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Identification and Functional Reconstitution of Phosphate: Sugar Phosphate Antiport of *Staphylococcus aureus*

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Summary. Resting cells of *Staphylococcus aureus* displayed a phosphate (P_i) exchange that was induced by growth with glucose 6-phosphate (G6P) or *sn*-glycerol 3-phosphate (G3P). P_i-loaded membrane vesicles from these cells accumulated $^{32}P_i$, 2-deoxyglucose 6-phosphate (2DG6P) or G3P by an electroneutral exchange that required no external source of energy. On the other hand, when vesicles were loaded with morpholinopropane sulfonic acid (MOPS), only transport of $^{32}P_i$ (and L-histidine) was observed, and in that case transport depended on addition of an oxidizable substrate (DL-lactate). In such MOPS-loaded vesicles, accumulation of the organic phosphates, 2DG6P and G3P, could not be observed until vesicles were preincubated with both P_i and DL-lactate to establish an internal pool of P_i. This *trans* effect demonstrates that movement of 2DG6P or G3P is based on an antiport (exchange) with internal P_i.

Reconstitution of membrane protein allowed a quantitative analysis of P_i-linked exchange. P_i-loaded proteoliposomes and membrane vesicles had comparable activities for the homologous $^{32}P_i : P_i$ exchange (K_t's of 2.2 and 1.4 mM; V_{max}'s of 180 and 83 nmol P_i/min per mg protein), indicating that the exchange reaction was recovered intact in the artificial system. Other work showed that heterologous exchange from either G6P- or G3P-grown cells had a preference for 2DG6P (K_t = 27 μM) over G3P (K_t = 1.3 mM) and P_i (K_t = 2.2 mM), suggesting that the same antiporter was induced in both cases. We conclude that $^{32}P_i : P_i$ exchange exhibited by resting cells reflects operation of an antiporter with high specificity for sugar 6-phosphate. In this respect, P_i-linked antiport in *S. aureus* resembles other examples in a newly described family of bacterial transporters that use anion exchange as the molecular basis of solute transport.

Key Words transport · anion exchange · sugar phosphate · chemiosmotic · phosphate · reconstitution/octylglucoside

Introduction

Early work by Mitchell [19–21] showed that nonmetabolizing cells of *Staphylococcus aureus* took up monovalent phosphate (P_i) from the medium in one-for-one neutral exchange with internal P_i. Although this reaction seemed to reflect a system that accumulated P_i during normal metabolic activity [21], later studies in other bacteria did not correlate the exchange of monovalent P_i with systems dedicated to net P_i transport. Substrate exchange was not associated with P_i transporters in either *Streptococcus faecalis* or *Escherichia coli* [8, 11, 12], and in both these organisms net P_i transport appeared to select for the divalent anion [11] in a reaction that required ongoing metabolism to generate either ATP or a proton-motive force [8, 11, 12]. On the other hand, when P_i-linked exchange resembling the *S. aureus* prototype was found, as in *Streptococcus lactis* [18] or *E. coli* [2], the reaction was mediated by a system whose function centered on the net transport of organic phosphates, not P_i. Such contradictory findings have led us to reexamine the associations between P_i exchange and P_i net transport in *S. aureus*. In the present work we confirm the original observation of a P_i exchange in this cell. Additional studies, in both membrane vesicles and proteoliposomes, now show that such exchange proceeds by a reaction normally linked to the transport of sugar phosphate. We conclude that this P_i-linked antiporter resembles other members of a newly described family of bacterial transport systems that use anion exchange as the molecular basis of solute transport.

Materials and Methods

Bacterial Strains and Growth Conditions

Staphylococcus aureus strains 5601 and 12715 were from the American Type Culture Collection, Rockville, MD., *S. aureus* strain 8709 (strain Duncan) was from the National Collection of Type Cultures, Central Public Health Laboratory, London, U.K. Cells were grown to stationary phase (13 to 16 hr) with vigorous aeration at 34°C in medium (pH 7) consisting of 1% (wt/vol) acid-hydrolyzed casein extract (Difco-Bacto), 0.1% yeast extract (Difco-Bacto), 100 mM NaCl, 10 mM NaH_2PO_4, 10 mM KCl, and 3 mM $MgCl_2$, supplemented with 1% sodium gluconate or lactate, 1% glycerol or 10 mM glucose 6-phosphate (G6P) or

glycerol 3-phosphate (G3P) (sodium salts). To screen or select for fosfomycin-resistance, solidified medium containing 10 mM G3P was supplemented with 200 μg/ml fosfomycin [8, 13]. Strain 12715 was fosfomycin-resistant on this medium, whereas strains 5601 and 8709 were fosfomycin-sensitive. Fosfomycin-resistant derivatives of 8709 appeared at a frequency of about 1 in 10^5 cells plated; one of these was chosen for further tests.

