Reconstitution of the Phosphoglycerate Transport Protein of Salmonella typhimurium*

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Operation of the phosphoglycerate transport protein (PgtP) of Salmonella typhimurium has been studied in proteoliposomes by using a technique in which membrane protein is solubilized and reconstituted directly from small volumes of cell cultures. When protein from induced cells was reconstituted into phosphate (Pi)-loaded proteoliposomes, it was possible to demonstrate a PgtP-mediated exchange of internal and external phosphate. For this homologous Pi;Pi antiport, kinetic analysis indicated a Michaelis constant ($K_m$) of 1 mM and a maximal velocity of 26 mmol/min mg of protein; arsenate inhibited with a $K_i$ of 1.3 mM, suggesting that PgtP did not discriminate between these two inorganic substrates. Pi;Pi antiport was also studied for their respective affinity for 3-phosphoglycerate and phosphoenolpyruvate, establishing for each of them a concentration gradient (in/out) of about 100-fold; phosphoenolpyruvate ($K_i = 70$ $\mu M$) rather than 3-phosphoglycerate ($K_i = 700$, $K_i = 900$ $\mu M$) was the preferred substrate for these conditions. We also concluded that such heterologous exchange was a neutral event, since its rate and extent were unaffected by the presence of a protonophore and unresponsive to the imposition of a membrane potential (positive or negative inside). In quantitative work, we found a stoichiometry of 1:1 for the exchange of Pi and 3-phosphoglycerate, and given an electroneutral exchange, this finding is most easily understood as the overall exchange of divalent Pi against divalent phosphoglycerate. These experiments established that PgtP functions as an anion exchange protein and that it shares important mechanistic features with the Pi-linked antiporers, GlpT and UhpT, responsible for transport of glycerol 3-phosphate and hexose 6-phosphates into Escherichia coli.

It is now appreciated that bacteria have a variety of carrier systems for the transport and accumulation of organic phosphates. PgtP, the phosphoglycerate permease, is one such example, serving Salmonella typhimurium during growth on 2- and 3PGA, P-enolpyruvate, and perhaps other three-carbon sugar phosphates (1). The initial descriptions of PgtP activity in cells (1) and membrane vesicles (2) indicated that its function was supported by substrate oxidation and that its operation required maintenance of a proton-motive force. For these reasons there was general agreement that PgtP operated as a proton/phosphoglycerate symporter. However, recent work indicates that PgtP function may be more complex. Thus, preliminary experiments showed that this system transports Pi, as well as phosphoglycerates (3), suggesting that PgtP may resemble GlpT and UhpT, the Pi-linked antiporers responsible for the uptake of glycerol 3-phosphate and hexose 6-phosphates, respectively, into Escherichia coli. Other Pi-linked antiporers are found in Streptococcus lactis (4, 5) and Staphylococcus aureus (6), and in all these cases, despite clear differences in specificity for an organic phosphate, Pi is not a low affinity substrate (reviewed in Ref. 3). The presently available sequence data also support the idea that a family of Pi-linked antiporers might include PgtP, since there is significant homology between the amino acid sequences of UhpT and GlpT (32% sequence identity) (7, 8) and between PgtP and GlpT or UhpT (31-37% identity) (9).

Although the preliminary biochemical studies and amino acid sequence comparisons justify the tentative conclusion that PgtP functions as a Pi-linked antipor, it is important to continue the analysis with direct tests. Indeed, with regard to the sequence analyses, the importance of direct tests is emphasized by experience gained in studies of LacY, the H+/lactose symporter of E. coli. An extensive mutational analysis of LacY indicates that changes in single amino acids can profoundly alter both phenotypic behavior and apparent biochemical mechanism (10). For example, in a Glu-325 $\rightarrow$ Ala replacement led to loss of an overt symport character with clear retention of an antipor or exchange capacity (11). In another instance (Gly-262 $\rightarrow$ Cys), "downhill" transport remained, but the coupling to $H^+$ was impaired (12). Therefore, while sequence similarity can indicate evolutionary relatedness, it need not predict the details of biochemical mechanism (uniport, antipor, symport); instead, one must arrive at such decisions on the basis of concrete experiment.

