In *Escherichia coli*, the UhpT transporter catalyzes the electroneutral accumulation of sugar 6-phosphate by exchange with internal inorganic phosphate (P$_i$). The substrate specificity of UhpT is regulated at least in part by constituents of an Asp$_{388}$-Lys$_{391}$ intrahelical salt bridge, and mutations that remove one but not both of these residues alter UhpT preference for organophosphate substrates. Using site-directed mutagenesis, we examined the role played by these two positions in the selection of the oxyanion countersubstrate. We show that derivatives having aliphatic or polar residues at positions 388 and 391 are gain-of-function mutants capable of transporting SO$_4^{2-}$ as well as P$_i$. These oxyanions share similar structures but differ significantly in the presence of a proton(s) on P$_i$. Our findings therefore lead us to suggest that the Asp$_{388}$-Lys$_{391}$ ion pair acts normally as a filter that prevents substrates lacking a proton that can be donated from occupying the UhpT active site.

The MFS is the largest known collection of evolutionarily related secondary transporters showing great diversity in both substrate specificity and kinetic mechanism (1–4). Despite this heterogeneity, members of the MFS appear to share a common architectural theme characterized by the presence of ~12 transmembrane segments that transverse the membrane in an α-helical conformation. The recent publication of high resolution structures for several of the best studied (yet unrelated) members of this superfamily (5–7) indicates that this structural theme is indeed correct, providing a framework by which to study helix relationships and domain function using site-directed mutagenesis and the modification chemistry it affords. We have applied these techniques to the study of UhpT, the P$_i$-linked hexose phosphate antiporter carrier of *Escherichia coli* (8–10), to identify determinants of substrate selectivity.

In previous work, TM11 of UhpT was shown to contain an intrahelical salt bridge with the constituents Asp$_{388}$ and Lys$_{391}$ that lie on the substrate translocation pathway (11). Further analysis indicated that these two positions play a direct role in determining substrate specificity (11, 12). Thus, derivatives having an uncompensated cationic charge at either position 388 or 391 are gain-of-function mutants in which substrate preference is strongly biased in favor of phosphoenolpyruvate, a substrate that carries one more negative charge than sugar 6-phosphate. If, however, an uncompensated anionic charge is placed at position 388, one observes behavior consistent with an increased preference for monovalent rather than divalent sugar 6-phosphate, as though the resident anion were acting as an anionic charge entering on sugar phosphate. Here, we provide evidence that when non-ionic residues occupy these two positions, UhpT can accept both P$_i$ and SO$_4^{2-}$, suggesting that this region also plays a role in discriminating between these two oxyanions.

**EXPERIMENTAL PROCEDURES**

**Strains and Plasmids—**Strain XL1-Blue (recA1 endA1 gyrA96 thi1 hsdR17 supE44 relA1 lac (F’ proAB lacZAM15 Tn10)) (Strategene Cloning Systems) was used for all cloning steps. Strain RK5000 (araD139 deoR-lacU169 relA1 rpsL150 thi gyrA219 ara-14 ilv-169 recT) (2056 recA1), a gift from R. J. Kadner, University of Virginia (13), served as the host for tests of function of plasmid-encoded UhpT. Plasmid pTrc (HisC$_5$S$_6$) encodes the N-terminal His$_{10}$-tagged, cytosteenless UhpT (11) that served as parent for the derivatives described in this study.

**Mutagenesis—**Site-directed mutagenesis was performed using the sequential polymerase chain reaction (14). Mutant uhpT alleles were sequenced at the Bio-synthesis and Sequencing Facility of the Johns Hopkins Medical School to confirm the desired mutation and to rule out the presence of other changes.

