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Crystallization and preliminary crystallographic analysis of rat monoamine oxidase A complexed with clorgyline

Jichun Ma,[a] Fumie Kubota,[a] Masato Yoshimura,[a] Eiki Yamashita,[a] Atsushi Nakagawa,[a] Akio Ito[b] and Tomitake Tsukihara[a]*

[a]Institute for Protein Research, Osaka University, Japan, and [b]Department of Chemistry, Faculty of Science, Kyushu University, Japan

Correspondence e-mail: tsuki@protein.osaka-u.ac.jp

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Monoamine oxidase (MAO) is an FAD-containing mitochondrial outer-membrane protein which catalyzes the degradation of several neurotransmitters in the central nervous system. The two subtypes of MAO, MAOA and MAOB, have similar primary sequences but different substrate and inhibitor specificities. The structure of human MAOB has recently been determined, but the structure of MAOA remains unknown. To clarify the mechanisms underlying their unique substrate and inhibitor recognition and thereby facilitate the development of new specific inhibitors to treat MAO-related neurological disorders, rat MAOA was crystallized in a complex with the specific inhibitor clorgyline. Diffraction data were collected to 3.2 Å resolution. The crystal belongs to the space group $P4_32_12$, with unit-cell parameters $a = b = 158.2$, $c = 258.4$ Å.

1. Introduction

Monoamine oxidase (MAO; EC 1.4.3.4) is a mitochondrial outer-membrane-bound flavoenzyme. It catalyzes the oxidative deamination of several important neurotransmitters, including serotonin (5-HT), norepinephrine (NE) and dopamine (DA). Therefore, MAO plays an important role in the regulation of normal central nervous system activity. Because MAO inhibitors can elevate the levels of neurotransmitters in the central nervous system, they are effective in the treatment of several psychiatric and neurological illnesses, including depression and Parkinson's disease.

The two subtypes of MAO, MAOA and MAOB, are 70% identical at the amino-acid level according to the deduced primary sequences for various species (Bach *et al.*, 1988; Ito *et al.*, 1988; Kuwahara *et al.*, 1990; Kwan & Abell, 1992). However, each enzyme has unique substrate and inhibitor specificities. MAOA is responsible for the degradation of 5-HT and is specifically inhibited by clorgyline (Johnston, 1968), while MAOB primarily degrades phenylethylamine (PEA) and benzylamine and is selectively inhibited by deprenyl (Knoll & Magyar, 1972). MAOA and MAOB knockout mice also exhibit distinct differences in neurotransmitter metabolism and behaviour (Shih *et al.*, 1999). MAOA knockout mice have elevated brain levels of serotonin, norepinephrine and dopamine and manifest aggressive behaviour, while MAOB knockout mice do not exhibit aggression but instead accumulate PEA in their tissues. As the two enzymes function differently, it is important to clarify in detail their mechanisms of catalysis and inhibition in order to develop new more effective specific inhibitors. Recently, Binda and coworkers reported the crystal structures of human MAOB with bound inhibitors (Binda *et al.*, 2002, 2003). However, there is still no direct structural evidence explaining the mechanisms of the different substrate and inhibitor recognition by MAOA and MAOB.

We previously expressed rat MAOA in yeast and purified the protein (Ma & Ito, 2002), providing the opportunity to crystallize this enzyme. MAOA from rat is one of three well characterized MAOAs (the others are human and bovine). It contains 526 amino-acid residues and has a molecular weight of approximately 59 kDa. The primary sequences of all three MAOAs are highly conserved, with approximately 90% identity in amino-acid residues. Rat MAOA and human MAOA are generally accepted to have the same tertiary structure. The comparison of the MAOA and MAOB structures should aid in understanding the unique substrate and inhibitor specificities of these two enzymes and facilitate the development of new specific inhibitors.

2. Methods and results

2.1. Protein expression, purification nd detergent exchange

His-tagged full-length rat MAOA was expressed in *Saccharomyces cerevisiae* BJ2168 using the yeast expression vector YEp51 as described previously (Ma & Ito, 2002) with some modifications. Briefly, transformed BJ2168 cells were cultured in 5 ml of leucine drop-out medium containing 2%(*w*/*v*) glucose as the carbon source at 303 K with vigorous shaking overnight. The culture was then transferred to 1 l of the same medium and

cultured in the same conditions until the OD at 600 nm reached approximately 6. This culture was then transferred to a fermenter containing 10 l of the same medium and cultured at 303 K while agitating at 300 rev min^{-1} with maximum aeration. After the OD at 600 nm reached approximately 7, the cells were collected and suspended in 10 l of new medium containing no glucose but 2%(*w*/*v*) galactose instead in order to induce MAOA expression. This medium was changed another two times after 12 and 24 h of induction and cultured for a further 12 h. Yeast cells were then collected by centrifugation at 1500*g* for 5 min, treated with Zymolyase and disrupted by sonication. After removal of cellular debris by low-speed centrifugation at 1200*g* for 20 min, the crude membrane fraction was collected by ultracentrifugation at 100 000*g* for 20 min. This membrane fraction was solubilized with 1%(*v*/*v*) Triton X-100 containing 10 m*M* imidazole pH 7.5, 10%(*v*/*v*) glycerol, 500 m*M* NaCl and 5 m*M* β-mercaptoethanol on ice for 1 h and then ultracentrifuged at 100 000*g* for 20 min. The resultant supernatant was applied to a nickel-chelating column and washed with the above buffer containing 0.1%(*v*/*v*) Triton X-100 and 80 m*M* imidazole. The column was then washed with the same buffer but with Triton X-100 replaced by 0.05%(*w*/*v*) dimethyldecylphosphine oxide (Anatrace Inc.) and 0.01% *N*-dodecylphosphocholine (FOS-choline 12; Anatrace Inc.). Finally, MAOA was eluted with the same buffer containing 500 m*M* imidazole.

