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STRUCTURE OF MACROMOLECULAR COMPOUNDS

Crystallization and Preliminary X-ray Diffraction Study of Phosphopantetheine Adenylyltransferase from *M. Tuberculosis* Crystallizing in Space Group $P3_2$

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Abstract—Crystals of *M. tuberculosis* phosphopantetheine adenylyltransferase were grown in microgravity by the capillary counter-diffusion method through a gel layer. The X-ray diffraction data set suitable for the determination of the three-dimensional structure at atomic resolution was collected from one crystal at the Spring-8 synchrotron facility to 2.00-Å resolution. The crystals belong to sp. gr. $P3_2$ and have the following unit-cell parameters: $a = b = 106.47$ Å, $c = 71.32$ Å, $\alpha = \gamma = 90°$, $\beta = 120°$. The structure was solved by the molecular-replacement method. There are six subunits of the enzyme comprising a hexamer per asymmetric unit. The hexamer is a biologically active form of phosphopantetheine adenylyltransferase from *M. tuberculosis*.

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INTRODUCTION

Bacterial phosphopantetheine adenylyltransferases (PPAT) are involved in the five-step coenzyme A (CoA) biosynthesis from pantothenate (vitamin B5), cysteine, and adenosine triphosphate (ATP) [1]. These enzymes catalyze the fourth, penultimate step of this process—the reversible transfer of an adenylyl group from ATP to 4'-phosphopantetheine accompanied by the release of pyrophosphate and the formation of dephosphocoenzyme A (dPCoA) [2]. It is assumed that PPAT catalyzes the reaction by orienting substrates in the proper way and stabilizing the transition state and that the functional groups of the enzyme are not directly involved in the acid-base or covalent catalysis [3].

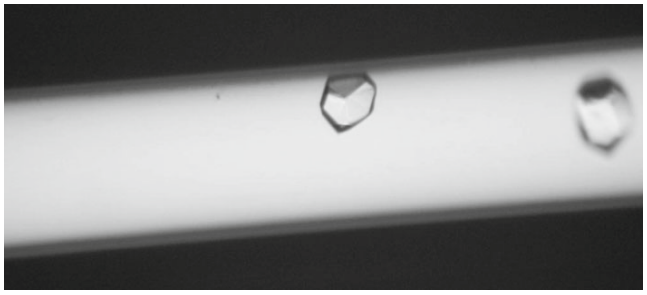
The phosphorylation of dPCoA in the final step of the process produces CoA. The CoA biosynthesis is an energy-consuming reaction, the rate of which is regulated by the feedback mechanism in the key steps. The PPAT-catalyzed reaction is a key step of the biosynthesis since, in the presence of CoA in an excess concentration, the latter forms a complex with PPAT, thus inhibiting the further reaction. Coenzyme A is involved in numerous metabolic reactions in the cell and is essential for the survival of the pathogenic mycobacterium, which is the causative agent of tuberculosis. Therefore, since PPAT regulates the rate of the CoA formation, it can serve as a target protein for the search for inhibitors of this enzyme, which are potent antituberculosis drugs. One substantial advantage of PPAT*Mt* as the target protein is the absence of a similarity between bacterial PPAT and the corresponding enzymes involved in the CoA biosynthesis in mammalian bodies.

The three-dimensional structures of PPAT in the apo form and in complexes with some functional ligands (ATP, CoA, dPCoA, PhP) were determined [4–8]. The crystal forms of the enzyme studied earlier by X-ray crystallography contain one, two, or four subunits per asymmetric unit, whereas the biologically active form of PPAT*Mt* is a hexamer.

In the present work, we obtained a new crystal form of PPAT containing one enzyme molecule per asymmetric unit. The crystals were grown in microgravity on the International Space Station by the counter-diffusion method. The X-ray data set was collected from these crystals to 2-Å resolution. The three-dimensional structure was solved by the molecular-replacement method. Structure refinement is currently underway.