Preparation of Membrane Vesicles

Membrane vesicles were prepared by osmotic lysis as described for *S. lactis* [3], except that lysostaphin (2.8 units/ml) was used in place of lysozyme and several low-speed centrifugations (1,000 *g* × 10 min) were used to remove residual intact cells before pelleting of vesicles. The method gave about 2 mg membrane protein/10^{12} cells, with 1,000–10,000 viable cells/mg protein. In most experiments, vesicles were prepared to contain 50 mM KP_i (pH 7), 10 mM $MgSO_4$ and 0.25 mM Na_3VO_4, but in some cases KP_i was replaced with 70 mM MOPS/K (pH 7), 10 mM K_2SO_4 [3]. Both vesicle types were stored at −70°C in their loading buffers until use. For assays of transport (*see below*), external P_i was removed by washing and resuspension using Buffer A (125 mM K_2SO_4, 20 mM MOPS/K (pH 7) and 0.25 mM Na_3VO_4). The $^{32}P_i$-accessible space [3, 4] of P_i-loaded vesicles gave an internal volume of 2.3 ± 0.2 μl/mg protein (mean ± SEM, 9 experiments) in agreement with earlier determinations [22].

Reconstitution of P_i-Linked Exchange

Protein from either right-side-out membrane vesicles (above) or French press particles [3, 4] was solubilized by 1.1% octyl-β-D-glucopyranoside in the presence of 3.7% acetone/ether washed *E. coli* phospholipid and 20% (vol/vol) glycerol [2, 4]. Reconstitution was performed by detergent-dilution into 100 mM KP_i (pH 7), 1 mM dithiothreitol [4]. After recovery of proteoliposomes by high speed centrifugation, debris was removed by low speed centrifugation and proteoliposomes were washed and finally resuspended in Buffer B (75 mM K_2SO_4, 20 mM MOPS/K, 2.5 mM $MgSO_4$, pH 7). Proteoliposomes retained 9 to 15% of the input membrane protein. Assays of $^{32}P_i$-accessible space [3, 4] indicated a trapped volume of 0.52 ± 0.06 μl/mg phospholipid (mean ± SEM, 14 measurements), as expected from earlier work [4].

Transport Assays

Transport was measured by filtration and washing on Millipore filters [3] after incubation in Buffer A (cells or membrane vesicles) or Buffer B (proteoliposomes) with 100 μM radiolabeled substrate and other indicated supplements. Cells were used at 1.7 mg (dry wt)/ml [19], vesicles were at 50 to 140 μg of protein/ml, and proteoliposomes were at 15 to 25 μg of protein/ml. When energy-dependent transport was monitored in membrane vesicles, 33 mM DL-lactate was used as the oxidizable substrate [22].

Measurements of $^{32}P_i$-accessible spaces (above) showed that a variable quantity of external P_i was introduced into assays of $^{32}P_i$ transport by P_i-loaded vesicles or proteoliposomes [3, 4]. In assays with membrane vesicles this unlabeled P_i was present at 135 ± 23 μM (mean ± SEM, 12 experiments); in assays with proteoliposomes this external pool corresponded to 260 ± 30 μM (mean ± SEM, 13 measurements). To correctly adjust $^{32}P_i$ specific activity in experiments that estimated kinetic parameters (Table 2), it was important to measure this P_i pool in the same experiment. Calculations of $^{32}P_i$ transport did not otherwise consider this additional pool.

Other Assays

The membrane potential established during oxidation of DL-lactate was estimated by distributions of ^{86}Rb in the presence of 0.5 μM valinomycin [1]. Total sugar 6-phosphatase activity was estimated by anion-exchange chromatography [3]. Protein was measured as described [4, 15]. The cell pool of P_i was estimated as outlined earlier [18].

Chemicals

2-Deoxy-D-[1-^{14}C]glucose 6-phosphate [52 mCi/mmol), L-[U-^{14}C] or [2-^{3}H]-*sn*-glycerol 3-phosphate (120 mCi/mmol or 7 Ci/mmol, respectively), L-[U-^{3}H]-histidine (11 Ci/mmol), ^{86}RbCl (1.4 Ci/mg), and $KH_2{}^{32}PO_4$ (1 Ci/mmol) were obtained from New England Nuclear Corp. Octyl-β-D-glucopyranoside was from Calbiochem. All other chemicals were from Sigma or Fisher Chemical Co.