In work described here, we have used biochemical techniques to reconstitute the PgtP transport system of S. typhimurium. Since the properties of the reconstituted activity conform to those expected if PgtP is a Pi-linked antipor, we have concluded that PgtP operates as an anion exchange protein.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strain and Growth Conditions—**S. typhimurium FR1, a derivative of wild-type strain LT-2, was used for the studies reported here. In preliminary work we found that induction of PgtP by growth on PGa also led to induction of GlpT (data not shown). Because this made it difficult to assess PgtP substrate specificity, we derived a GlpT-negative mutant by selection for resistance to fosfomycin (phosphonomycin), an antibiotic taken up by PgtP via the PgtP exchange mechanism (13, 14). Wild-type cells were seeded at high density on minimal plates containing 0.5% glycerol (v/v), and drug-resistant cells were then selected in the presence of fosfomycin.
colonies were chosen from within the zone of growth inhibition surrounding crystals of fosfomycin. Phosphotyptic tests showed that one of these mutants, designated S. typhimurium FR1, lacked GlpT activity but was otherwise normal for P₃-linked exchanges. Thus, FR1 no longer took up [¹⁴C]glycerol-3-P after growth on glycerol or 3PGA, yet was P₃-linked and PgtP transport systems had their normal induction patterns.

For experiments involving reconstitution of P₃-linked transport, cells were inoculated from an overnight broth (15) culture and grown aerobically using medium 83 (Ref. 16) supplemented with 0.1% casamino acids, 1 µg/ml thiamine and 1 mM MgSO₄. PgtP-induced cells were obtained by using 0.5% (w/v) 3PGA as a carbon source, while uninduced control cells were grown with 0.5% glucose (w/v). When the late-exponential phase of growth was reached (Asc₅₀ of 0.7–0.8), a 10 ml culture volume (or multiples thereof) was harvested by centrifugation, and the cell pellet was rapidly frozen and stored at −70°C for subsequent use. In a few experiments we also examined the [³²P] transport activity of intact cells, and in these cases cells were grown as usual but in capped tubes to maintain a relative anaerobiosis. For S. typhimurium and E. coli this procedure decreases the expression of constitutive P₃ transport systems relative to that of the inducible P₃-linked exchanges (3), as judged by the plateau level of inhibition by excess PgtP substrates; for these conditions about 50–60% of the [³²P] taken up in 1 min was attributable to activity of PgtP.

**Solubilization and Reconstitution**—Assays of transport by proteoliposomes were based on a new method developed to solubilize and reconstitute membrane proteins directly from small culture volumes (18). The cell pellet was osmotically sensitized and resuspended at 37°C to one-half the original volume with 0.3 mg/ml lysozyme. After 10 min, the osmotically sensitized cells were pelleted, and lysis was completed by resuspension in distilled water. This was followed by a recentrifugation to isolate broken cells, after which protein was solubilized by addition (in order) of 40 mM KPi (pH 7), 20% glycerol, 0.2% acetone/ether-washed E. coli phospholipid, 4 mM MgSO₄, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 1.2% octyl β-D-glucopyranoside, all in a final volume of 350 µl. Following a 20-min incubation on ice, the insoluble material was removed by recentrifugation, and 250 µl of the clarified extract (−450 µg of protein) was incubated with an additional 1 µg of sonicated E. coli phospholipid for reconstitution by detergent-dilution (17, 18). Proteoliposomes were prepared at room temperature by a 20-fold dilution into a suitable loading buffer (see figure legends); the lipid-to-protein weight ratio should have been about 21:1 for these conditions, assuming the usual recoveries of protein and lipid (17, 18) and an endogenous lipid-to-protein weight ratio of 1. For routine work, proteoliposomes were collected by centrifugation, washed, and finally suspended in 250 µl of buffer A, which contained 20 mM MOPS/K (pH 7), 75 mM K₂SO₄, and 2.5 mM MgSO₄. If potassium was to be avoided, proteoliposomes were isolated by addition of 125 mM KPi buffer, B, which contained 20 mM MOPS/N-methylglucamine (pH 7), 75 mM N-methylglucamine-SO₄, and 2.5 mM MgSO₄. Control experiments confirmed that these general methods were satisfactory for the study of PgtP function in proteoliposomes. In particular, omission of sonicated lipid from the reconstitution mixture led to a null response, excluding any contribution to the signal by residual intact cells or membrane vesicles (data not shown).