**Whole Cell Transport Assays—**Unless noted otherwise, overnight cultures were diluted 200-fold into M63 minimal medium, pH 7 (15), containing thiamine (2 μg/ml), required amino acids (50 μg/ml), antibiotics (100 μg/ml streptomycin, 100 μg/ml ampicillin), and 0.2% (w/v) glucose as a carbon source. The high P$_i$ content of M63 ensured maximal repression of other P$_i$ transporters (Pet and Pit) so that most P$_i$ transport occurred via UhpT (11, 16). Cells were grown at 37 °C to a density of 2–5 × 10$^8$ cells/ml, harvested by centrifugation, and then washed twice and resuspended in buffer A (50 mM MOPS/K, pH 7) at an optical density at 660 nm of 1.4, equivalent to about 2 × 10$^9$ cells/ml. After equilibration at room temperature, tests of Glc-6-P, SO$_4^{2-}$, or P$_i$ transport were initiated by adding a 0.1 or 0.05 volume of labeled substrate to a final concentration of 50 μM, 1 mM, or 100 μM, respectively. At indicated times, aliquots were removed for filtration on Millipore filters (0.45-μm pore size) followed by two washes with 5 ml of buffer A.

**Protein Purification, Reconstitution, and Transport Assays—**His$_{10}$-tagged, cytosteenless UhpT and its D383V/K391C derivative were purified by nickel-nitrilotriacetic acid metal affinity chromatography. Overnight cultures were diluted 50-fold into 400 ml of M63 medium (as above) and grown at 37 °C to OD$_{660}$ 1.0–1.2. To induce UhpT expression, isopropyl 1-thio-β-D-galactopyranoside was added to 0.5 mM, and cells were harvested after 2 h. Cells were then washed twice with 20 ml of 100 mM potassium P$_i$, pH 7.5, and resuspended in 20 ml of 100 mM potassium P$_i$, pH 7, containing 1 mM phenylmethylsulfonyl fluoride and 200 μg of DNase I. Cells were disrupted in the cold using a French pressure cell (16,000 p.s.i.), and after removal of unchanged cells and cell debris by low speed centrifugation (12,000 × g for 30 min), membranes were pelleted (150,000 × g for 1 h) and resuspended in 8 ml of buffer B (100 mM potassium P$_i$, 20% glycerol, 5 mM β-mercaptoethanol, 200 mM...
Anion Selectivity in UhpT

RESULTS

Isolation of Mutants That Transport SO₄—Within the MFS, UhpT belongs to a coherent family of organophosphate transporters and receptors, each having high specificity for some organic phosphate ester and low affinity for Pi (1, 3, 8). We showed previously that both Asp³⁸⁸ and Lys³⁹¹, normally associated in a TM11 intrahelical salt bridge, can play a direct role in determining substrate specificity (11, 12). For example, elimination of the anionic partner, which introduces an uncompensated positive charge in this region, alters UhpT substrate bias so as to favor organophosphate substrates with increased electronegative character. Such findings prompted us to ask whether this region might also play a role in discriminating between the low affinity Pi substrate and other oxyanions, such as SO₄, that are normally excluded.

In the present study we explored the role of TM11 in oxyanion selectivity, beginning with an analysis of the UhpT D388C/K391C variant, a mutant whose behavior is markedly affected by the presence of SO₄. Thus, in the absence of salts and in the presence of KCl, this variant and its parent transported ³²P with comparable efficiency; by contrast, ³²P transport by the mutant was strongly inhibited when K₂SO₄ was added to the assay medium (Fig. 1). This finding suggests that SO₄ is a substrate for or an inhibitor of transport by the D388C/K391C variant, and because this mutant displays normal levels of Pi, self-exchange in the absence of salts (Figs. 1 and 4, Table I), we could address this issue by measuring the relative effectiveness with which added SO₄ caused loss of internal Pi during heterologous exchange (Fig. 2). As expected, the addition of both Glc-6-P and Pi led to a marked loss of internal phosphate from cells carrying either cysteineless or D388C/K391C UhpT, whereas SO₄ induced significant Pi efflux only in the D388C/K391C mutant. These findings strongly argue that D388C/K391C is a gain-of-function mutation that allows UhpT to recognize and transport both Pi and SO₄. This supposition was confirmed by the finding that this mutant, and not its parent, was able to transport SO₄ (Fig. 3).

