MAD Data Collection on a Samarium Derivative of In1B, a Surface-Attached Protein of the Bacterial Pathogen *Listeria monocytogenes*

M. Marino, P. Ghosh
University of California, San Diego, CA, U.S.A.

Introduction

InlB is a 67 kD surface-attached protein of the bacterial pathogen *Listeria monocytogenes* that promotes bacterial invasion of diverse mammalian cell types. The protein triggers an intracellular signaling cascade involving activation of phosphoinositide 3-kinase. The structure of the leucine-rich repeat domain of InlB demonstrated the presence of two unsuspected calcium binding sites.[1] Based on the presence of these sites, we designed a multiwavelength anomalous dispersion (MAD) experiment utilizing samarium, a lanthanide that has been shown to substitute for calcium, to determine the x-ray crystal structure of intact InlB.

Methods and Materials

Crystals of intact InlB were derivatized with $SmCl_3$, soaked in an erythritol-containing cryobuffer, and flash-cooled at ~100 K. Several MAD data sets utilizing three wavelengths (peak, inflection, and remote) corresponding to the samarium L_{III} edge were collected at SBC 19-ID.

Results

Analysis of the MAD experiment using SOLVE indicated that both anomalous and dispersive difference signals yielded useful phasing information. Furthermore, the extent of resolution from native InlB crystals, which are anisotropic, was extended from the previous limit of ~3.2 Å to 2.8 Å resolution. Phasing information using the samarium derivative appears to extend to ~3.0 Å resolution, and, given the high solvent content of the crystals of 75%, should yield a structural determination with the aid of solvent flattening and phase extension. Initial electron density maps have been calculated and model building is in progress.

Acknowledgments

Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. We thank Rongguang Zhang and the staff of SBC for advice and help during data collection.

References

[1] M. Marino, L. Braun, P. Cossart, and P. Ghosh Molecular Cell **4**, 1063-1072 (1999).