**Assays of Transport**—Transport assays were performed at 21°C by addition of prewarmed aliquots of cell or proteoliposome suspensions to a reaction mixture containing 100 µM [³²P] or [¹⁴C]-labeled 3PGA or P₃-linked substrates along with other additives as specified in the individual experiments. Assays were terminated by filtration through wet 0.22 µm GSTP Millipore filters, followed by a wash with 10 ml of buffer A, with conditions arranged so as to achieve about 4 or 8 µg of protein per filter, respectively, for transport assays of [³²P] or the [¹⁴C]-labeled substrates. There were two potential sources of error in quantitation of these assays. First, we did not directly measure protein in proteoliposomes, but assumed that one-fourth of the solubilized material became incorporated, as in earlier work (17). Second, a residue of external loading buffer may affect the specific activity of transport substrates, and while this did not change the qualitative information obtained in these experiments, when quantitative information was required, the measurement of residual external material was performed as given in Fig. 1B.

**Other Procedures**—Protein in the solubilized extract was determined by a modification of the procedure of Schaffner and Weissman. Total content of intact cells was taken as 205 µg of protein/ml for Asc₅₀ of 1 (20).

**Chemicals**—[³²P]KH₂PO₄, (1 Ci/mol) was obtained from Dú Pont New England Nuclear; the [¹⁴C]-3PGA (38.6 Ci/mol) was from Amersham Corp. [¹³C]-3PGA (55 Ci/mol), which was a generous gift of H. R. Kaback (UCLA Howard Hughes and Molecular Biology Institutes), was synthesized at the Roche Research Center, Nutley, NJ, by Yu-Ying Liu, under the direction of A. Liebman. With the exception of octyl β-D-glucopyranoside (Calbiochem) and 0.2% crude lipid (Avanti Polar Lipids, Inc., Birmingham, AL), all other materials were purchased from Sigma, Fisher, or Boehringer Mannheim.

**RESULTS**

**Reconstitution of the Homologous and Heterologous Exchange Reactions**—To test the idea that PgtP functions as an anion exchange carrier, we required a stable in vitro system in which the composition of the internal compartment could be readily manipulated. Accordingly, we concentrated on studies of reconstituted preparations, and in initial work focused on those features of P₃-linked exchange which have proven diagnostic in other instances. For example, if PgtP is a P₃-linked anion exchange carrier, one expects to find both homologous P₃:P₃ exchange and the heterologous antiport of P₃ against a suitable organic phosphate (P₃-enolpyruvate, 3PGA, etc.). The presence of an appropriate P₃:P₃ exchange is documented in Fig. 1, which summarizes experiments using proteoliposomes prepared to contain 100 mM KP, as the internal substrate. In these cases it was evident that P₃-loaded proteoliposomes prepared using 3PGA-induced cells took up [³²P] from the medium and that this reaction was readily reversed on addition of unlabeled P₃ (or arsenate; not shown) (Fig. 1A). MOPS-loaded particles did not accumulate [³²P], nor was there a positive response when liposomes (no protein) were studied (not given), and from these observations we concluded that such [³²P] transport was mediated by an exchange with internal P₃. This reaction was clearly attributable to PgtP, since [³²P] accumulation required use of protein from 3PGA-induced cells, and since substrates of PgtP (2PGA, 3PGA, P₃-enolpyruvate) blocked [³²P] transport while substrates of UhpT (glucose-6-P) or GlpT (glycerol-3-P) were without effect (Fig. 1A). Taken together, these findings offer qualitative evidence establishing that PgtP catalyzes the exchange of external and internal P₃.