Having found that the D388C/K391C mutation enabled UhpT to transport SO₄, we next asked whether this gain-of-function phenotype reflects the presence of cysteine at these two positions or the absence of the constituents of the Asp³⁸⁸-Lys³⁹¹ salt bridge. Analysis of UhpT relatives suggests that although some residues within TM11 are highly conserved, the Asp³⁸⁸-Lys³⁹¹ ion pair is restricted to family members that transport sugar 6-phosphate (11, 19). By contrast, in examples that do not transport sugar 6-phosphate, either aliphatic (Ala or Val) or polar residues (Asn, Ser, or Thr) are found at these two positions. With this in mind, we generated UhpT double mutants in which Asp³⁸⁸ was replaced by Ala, Cys, or Val and Lys³⁹¹ was replaced with either a Cys or Thr. All of these variants, unlike their parent, are able to transport Glc-6-P and Pi as well as SO₄ (Fig. 4). A more detailed analysis indicated that removal of the Asp³⁸⁸-Lys³⁹¹ salt bridge, although associated with modest changes in the affinities for Glc-6-P or Pi, led to a marked increase in the ability to accept SO₄ from an unquantifiably low level to Kᵣ values in the range of 10–35 mM (Fig. 5, Table I). We believe these observations are consistent with the idea that the Asp³⁸⁸-Lys³⁹¹ ion pair in UhpT normally aids in defending the active site against SO₄.

Characterization of UhpT SO₄ Transport—Among the gain-of-function mutants described above, the D388V/K391C derivative exhibits the highest affinity for SO₄ (Table I), making this variant the most convenient model with which to characterize this novel transport reaction. Our first tests analyzed the response to changes in external pH. We found that acidification diminished the activity of both the parental and mutant...
Anion Selectivity in UhpT

Table I  Transport properties of UhpT mutants displaying increased affinity for $SO_4$

| UhpT derivative | $K_m$ ($\mu$M) | $V_{max}$ (nmol/mg protein/min) | $K_m$ ($\mu$M) | $V_{max}$ (nmol/mg protein/min) | $K_m$ ($\mu$M) | $V_{max}$ (nmol/mg protein/min) |
|-----------------|----------------|---------------------------------|----------------|---------------------------------|----------------|---------------------------------|
| Cysteineless UhpT (parent) | 38 ± 7.6 | 41 ± 7.0 | 520 ± 14 | 50 ± 4.1 | nil | nil |
| D388C/K391C | 78 ± 8.1 | 53 ± 6.7 | 320 ± 25 | 22 ± 1.5 | 31 ± 3.2 | 22 ± 3.0 |
| D388V/K391C | 110 ± 6.6 | 60 ± 0.84 | 95 ± 14 | 26 ± 1.2 | 13 ± 0.63 | 35 ± 0.81 |
| D388A/K391T | 75 ± 1.6 | 62 ± 2.2 | 100 ± 16 | 57 ± 1.4 | 29 ± 2.0 | 54 ± 5.5 |
| D388A/K391C | 99 ± 10 | 67 ± 4.9 | 120 ± 25 | 54 ± 1.6 | 30 ± 1.2 | 20 ± 1.9 |
| D388C/K391T | 69 ± 0.63 | 54 ± 2.1 | 200 ± 24 | 48 ± 1.1 | 34 ± 1.5 | 55 ± 3.4 |

Note: Rates of transport were measured as described under “Experimental Procedures,” and kinetic constants were determined by fitting initial transport rates to the Michaelis-Menten equation (18) using nonlinear regression analysis. $K_m$ and $V_{max}$ values are means ± S.E. for three separate trials and are shown as means ± S.E.

Fig. 2. Substrate transport by UhpT and its D388C/K391C derivative. $P_i$ transport by parental cysteineless (A) and D388C/K391C (B) variants was assayed as described under “Experimental Procedures.” Cells were incubated with radiolabeled $P_i$ at the time point indicated by the arrow; the control tube was divided into five portions, and each portion was given either additional buffer A (●) or buffer A containing unlabeled 1 mM $P_i$ (▲), 1 mM Glc-6-P (■), 10 mM KCl (○), or 10 mM K$_2$SO$_4$ (○). Data are from three separate trials and are shown as means ± S.E.

