Structures of Enzymes in the Alginate Biosynthetic Pathway of the Pathogen *Pseudomonas aeruginosa*

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Introduction

Cystic Fibrosis (CF) patients are prone to secondary infections in the lungs. One of the infectious organisms is *Pseudomonas aeruginosa*. In the CF lung, this organism transforms into a mucoidy state that overproduces its natural alginate coating—a polysaccharide. 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] These proteins have not been structurally characterized, and the understanding of their mechanisms would be greatly enhanced by the structural knowledge obtained.

Methods and Materials

In this study we have obtained large single crystals of two native recombinant proteins involved in the alginate pathway for x-ray diffraction studies. These are the phosphomannomutase (PMM)[2] and GDP-mannose dehydrogenase (GMD) proteins. Selenomethionine-substituted protein crystals or heavy-atom-soaked crystals will be used for multiple-wavelength anomalous dispersion (MAD) phasing experiments. Protein complexes, with substrates and cofactors, have been obtained by crystal-soaking experiments. All data collection was carried out on beamline SBC 19-ID at 100-110K.

Results

We have collected six data sets of PMM soaked with various metal ions and or substrates to a maximum resolution of 1.9 Å and maximum completeness of 96%. The space group was $P2_12_12_1$ with unit cell dimensions a = 71.4 Å, b = 73.0 Å and c = 92.9 Å. The mosaicity of these data sets ranged form 0.45 to 1.0°. These are being evaluated by difference Fourier maps using the native structure, previously determined by MAD phasing to 1.7 Å resolution. For the native GMD protein we collected three data sets and one SAD data set of the SeMet substituted protein. The native GMD crystals diffracted to 1.75 Å in space group $P4_1$ or $P4_3$ with unit cell axes of a = b = 82.5 Å, c = 310.0 Å. The mosaicity is as low as 0.12° and a maximum completeness of 99.7%. The SeMet GMD diffracted to 3.1 Å and with a mosaicity of 1.0°. The space group was $P2_1$ with unit cell dimensions of a = c = 94.4 Å , b = 218.6 Å, $\alpha = \gamma = 90°$, $\beta = 91.9°$. All data sets were collected at a wavelength of 0.979 Å with R_{merge} ranging from 3.2% to 7.2% except for the SeMet GMD data set, which has an average an R_{merge} of 20%.

Discussion

We are progressing rapidly through the structural analysis of PMM and its complexes. Progress on the GMD project has been slowed by the discovery that the native crystal form is merohedrally twinned and that the twinning fraction varies with each individual crystal. The long unit cell axis has also slowed progress due to the need for a 2θ-arm on the detector to collect high-resolution data. Attempts to obtain untwinned crystals are in progress. Alternate forms of SeMet GMD crystals are actively being characterized for improved diffraction.

Acknowledgments

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References

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