Translocation of Metal Phosphate via the Phosphate Inorganic Transport System of *Escherichia coli*†

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ABSTRACT: P_i transport via the phosphate inorganic transport system (Pit) of *Escherichia coli* was studied in natural and artificial membranes. P_i uptake via Pit is dependent on the presence of divalent cations, like Mg^{2+}, Ca^{2+}, Co^{2+}, or Mn^{2+}, which form a soluble, neutral metal phosphate ($MeHPO_4$) complex. P_i-dependent uptake of Mg^{2+} and Ca^{2+}, equimolar cotransport of P_i and Ca^{2+}, and inhibition by Mg^{2+} of Ca^{2+} uptake in the presence of P_i, but not of P_i uptake in the presence of Ca^{2+}, indicate that a metal phosphate complex is the transported solute. Metal phosphate is transported in symport with H^+ with a mechanistic stoichiometry of 1. Pit mediates efflux and homologous exchange of metal phosphate, but not heterologous metal phosphate exchange with P_i, glycerol-3P, or glucose-6P. The metal phosphate efflux rate increased with pH, whereas the rate of metal phosphate exchange was essentially pH independent. Metal phosphate uptake was inhibited at low internal pH. Efflux was inhibited by a proton motive force (interior negative and alkaline), whereas exchange was inhibited by the membrane potential only. These results have been evaluated in terms of ordered binding and dissociation of metal phosphate and proton on the outer and inner surface of the cytoplasmic membrane.

Escherichia coli possesses four systems via which P_i can enter the cell (Rosenberg, 1987). The two major systems, the phosphate-specific transport system (Pst) and the phosphate inorganic transport system (Pit) are highly specific for P_i and were originally described by Medveczky and Rosenberg (1971) and by Willsky et al. (1973). In addition, two transport systems, designated GlpT and UhpT, accept P_i as a low-affinity analog of glycerol-3P (Hayashi et al., 1964) and glucose-6P (Winkler, 1966; Pogell et al., 1966), respectively. The Pst system is an inducible, periplasmic binding protein-dependent solute ATPase that accumulates $H_2PO_4^-$ and HPO_4^{2-} at the expense of ATP (Gerdes et al., 1977; Rosenberg et al., 1979; Bishop et al., 1989; Luecke & Quiocho, 1990). Pit, GlpT, and UhpT are chemiosmotic carriers. GlpT and UhpT belong to a family of P_i-linked antiporters which are induced in the presence of the phosphorylated solute and which mediate electroneutral exchange of $H_2PO_4^-$, organic phosphate anions, or both. P_i-linked exchange carriers of *E. coli*, *Lactococcus lactis*, and *Staphylococcus aureus* have been well characterized in cells, membrane vesicles, and reconstituted systems [for review, see Maloney et al. (1990)]. Pit has received much less attention over the past 20 years. Studies in the 1970s and early 1980s with wild-type cells (Medveczky & Rosenberg, 1971; Willsky & Malamy, 1980), Pst-deficient mutants (Rosenberg et al., 1977, 1979), and membrane vesicles (Konings & Rosenberg, 1978) of *E. coli* indicated that Pit is a constitutive system which probably catalyzes an electrogenic $n H^+/P_i$ symport. Proton motive force (Δp)[1]-driven uptake of P_i was not observed in membrane vesicles prepared from the Pit-deficient *E. coli* strain K-10 (Konings & Rosenberg, 1978).

Recent studies on P_i transport in *Acinetobacter johnsonii* 210A revealed the presence of two transport systems which show a strong analogy with the Pst and Pit system of *E. coli* (Van Veen et al., 1993a). Experiments aimed to clarify the mechanism of the secondary P_i transport system of *A. johnsonii* 210A point to an electrogenic symport of a proton and a neutral metal phosphate ($MeHPO_4$) chelate which is formed by complexation of divalent metal ions and P_i (Van Veen et al., 1993b). In view of the apparent similarities between P_i transport in *A. johnsonii* 210A and *E. coli*, these results have led us to reevaluate and reexamine P_i transport via Pit. The substrate specificity and mechanism of Pit were characterized in membrane vesicles and proteoliposomes in which the transport protein was successfully reconstituted. In this paper, evidence will be presented for an electrogenic metal phosphate/proton symport mechanism. The effects of pH and Δp on the different modes of metal phosphate transport via Pit are consistent with the ordered binding model which was recently put forward for the secondary phosphate transport system of *A. johnsonii* 210A (Van Veen et al., 1993b).

