Selective molecular transport through the protein shell of a bacterial microcompartment organelle

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Bacterial microcompartments are widespread prokaryotic organelles that have important and diverse roles ranging from carbon fixation to enteric pathogenesis. Current models for microcompartment function propose that their outer protein shell is selectively permeable to small molecules, but whether a protein shell can mediate selective permeability and how this occurs are unresolved questions. Here, biochemical and physiological studies of structure-guided mutants are used to show that the hexameric PduA shell protein of the 1,2-propanediol utilization (Pdu) microcompartment forms a selectively permeable pore tailored for the influx of 1,2-propanediol (the substrate of the Pdu microcompartment) while restricting the efflux of propionaldehyde, a toxic intermediate of 1,2-propanediol catabolism. Crystal structures of various PduA mutants provide a foundation for interpreting the observed biochemical and phenotypic data in terms of molecular diffusion across the shell. Overall, these studies provide a basis for understanding a class of selectively permeable channels formed by nonmembrane proteins.

microcompartment | protein channel | carboxysome | Salmonella | B12

The complex behavior of biological systems depends fundamentally on the controlled movement of molecules between cellular compartments. Such processes occur in a wide range of biological contexts through the movement of ions and small molecules across lipid bilayers via proteins—channels and pumps—embedded in the bilayer. Achievements in understanding molecular transport in transmembrane systems have contributed to scientific disciplines from cell biology and physiology to membrane biophysics (1, 2). Interestingly, there exists a second type of system for molecular transport through proteins that is fundamentally different and much less understood. Hundreds of species of bacteria produce large subcellular organelles known as microcompartments (MCPs), which consist of metabolic enzymes encapsulated within proteinaceous shells reminiscent of viral capsids (reviewed in ref. 3). For MCPs to function, substrates and products must move across their outer protein shell, which lacks any lipid-based membrane. In the last several years, 3D structures of the proteins that comprise MCP shells have revealed narrow pores through their centers that have been hypothesized to be the routes by which substrates enter (and products escape from) MCPs (4; reviewed in ref. 5). However, experimental evidence to support this key hypothesis and the molecular principles involved is lacking.

The overarching function of MCPs is to optimize metabolic pathways that have toxic or volatile intermediates. MCPs are present across at least 11 different bacterial phyla, where they carry out diverse metabolic processes (6–12). The carboxysome MCP is used to enhance CO2 fixation in nearly all bacteria that use the Calvin cycle, and it has been estimated that 25% of the carbon fixation on Earth occurs within this proteinaceous bacterial organelle (9). The 1,2-propanediol utilization (Pdu) and ethanolamine utilization (Eut) MCPs are used to optimize 1,2-propanediol (1,2-PD) and ethanolamine catabolism, respectively (13–15), and the degradation of these compounds is thought to promote enteric pathogenesis (12, 16, 17). Although the Pdu and Eut MCPs, the carboxysome, and other metabolically diverse MCPs of unknown function encapsulate distinct sets of enzymes, all have shells built from homologous proteins suggesting they operate by conserved functional principles. Most models of MCP function propose that the protein shell acts as a diffusion barrier that allows passage of substrates (and products) while limiting the escape of a toxic or volatile metabolic intermediate such as CO2 or toxic aldehyde (9, 18), but selective permeability by MCP shells has not been established experimentally.

The shells of MCPs are assembled primarily from a family of small proteins that have so-called bacterial microcompartment (BMC) domains (5). Many BMC domain proteins form flat, hexagonally shaped oligomers that tile into extended sheets that form the basis of the MCP shell (4, 19, 20) (Fig. 1). In most cases, MCP shells are composed of four to eight different types of functionally diversified BMC domain proteins, some of which have pores proposed to mediate the selective movement of metabolites across the shell (4, 7, 8, 18). For example, the PduA shell protein from the Pdu MCP has a small central pore (~6 Å) that is lined with numerous hydrogen-bond donors and acceptors, leading to a suggested role in the preferential movement of 1,2-PD over the less polar propionaldehyde (a toxic intermediate) (21).