If PgtP mediates simple P₃:P₃ exchange, the steady state distribution of [³²P] between external and internal pools should also reflect the partitioning of total P₃. Consequently, one expects that if the external pool is expanded by known amounts, there will be a corresponding increase in the ratio of external to internal [³²P]. This expectation was fulfilled (Fig. 1B), providing quantitative support for the idea of a PgtP-mediated P₃-linked exchange. Such data (Fig. 1B) also gave information about the P₃-accessible space, which, in these experiments, corresponded to 3.3 ± 0.9 nmol P₃/mg lipid and 0.6 ± 0.1 µl/mg protein, assuming an 80% recovery of the lipid added during reconstitution (18), and with a recovery of protein as noted under “Experimental Procedures” (Fig. 1B, legend). Since this P₃-accessible space was a small fraction of the total internal P₃ pool (~100 nmol P₃/mg lipid) (18), it seemed likely that in these experiments PgtP-mediated exchange was recovered at the level of single functional units distributed in single proteoliposomes.

Additional tests examined the possibility that PgtP carries out the heterologous exchange of P₃ and sugar phosphates leading to a net accumulation of substrate. In these cases (Fig. 2), it could be shown that P₃-loaded proteoliposomes took up both 3PGA and P₃-enolpyruvate (Fig. 2A), establishing concentration gradients (in/out) of about 100 (assuming a P₃-accessible space of 0.6 µl/mg protein), without provision of an external source of energy. The reciprocal experiment showed that 3PGA-loaded proteoliposomes accumulated [³²P]
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Fig. 1. Homologous Pi:P_i exchange mediated by PgtP. A, protein was solubilized from uninduced (Δ) or 3PGA-induced cells (all other symbols) and P_i-loaded proteoliposomes were formed as described under "Experimental Procedures." Transport of _32P_i, was estimated in buffer A using 100 μM _32P_i, with no further additions (○, Δ) or with 3 mM added sugar phosphate, as indicated: glycerol-3-P (○); glucose-6-P (■); 2PGA (●); 3PGA (△), or P-enolpyruvate (○). The arrow indicates the time of addition of 30 mM KPi. B, in a similar experiment proteoliposomes were incubated for 90 min with 100 μM _32P_i alone and additional unlabeled P_i to give the indicated final concentrations. As noted elsewhere (18), in this method of presentation the horizontal intercept gives residual external P_i carried into the experiment from the proteoliposomal stock (108 ± 6 μM here and in three similar experiments), whereas the reciprocal of the slope of the line gives information about the _32P_i-accessible space (see text).

(190-fold) (Fig. 2B), and in both instances, the incorporated material was retained in the presence of a proton conductor (see below), yet readily released by addition of unlabeled substrate. The latter trials also identified the required inducer and substrate specificities by showing that heterologous antiport was recovered only from 3PGA-induced cells and that addition of an alternate substrate (P-enolpyruvate) could block the reaction. As a result of these tests, we concluded that PgtP displays heterologous exchange reactions of the kind associated with P_i-linked antiport.

Kinetic Analysis—Having established the presence of PgtP-specific homologous and heterologous exchanges, we next used kinetic studies to obtain quantitative information concerning the various exchange substrates. Those data (Table I) include the Michaelis constant (K_M) and maximal velocity (V_max) for P_i and 3PGA transport by P_i-loaded proteoliposomes, but for the most part substrates were ranked according to the inhibition constant (K_i) measured by using an unlabeled test compound as a competing substrate in the assay of _32P_i transport (4, 21). This method of evaluating different antiport substrates had proven satisfactory in other cases of P_i-linked exchange (3, 4, 21).