2.2. Crystallization

The purified protein, with a concentration of 15 mg ml^{-1}, was dialyzed against buffer containing 20 m*M* sodium phosphate pH 7.5, 200 m*M* NaCl, 10%(*v*/*v*) glycerol, 0.05%(*w*/*v*) dimethyldecylphosphine (Anatrace Inc.) and 0.01%(*w*/*v*) FOS choline-12 (Anatrace Inc.). Clorgyline was added to the protein solution at a molecular ratio of protein to clorgyline of 1:5 prior to crystallization. The best crystal was obtained using the hanging-drop vapour-diffusion method at 277 K with a reservoir containing 12%(*w*/*v*) polyethylene glycol 2000 monomethylether, 100 m*M* sodium acetate, 100 m*M* sodium phosphate buffer pH 6.2 and 26%(*v*/*v*) glycerol. The drop was made by mixing 2 µl protein sample and 2 µl reservoir solution. The crystals grew to dimensions of 0.2 × 0.2 × 1.0 mm within three weeks.

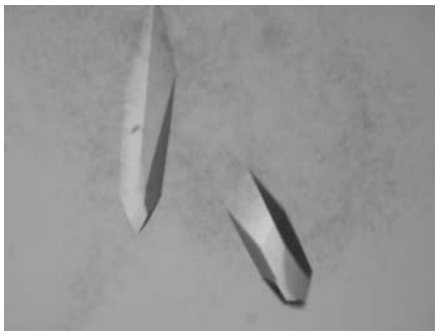

Figure 1
Crystals of rat MAOA complexed with clorgyline. The dimensions of the crystals are approximately 0.2 × 0.2 × 1.0 mm.

2.3. Data collection and processing

Diffraction data were collected at 100 K using beamline BL44XU at the SPring-8 synchrotron-radiation facility, Japan. Imaging plates (DIP6040) were used as detectors. Diffraction-image data were indexed and integrated with *MOSFLM* (Leslie, 1999) and scaled using *SCALA* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

To crystallize rat MAOA, we first searched for detergents that could best solubilize and stabilize the protein. Results showed that MAOA could be solubilized by several detergents without losing its activity. These detergents were used to replace Triton X-100 in the final step of purification. Protein in 20 m*M* sodium phosphate buffer pH 7.5, 200 m*M* NaCl, 5 m*M* β-mercaptoethanol and detergent at a concentration twice its CMC were used for crystallization screening with the MembFac Kit (Hampton Research) by the hanging-drop vapour-diffusion method. Among dozens of samples, those solubilized in *N*-dodecyl-*N*,*N*-dimethylamine-*N*-oxide (LDAO; Anatrace Inc.) or dimethyldecylphosphine oxide (Anatrace Inc.) formed crystals in several conditions containing PEG 4000 or PEG 6000 as precipitants at 277 K. LDAO was used as a detergent for crystallizing MAOB (Binda *et al.*, 2002), but we were unable to obtain high-quality MAOA crystals with this detergent. These crystals diffracted to only approximately 6 Å without further improvement, while dimethyldecylphosphine oxide performed better after optimization. Subsequently, FOS choline-12 was found to help stabilize MAOA and was included in the protein sample. The best crystals (Fig. 1) were obtained using the conditions described in §2. We also tried to crystallize MAOA without the inhibitor and indeed obtained crystals under the same conditions.

Table 1
Summary of data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

Beamline	BL44XU
Wavelength (Å)	0.9
Space group	$P4_32_12$
Unit-cell parameters (Å)	a = 158.2, b = 158.2, c = 259.2
Resolution range (Å)	16.20–3.20 (3.37–3.20)
Completeness (%)	99.2 (99.3)
No. of unique reflections	54007 (7825)
Redundancy	8.3 (8.6)
$R_{\rm merge}$	0.119 (0.614)
$I/\sigma(I)$	3.2 (1.3)

However, these crystals only diffracted to a low resolution (>4 Å) and further improvement of their quality was not successful. This suggests that the binding of the inhibitor causes a more compact MAOA structure, leading to a better crystal packing.

These results suggest also that the detergent played a critical role in the crystallization of MAOA, as it does in the crystallization of many other membrane proteins. It essentially decides whether a membrane protein forms a crystal and influences its quality. We noted that further purification of MAOA using other chromatography methods seemed to adversely affect MAOA crystallization. Over-purification results in either no crystals or poor-quality crystals, suggesting that the properties of the protein have changed during these procedures. These findings should be considered in the crystallization of other membrane proteins.

Primary analysis of the X-ray diffraction data (Table 1) show that the MAOA crystal belongs to the space group $P4_12_12$ or $P4_32_12$, according to diffraction patterns and systematic extinctions. The unit-cell parameters are $a = b = 158.2$, $c = 258.4$ Å. Since the molecular weight of rat MAOA with a His tag is about 60 kDa, it is assumed that there are four molecules in one asymmetric unit, giving a reasonable Matthews coefficient $V_{\rm M}$ = 3.37 Å^3 Da^{-1} and a solvent content of 63.48%. Using the molecular-replacement method, we located four molecules in the $P4_32_12$ cell. The structure details will be available soon.

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