Structure of the ADP-Glucose Pyrophosphorylase

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Introduction

ADP-glucose pyrophosphorylase (ADPGlc PPase, EC 2.7.7.27) catalyzes the synthesis of ADP-glucose from ATP and glucose-1-phosphate, releasing pyrophosphate as a product.[1] ADPGlc Ppase plays the key regulatory role in the biosynthesis of starch in plants[2] and of glycogen in bacteria.[3] Despite being extensively studied for decades, there is not a three-dimensional structure available for ADPGlc PPase from any organism. Earlier attempts to determine the structure of ADPGlc PPase from *E. coli* were unsuccessful due to extremely rapid decay and the fact that crystals diffracted to low resolution.[4] Data on two forms of seleno-methionine-substituted *E. coli* ADPGlc PPase crystals were collected at the SBC 19-ID beamline.

Methods and Materials

Enzymes were expressed and purified by the Dr. Jack Preiss lab, Michigan State University.

Results

Crystal form #1 (space group P21212) was derived from the conditions reported previously,[4] and the best diffracting crystals were grown in 18% PEG8000, 0.2 M $(NH_4)_2SO_4$, 0.1 M Na-HEPES, pH 7.1. Crystals were frozen in cryo-protectant including 20% glycerol in the well solution. Crystals diffracted to 3.3 Å.

Crystal form #2 (space group P622?) was derived from conditions reported,[5] and the best diffractng crystals were grown in 1.0 M $(NH_4)_2SO_4$, 0.2 M NaAc, 0.1 M Na-HEPES, pH 7.5. Crystals were frozen in cryo-protectant including 30% glycerol in the well solution. Crystals diffracted only to 3.3 Å.

Crystal form #3 (Space group P21212). These crystals diffract to about 3.0 Å resolution. Complete four-wavelength MAD data was collected on this form. The complexity of the unit cell (18-20 monomers/A.S.) has impeded efforts to determine the structure.

Discussion

It can be seen from the results that obtaining a three-dimensional structure of ADPGlc PPase is a challenging task. Despite a large effort in crystal growth, data collection and processing, we have not yet been able to produce an electron density map due to lack of good-quality data. The structure could be solved based on either crystal form #1 ($P2_12_12_1$) or crystal form #2 (P622?) by obtaining additional source of phasing information from multiple isomorphous replacement. The weak diffraction of these crystals requires the powerful beamline at SBC.

References

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