MATERIALS AND METHODS

The enzyme PPAT from *M. tuberculosis* (the molecular weight of the enzyme subunit is 17 028 Da), which was used for the design and conduction of the



Crystal grown in microgravity and used for X-ray data collection at the synchrotron radiation source. The crystal size is 0.2–0.3 mm.

protein-crystal-growth experiment in microgravity, was produced as described in [7].

Crystal Growth

Crystals were grown by the capillary counter-diffusion method through a gel layer according to a procedure described in [9, 10]. A protein solution contained 10 mg/mL of PPAT*Mt*, 10 mM HEPES, pH 8.0, 0.15 M NaCl, and 14 mM ATP. The reservoir solution was composed of 0.1 M NaAc, pH 5.0, 10 mM $MgCl_2$, 5 mM HEPES, pH 8.0, 0.075 M NaCl, 14 mM ATP, and 1.1 M ammonium sulfate. The crystals were grown up to 0.3 mm (figure).

The crystals were frozen before exposure to X-rays using a cryoprotectant solution, which contained the same components as the reservoir solution and 15% of glycerol.

X-ray Diffraction Data Collection and Processing

The X-ray diffraction data set was collected from the grown crystals on the BL41XU beamline at the Spring-8 synchrotron facility at 100 K using the MAR225HE CCD detector. The X-ray data were obtained from one crystal by the rotation method at a wavelength of 0.8 Å. The crystal-to-detector distance was 110 mm, the oscillation angle was 0.5°, and the rotation angle was 180°. The X-ray diffraction intensities were processed using the HKL-2000 program suite [11]. The crystals belong to sp. gr. $P3_2$ and have the following unit-cell parameters: $a = b = 106.47$ Å, $c = 71.32$ Å, $\alpha = \gamma = 90°$, $\beta = 120°$. The X-ray data set was processed to 2.0-Å resolution. The X-ray-data-collection statistics are given in the table.

Structure Solution

The X-ray diffraction data were employed to solve the three-dimensional structure of the enzyme by the molecular-replacement method using the PHASER program [12]. The atomic coordinates of the unliganded enzyme (PDB_ID: 4E1A) were used as the starting model. The structure-solution statistics are given in the table.

Crystallographic data and X-ray-data-collection statistics for the crystal of PPAT*Mt*

Sp. gr.	$P3_2$
$a = b$, c, Å; $\alpha = \gamma$, β, deg	106.47, 71.32; 90, 120
T, K	100
λ, Å	0.8
Resolution, Å	20.00–2.00 (2.05–2.00)*
Number of unique reflections	54681 (3697)
Redundancy	2.53 (2.16)
Completeness, %	94.3 (87.9)
$I/\sigma(I)$	8.4 (2.6)
Rmrgd-F (%)	8.5 (54)

* The data for the last resolution shell are given in parentheses.

RESULTS AND DISCUSSION

The crystals suitable for a determination of the three-dimensional structure of PPAT from *M. tuberculosis* were grown in microgravity on the International Space Station by the counter-diffusion method through a layer of agarose gel using ammonium sulfate as the precipitant. The crystals were grown employing the Japan Aerospace Exploration Agency (JAXA) facilities [10]. Crystallization units were packed in special boxes and delivered to the International Space Station, where the crystal growth occurred in a thermostat at 20°C.

The X-ray diffraction data set collected from the grown crystals at the Spring-8 synchrotron facility is suitable for the determination of the structure of the enzyme at 2.0-Å resolution.

The solvent content of the unit cell estimated by Matthews's method [13] is 46.16% of the unit-cell volume. The Matthews coefficient is 2.28 $Å^3$/Da. There are six subunits of the enzyme per asymmetric unit. The subunits form a hexamer, which is the biologically active form of *M. tuberculosis* PPAT. This crystal form of PPAT was obtained for the first time. The structure of the enzyme is currently refined at 2-Å resolution.

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