Fig. 3. $SO_4$ transport by UhpT and its D388C/K391C derivative. Transport of $SO_4$ by parental cysteineless ( ■ ) and D388C/K391C (●) UhpT-expressing strains was assayed as described under “Experimental Procedures.” Data are from three separate trials and are shown as means ± S.E.

(D388V/K391C) proteins. For $P_i$ transport, this reflects reduced turnover because, as pH fell from 7.5 to 5.2, $V_{max}$ values were reduced 10–50-fold with little change (1–2-fold) in the $K_m$ for $P_i$ (not shown) (20). For $SO_4$ transport, however, we observed significant changes in both kinetic parameters ($K_m$, $V_{max}$) over this same pH range (Fig. 6). In particular, as assay pH became more acidic, the $K_m$ for $SO_4$ transport by the mutant decreased significantly, showing a nearly 10-fold drop from its value of 18 $\mu$M at pH 7.5 to near 2 $\mu$M at pH 6.1 and below (Fig. 6). Because $SO_4$ exists as a divalent species and E. coli maintains a slightly alkaline cytoplasmic pH under physiological conditions (21), one would not expect this change in affinity to arise from a change in stoichiometry for the exchange of external $SO_4$ and internal substrate ($P_i$ or Glc-6-P). Instead, this decrease in affinity is better explained by changes in the protonation state of a residue on the translocation pathway. Because $SO_4$ affinity in the D388V/K391C variant is greatest at a pH near 6.1, this role may be played by histidine ($pK_a$ 6.0). Analysis of the UhpT primary sequence suggests His$^{168}$ (in TM5) as a possible candidate in this regard. His$^{168}$ is conserved within the family of organophosphate transporters and receptors, and crystallography of GlpT, the glycerol 3-phosphate antiporter of E. coli, shows this residue positioned close to the two essential arginines known to interact with UhpT substrates (7).

We next examined the homologous and heterologous exchange reactions in the D388V/K391C variant relative to its parent. As illustrated in Fig. 7, both proteins mediated $P_i$ self-exchange and the heterologous exchange of internal $P_i$ with external Glc-6-P; only in the gain-of-function derivative was $P_i$ efflux induced by the addition of $SO_4$ to the external medium (Fig. 7B). Further study indicated that the mutant also displays $SO_4$ efflux when either Glc-6-P, $P_i$, or $SO_4$ acts as the external countersubstrate. We noted, however, that $P_i$, despite having a relatively high affinity for this UhpT variant, provoked low rates of $SO_4$ efflux relative to those found with SO$_4$ and Glc-6-P. We attribute this to a futile $P_i$ self-exchange that obscures the net heterologous reaction ($P_i$ for SO$_4$). This phenomenon is often found when two substrates with widely different affinities for exchange are used (8, 22). In the present case, this phenomenon would reflect that as (unlabeled) $P_i$ accumulates, this relatively high affinity substrate competes...
successfully with (labeled) SO$_4$ for efflux. On the other hand, because Glc-6-P is readily metabolized, exogenous Glc-6-P would not elicit this response when added to intact cells. To circumvent the complexities associated with evaluating UhpT-mediated SO$_4$ exchange in whole cells, we purified and reconstituted both the D388V/K391C protein and its parent. Proteoliposomes were prepared to contain P$_i$ as the sole internal substrate, and homologous as well as heterologous P$_i$-linked exchanges were documented (Fig. 8). As anticipated from work with intact cells, we found comparable behavior for both mutant and parental proteins in regard to P$_i$ and Glc-6-P transport; SO$_4$ transport, which could be blocked by both P$_i$ and Glc-6-P, was noted only in preparations containing the mutant protein (Fig. 8C). These findings conform to the general behavior of the gain-of-function mutant as tested in intact cells and, when taken together with our other findings, lead us to conclude that the region in and around positions 388 and 391 serves as one determinant of UhpT oxyanion selectivity.