EXPERIMENTAL PROCEDURES

Membrane Vesicles and Proteoliposomes. Cells of *E. coli* K-12 strain PC 1012[2] (*pit*$^+$ *pst*$^+$) were grown aerobically at

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[1] Abbreviations: Δp, protonmotive force; metal phosphate, $MeHPO_4$; CCCP, carbonyl cyanide (3-chlorophenyl)hydrazone; $Z\Delta$pH, transmembrane pH gradient (in mV); $\Delta\psi$, membrane potential; $Z\Delta u_{MeHPO4}$, transmembrane metal phosphate concentration gradient (in mV); Hepes, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); PQQ, 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid.

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37 °C to an A_{660} of 0.6 in minimal glucose medium (Gerdes & Rosenberg, 1974) supplemented with 5 mM sodium phosphate to repress the synthesis of the Pst system. Membrane vesicles were prepared as described (Kaback, 1971) in 10 mM potassium Pipes (pH 7.0) containing 0.1 mM $MgSO_4$ and finally suspended in 50 mM potassium Pipes (pH 7.0) supplemented with 10 mM $MgSO_4$. Solubilization of membrane vesicles with *n*-octyl β-glucopyranoside and reconstitution of membrane proteins by detergent dilution were performed by the procedures described (In 't Veld et al., 1992; Van Veen et al., 1993b).

Transport Assays. Δ*p*-driven uptake of [^{32}P]P_i in membrane vesicles and the determination of the membrane potential (Δψ) using a tetraphenylphosphonium ion-selective electrode (Shinbo et al., 1978) were performed as described (Van Veen et al., 1993a,b) in 50 mM potassium Pipes (pH 7.0) or 20 mM potassium Mes–Pipes–Hepes (pH 6.0–8.0) (MPH buffer), supplemented with 10 mM $MgSO_4$. The procedure for uptake of [^{32}P]P_i or $^{45}Ca^{2+}$ in proteoliposomes driven by an artificial Δψ and/or pH gradient (ΔpH) was essentially as described previously (Van Veen et al., 1993b) using sodium Pipes-based buffers, pH 7.0, for dilution. For metal phosphate efflux and exchange, proteoliposomes in 20 mM potassium Pipes (pH 7.0) containing 100 mM potassium acetate (PPA buffer) or MPH containing 100 mM potassium acetate (MPHA buffer) were preloaded for 3 h at 20 °C with [^{32}P]P_i and divalent cations as specified in the legends to figures. Loaded proteoliposomes were subsequently diluted 100-fold into buffer [PPA plus 20 μM carbonyl cyanide (3-chlorophenyl)hydrazone (CCCP), MPHA plus 20 μM CCCP, or the appropriate buffers to impose artificial diffusion gradients] without P_i (efflux), with an equimolar concentration of nonlabeled potassium phosphate (homologous exchange), or with organic phosphate anions (heterologous exchange). Dilution buffers were supplemented with divalent cations as described in the legends to figures. Transport of [^{32}P]P_i (1.7 TBq/mol) and $^{45}Ca^{2+}$ (2.1 TBq/mol) was assayed at 30 °C by the filtration method (Kaback, 1974). [^{32}P]P_i (carrier-free) and $^{45}CaCl_2$ (14.8 TBq/mol) were purchased from Amersham, United Kingdom. Uptake of Mg^{2+} was monitored at 30 °C in proteoliposomes loaded with 5 mM Mag-Quin-2 by freeze–thaw sonication (Driessen et al., 1985). The fluorescence intensity at 490 nm was measured at an excitation of 335 nm with slit widths of 4 and 7.5 nm, respectively. Mag-Quin-2 was obtained from Molecular Probes, Inc., Eugene, OR.