Results

Studies with Intact Cells

The experiment shown by Fig. 1 describes P_i transport and exchange by cells of *S. aureus* suspended in a buffered salts solution without metabolizable substrate. For each strain examined, P_i incorporation was monitored following addition of 100 μM $^{32}P_i$ to the medium. Despite the absence of an added energy source, transport of $^{32}P_i$ was readily observed in strain 5601, and also in strain 8709, the cell in which $^{32}P_i$: P_i exchange was first described [19–21]. In each case, $^{32}P_i$ appeared to have entered the free cellular pool of P_i (25 to 50 mM; *data not shown*), since addition of unlabeled P_i immediately reversed the direction of isotope flux.

Although $^{32}P_i$ transport and exchange could be observed in strains 5601 and 8709, no exchange was found using strain 12715 (Fig. 1) or the fosfomycin-resistant derivative of strain 8709 (*not shown*), even though the latter had internal P_i pools of normal size (*data not given*). These negative responses, together with the observation (Materials and Methods) that strain 12715 was fosfomycin-resistant and the report that such resistance correlates with loss of sugar phosphate transport [8, 13], suggested that $^{32}P_i$ incorporation by the strains 8709 and 5601 might reflect a pathway of sugar phosphate rather than $^{32}P_i$ transport. For this reason, we next examined strain 8709 in experiments using cells grown with different supplemental carbon sources. Those trials showed that exchange was most active in cells grown with

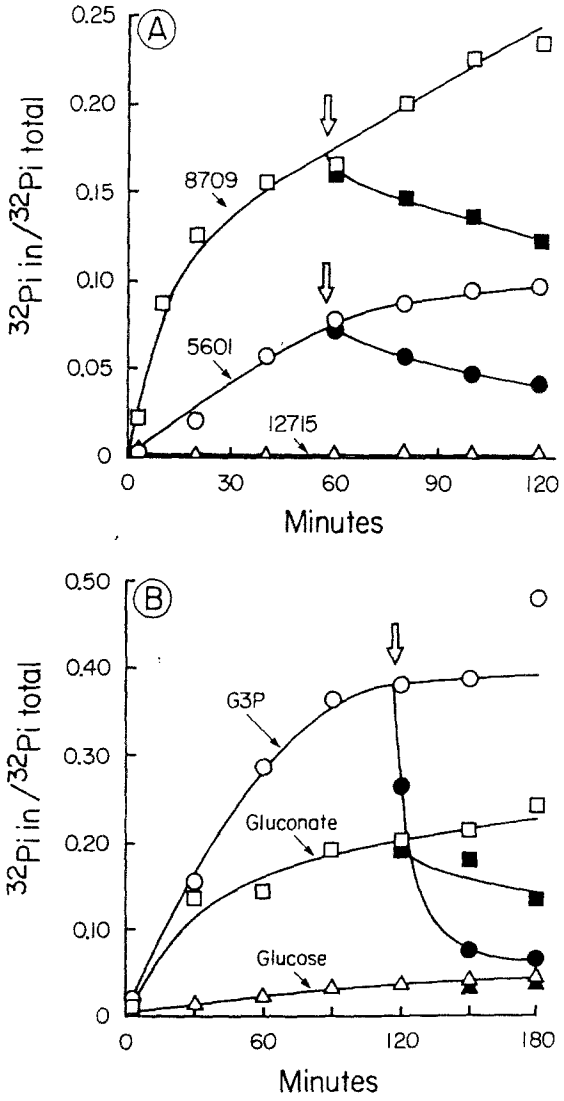

Fig. 1. P_i transport and exchange by intact cells of *S. aureus*. $^{32}P_i$ transport was measured using washed cells suspended in Buffer A with 100 μM $^{32}P_i$. The arrow indicates addition of 5 mM KP_i. (*A*) Cells were grown with gluconate as supplement. Symbols: □, strain 8709; ○, strain 5601; △, strain 12715. (*B*) $^{32}P_i$ transport was determined as in *A*, using strain 8709 grown with the indicated supplements