Significance

Here, we describe a type of selective channel formed by a nonmembrane protein that is used to control metabolic movement between cellular compartments. In this case, the channel controls the movement of metabolites between the bacterial cytoplasm and the lumen of a bacterial microcompartment, a primitive organelle that is bounded by a protein shell and lacks any lipid bilayer. These studies are the first to our knowledge to prove selective molecular transport through a protein-based barrier. These findings broaden our understanding of how cells control the movement of molecules between cellular compartments, which is fundamental to biological systems. It also solves a key question about the function of bacterial microcompartments, which themselves impact many diverse and important biological processes.


The authors declare no conflict of interest.

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Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 4RBT [PduA-540], 4RBU [PduA-5405SG], 4QIF [PduA-540H], 4QIG [PduA-540C], and 4RBU [PduA-540Q]).

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addition, a subgroup of BMC proteins have been crystallized in two distinct conformations where the central pore is either fully closed or opened widely (12–15 Å), suggesting that a gating mechanism might control the movement of larger molecules (such as enzymatic cofactors) across the MCP shell (22, 23). However, no physiological or biochemical studies demonstrating transport or selective movement specifically through any MCP pore have been reported. As a result, the idea that the MCP protein shell is capable of mediating selective diffusion has lacked a clear experimental basis. Furthermore, a recent alternative model for MCP function proposes that enzymes embedded in or tightly associated with the shell could move metabolites into MCPs by vectorial catalysis, in which case functional pores might not be required for metabolite movement (24).

Here, we use the known structure of PduA—a canonical, hexameric BMC-type shell protein in the Pdu MCP—to design a series of mutant shell proteins having central pores with altered sizes and physicochemical properties. A combination of physiological studies on mutant bacteria, biochemical studies on isolated mutant MCPs, and crystal structure studies on the mutant shell proteins, show that the PduA pore serves as a key route for entry of the metabolic substrate (1,2-PD), and that the chemical properties of the PduA pore are tuned to limit the escape of the toxic propionaldehyde intermediate.

**Results**

**PduA Pore Mutants and Their Structures.** The PduA protein is a major component of the shell of the Pdu MCP, whose function is diagrammed in Fig. 1 (25). To investigate the role of the pore in molecular transport, we made eight PduA pore variants in *Salmonella enterica* serovar Typhimurium LT2 via chromosomal mutations: PduA-S40T, PduA-S40Q, PduA-S40C, PduA-S40L, PduA-S40A, PduA-S40H, and PduA-S40M, and an insertion mutation (PduA-S40GSG) that was intended to occlude the pore by the addition of two glycine residues (one on each side of S40). For all eight PduA mutants, electron microscopy and SDS/PAGE showed that the mutated shell protein assembled into normal-appearing MCPs with protein compositions similar to wild-type, indicating that these mutations did not cause major structural changes (SI Appendix, Figs. S1 and S2).

To characterize the detailed structural effects of the pore mutations, X-ray crystallographic studies were undertaken on the individual shell protein mutants. Crystal structures of five of the PduA mutants were successfully determined (PduA-S40L, PduA-S40H, PduA-S40C, PduA-S40Q, and PduA-S40GSG) in the background context of a K26A edge mutation that mitigates crystallographic problems that often arise from the side-by-side aggregation of hexameric units (SI Appendix, Table S1) (26). In all five crystal structures elucidated, the protein assembled into homohexamers (despite the potentially disrupting effects of having six closely spaced changes in the native pore region) and revealed differences relative to the wild-type PduA structure, mainly in the pore region as intended (Fig. 2 and SI Appendix, Appendix, Figs. S1 and S2).

Fig. 1. Structure and function of the propanediol utilization (Pdu) bacterial microcompartment. A few thousand shell proteins (mostly of the BMC family) encapsulate a series of enzymes for metabolizing 1,2-PD. The protein shell of the Pdu MCP has been hypothesized to be selectively permeable allowing substrates such as 1,2-PD to enter through small pores in the center of hexameric shell proteins while restricting the efflux of propionaldehyde, which is toxic to the cell. For clarity, the reaction scheme has been simplified by omitting some of the steps involved in coenzyme B12 recycling.