**FIG. 4. Transport of Glc-6-P, P$_i$, and SO$_4$.** Transport of Glc-6-P (A), P$_i$ (B), and SO$_4$ (C) by the parental cysteineless UhpT and its derivatives is shown. Rates of transport were monitored by a 5-min incubation of cells with labeled substrate as described under “Experimental Procedures.” Values shown are means ± S.E. for three independent experiments.

**FIG. 5. Kinetics of SO$_4$ transport.** Rates of SO$_4$ transport were estimated as described under “Experimental Procedures” for cells expressing parental cysteineless UhpT (□) and its D388C/K391C (▲), D388V/K391C (●), D388A/K391T (△), D388A/K391C (○), and D388C/K391T (■) derivatives. Data from three independent trials are shown as means ± S.E. $K_m$ and $V_{max}$ values for UhpT variants under these conditions are shown in Table I.

UhpT-mediated SO$_4$ exchange in whole cells, we purified and reconstituted both the D388V/K391C protein and its parent. Proteoliposomes were prepared to contain P$_i$ as the sole internal substrate, and homologous as well as heterologous P$_i$-linked exchanges were documented (Fig. 8). As anticipated from work with intact cells, we found comparable behavior for both mutant and parental proteins in regard to P$_i$ and Glc-6-P transport; SO$_4$ transport, which could be blocked by both P$_i$ and Glc-6-P, was noted only in preparations containing the mutant protein (Fig. 8C). These findings conform to the general behavior of the gain-of-function mutant as tested in intact cells and, when taken together with our other findings, lead us to conclude that the region in and around positions 388 and 391 serves as one determinant of UhpT oxyanion selectivity.

**DISCUSSION**

Instances of altered substrate specificity within the MFS have been demonstrated for a number of transporters. For example, point mutations in LacY, the lactose permease of *E. coli*, can lead to a preference for maltose (23), malto-oligosaccharides (24), sucrose (25), or arabinose (26). Similarly, single amino acid changes in the CitA citrate transporter of *Salmonella typhimurium* and the MelY melibiose-H$^+$ symporter of *Enterobacter cloacae* alter substrate specificity so as to allow isocitrate and maltose transport, respectively (27, 28). In earlier work with UhpT, we reported comparable effects arising from mutations (at positions 388 and 391) in TM11 that can lead to a bias favoring phosphoenolpyruvate and 3-phosphoglyceric acid over the normal sugar 6-phosphate substrates (11, 12). Such precedents argue that small structural perturbations may result in physiologically significant differences in substrate preference. Here, we expand upon such work by identifying alterations of the TM11 intrahelical salt bridge (Asp388-Lys391) that allow UhpT to transport SO$_4$.

The notion that positions in TM11 might act as determinants of both UhpT organophosphate and oxyanion substrate specificity, and thus line the transport pathway, is in agreement with both bioinformatic information and the known structures of members of the MFS. Thus, the general helix-packing model proposed by Goswitz and Brooker (29) suggests that TM11 is within the core of potential pathway-lining segments of MFS transporters. Further, all three solved MFS transporter structures (GlpT (7) and lactose permease (LacY) (6) of *E. coli* and the oxalate:formate exchange protein (OxlT) (5) of *Oxalobacter formigenes*) confirm that residues on TM11 form part of the transport pathway. Interestingly, by using the GlpT structural template, a homology model of UhpT in which Asp$^{388}$ and...
Lys<sup>391</sup> orient toward the transport pathway can be derived.<sup>2</sup> Although this model (and the GlpT structure) depicts a conformation that faces the cytoplasmic membrane, it seems likely that during the global conformational changes that occur as UhpT (GlpT) fluctuates between cytoplasm and periplasm open states, these positions could be exposed to the substrate translocation path at some, if not all, times during the reaction cycle. This supposition is reinforced by our biochemical studies with both lysine- and thiol-specific probes that indicate that a large tract of TM11, including the surface containing positions 388 and 391, lies on the aqueous permeation pathway within UhpT (12, 30).