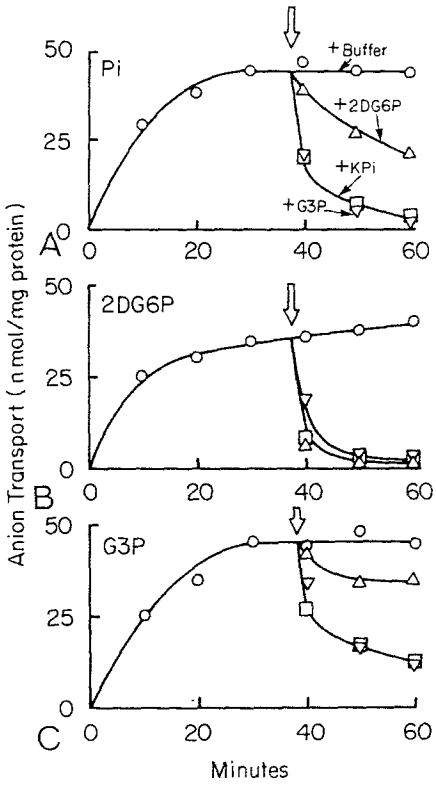

Fig. 2. P_i and sugar phosphate accumulation by P_i-loaded membrane vesicles of *S. aureus*. P_i-loaded membrane vesicles were derived from cells of strain 8709 grown in the presence of G6P. Vesicles were placed in Buffer A with labeled substrates added to 100 μM. After 37 min (arrow) each assay mixture was divided into four portions, and each portion received either buffer (○) or 5 mM unlabeled P_i (□), 2DG6P (△) or G3P (▽). (*A*) $^{32}P_i$ transport. (*B*) [^{14}C]-2DG6P transport. (*C*) [^{14}C]-G3P transport. Vesicles derived from G3P-grown cells were also tested in this experiment, with identical results (*not given*)

G3P (or G6P), moderately active in cells exposed to gluconate (or lactate), but at a low level after growth with glucose (or glycerol or no added carbohydrate) (Fig. 1*B*). These findings made it likely that *S. aureus* strain 8709 harbors an inducible P_i-linked anion exchange of the kind found in *S. lactis* [18] or *E. coli* [2]. Direct tests of this idea were made possible by experiments using membrane vesicles.

P_i-Linked Exchange in Membrane Vesicles

Initial studies examined P_i-loaded right-side-out vesicles derived from cells of strain 8709 grown with G6P; G3P-grown cells were also examined, but since the two cell types gave identical responses, only the former are shown. In either case, P_i-loaded vesicles took up $^{32}P_i$, 2-deoxyglucose 6-phosphate (2DG6P, a nonmetabolizable analogue of G6P) or G3P without requiring an external source of energy (Fig. 2). Further work indicated that both 2DG6P and G3P had been transported intact, since no free sugar (<3%) was detected by anion exchange chromatography [3] of material extracted from vesicles at the steady state. Therefore, incorporation of these substrates at 40 to 50 nmol/mg protein corresponded to apparent internal concentrations of 20 to 25 mM and substrate accumulation ratios of 200 to 250-fold. Three observations suggested that such transport (Fig. 2) was linked to an exchange with intravesicular P_i. First, when internal P_i was replaced by MOPS, accumulation of these substrates did not occur without an added energy source (*see below*). Second, a protonophore, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) had

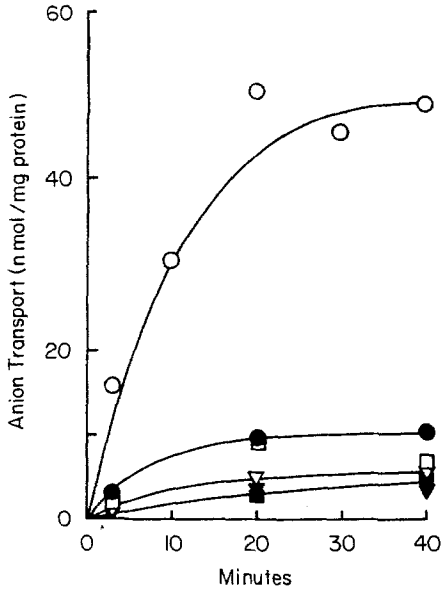

Fig. 3. Transport of P_i and sugar phosphates by MOPS-loaded membrane vesicles. MOPS-loaded vesicles were prepared from cells of strain 8709 grown in the presence of G6P. Vesicles were suspended in Buffer A with labeled substrates added to 100 μM and in the presence (open symbols) or absence (filled symbols) of 33 mM DL-lactate. Symbols: (●, ○) $32P_i$ transport; (■, □) 2DG6P transport; (▼, ▽), G3P transport