The data of Table I indicate a K_M of 1 mM for the homologous P_i:P_i exchange reaction and an equivalent value (1.3 mM) for the K_i of its inhibition by arsenate. These estimates agree well with those obtained for P_i and arsenate interactions with the related antiport systems, GlpT and UhpT (K_M and K_i values of 1-1.2 mM; Refs. 3, 22), and it seems likely that, in common with GlpT and UhpT (3, 23), PgtP fails to distinguish between these inorganic substrates. In trials using the organic substrates, we began with the assumption that 2PGA, 3PGA, and P-enolpyruvate have equivalent kinetic properties, since preliminary work with intact cells had pointed to each of these as having relatively high affinity (K_i values of 30-60 μM) (Table I; Ref. 1). However, these initial findings did not

Fig. 2. Heterologous exchanges mediated by PgtP. A, P_i-loaded proteoliposomes prepared from 3PGA-induced cells were exposed to 100 μM _14C-labeled P-enolpyruvate (○) or 3PGA (●) for conditions as described in Fig. 1A; 30 mM KPi was added at the time indicated by the arrow. B, proteoliposomes from 3PGA-induced (○, Δ) or uninduced (□) cells were prepared to contain 3PGA by using a loading buffer composed of 10 mM MOPS/K, 30 mM 3PGA, and 65 mM K_2SO_4 (pH 7). After resuspension in buffer B, the washed proteoliposomes were tested for _32P_i transport as described for Fig. 1A, in the absence (○, □) or presence (△) of 3 mM P-enolpyruvate.
predict the results of work with reconstituted preparations. Instead, PgpP activity in proteoliposomes showed a striking preference for P-enolpyruvate over the other organic substrates ($K_i$ values of 700 vs. 800 $\mu$M). As noted below, this discrepancy may reflect the interconversion of these compounds in the periplasmic space of intact cells.

In studies of UhpT in E. coli and other cells, it was found that the maximal velocity of homologous P$_i$P exchange exceeded that of the heterologous P$_i$:glucose-6-P antiport by about 5-fold (3). This also appears true in the case of PgpP function. For example, direct measurements gave a velocity ratio of 5.4 for the P$_i$:P$_i$ and P$_i$:3PGA exchanges (Table I). In addition, using the other data of Table I ($K_i$, $K_a$), we calculated this velocity ratio for experiments in which the homologous and heterologous exchanges had been measured in the same batch of proteoliposomes. Those comparisons give a relative velocity ratio of 6.5±1.1 (eight trials), consistent with the idea that for PgpP the homologous exchange is inherently more rapid than heterologous antiport.

Effect of Ionophores—Other experiments sought to determine whether PgpP catalyzed an electroneutral or electrogenic reaction. To address this issue we examined the antiport of P$_i$ and 3PGA in the presence of a membrane potential, using conditions known to accelerate or retard an electrogenic anion exchange according to the polarity of the imposed electrical gradient (24). The use of valinomycin, along with suitably oriented potassium gradients, established an internally negative (Fig. 3A) or positive potential (experiment not shown), neither of which affected the rate or extent of $^{32}$P$_i$ accumulation by 3PGA-loaded proteoliposomes. The reciprocal trial studied $^{[14]C}$3PGA movement into P$_i$-loaded particles (Fig. 3B), and for that case, too, imposition of a membrane potential (positive inside) was without effect. These negative findings suggested that such P$_i$:3PGA antiport was electroneutral, and this interpretation was strengthened by finding that, in the absence of potassium gradients, the protonophore, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (used at 5 $\mu$M) had no effect on $^{32}$P$_i$ transfer into either P$_i$- or 3PGA-loaded proteoliposomes (data not shown).