Our current findings not only substantiate earlier work that places positions 388 and 391 on the transport pathway but also provide insight into the mechanism by which UhpT discriminates between P<sub>i</sub> and SO<sub>4</sub><sup>2-</sup>. Despite the structural similarity of these oxyacids, UhpT has a marked preference for P<sub>i</sub> relative to SO<sub>4</sub><sup>2-</sup> (Fig. 4, Table I), suggesting the presence of a molecular “filter” that discriminates between the two. The nature of such a filter may now be at least partially explained by analogy with the periplasmic P<sub>i</sub>-binding protein of <i>E. coli</i>. In that case, crystallography shows that the stringent specificity of this receptor for P<sub>i</sub> is because of a single carboxylate group that not only accepts a proton donated by P<sub>i</sub> but also disallows SO<sub>4</sub><sup>2-</sup> binding via charge repulsion (31, 32). Because UhpT-mediated SO<sub>4</sub><sup>2-</sup> transport is only observed when non-ionic residues occupy both positions 388 and 391, one role of Asp<sup>388</sup> may be to aid in defending the active site against SO<sub>4</sub><sup>2-</sup>. We suggest that this is accomplished through charge stabilization. Although contributing to the inability of fully ionized SO<sub>4</sub><sup>2-</sup> to achieve an electrostatic balance at the substrate binding site, Asp<sup>388</sup> assists in the recognition of P<sub>i</sub> via acceptance of a hydrogen bond. The idea that such a function can be attributed to this residue is strengthened by the earlier finding (12) that the protonation state of Asp<sup>388</sup> in the K391C UhpT variant aids in determining whether mono- or divalent sugar phosphate is transported. We also note that the Asp<sup>388</sup>-Lys<sup>391</sup> ion pairing is restricted to members of the organophosphate transporter family that accept sugar 6-phosphate, yet SO<sub>4</sub><sup>2-</sup> is rejected as a countercsubstrate for all members of this family (8). It seems likely, therefore, that the discrimination provided by TM11 in UhpT is supplemental to a more general oxyanion exclusion mechanism shared by all family members.

Together with previous studies, the work presented here allows elements required for UhpT function and specificity to be added to the scaffolding provided by recent publication of MFS transporter structures. For example, Arg<sup>275</sup> and Arg<sup>45</sup>, both essential and conserved throughout the UhpT family (19), lie at the center of the substrate translocation pathway and take part in recognizing the anionic phosphoryl group of the organosubstrate (7, 12). The constituents of the Asp<sup>388</sup>-Lys<sup>391</sup> salt bridge, on the other hand, are positioned near the cytoplasmic edge of the pathway (12) and, although not required for sugar phosphate transport function, appear to act as supplemental oxyanion and organophosphate charge filters. The location of these specificity determinants ensures that the inorganic substrate only binds to the active site when it is capable of both donating a proton (i.e. mono- or divalent phosphate groups) and establishing an electroneutral environment within the binding pocket. Only when these conditions are satisfied is UhpT capable of facilitating substrate exchange through interconversion between the cytoplasmic and periplasm open conformations.

**REFERENCES**

1. Pao, S. S., Paulsen, I. T., and Saier, M. H. (1998) Microbiol. Mol. Biol. Rev. 62, 301–360
2. Marger, M. D., and Saier, M. H. (1993) Trends Biochem. Sci. 18, 13–20
3. Henderson, P. J. F. (1993) Curr. Opin. Cell Biol. 5, 708–721.
4. Saier, M. H. (2000) Microbiol. Mol. Biol. Rev. 64, 354–411
5. Hirai, T., Heymann, J. A. W., Shi, D., Sarker, R., Maloney, P. C., and Subramaniam, S. (2002) Nat. Struct. Biol. 9, 597–600
6. Abramson, J., Smirnova I., Kasho, V., Verner, G., Kabaek, H. R., and Iwata, S. (2005) Science 310, 610–615
7. Huang, Y., Lemieux, M. J., Song, J., Auer, M., and Wang, D.-N. (2003) Science 301, 616–620
8. Maloney, P. C., Ambudkar, S. V., Anantharam, V., Sonna, L. A., and Varad-
hachary, A. (1990) Microbiol. Rev. 54, 1–17

