Structural Studies of Alginate Pathway Enzymes from *Psuedomonas aeruginosa*

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Introduction

Cystic fibrosis (CF) patients are prone to secondary infections in the lungs. The prevalent causative organism is *Psuedomonas aeruginosa*, also a leading agent in hospital-acquired infections. In the CF lung, this organism transforms into a mucoidy state that overproduces the natural polysaccharide coating, alginate. In this state, *P. aeruginosa* is resistant to antibiotics and the innate immune response of the lung. Various proteins are involved in the synthesis of alginate [1], and the structure and mechanisms of these proteins would greatly enhance our understanding of the alginate pathway.

To date, we have crystallized two proteins from the pathway and are presently working on two more. The first enzyme worked on was phosphomannomutase (*algC*), and its structure has now been solved [2]. This report primarily concerns the pivotal enzyme in the pathway, GDP-mannose dehydrogenase (GMD).

Methods and Materials

In this study, we have obtained large single crystals of GMD by hanging-drop vapor diffusion crystallization of native recombinant protein. The seleno-methionine (SeMet) substituted protein was obtained by methionine pathway suppression and supplemental addition of SeMet during production of the enzyme [3]. The presence of selenium in the protein was confirmed by matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS). The multiwavelength anomalous diffraction (MAD) phasing experiments were carried out by using seleno-methionine substituted protein. All protein samples used were cryoprotected in mother liquor containing 50% v/v saturated sucrose.

Halide soaks were used to help phase the structure [4]. This was achieved by spiking the cryoprotectant with 20 mM of sodium bromide. Heavy atoms other than selenium and bromide were soaked into the crystals before cryoprotection. A second protein PutA was soaked in bromide as a further test of this technique. The presence of heavy atoms (Se, Hg, and Br) was confirmed by fluorescence scans on the crystals before data collection. All data were collected at 100-110K.

Results

We have now collected two progressively-higher-resolution native data sets (1.75 and 1.55 Å) and one complete three-wavelength MAD data set to 2.0 Å. We also have a 2.5-Å mercury soak data set. The crystals grow in a P4(3) space group with unit cell edges of 82 and 310 Å. The bromide soak for GMD did not give usable results. However, the PutA protein did produce results that enabled the solution of that structure [5].

The structure of GMD was recently solved by MAD phasing by using SOLVE/RESOLVE [6]. The initial model was built by using RESOLVE version 2.02 [7]. Subsequent refinement and rebuilding are in progress.

Discussion

The facilities at Structural Biology Center (SBC) beamline 19-ID have enabled us to solve the GMD structure for two reasons: the ability to (1) tune the wavelength and (2) swing the detector on 2-θ axis to obtain the high-resolution data. The high-resolution structure of GMD will enable us to help explain the idiosyncratic kinetic behavior of this protein [8]. A high-resolution structure will enable inhibitor design to be carried out with greater confidence than would a lower-resolution structure.

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