**Fig. 2.** Three-dimensional crystal structures of PduA and pore mutants. PduA hexamer assemblies are shown in cartoon format (Left) with close-up view of the pore region (Middle) and overlaid with wild-type (Right). From top to bottom: Wild-type (WT) PduA (PDB ID code: 3NGK, cyan), PduA-S40A (model, marine blue), PduA-S40L (PDB ID code: 4RBT), PduA-S40GSG (PDB ID code: 4RBV), PduA-S40H (PDB ID code: 4QIF), PduA-S40C (PDB ID code: 4QIG), and PduA-S40Q (PDB ID code: 4RBU).
The pore of PduA-S40L is considerably more hydrophobic, but not more occluded than the wild type. The S40H mutant has a pore whose natural sixfold symmetry is broken, but which is likewise not occluded. The S40C mutant shows polymorphic behavior; three distinct instances of the hexamer occur in the crystal form, and the pore can be open or occluded depending on the cysteine side chain orientations and whether disulfide bonds are formed between them. The S40GSG insertion mutation has a fully occluded pore (which was the intended outcome) in two of three distinct hexamers of the crystal structure. The S40Q mutant also has an occluded pore in both distinct hexamers of the crystal structure (SI Appendix, Table S2). Three other mutants (S40A, S40M, and S40T) formed essentially normal-appearing MCPs but were not characterized by crystallography. Models of those mutants were generated by conservative computer modeling. In particular, the S40A mutant, whose physiological behavior was notable, was modeled simply by removing the serine hydroxyl group. The PduA-S40A model has a central pore that is not significantly larger than wild type (despite loss of the hydroxyl groups), but which is less polar (Table 1 and SI Appendix, Table S2 and Fig. S6).

**Effects of PduA Mutations on Shell Function.** Prior studies showed that mutational impairment of the shell of the Pdu MCP results in faster growth of *Salmonella* on 1,2-PD at limiting B₁₂ concentrations, whereas shell defects lead to propionaldehyde toxicity at saturating B₁₂ (27, 28). When B₁₂ is saturating, diol dehydratase rapidly converts 1,2-PD to propionaldehyde, which leaks from defective MCPs causing toxicity and growth inhibition (27–29). At limiting B₁₂, a broken or damaged shell results in faster growth due to the increased availability of enzyme substrates and cofactors to the 1,2-PD degradative enzymes encased within the MCP (27, 29). No aldehyde toxicity occurs at limiting B₁₂ due to the lower rate of propionaldehyde formation by diol dehydratase. Hence, growth rate at saturating B₁₂ provides a means to measure the extent of propionaldehyde leakage from the MCP, whereas growth rate at limiting B₁₂ allows an estimate of the permeability of the shell to substrates (27, 29).

In our initial tests, we looked at the effects of the eight PduA pore mutants (described above) on shell permeability by measuring growth of *Salmonella* on 1,2-PD at limiting B₁₂, PduA-S40T, PduA-S40A, PduA-S40H, and PduA-S40M mutants grew similarly to wild type (SI Appendix, Fig. S3), indicating no significant change in shell permeability. In contrast, the PduA-S40L, PduA-S40C, PduA-S40Q, and PduA-S40GSG mutants grew more slowly than wild type on 1,2-PD (SI Appendix, Fig. S3). This finding suggested that the PduA-S40L, PduA-S40C, PduA-S40Q, and PduA-S40GSG pore mutations resulted in a shell that is less permeable to substrates. Notably, three of these slow-growing mutants (PduA-S40GSG, PduA-S40Q, and PduA-S40C) had occluded pores in the crystal structures. Moreover, additional tests with the PduA-S40L, PduA-S40GSG, PduA-S40C, and PduA-S40Q mutants showed that increasing the 1,2-PD concentration from 0.4 to 2.4% corrected their slow-growth phenotype, consistent with an impaired diffusion of 1,2-PD into the MCP that could be offset by a much higher concentration gradient (SI Appendix, Fig. S4).

To more directly test whether PduA pore mutations impaired the movement of 1,2-PD across the shell of the Pdu MCP, we measured the coenzyme B₁₂-dependent diol dehydratase (DDH) activity in purified MCPs. DDH is an MCP lumen enzyme that catalyzes the first step of 1,2-PD degradation (the conversion of 1,2-PD to propionaldehyde); hence, its activity depends on the diffusion of 1,2-PD across the MCP shell (13). MCPs purified from PduA-S40L, PduA-S40GSG, and PduA-S40Q mutants had ∼50%, 62%, and 75% DDH activity compared with the wild-type level, whereas MCPs from PduA-S40T, PduA-S40C, PduA-S40A, PduA-S40H, and PduA-S40M mutants had activities similar to wild type (Table 2). The markedly reduced DDH activity of the PduA-S40L, PduA-S40Q, and PduA-S40GSG mutants is most simply explained by altered structural features that block the influx of 1,2-PD and/or that impair the egress of propionaldehyde (which might lead to product inhibition). The former explanation is most consistent with the experiments noted above in which increased 1,2-PD concentration mitigated the growth defect.