9. Maloney, P. C., and Wilson, T. H. (1996) in Escherichia coli and Salmonella: Cellular and Molecular Biology (Neidhardt, F. C., Curtiss, R., Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) pp. 1130–1148, American Society of Microbiology, Washington, D. C.

10. Sonna, L. A., Ambudkar, S. V., and Maloney, P. C. (1988) J. Biol. Chem. 263, 6625–6630

11. Hall, J. A., Fann, M.-C., and Maloney, P. C. (1999) J. Biol. Chem. 274, 6148–6153

12. Hall, J. A., and Maloney, P. C. (2001) J. Biol. Chem. 276, 25107–25113

13. Weston, L. A., and Kaufner, R. J. (1987) J. Bacteriol. 169, 3546–3555

14. Ho, S. N., Hunt, H. D., Morton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 51–59

15. Miller, J. H. (1972) Experiments in Molecular Genetics, p. 431, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

16. Rosenberg, H. (1987) in Ion Transport in Prokaryotes (Rosen, B. P., and Silver, S., eds) pp. 205–248, Academic Press, Inc., San Diego, CA

17. Brown, R. E., Jarvis, K. L., and Hyland, K. J. (1989) Anal. Biochem. 180, 136–139

18. Segel, I. H. (1975) Enzyme Kinetics, p. 210, John Wiley & Sons, Inc., New York

19. Fann, M.-C., Davies, A. H., Varadhachary, A., Kuroda, T., Sevier, C., Tsuchiya, T., and Maloney, P. C. (1998) J. Membr. Biol. 164, 187–195

20. Maloney, P. C., Ambudkar, S. V., Thomas, J., and Schiller, L. (1984) J. Bacteriol. 158, 997–1005

21. Padan, E., Zilberstein, D., and Rottenberg, H. (1976) Eur. J. Biochem. 63, 533–541

22. Sonna, L. A., and Maloney, P. C. (1988) J. Membr. Biol. 101, 267–274

23. Brooker, R. J., and Wilson, T. H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3959–3963

24. Olsen, S. G., Greene, K. M., and Brooker, R. J. (1993) J. Bacteriol. 175, 6269–6275

25. King, S. C., and Wilson, T. H. (1990) J. Biol. Chem. 265, 9638–9644

26. Goswitz, V. C., and Brooker, R. J. (1993) Membr. Biochem. 10, 61–70

27. Shimamoto, T., Negishi, K., Tsuda, M., and Tsuchiya, T. (1996) Biochem. Biophys. Res. Commun. 226, 481–487

28. Shinnick, S. G., Perez, S. A., and Varela, M. (2003) J. Bacteriol. 185, 3672–3677

29. Goswitz, V. C., and Brooker, R. J. (1995) Protein Sci. 4, 534–537

30. Hall, J. A., and Maloney, P. C. (2002) J. Bacteriol. 184, 3756–3758

31. Quiocho, F. A. (1996) Kidney Int. 49, 943–946

32. Luecke, H., and Quiocho, F. A. (1999) Nature 347, 492–496

Anion Selectivity in UhpT
Altered Oxyanion Selectivity in Mutants of UhpT, the P_{i}-linked Sugar Phosphate Carrier of *Escherichia coli*

Jason A. Hall and Peter C. Maloney

*J. Biol. Chem.* 2005, 280:3376-3381.
doi: 10.1074/jbc.M409965200 originally published online November 19, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M409965200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 15 of which can be accessed free at
http://www.jbc.org/content/280/5/3376.full.html#ref-list-1