no effect on the transport of $^{32}P_i$ or sugar phosphate by P_i-loaded vesicles, yet FCCP did not block energy-dependent transport by MOPS-loaded vesicles (*see below*). Finally, addition of any single substrate (P_i, 2DG6P, G3P) caused a prompt efflux of each of the others (Fig. 2). Accordingly, the simplest interpretation of data given in Fig. 2 is that G6P (G3P)-grown cells have a P_i-linked antiport that uses either P_i, 2DG6P or G3P as a substrate. We also infer that this exchange is electroneutral, because ionophores (FCCP, valinomycin, valinomycin *plus* nigericin) did not significantly enhance or depress initial rates of substrate transport, nor did their delayed addition alter steady-state incorporation (three experiments as in Fig. 2, *data not shown*). Moreover, it is likely that antiport responds to 2DG6P with highest affinity, since 2DGGP was least effective in provoking net substrate efflux (and *see* Table 2, below).

Effect of a Proton-Motive Force

Accumulation of 2DG6P and G3P by P_i-loaded vesicles was consistent with the presence of P_i-linked antiport. However, because symport reactions may also catalyze substrate exchange, it could also be argued that P_i-linked antiport (Fig. 2) was mediated by nH$^+$/G6P symport which (for unknown reasons) operated only in an exchange mode in the "un-

Table 1. Substrate accumulation by MOPS/SO_4-loaded vesicles[a]

	$^{32}P_i$	[^{14}C]2DG6P
	(nmol/mg protein)	
Additions		
None	4.8 ± 1.2	0.8 ± 0.3
+ 33 mM DL-lactate	23.9 ± 4.9	3.0 ± 0.9
+ 0.1 mM KP_i	—	3.8 ± 1.1
+ 0.1 mM KP_i, 33 mM DL-lactate	—	21.9 ± 5.1

[a] Steady-state levels of substrate accumulation are reported as mean values ± SEM from 8 (P_i) or 5 (2DG6P) experiments as described in Figs. 3 and 4.

energized" state. To evaluate this alternative, it was necessary to examine sugar phosphate transport in the absence of internal P_i and for conditions that established a proton-motive force. Control experiments showed that such studies could be done using MOPS-loaded vesicles during the oxidation of DL-lactate. Assays of steady-state ^{86}Rb distributions in the presence of valinomycin [1] verified the presence of a membrane potential (−68 to −90 mV) for these conditions. And in other control experiments, it was shown that addition of DL-lactate allowed both P_i- and MOPS-loaded vesicles to accumulate L-histidine 12 to 15-fold above medium levels; no L-histidine accumulation was found in the presence of 5 μM FCCP (*data not shown*).

Having confirmed that MOPS-loaded vesicles sustain a proton-motive force, we studied both net P_i transport and P_i-linked exchanges in the presence of a driving ion-motive gradient. That work revealed a fundamental difference in the capacity of MOPS-loaded vesicles to transport the inorganic and organic substrates. Whereas oxidation of DL-lactate supported net transport and accumulation of P_i (to 45 nmol/mg protein), no significant accumulation of 2DG6P or G3P was found (Fig. 3; *see also* Table 1). These observations suggested that vesicles from G6P-grown cells had two distinct kinds of membrane carriers—a P_i transporter coupled to H$^+$ movements (perhaps electrogenic nH$^+$/P_i symport as in other bacteria [6, 10, 17]), and also an anion exchange that moves P_i and sugar phosphates independently of the H$^+$ circulation. This would reconcile the otherwise paradoxical findings of 2DG6P (and G3P) accumulation by P_i-loaded vesicles (Fig. 2), but not by the MOPS-loaded vesicles that transported other substrates (Fig. 3 and text).

If 2DG6P and G3P are accumulated by an exchange reaction, then MOPS-loaded vesicles should transport 2DG6P when a suitable internal counter-substrate is made available. To test this prediction, MOPS-loaded vesicles were preincubated with P_i and DL-lactate to establish an internal pool of P_i by H$^+$-coupled transport (Fig. 4*A*); only after this

