was closed in the closed form (Fig. 6C), in which the proximal α-helix was rotated ∼15° (as viewed from the heme) and the 3_{10} helix deviated ∼5 Å to cover the channel entrance. The conformational change from the open to the closed form resulted from the sliding of Phe-306 by 3 Å vertically to the heme plane, accompanied by rotation of the proximal α-helix. This movement of Phe-306 disconnected the substrate-binding cavity from the substrate access/product release channel (Fig. 6A). Thus, the 3_{10} helix and side chain of Phe-306 act as a gate for the channel, modulating substrate access and product release.

Substrate-bound OxRE displayed different populations of two conformations, depending on crystal preparation method. PrOx-bound OxRE prepared by soaking the OxRE crystal in PrOx for 30 s contained two dimers (AB and CD) in an asymmetric unit. Although the A- and C-chains had open conformation, the B- and D-chains had mixtures of open and closed forms, with estimated occupancies of 75 and 25% for the open and closed forms, respectively (supplemental Fig. 7A). Box-bound OxRE prepared by the soaking method had a similar population of the two forms as the PrOx-bound OxRE (supplemental Fig. 7B). However, Box-bound OxRE prepared by co-crystallization had mainly the closed conformation (occupancy >90%) for all chains in the asymmetric unit. These results suggest that the open and closed forms are in equilibrium in solution; and that the substrate binding can shift the equilibrium from the open to the closed form. As complete equilibration may not be achieved in the crystal prepared by soaking in PrOx or Box, the substrate-bound OxREs prepared by the soaking method will have a mixed conformation.

**Reaction Mechanism of OxRE**—The apparent role of the heme in OxRE is to tether the substrate in the catalysis by aldoxime dehydratase. The crystal structures determined in this study reveal that hydrogen bonding between the OH group of aldoxime and the side chains of Ser-219 and His-320 controls the specific orientation of the heme-bound substrate suitable for the elimination of the OH group of aldoxime and that these residues and the heme create a prefixed site for substrate recognition and binding. An induced-fit type conformational change was not observed upon substrate binding at the distal heme pocket, the substrate-binding site in OxRE.

His-320 also serves as a catalytic residue for the elimination of the substrate OH group to form H_2O, consistent with previous mutagenesis studies (7–10). The H320A mutant lacked enzymatic activity (Table 2). A hydrogen-bond network existed among Glu-143, Arg-

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**TABLE 2**

**Enzymatic activities of OxRE and its mutants**

| Specific activity | Relative activity |
|------------------|------------------|
| turnovers/heme/min | % |
| Wild type | 2840 ± 180 | 100 |
| E143Q | 410 ± 6 | 14 |
| R178Q | 1030 ± 44 | 36 |
| S219A | 670 ± 8 | 23 |
| F306A | 940 ± 27 | 33 |
| H320A | 310 ± 16 | 11 |

*The activity measurements were carried out at least three times for each sample.*

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**FIGURE 7.** The reaction scheme of aldoxime dehydration by OxRE.
Crystal Structure of OxdRE

178, and His-320 that fixed the proper orientation of His-320 toward the heme-bound substrate. Based on analyses of the resonance Raman spectra of CO-bound aldoxime dehydratase from Pseudomonas chlororaphis B23 (OxdA), Konishi et al. (9) have proposed that the catalytic His, which corresponds to His-320 in OxdRE, is in imidazolium form in the resting state, in which both of the nitrogen atoms of the imidazole ring are protonated. The hydrogen bond between Glu-143 and His-320 will play an important role not only for control of the proper orientation of His-320 but also for stabilization of the imidazolium form of His-320. Taken together, we propose the reaction scheme as shown in Fig. 7.

Previous studies using a cavity mutant of OxdRE revealed that electron donation from the proximal ligand to the heme iron accelerates the dehydration reaction (27). The hydrogen bond between His-299 and the oxygen atom of the main chain will strengthen the imidazolate character and electron donation ability of the proximal His, partially regulating the enzymatic activity of OxdRE. These results suggest another functional role for the heme in which the substrate coordination polarizes the N=C bond, assisting in product formation.

Mutagenesis studies confirmed the functional role of Glu-143, Arg-178, Ser-219, and His-320 deduced from the structural analyses. Mutation of Glu-143, Arg-178, Ser-219, His-320, or Phe-306 resulted in a 60–90% loss in enzymatic activity (Table 2). E143Q OxdRE displayed similar activity to that of H320A OxdRE, which lacked the catalytic residue. This result indicates that the proper orientation of His-320, which is mainly achieved by a hydrogen bond with Glu-143, is crucial for the enzymatic activity of OxdRE. The effect of the mutation of Phe-306 suggests that disconnection of the substrate-binding cavity from the substrate access channel and bulk solution, which is induced by substrate binding to the heme, controls the hydrophobicity/hydrophilicity of the distal heme pocket for the reaction to proceed efficiently.