RESULTS

Kinetic Analysis of P_i Uptake. P_i was accumulated about 30-fold in membrane vesicles from *E. coli* suspended in Pipes buffer when a Δ*p* of −109 mV was established by oxidation of glucose via the membrane-bound, pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase. Initial rates of Δ*p*-driven uptake were determined from time points taken during the first 60 s of linear uptake of P_i between 1 and 300 μM. Kinetic analysis using Lineweaver–Burk plots revealed the presence of one P_i transport system with an apparent K_t of 11.9 μM and a V_{max} of 0.74 nmol/(min·mg of protein) (data not shown). This K_t corresponds well with the reported K_t of 9.2 μM for the Pit system in cells (Medveczky & Rosenberg, 1971).

Substrate Specificity. Divalent cations are required for binding of apo-glucose dehydrogenase to its prosthetic group PQQ and may influence the magnitude or composition of the Δ*p* generated by glucose oxidation (Van Schie et al., 1987). Such an influence of divalent cations on the stability and magnitude of an artificially imposed Δ*p* in proteoliposomes was not observed (Van Veen et al., 1993b). However, uptake of 50 μM P_i, driven by an imposed Δ*p* in proteoliposomes in which Pit protein was reconstituted, was inhibited to equilibration levels in the presence of 0.5 mM EDTA. Furthermore, the rate of P_i uptake was stimulated from 1.4 nmol/(min·mg of protein) in the absence of added cations to 3.7, 4.0, 6.9, and 7.5 nmol/(min·mg of protein) in the presence of 2 mM Mg^{2+}, Ca^{2+}, Co^{2+}, or Mn^{2+}, respectively. These cations form a soluble, electroneutral metal phosphate complex ($MeHPO_4$) with 31% (Ca^{2+}), 36% (Mg^{2+}), 70% (Co^{2+}), and 87% (Mn^{2+}) of the P_i present in the incubation (Sillén & Martell, 1964). This complexation could be detected with the Mg^{2+} indicator Mag-Quin-2 which undergoes a fluorescence excitation intensity enhancement upon binding of Mg^{2+}. Addition of 100 mM P_i to a solution of 520 μM probe and 400 μM Mg^{2+} at pH 7.0 resulted in a 4-fold decrease of the fluorescence intensity, corresponding to a decrease of the Mg^{2+}/probe complex concentration from 130 to 18 μM, due to the formation of about 350 μM $MgHPO_4$ (data not shown). In view of the extensive complexation of P_i and Me^{2+}, the strict metal dependency of P_i uptake may be interpreted as the translocation of metal phosphate rather than P_i. This was investigated by measuring the uptake of Mg^{2+} and Ca^{2+} in the presence and absence of P_i. Mg^{2+} transport was monitored in proteoliposomes containing Mag-Quin-2. A significant increase in fluorescence intensity due to uptake of Mg^{2+} was only observed in the presence of a Δψ (inside negative) when P_i was present in the incubation mixture (Figure 1). Similar results were obtained for Ca^{2+} uptake. The rate of Δ*p*-driven uptake of $^{45}Ca^{2+}$ in proteoliposomes was low in P_i-free buffer (Figure 2). In the presence of P_i a considerably higher rate of Ca^{2+} uptake was observed. [^{32}P]P_i uptake was measured under identical conditions. The same initial rate and steady-state level of accumulation of P_i as Ca^{2+} was found, suggesting a Ca^{2+}/P_i ratio of 1. Ca^{2+} uptake but not P_i uptake was strongly inhibited by excess Mg^{2+}. These uptake experiments provide strong evidence for the transport of Ca^{2+} or Mg^{2+} via a metal phosphate complex. The substrate specificity of Pit was further studied in efflux and exchange experiments under conditions in which no Δ*p* was imposed. At pH 7.0, the efflux of ^{32}P-labeled magnesium phosphate from proteoliposomes was stimulated by the addition of an equimolar amount of