It was notable that overall the DDH activities of MCPs purified from the PduA pore mutants correlated well with the growth rates of those mutants on 1,2-PD, providing both in vivo and in vitro evidence that certain PduA pore variants limit substrate influx. The PduA-S40C mutant, however, provided one interesting exception. It showed normal DDH activity in vitro for purified MCPs, although it showed a reduced growth rate on 1,2-PD (Table 2 and SI Appendix, Fig. S3). To test whether the in vitro activity of this mutant might be lowered by environmental conditions likely to occlude the pore, we added iron (FeCl₃) under reducing conditions (adding DTT) with the expectation that the multiple proximal cysteine residues might bind iron at the center of the pore. Indeed, under these conditions DDH activity for the S40C mutant dropped to 66% (similar treatment lowered the activity in the wild-type case to 87%) (SI Appendix, Table S3). This finding provided further evidence that occluding the pore of the PduA shell protein limits influx into the

**Table 1. Pore diameter and hydrophobicity**

<table>
<thead>
<tr>
<th>PduA mutant</th>
<th>Pore diameter, Å</th>
<th>Pore segment</th>
<th>Solvation free energy difference in the pore, kcal mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>5.6</td>
<td>I38-L42</td>
<td>—</td>
</tr>
<tr>
<td>PduA-S40A</td>
<td>5.9</td>
<td>I38-L42</td>
<td>−2.1 ± 0.5</td>
</tr>
<tr>
<td>PduA-S40L</td>
<td>5.5</td>
<td>I38-L42</td>
<td>4.7 ± 0.7</td>
</tr>
<tr>
<td>PduA-S40GSG</td>
<td>1.9</td>
<td>I38-L44</td>
<td>−1.3 ± 0.9</td>
</tr>
<tr>
<td>PduA-S40Q</td>
<td>0.5</td>
<td>I38-L42</td>
<td>−0.2 ± 0.1</td>
</tr>
<tr>
<td>PduA-S40H</td>
<td>4.3</td>
<td>I38-L42</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

In cases where multiple instances of a hexamer were present in a crystal structure, the reported pore diameters and solvation-free energies are average values.

**Table 2. DDH activity of purified mutant MCPs**

<table>
<thead>
<tr>
<th>Type of purified MCPs</th>
<th>Specific activity, μmol min⁻¹ mg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (LT2)</td>
<td>28.4 ± 0.6</td>
</tr>
<tr>
<td>PduA-S40L</td>
<td>14.8 ± 0.8</td>
</tr>
<tr>
<td>PduA-S40GSG</td>
<td>17.5 ± 0.5</td>
</tr>
<tr>
<td>PduA-S40Q</td>
<td>21.2 ± 0.7</td>
</tr>
<tr>
<td>PduA-S40H</td>
<td>28.5 ± 0.4</td>
</tr>
<tr>
<td>PduA-S40T</td>
<td>29.4 ± 0.4</td>
</tr>
<tr>
<td>PduA-S40M</td>
<td>29.2 ± 0.2</td>
</tr>
<tr>
<td>PduA-S40N</td>
<td>27.8 ± 0.8</td>
</tr>
<tr>
<td>PduA-S40A</td>
<td>26.1 ± 0.8</td>
</tr>
<tr>
<td>PduA-S40C</td>
<td>27.1 ± 0.2</td>
</tr>
</tbody>
</table>

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MCP and also suggested that the inconsistency between growth test and DDH assays (noted above) might be due to differences between in vivo and in vitro conditions.

Control experiments were performed to rule out ancillary effects of pore mutations. If the PduA-S40L and PduA-S40GSG mutants reduced DDH activity by impeding the movement of 1,2-PD across the MCP shell (rather than by some other unanticipated effect), then breaking the MCP should return the DDH activity to wild-type levels. The measured activities of wild type, PduA-S40L, and PduA-S40GSG MCPs following disruption using standard protocols were all very similar (SI Appendix, Table S4).

