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Investigation of Photo-Activation Process of Nitlile Hydratase Crystallized into a New Crystal Form

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Nitrile Hydratase (NHase) is an enzyme of *Rhodococcus* sp. N-771, which hydrates nitriles to their corresponding amides. NHase consists of α and β subunits each with molecular weight of 23kDa. The reaction center is a non-heme iron surrounded by a motif of α subunit, Cys109-Ser-Lue-Cys112-Ser-Cys114. Cys112α and Cys114α are posttranslationally modified to Cystein sulfinic acid (Cys112-SO_2H) and Cystein sulfenic acid (Cys114-SOH), respectively, and are considered to be relating directly with the enzymatic reaction of NHase. The iron center combines with an endogenous nitric oxide (NO) at the sixth coordination site in the inactive state (*Nature Struc. Biol.* **5**, 347-351 (1998)). Upon visible light irrumination, the inactive enzyme changes to the active form accompanied with dissociation of the NO molecule. The active NHase binds a water molecule (or OH^- anion) at the sixth coordination site, and possesses a relatively wide reaction cavity around the water molecule. In order to investigate the photo-activation process and enzymatic reaction of NHase including the roles of the two cystein residues posttranslationally modified, we tried to collect diffraction data sets with large angle oscillation (LOT) technique using the automated X-ray detector of large-format imaging plate (IP), which was installed in the BL41XU experimental hutch. The sample crystal used in this study was a new form which crystallized in monoclinic system and diffracted up to atomic level resolution, over 1.0 A. This crystal form was very useful for our purpose because of its high quality and ability of photodissosiation of NO in crystal. Two data sets collected by the LOT technique and the IP detector are listed in table and structure determination is in progress.

	inactive form at 100K	1 hr incubation at 160 K after activation
resolution	20-1.8 (1.86-1.8)	20-2.0 (2.06-2.0)
obs. reflection	87193	30984
redundancy	2.4	1.5
completeness	91.9(96.2)	93.6(96.5)
I/sigma	14.6(7.6)	13.1(6.3)
R-merge (%)	8.9(14.0)	11.1(21.6)

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Crystal Structure Analysis of Glycerol-3-Phosphate Acyltransferase (GPAT)

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The ratio of saturated to unsaturated fatty acid incorporation varies tremendously in plants, and is an important determinant of a plant's ability to tolerate chilling temperatures. The stromal glycerol-3-phosphate acyltransferase (GPAT) is responsible for the selective incorporation of saturated and unsaturated fatty acyl chains into chloroplast membranes. In order to clarify the recognition mechanism for the fatty acids, tertiary structure of the stromal GPAT from squash (*Cucurbita moscata*) was determined by X-ray crystallography.

The squash GPAT expressed in *Escherichia coli* was crystallized by the vapor diffusion method in 20mM Tris-HCl (pH 8.0) containing 1.7 M ammonium sulfate. The crystal belongs to a space group $P2_12_12_1$ (a = 60.1 Å, b = 63.7 Å, and c = 116.2 Å) and diffracted beyond 2.0Å resolution. The crystal structure was determined by the multi-wavelength anomalous dispersion (MAD) method using selenium labeled protein. The present refined atomic model has an R-factor of 0.234 (R_{free} = 0.275) between 6.0 and 1.9 Å resolution. The polypeptide chain of squash GPAT was folded into 14 α helices, five short 3_{10} helices, and nine β strands. The structure was composed of two domains: the helical domain (residues 1-80) and the α/β domain (residues 81-368) (Figure 1). The core of the α/β domain was an open twisted α/β structure with five parallel β strands that is unique in comparison with other known structures of acyltransferase. Additionally, it was found that GPAT belongs to new protein folding family, judging from the multiple alignment of 3D structural neighbors search. Putative binding sites of substrates (CoA and glycerol-3-phosphate(G3P)) exist in a pocket in the α/β domain. On the other hands, substrate (acyl-chain) selective site may be situated in reverseed side of CoA and G3P binding sites. Further crystallographic refinement and crystallization of GPAT/ substrates complex are now in progress.

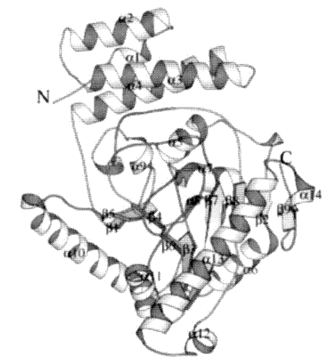

Figure 1 Overall Structure of squash GPAT.