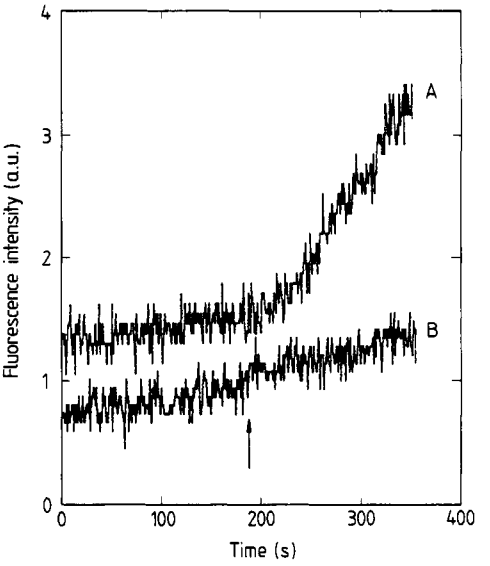

FIGURE 1: Time course of the fluorescence emission of Mag-Quin-2-loaded proteoliposomes representing the uptake of 200 μM Mg^{2+} in the presence (A) and absence (B) of 5.4 mM P_i. At the arrow, 1 nmol of valinomycin/mg of protein was added to impose a potassium diffusion potential (interior negative) of −138 mV.

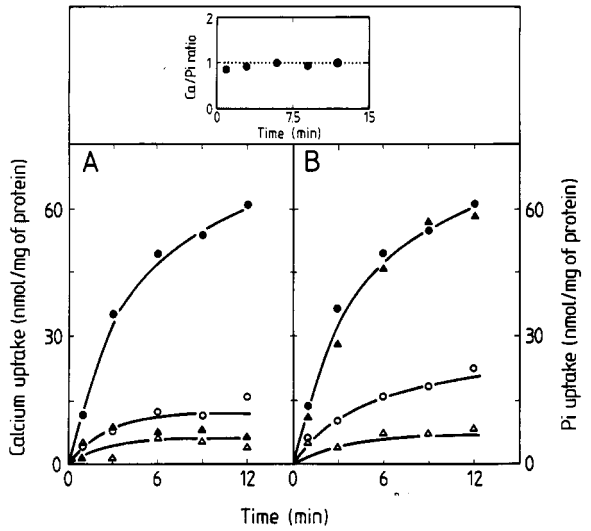

FIGURE 2: Δp-driven uptake of Ca^{2+}, P_i, and $CaHPO_4$ in proteoliposomes. Uptake of 100 μM $^{45}Ca^{2+}$ (A) or 100 μM [^{32}P]P_i (B) (○); uptake of 100 μM $^{45}Ca^{2+}$ plus 100 μM $^{45}CaHPO_4$ (A) or 100 μM [^{32}P]P_i plus 100 μM $CaH^{32}PO_4$ (B) in the absence (●) and presence of 10 mM Mg^{2+} (▲) or 0.5 mM EDTA (△). The Ca/P_i ratio for uptake of $CaHPO_4$ (●, panels A and B) is presented in the inset.

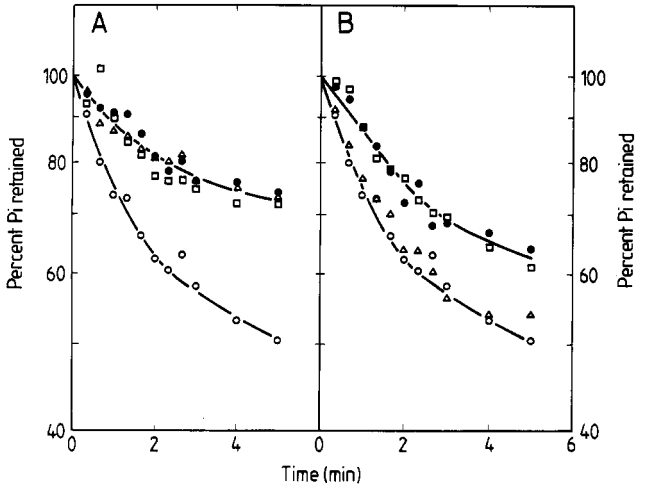

FIGURE 3: Efflux and exchange via Pit. Proteoliposomes in PPA buffer were loaded with 50 μM [^{32}P]P_i and 2 mM $MgSO_4$. Exit of [^{32}P]P_i was monitored following 100-fold dilution of a suspension into PPA buffer containing 20 μM CCCP and the following additions: (A) 2 mM Mg^{2+} in the absence (●) and presence of 50 μM P_i (○), 10 mM glycerol-3P (△), or glucose-6P (□); (B) 0.5 mM EDTA in the absence (●) and presence of 50 μM P_i (□), or 50 μM P_i in the presence of 2 mM Mn^{2+} (△) or 2 mM Mg^{2+} (○).