**Movement of Propionaldehyde Through the PduA Pore.** The hypothesized role of the Pdu MCP shell is to retain propionaldehyde while allowing 1,2-PD entry. We therefore tested the effects of selected PduA pore mutants on the efflux of propionaldehyde from the Pdu MCP by measuring the amount of propionaldehyde that diffused out of the MCP, through the bacterial cell membrane, and into the culture medium during growth of *Salmonella* on 1,2-PD as described (28) (Fig. 3). Simultaneously, we measured growth rate and 1,2-PD consumption. The PduA-S40L and PduA-S40GSG mutants (whose pores were shown to diminish 1,2-PD uptake) consumed 1,2-PD more slowly than wild type.

![Fig. 3](image-url)
type, but excreted propionaldehyde in similar amounts. Most notably, the PduA-S40A mutant consumed 1,2-PD at a rate similar to wild type, but excreted approximately 2.5-fold more propionaldehyde. Increased excretion led to a marked inhibition of growth (from 12 to 30 h) as a result of propionaldehyde toxicity as was previously observed for deletion mutations that disrupt the MCP shell (27) (Fig. 3). These results indicated that the wild-type PduA pore provides a key route for inward diffusion of 1,2-PD, and that its detailed structural features are important for restricting the egress of propionaldehyde to mitigate aldehyde toxicity.

A second test used to assess the permeability of PduA pore variants to propionaldehyde was to grow Salmonella on 1-propanol under conditions where growth was limited by diffusion of propionaldehyde, produced by oxidation of 1-propanol in the cytoplasm, into the Pdu MCP (SI Appendix, SI Materials and Methods). These tests indicated that the relative rate of propionaldehyde movement into the Pdu MCP was PduAS40A > PduAS40L > wild type ∼ PduAS40T (SI Appendix, Fig. S5). The modest increase in the permeability of S40L compared with wild type for propionaldehyde influx (SI Appendix, Fig. S5) can be reconciled with the unaffected rate of aldehyde efflux during growth on 1,2-PD (Fig. 3) by a reduced rate of internal aldehyde production (by diol dehydratase) in this mutant (Table 2). Most importantly, however, this finding was the second experiment that indicated the S40A mutant has an increased permeability to propionaldehyde compared with wild type.

Effects of Polarity of the PduA Pore on Small Molecule Diffusion. Some of the observed data on transport through mutant PduA pores (summarized in SI Appendix, Table S6) are easily interpreted, such as the blockage of 1,2-PD influx in the fully occluded S40GSG mutant and in the pore-constricted S40Q mutant, whereas the behavior in other cases calls for a more detailed analysis. Two observations in particular are worth examining. First, the S40L mutant did not produce a pore with a narrower diameter, yet influx of 1,2-PD was diminished. Second, the S40A mutation did not significantly increase the minimal radius of the pore, yet propionaldehyde efflux was enhanced. We examined the physicochemical properties of the mutant pores to shed light on these phenomena. We evaluated the polarity of the pore in the different structures according to a calculated estimate of the difference in free energy of solvation between 1,2-PD and propionaldehyde. The 1,2-PD is precluded, the hydrophobic nature of the S40L pore likely creates narrows at a more focused point. Although the pore is not occluded, the hydrophobic nature of the S40L pore likely creates an energetic barrier for the 1,2-PD substrate. The 1,2-PD is precluded, the hydrophobic nature of the S40L pore likely creates narrows at a more focused point.

The Movement of Large Cofactors into Purified Mutant and Wild-Type MCPs. Prior studies indicated that PduP, which catalyzes the conversion of propionaldehyde + HS-CoA + NAD⁺ → propionyl-CoA and NADH, localizes to the lumen of the Pdu MCP (31). Therefore, measurement of PduP activity provides a way to assess the movement of the large cofactors, HS-CoA and NAD⁺, across the MCP shell. Enzyme assays on purified MCPs (both broken and intact) showed that wild-type and mutant MCPs all had similar levels of PduP activity (approximately 2.8 μmol·min⁻¹·mg⁻¹) when assayed under conditions identical to those used to show that the PduA-S40L and PduA-S40GSG mutations restricted the influx of 1,2-PD (SI Appendix, Table S4). This finding suggests that large cofactors cannot enter mutant Pdu MCPs capable of restricting the movement of 1,2-PD and propionaldehyde. The absence of effect with mutants in PduA supports earlier hypotheses that large cofactors cross the MCP through other specialized shell proteins that have gated pores that open and close when triggered for specific transport (22, 23, 32).