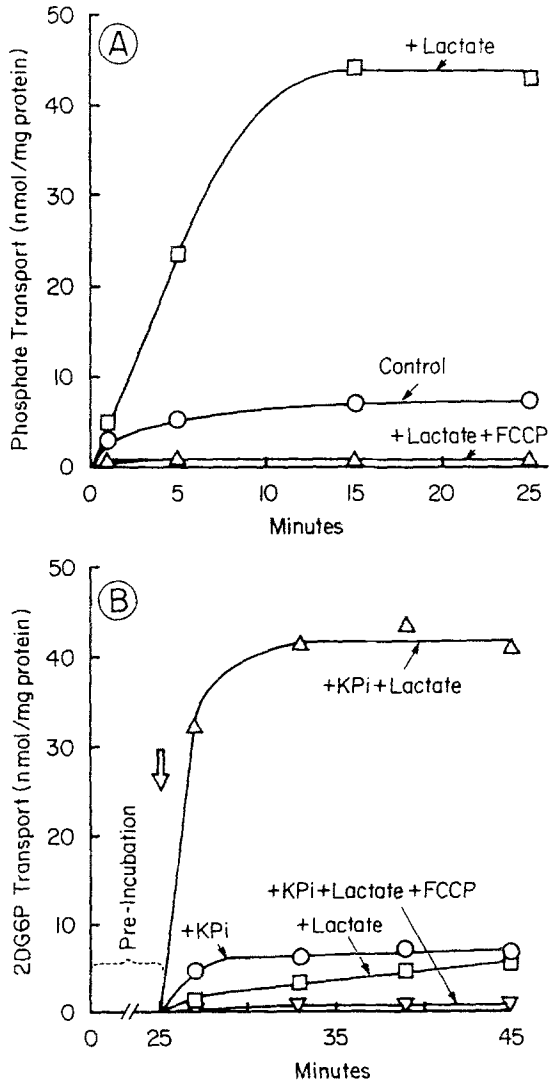

Fig. 4. Sugar phosphate accumulation requires internal P_i. MOPS-loaded vesicles made from G6P-grown cells of strain 8709 were suspended in Buffer A. (*A*) In the first part of the experiment 100 μM $^{32}P_i$ was added to vesicles which had received either no further additions (○), 33 mM DL-lactate (□), or 33 mM DL-lactate *plus* 1 μM carbonylcyanide-*p*-trifluromethoxy phenylhydrazone (FCCP) (△). (*B*) In the second part of the experiment 2DG6P (100 μM) was added *after* vesicles had been preincubated (25 min, 23°C) with 100 μM P_i (○), 100 μM P_i *plus* 33 mM DL-lactate (△), 33 mM DL-lactate alone (□), or 100 μM P_i *plus* 33 mM DL-lactate *plus* 1 μM FCCP (▽)

preincubation were vesicles presented with sugar phosphate (Fig. 4*B*; Table 1). Clearly, vesicles previously exposed to *both* P_i and DL-lactate accumulated sugar phosphate, while preincubation with either P_i or DL-lactate *alone* failed to support transport. Since 2DG6P (and G3P [data not given]) transport was found only when external P_i could be taken inside, this experiment gave direct confirmation of anion exchange by showing that 2DG6P and G3P accumulation depended on an appropriate *trans* substrate.

Table 2. Michaelis constants for P_i-linked exchange in proteoliposomes[a]

Growth supplement	Test substrate			
	P_i	2DG6P K_t (mM)	G3P	
G6P	2.2 ± 0.4 (3)	0.029 ± 0.003 (4)	1.0	(2)
G3P	—	0.025 ± 0.003 (3)	1.6	(2)
All trials	2.2 ± 0.4 (3)[b]	0.027 ± 0.002 (7)[b]	1.3 ± 0.2 (4)[b]	

[a] The Michaelis constant (K_t) for P_i, 2DG6P or G3P transport was determined for P_i-loaded proteoliposomes using material derived from cells grown with G3P or G6P (*see also* Fig. 5*B*). Data are given as mean values (±SEM) determined in the indicated number of experiments.

[b] Corresponding maximal velocities were 180 ± 15, 46 ± 7 and 39 ± 6 nmol/min per mg protein for P_i, 2DG6P and G3P transport, respectively.

Reconstitution of P_i-Linked Exchanges

For purposes of quantitative description, the anion exchange identified in membrane vesicles was reconstituted into proteoliposomes using a protocol established for the recovery of membrane transport reactions from *S. lactis* and *E. coli* [2, 4]. The experiment in Fig. 5*A* shows reconstitution of the heterologous P_i:2DG6P exchange from G6P-grown cells of *S. aureus*, strain 8709. P_i-loaded proteoliposomes of the wild-type strain, but not its fosfomycin-resistant derivative, took up either 2DG6P (Fig. 5*A*), G3P or $^{32}P_i$ (*data not shown*), and in each instance the accumulated material was promptly lost on addition of any one of the other compounds (Fig. 5*A* and other data, not given).

