Structural and Functional Properties of Transporter Solute Binding Proteins

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Project Goals: The Argonne "Environment Sensing and Response" Scientific Focus Area (ESR-SFA) program addresses the hypothesis that cellular behavior can be modeled through an understanding of the biological interface with the environment and the cellular responses that originate from the cell/environment interaction. The overall objective of the ESR-SFA program is to identify the molecular basis of cellular transport and sensory pathways that mediate the response to environmental nutrients. We recently identified four possible solute binding proteins (SBPs) in *R. palustris* that demonstrated binding to aromatic lignin monomers. We used molecular crystallography to examine the molecular basis for the aromatic compound binding specificity of these proteins and assess the feasibility for the development of sequenced based prediction methods to determine ligand binding specificity.

Rhodopseudomonas palustris metabolizes aromatic compounds derived from lignin-degradation products and has the potential for bioremediation of xenobiotic compounds. In this study, we exploit the ligand binding specificity of solute binding proteins (SBPs) to evaluate the transport specificity of ABC transporters for lignin degradation products for multiple transporters. We recently identified four possible SBPs in *R. palustris* CGA009 that demonstrated binding to aromatic ligands similar to lignin monomers. To expand on these initial results, we selected homologues from *R. palustris* strains HaA2 and BisB5, *B. japonicum* USDA110 and *S. meliloti* 1021 and subjected targets to a directed screen against potential aromatic ligands. A fluorescent thermal shift (FTS) assay was used to screen for ligands of purified SBPs using a library of 46 compounds. Individual proteins exhibited binding to multiple structurally similar molecules, but also demonstrated clear ligand preferences arising from carbon chain length and ring modifications. Arrangement of FTS data by reference SBP group and common chemical features of the ligands enables organization of the binding capabilities into distinct groups of protein-ligand interactions. Overlay of the chemical specificity on an unrooted phylogenetic tree suggests a consistent relationship of the binding profile inferred by the FTS assay with the phylogenetic relatedness.

To establish a molecular basis for the aromatic compound binding specificity of these proteins, 13 proteins were produced in a bacterial expression system, purified and routed to crystallization screens in the presence and absence of ligands. Each protein was screened with either no ligand (apo) or one of two preferred ligands (ligand1, ligand 2) indicated by induction of the highest melting temperature stabilization in FTS assays. These 39 combinations yielded multiple crystals, 9 of which gave refraction patterns suitable for solving structures. Solved structure resolutions are generally less than 2 Å and all structures indicate SBPs in a closed formation with a ligand in the binding pocket. Most targets were solved as monomers, however two targets exhibited dimers in the space group. In cases where an FTS-identified ligand was found in the active site, structure resolution was high enough to calculate hydrogen bond coordination in the residues that comprise the binding pocket. Based on these structures, ligand

coordination differs between the two groups described by phylogenetic and *in vitro* functional analysis of these targets.

The crystal structures of these novel aromatic binding proteins from *R. palustris* confirmed the inferred functional assignment as binding proteins for aromatic compounds. The variances in binding pocket geometry and thermodynamic parameters for these SBPs are consistent with the observed sequence variance and sequence phylogeny for this protein family. This information will enable an understanding of the molecular mechanisms that underlie microbial interactions and thereby facilitate the development of system-level models predictive of cellular response to environmental conditions or changes.

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