Exchange Stoichiometry—The finding of an electroneutral heterologous exchange places restrictions on the ionic species that might reasonably participate in antiport, since at pH 7 monovalent and divalent P$_i$ are present in about equal quantity (pK$_2$ = 6.8), yet 3PGA is predominantly divalent and trivalent (pK$_3$ = 6). Accordingly, an exchange ratio of 2 (or 3) P$_i$ per 3PGA would imply use of monovalent P$_i$, as demonstrated for the UhpT systems of S. lactis, S. aureus (21, 23) and as inferred for E. coli (3, 25), while an equimolar 1:1 exchange ratio would suggest an exchange based on divalent anions, as noted for P$_i$:triose phosphate exchange in chloroplasts (26, 27). With this in mind, our final experiments were designed to measure the stoichiometry of heterologous exchange, and for this purpose, we prepared proteoliposomes loaded with 30 mM 3PGA. To label the 3PGA-accessible pool, proteoliposomes were preincubated with 15 $\mu$M $^{[14]C}$3PGA until steady state was attained (100 min), at which point 5 mM $^{32}$P$_i$ was added to initiate the heterologous reaction (Fig. 4A). In this and in one additional set of experiments, the slope of the relationship between 3PGA lost and P$_i$ gained...
port, the GlpT and UhpT proteins of preliminary biochemical studies (3) and by the sequence homology between PgtP and two known examples of such antiporters. This last feature was of special interest, since the Pi:triose phosphate transporter of the chloroplast envelope membrane is thought to be electrogenic (27), and its substrate specificity and in its apparent preference for divalent P\(_i\) (26, 27; Fig. 4 and text).

We have concluded that in both its qualitative and quantitative behavior, PgtP functions as a P\(_i\)-linked antiporter. This decision therefore raises the question of why PgtP shows a proton-coupled phenotype in other settings, such as in intact cells or membrane vesicles (1, 2). The likely explanation for this behavior involves simultaneous operation of PgtP-mediated antiport and of a separate proton-linked P\(_i\) symport. This latter reaction is constitutively expressed by a number of bacteria (3), and its activity is demonstrable in several membrane vesicle preparations (6, 25). It is likely, therefore, that in both cells and vesicles there is accumulation of P\(_i\) in symport with protons and that this indirectly supports PgtP function by the subsequent exchange of internal P\(_i\) for external phosphoglycerate. Although speculative in the example of PgtP, this scenario is well documented for UhpT function in vesicles from E. coli (25) and S. aureus (6). Such considerations illustrate that the operation of P\(_i\)-linked antiport may be complex (discussed in Ref. 3), and for this reason an understanding of the biochemical basis of PgtP activity, as described here, is important to a realistic appraisal of its potential functions in bacterial cell biology. As well, we note that the incorporation of P-enolpyruvate by action of PgtP has in the past been used to influence intracellular or intravesicular levels of ATP (2, 28). It should be now possible to enlarge the number of situations in which this intervention can be used on a rational basis.

A noteworthy finding in our studies has been the conflict between data gathered in intact cells and proteoliposomes with regard to substrate specificity. Studies with intact cells (Table I, Ref. 1) suggest that the organic substrates (P-enolpyruvate, 2PGA, 3PGA) should have equivalent kinetic properties, yet in proteoliposomes the affinity for phosphoglycerates is considerably diminished while that of P-enolpyruvate is maintained (Table I). A similar discrepancy has been noted for certain substrates of UhpT. In that case, work with intact cells or membrane vesicles would classify glucose-1-P as a high affinity substrate (\(K_t < 100 \mu M\)) (29), yet the study of proteoliposomes shows glucose-1-P to have a low affinity (\(K_t > 1 \text{ mm}\)) (3). We propose that these kinds of observations are most simply understood as a result of a processing by enzymes in the periplasmic space of the Gram-negative cell. For example, 3PGA might be transformed into 2PGA by a phosphoglycerate mutase, with subsequent conversion of 2PGA to P-enolpyruvate by an enolase (2-phospho-D-glycerate hydrolase). While there is no specific evidence that the presently characterized forms of these enzymes are also found in the periplasm, it should be recalled that induction of UhpT by external fructose 6-phosphate is due to the activity of a periplasmic form of phosphoglucose isomerase (30, 31). If this general argument is true, then, substrate (and inducer) specificity for these membrane proteins can be determined with biochemical resolution only in proteoliposomes, or perhaps membrane vesicles. This point may be especially important in the analysis of metabolically reactive materials such as phosphate esters. There is, as well, the further implication that the Gram-negative periplasm may be responsible for a significant premetabolic processing.