Selectivity of the PduA Pore for 1,2-PD and Glycerol. A multiple sequence alignment showed that the serine at position 40 of PduA is widely conserved among diverse genera of bacteria except for the Lactobacilli, where all species examined had a histidine at that position (SI Appendix, Fig. S7). Interestingly, previous studies showed that Lactobacillus uses the Pdu MCP for the catabolism of both 1,2-PD and glycerol (33, 34). In contrast, Salmonella uses the Pdu MCP for 1,2-PD degradation but catabolizes glycerol by alternative pathways. To test whether the H40 amino acid of the Lactobacillus PduA protein might be an adaptation that allows movement of both glycerol and 1,2-PD into the Lactobacillus Pdu MCP, we constructed a chromosomal PduA-S40H mutation in Salmonella, and measured its effects on the influx of 1,2-PD and glycerol into the Pdu MCP. The PduA-S40H mutation had no significant effect on growth of Salmonella on 1,2-PD or on the DDH activity of purified MCPs (SI Appendix, Fig. S3 and Table 2), indicating that H40 allowed normal entry of 1,2-PD into the Pdu MCP under the conditions used. To examine molecular transport in the PduA-S40H mutant in vitro, we looked at glycerol inhibition of DDH in purified Pdu MCPs; glycerol is both a substrate and an inhibitor of DDH (35). The DDH activity of purified PduA-S40H MCPs was inhibited by glycerol at a five times lower concentration compared with wild-type MCPs, indicating substantially greater permeability toward glycerol (Table 3). Conversely, DDH inhibition in the PduA-S40L and PduA-S40GSG mutants required five times higher concentrations of glycerol. This finding indicated a change in the relative permeability of the pore to 1,2-PD and glycerol, which compete for the DDH active site. We also found that purified recombinant DDH (SI Appendix, Fig. S2) was particularly sensitive to glycerol inhibition, further indicating the selectivity of the MCP shell in general (Table 3). Collectively, these results emphasize the role of the MCP shell and the PduA in dictating transport selectivity in the Pdu MCP.

Discussion

A fundamental question about bacterial MCPs is how their protein shells restrict the efflux of volatile/toxic pathway intermediates while allowing enzyme substrates, cofactors, and products to pass. In this report, we show that the PduA protein forms selectively permeable pores across the shell of the Pdu MCP that allow the retention of the propionaldehyde intermediate while

Table 3. DDH inhibition by glycerol in MCPs purified from different pore mutants

<table>
<thead>
<tr>
<th>Type of purified MCP</th>
<th>IC₅₀ of glycerol, mM</th>
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<tbody>
<tr>
<td>Wild-type (LT2)</td>
<td>291.7 ± 2.2</td>
</tr>
<tr>
<td>PduA-S40L</td>
<td>1,560 ± 50.1</td>
</tr>
<tr>
<td>PduA-S40GSG</td>
<td>1,410 ± 35.9</td>
</tr>
<tr>
<td>PduA-S40H</td>
<td>55.3 ± 1.7</td>
</tr>
<tr>
<td>Purified DDH</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>
allowing influx of the 1,2-PD substrate. These studies demonstrate that the pores of MCP shell proteins are selectively permeable to small molecules. In the work presented here, crystal structures of several of the PduA pore mutants provided a framework for interpreting the phenotypic data in terms of physical properties of the pore. Mutants unable to take up the 1,2-PD substrate could be correlated with pores that were fully occluded or substantially more hydrophobic. The behavior and structure of the S40A mutant shed light on the importance of the detailed properties of the pore for aldehyde retention. In this case, the removal of (six copies of) a hydroxyl group in the pore does not have a significant effect on aldehyde retention. In this case, the removal of (six copies of) a hydroxyl group in the pore does not have a significant effect on aldehyde retention.

**Methods and Materials**

MCP purification, enzyme assays, and growth studies were performed as described (27, 31, 37). Site-directed mutants were made by using standard 

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