The technique of reconstitution was next used to study the kinetic parameters of P_i-linked exchange for cells grown with either G6P or G3P (Fig. 5*B*; Table 2). The maximal velocity of homologous $^{32}P_i$:P_i exchange was comparable for P_i-loaded vesicles (83 ± 15 nmol/min per mg protein; 6 experiments, *not shown*) and proteoliposomes (180 ± 15 nmol/min per mg protein, Table 2), indicating that the antiporter was not adversely affected by reconstitution using phospholipids from *E. coli* (*see* [4]). Moreover, the Michaelis constant (K_t) for $^{32}P_i$ transport into P_i-loaded proteoliposomes was the same as that estimated using either membrane vesicles (1.4 ± 0.2 mM, 6 experiments; *not shown*) or intact cells (1.6 mM [20]). The Michaelis constant for G3P transport was also in this range (K_t = 1.3 mM), but a lower value for K_t was derived for transport of

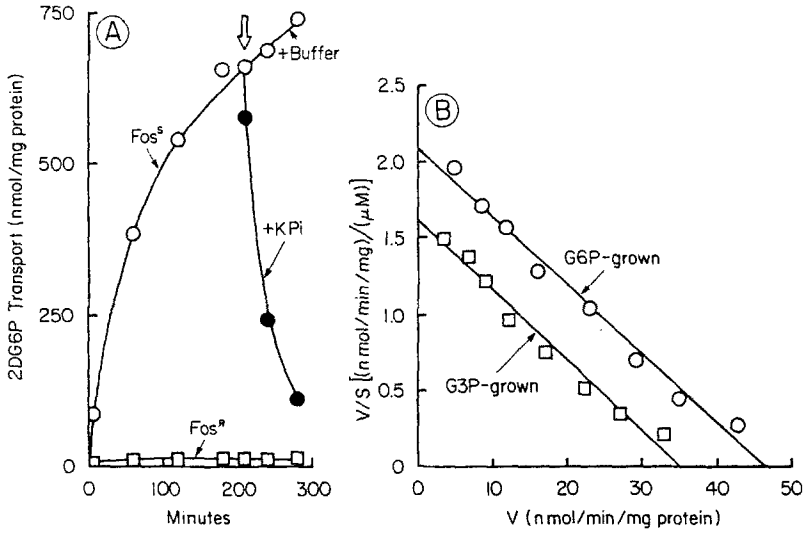

Fig. 5. Reconstitution of P_i-linked exchanges from *S. aureus*. (*A*) Membrane protein from G6P-grown cells of strain 8709 was used to prepare proteoliposomes loaded with 100 mM KP_i. Washed proteoliposomes were suspended in Buffer B before addition of 100 μM [^{14}C]-2DG6P. The arrow shows the time of addition of 5 mM KP_i. (*B*) In a separate experiment, initial rates of [^{14}C]-2DG6P transport were determined for P_i-loaded proteoliposomes made from cells grown in G6P (○) or G3P (□)

2DG6P, in both proteoliposomes (K_t = 0.027 mM) and vesicles (K_t = 0.037 mM, 2 experiments). This order of preference (G6P > G3P, P_i) was consistent with studies of efflux from P_i-loaded vesicles (Fig. 2 and text), and because similar values were found for both G6P- and G3P-grown cells (Fig. 5*B*), it is probable that the same antiporter is induced by growth with either organophosphate.

Discussion

The work described here confirms the earlier observation [19–21] of a P_i exchange in *S. aureus* and extends this finding to demonstrate that such exchange is mediated by an inducible and electroneutral P_i-linked antiport that has high specificity for sugar 6-phosphate. This conclusion is supported by experiments using membrane vesicles as well as proteoliposomes, each of which was able to take up and sustain high gradients of P_i, 2DG6P or G3P so long as there was a suitable internal countersubstrate. Perhaps most informative were studies with vesicles made in the absence of P_i. In response to DL-lactate oxidation, MOPS-loaded vesicles generated a membrane potential and accumulated both L-histidine and $^{32}P_i$ (Figs. 3, 4 and text). Nevertheless, these vesicles could not transport 2DG6P or G3P (Fig. 3) *until* P_i had been placed inside (Fig. 4). This *trans* stimulation is not expected of ion-coupled symport (nH^+/2DG6P symport), but it is an obvious part of any exchange mechanism (P_i : 2DG6P antiport).