A second significant aspect of our work lies in its use of a new technique to reconstitute membrane proteins from small volumes of cell cultures (17). Until the present work, this approach had not been used to characterize a transport

\[ \text{FIG. 4. Stoichiometry of exchange of P\(_i\) and 3PGA}. \] A. proteoliposomes from 3PGA-grown cells were prepared to contain 30 mM 3PGA, 30 mM MOPS/K (pH 7), 15 mM K\(_2\)SO\(_4\), and 1 mM dithiothreitol. In three pairs of tubes, the 3PGA-loaded particles were preincubated with 15 \(\mu\)M labeled or unlabeled 3PGA. At steady state (100 min), the tubes were given 5 mM P\(_i\) or \(^{32}\text{P}\)\(_i\), respectively, and at the indicated times samples were taken to correlate the loss of 3PGA with the gain of P\(_i\); results are shown as the mean \pm S.E. of the triplicate determinations. B, 3PGA lost is correlated with P\(_i\) gained.

(Fig. 4B) was 1.1, suggesting that the heterologous P:3PGA antiport involved a simple one-for-one exchange of substrates, consistent with the exchange of divalent phosphate with divalent 3PGA.

**DISCUSSION**

In this article we describe the reconstitution and partial characterization of PgtP, the phosphoglycerate transport protein of *S. typhimurium*. The object of these experiments has been to test the idea that PgtP functions as a P\(_i\)-linked antiport system, a suggestion which was prompted by both preliminary biochemical studies (3) and by the sequence homology between PgtP and two known examples of such antiport, the GlpT and UhpT proteins of *E. coli* (9). The mechanistic resemblance of PgtP to these other P\(_i\)-linked antiporters is now supported by a number of important biochemical findings. In particular, and in common with the other examples, PgtP displays the appropriate homologous and heterologous exchange reactions, involving both inorganic (phosphate, arsenate) and organic (P-enolpyruvate, 2PGA, 3PGA) substrates (Figs. 1 and 2). A similarity between PgtP and UhpT or GlpT is also apparent in the quantitative aspects of the kinetics of these events (Table I and text). We also demonstrated that PgtP catalyzes an electrically neutral antiport, as do GlpT and UhpT. This last feature was of special interest, since the P:triose phosphate transporter of the chloroplast envelope membrane is thought to be electrogenic (27), and since that protein resembles PgtP in its exchange cha-
system; our experience suggests these methods have the sensitivity needed to be of value in such circumstances. To illustrate this, it is useful to outline some of the quantitative aspects of the reconstitution of PgtP. For example, of the total available internal P pool, only a small part was accessible to PgtP-mediated P exchange (100 \text{ versus} 3.3 \text{ nmol P/mg lipid}). If one assumes an overall particle density of $2 \times 10^{13}$ proteoliposomes/mg protein (18) and that solubilization and reconstitution leads to a dispersion of monomeric PgtP among proteoliposomes, as is true for UhpT, the methods used here. We anticipate, then, that such technology is well suited to the study of membrane systems; our experience suggests these methods have the sensitivity needed to be of value in such circumstances. To illustrate this, it is useful to outline some of the quantitative aspects of the reconstitution of PgtP. For example, of the total available internal P pool, only a small part was accessible to PgtP-mediated P exchange (100 \text{ versus} 3.3 \text{ nmol P/mg lipid}). If one assumes an overall particle density of $2 \times 10^{13}$ proteoliposomes/mg protein (18) and that solubilization and reconstitution leads to a dispersion of monomeric PgtP among proteoliposomes, as is true for UhpT (32), then PgtP is recovered at a level of about $6.6 \times 10^{10}$ monomers/mg lipid.

And given a bulk lipid:protein weight ratio of 21:1 (see “Experimental Procedures”) and a $M_r$ of 45,000 for PgtP (9), this system would represent about 0.01% of proteoliposomal protein. This level of recovery illustrates the high sensitivity of the methods used here. We anticipate, then, that such technology is well suited to the study of membrane systems expressed at their normally induced levels. There is, of course, no reason why such methods should be restricted to bacterial cells.

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