Crystal Structures of Complexes of the *B. subtilis* Maf Protein with Ligand

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Introduction

Structural genomics efforts are beginning to produce 3-D structures for functionally uncharacterized proteins. The structures may furnish insight into the biolgical functions of these proteins. The potential benefits of 3-D structural information regarding such proteins are particularly obvious when the corresponding genes are conserved during evolution, implying an important function, and no functional classification can be inferred from their sequences. The *Bacillus subtilis* Maf protein is representative of a family of proteins that has homologs in many of the completely sequenced genomes from archaea, prokaryotes, and eukaryotes but whose function is unknown. A previously determined crystal structure, in combination with multiple sequence alignment, revealed a putative active site [1]. Phosphate ions present at this site suggested a phosphorylated ligand [1].

Materials and Methods

The *Bacillus subtilis* Maf protein was prepared and crystallized as described previously [1] or crystallized in the presence of a potential ligand. Complexes with ligands were also formed by soaking crystals in solutions containing the ligand of interest. X-ray diffraction data were collected at 100K on the 5-IDB station of the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) with a Mar165 charged-coupled device (CCD) detector. Data sets were collected for complexes with 2´-deoxy-inosine 5´-monophosphate, inosine 5´-diphosphate, 2´-deoxy-adenosine 5´-triphosphate, 2'-deoxy-guanosine 5´-triphosphate, 2´-deoxy-guanosine 5´-diphosphate, 2´-deoxy-uridine 5´-triphosphate, NADP, Coenzyme A, thiamine phosphate, and 1,6-bisphophoglucose. The data were integrated and merged with DENZO/SCALEPACK.

Results and Discussion

An array of phosphate-containing ligands were either co-crystallized with the Maf protein, soaked into existing crystals as single components, or soaked in cocktail mixtures and the structures determined. Difference electron density maps indicated the presence of bound ligands. The *B. subtilis* Maf protein was shown to bind a variety of phosphorylated ligands. In each case, a phosphoryl group interacts with residues that are conserved among homologs of the Maf protein from other species. However, interactions with other portions of the tested compounds were generally minimal, suggesting that none of the tested compounds represent a biologically relevant ligand

This approach did identify compounds that interact with the functionally uncharacterized Maf protein. The binding sites for the compounds were in the putative active site cleft [1] and demonstrated the importance of the phosphoryl group to binding. The biological function of this family of proteins, however, remains elusive.

Acknowledgments

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Reference

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