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Crystal Structure Analysis of Enzymes Involved in Biosynthesis of Marihuana Compounds

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Introduction

Cannabinoids, which are produced predominantly in *Cannabis sativa*, are unique secondary metabolites consisting of alkylresorcinol and monoterpene groups. More than 60 cannabinoids have been isolated from marijuana or fresh *Cannabis* leaves, and their biosynthetic pathways have been extensively investigated.

We demonstrated that the major cannabinoids (tetrahydrocannabinolic acid and cannabidiolic acid) are biosynthesized by novel FAD-dependent oxidases named tetrahydrocannabinolic acid synthase (THCA synthase) [1] and cannabidiolic acid synthase (CBDA synthase) [2], respectively, from the common precursor cannabigerolic acid which is produced from olivetolic-acid (OLA) and geranylpyrophosphate. We have recently cloned the gene coding for a plant type III polyketide synthase, named polyketide synthase-1 (PKS-1), which catalyzes OLA production. Here we report the X-ray diffraction studies of THCA synthase and PKS-1.

THCA synthase

Single crystals of THCA synthase suitable for X-ray diffraction measurement with the dimension of 0.1×0.1×0.1 mm were obtained in 0.09 M HEPES buffer (pH 7.5) containing 1.26 M sodium citrate [3]. This crystal belonged to the primitive cubic space group *P*432, with unit-cell parameters $a=b=c=178.2$ Å. The calculated Matthews coefficient was approximately 4.1 $Å^3$ Da^{-1} assuming the presence of one molecule of THCA synthase in the asymmetric unit. Diffraction data was collected at NW12A in PF-AR, and was completed up to 2.6 Å resolution with completeness of 97.5% and R_{merge} of 0.99. The structure was solved by molecular replacement using the coordinates of glucooligosaccharide oxidase (PDB ID: 2AXR). The final model of the THCA synthase (residues 28-545) containing a FAD molecule, five N-linked sugar chains, and 43 waters was refined to a R_{cryst}/R_{free} of 0.207/0.259 at 2.6 Å resolution.

Overall structure of THCA synthase is shown in Figure 1. The polypeptide chain of THCA synthase is folded into 14 α-helices, six short 3_{10}-helices, and 16 β-strands. THCA synthase consists of two domains. The FAD binding domain (residues 28-253 and 476-545) folds into two α + β subdomains. The FAD molecule is positioned between two subdomains (colored by blue and magenta in Figure 1). The isoalloxazine ring of FAD is cross-linked to the enzyme at two sites (His114 and Cys176). The other domain is colored by green in Figure 1. This domain (residues 254-475) is composed of an anti-parallel β sheet and several helices. Crystallization of substrate complex is now in progress.

PKS-1

The diffraction quality crystals of PKS-1 were obtained under the condition, 100 m*M* HEPES buffer (pH 7.5) containing 200 m*M* calcium acetate and 20% (*w*/*v*) PEG 3350 [4]. The typical crystal size of each condition was approximately 0.02×0.02×0.3 mm. This Crystal belonged to space group *P*1 with the unit-cell parameters of a = 54.3, b =59.3, c = 62.6 Å, α=69°, β=81°, γ=80°. The calculated Matthews coefficient was approximately 2.2 $Å^3$ Da^{-1} assuming the presence of two molecules of PKS-1 in the asymmetric unit. Diffraction data was collected at BL-5A in PF, and was completed up to 1.65 Å resolution with completeness of 90.4% and R_{merge} of 0.52. The structure was solved by molecular replacement using the coordinates of chalcone synthase (PDB ID: 1BI5). Crystallographic refinement is now in progress.

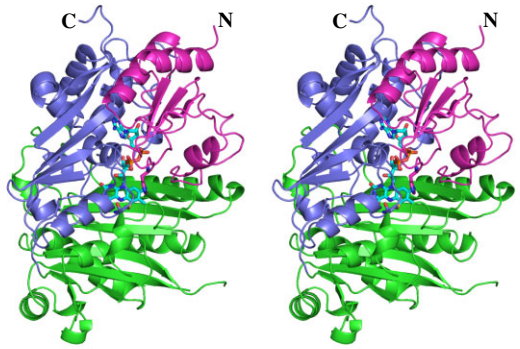

Figure 1. Overall structure of THCA synthase. FAD molecule is indicated as stick model.

References

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