Anion exchange in *S. aureus* has features in common with the P_i-linked antiporters recently identified in *E. coli* and *S. lactis*. In regard to substrate specificity, for example, antiport in *S. aureus* resembles the *uhpT*-encoded system of *E. coli*—each system prefers G6P and has a rather low affinity for P_i and G3P [2]. This contrasts with substrate specificity for the streptococcal exchange, which does not recognize G3P [18], and with the *E. coli* *glpT*-encoded antiport, which shows high affinity for G3P and no response to sugar 6-phosphate [2, 8]. In other respects, however, the staphylococcal exchange compares favorably with its streptococcal counterpart. Thus, in *S. aureus* the kinetic study in proteoliposomes (Table 2) gave a 4 : 1 ratio for maximal velocities of homologous (P_i : P_i) and heterologous (P_i : 2DG6P) exchanges; this 4 : 1 ratio is also characteristic of the exchange reaction in *S. lactis* [4]. And with respect to ionic selectivity, it is worth noting that in both *S. aureus* [20] and *S. lactis* [5, 18] antiport uses only monovalent P_i during the homologous $^{32}P_i$: P_i exchange. This unusual selectivity has important implications for exchange stoichiometry and kinetic mechanism as well as for physiological function [5, 16, 17].

In several ways, understanding P_i transport and exchange in *S. aureus* helps to clarify general relationships among P_i and sugar phosphate transport systems in bacteria (Fig. 6). For example, early studies with P_i-loaded cells or membrane vesicles of *E. coli* were interpreted to support the idea of nH^+/G6P symport [9, 14]. While this interpretation is not correct [16, 17, 23], its origin is understandable, since G6P accumulation can be linked indirectly to H^+ movements *via* a P_i circulation (Fig. 6; [3]). We have presumed that such a recycling of P_i is responsible for the increased accumulation of sugar phosphate observed when P_i-loaded vesicles are given DL-lactate ([7]; *data not shown*). Analysis of the

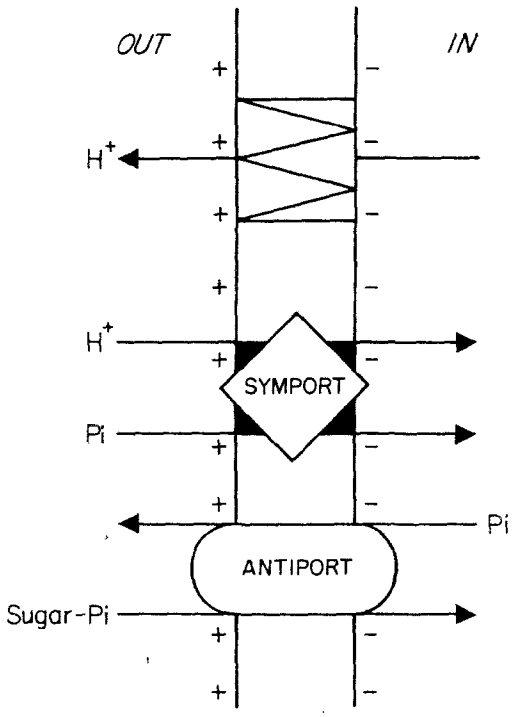

Fig. 6. Physiological relationships among P_i and sugar phosphate transporters in *S. aureus*. *See* text for details

interactions of these systems (Fig. 6) is made all the more complex by their apparently different responses to a proton-motive force. Thus, P_i-linked antiport operates independently of external ion-motive gradients (Figs. 2 and 5), yet nH^+/P_i symport seems to function only if a proton-motive force (or a membrane potential) exists. This behavior is suggested by the fact that net P_i efflux did not occur when vesicles loaded with 50 mM KP_i were suspended in 0.1 mM KP_i, even when proton conducting ionophores were present (Fig. 2 and *data not shown*). Regulation of this sort also appears to occur in the resting cell, since in *E. coli* [8], *S. lactis* [18] and *S. aureus* [this work], all $^{32}P_i$ transport is mediated by systems normally dedicated to transport of organphosphates. Indeed, with classification of the exchange reaction in *S. aureus*, all known examples of $^{32}P_i : P_i$ exchange may now be attributed to members in the family of P_i-linked antiport [2, 5, 8, 16–18]. As a result, we suggest that in the absence of metabolic activity, $^{32}P_i$ movement across bacterial membranes will be diagnostic of P_i-linked exchange.

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