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REFERENCES
1. Kato, Y., Ooi, R., and Asano, Y. (1998) Arch. Microbiol. 170, 85–90
2. Kato, Y., Ooi, R., and Asano, Y. (2000) Appl. Environ. Microbiol. 66, 2290–2296
3. Xie, S. X., Kato, Y., Komeda, H., Yoshida, S., and Asano, Y. (2003) Biochemistry 42, 12056–12066
4. Oinuma, K., Hashimoto, Y., Konishi, K., Goda, M., Moguchi, T., and Higashibata, H., and Kobayashi, M. (2003) J. Biol. Chem. 278, 29600–29608
5. Asano, Y. (2002) J. Biotechnol. 94, 65–72
6. Banerjee, A., Sharma, R., and Banerjee, U. C. (2002) Appl. Microbiol. Biotechnol. 60, 33–44
7. Kobayashi, K., Yoshioka, S., Kato, Y., Asano, Y., and Aono, S. (2005) J. Biol. Chem. 280, 5486–5490
8. Konishi, K., Ishida, K., Oinuma, K., Ohta, T., Hashimoto, Y., Higashibata, H., Kitagawa, T., and Kobayashi, M. (2004) J. Biol. Chem. 279, 47619–47625
9. Konishi, K., Ohta, T., Oinuma, K., Hashimoto, Y., Kitagawa, T., and Koba-
yashit, M. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 564–568
10. Oinuma, K., Kunita, H., Ohta, T., Konishi, K., Hashimoto, Y., Higashibata, H., Kitagawa, T., Shiro, Y., and Kobayashi, M. (2005) FEBS Lett. 579, 1394–1398
11. Sakai, K., Matsui, Y., Koyuma, T., Shiro, Y., and Adachi, S. (2002) J. Appl.
Crystallogr. 35, 270–273
12. Otwinowski, Z., Borek, D., Majewski, W., and Minor, W. (2003) Acta
Crystallogr. A 59, 228–234
13. Kabsch, W. (1993) J. Appl. Crystallogr. 26, 795–800
14. Sheldrick, G. M. (2008) Acta Crystallogr. A 64, 112–122
15. Pape, T., and Schneider, T. R. (2004) J. Appl. Crystallogr. 37, 843–844
16. Hendrickson, W. A. (1991) Science 254, 51–58
17. Morris, R. J., Zwart, P. H., Cohen, S., Fernandez, F. J., Kakaris, M., Kirillova, O., Vonrhein, C., Perrakis, A., and Lamzin, V. S. (2004) J. Synchrotron
Radiat. 11, 56–59
18. Emsley, P., and Cowtan, K. (2004) Acta Crystallogr. D. Biol. Crystallogr. 60, 2126–2132
19. Winn, M. D., Murshudov, G. N., and Papiz, M. Z. (2003) Methods Enzymol. 374, 300–321
20. Vriend, G. (1990) J. Mol. Graph. 8, 52–56
21. Lebedev, A. A., Vagin, A. A., and Murshudov, G. N. (2008) Acta Crystallogr. D. Biol. Crystallogr. 64, 33–39
22. Kato, Y., Yoshida, S., Xie, S. X., and Asano, Y. (2004) J. Biosci. Bioeng. 97, 250–259
23. Holm, L., and Sander, C. (1997) Nucleic Acids Res. 25, 231–234
24. Kawabata, T. (2003) Nucleic Acids Res. 31, 3367–3369
25. Zhang, C., and Kim, S. H. (2000) J. Mol. Biol. 299, 1075–1089
26. Ebihara, A., Okamoto, A., Kousumi, Y., Yamamoto, H., Masui, R., Ueyama, N., Yokoyama, S., and Kuramitsu, S. (2005) J. Struct. Funct. Genomics 6, 21–32
27. Kobayashi, K., Kubo, M., Yoshioka, S., Kitagawa, T., Kato, Y., Asano, Y., and Aono, S. (2006) ChemBioChem. 7, 2004–2009
28. DeLano, W. L. (2005) Drug Discovery Today 10, 213–217
29. Kleywegt, G. J., and Jones, T. A. (1994) Acta Crystallogr D Biol Crystallogr. 50, 178–185