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Crystallographic analysis of cytochrome P450 MoxA from *Nonomuraea recticatena* (CYP105)

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Introduction

Cytochrome P450 monooxygenases (P450s) are a hemoprotein catalyzing a wide variety of oxidative reactions, i.e., biosynthesis of steroid hormones, lipids, and complex antibiotics such as polyketides, detoxification of xenobiotics, drug metabolism, and bioconversion of recalcitrant molecules to utilize them as carbon sources. The catalytic abilities of P450s are highly attractive in that they involve a high regio- and stereo-selectivity, thus introducing the possibility of numerous biotechnological applications.

P450moxA from the rare actinomycete *Nonomuraea recticatena* IFO 14525 was first identified as an enzyme that exhibited compactin hydroxylase activity. Sequence similarity search indicated that P450moxA belongs to the CYP105 family [1]. Interestingly, tests for acceptable substrates have revealed that P450moxA also acts on a wide range of hydrophobic compounds such as testosterone and oleanolic acid. The remarkably broad substrate specificity of P450moxA is reminiscent of the mammalian liver microsomal P450s, which also exhibit broad substrate specificity for metabolizing a vast array of xenobiotics (drugs). Here we report the X-ray structure of P450moxA in substrate-free form at 2.8 Å resolution.

Methods

The recombinant P450moxA was overexpressed by *Escherichia coli*, and purified by Ni affinity chromatography. The crystals of P450moxA were obtained by the hanging-drop vapor-diffusion method, using reservoir solution containing 0.1 M MES, pH 6.5, 8% ethyleneglycol, 3.5-6% PEG 8000, and 15-20% glycerol. The crystals belong to the space group $P2_12_12_1$ with unit-cell dimensions of a = 82 Å, b = 83 Å and c = 176 Å. The structure of P450moxA was determined by the molecular replacement using CYP107A1 as a search model (PDB code, 1JIN). The model refinement was performed using the program CNS. Atomic coordinates and structure factors have been deposited in Protein Data Bank under accession number 2Z36. The detail experimental procedures were described elsewhere [2].

Results and Discussion

The crystal structure of P450moxA was determined at a resolution of 2.8 Å with an R and R-free factor of 23.0% and 27.4%, respectively. The asymmetric unit contains two molecules, and no conformational differences were observed between them. P450moxA is structurally the most homologous to fungal nitric oxide reductase (CYP55A1), while the residues creating substrate-binding pocket of these two P450s are not conserved, suggesting the considerable functional divergence. The current structure analysis also reveals that the MES molecule occupies the substrate-binding site. It is interesting to note that the morpholine ring O1 and C4 of the MES are located at a distance of approximately 4.5 Å from the heme iron. The unexpected binding of the MES molecule might reflect the ability of P450moxA with regard to capturing a broad range of structurally diverse compounds. The structure of P450moxA may serve as a model for further structural study and molecular dynamics simulation analysis of CYP105 and related P450s.

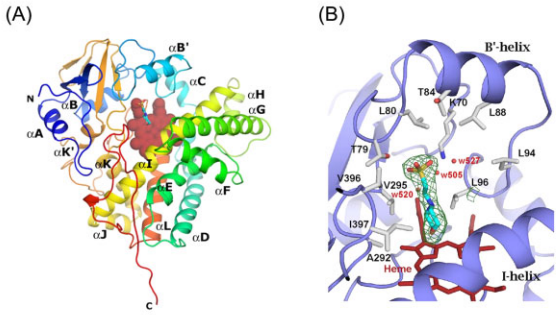

Fig.1 P450moxA structure. (A) Ribbon representation of the overall structure and (B) substrate binding pocket accommodating MES molecule.

References

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