nonlabeled magnesium or manganese phosphate to the external medium (Figure 3). This stimulation was not observed in the presence of a 200-fold excess of glycerol-3P or glucose-6P (Figure 3A) or an equimolar amount of P_i plus 0.5 mM EDTA (Figure 3B). Besides uptake of metal phosphate, Pit obviously mediates efflux and homologous exchange of metal phosphate, the latter reaction being faster at pH 7.0, but not heterologous exchange of metal phosphate and P_i, glycerol-3P, or glucose-6P.

Energy Coupling to Metal Phosphate Uptake. The driving force for uptake of metal phosphate was analyzed in proteoliposomes which were subjected to artificial gradients of protons and/or potassium ions. In the presence of a $\Delta\psi$ of −120 mV or a $-Z\Delta$pH of −120 mV, metal phosphate (50 μM P_i plus 10 mM Mg^{2+}) was taken up at a rate of 1.6 and 3.5 nmol/(min·mg of protein), respectively. The effects of $\Delta\psi$ and ΔpH were additive. Imposition of a Δp of −240 mV resulted in metal phosphate uptake at a rate of 4.8 nmol/(min·mg of protein). In the absence of a gradient, no metal phosphate uptake was observed. The metal phosphate/proton stoichiometry was determined in membrane vesicles at pH 7.8, from the steady-state accumulation level of metal phosphate (50 μM P_i plus 10 mM Mg^{2+}) in the presence of 0.1 nmol of nigericin/mg of protein ($\Delta p = \Delta\psi$). The $\Delta\psi$ was varied by titration with 0.01–0.1 nmol of valinomycin/mg of protein. At thermodynamic equilibrium in the absence of a ΔpH, n equals $-Z\Delta\bar{u}_{MeHPO4}/\Delta\psi$, in which n represents the number of protons translocated in symport with metal phosphate and $Z\Delta\bar{u}_{MeHPO4}$ represents the transmembrane metal phosphate concentration gradient. A linear relationship was observed between $-Z\Delta\bar{u}_{MeHPO4}$ and the $\Delta\psi$ in which n was 0.91, indicating a symport of a neutral $MeHPO_4$ complex and one proton (data not shown).

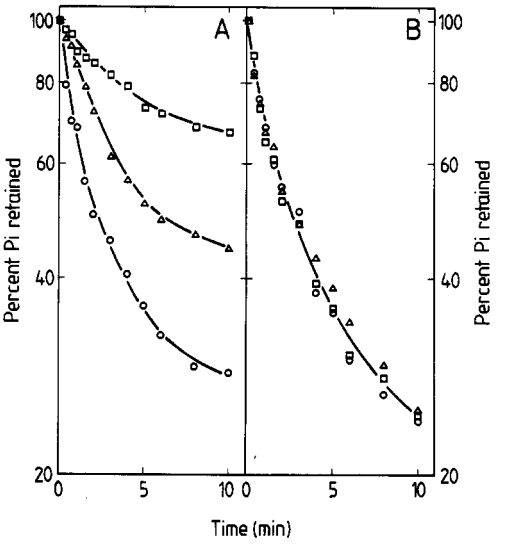

FIGURE 4: Effect of pH on metal phosphate efflux and exchange. Proteoliposomes were equilibrated in MPHA buffer [pH 6.3 (□), 7.0 (△), or 7.7 (○)] containing 150 μM [^{32}P]P_i and 10 mM Mg^{2+} and subsequently diluted 100-fold into MPHA buffer of the same pH containing 10 mM Mg^{2+} and 20 μM CCCP without (A, efflux) or with 150 μM P_i (B, exchange).

Effect of pH and Δp on Metal Phosphate Efflux and Exchange. The effect of pH on metal phosphate efflux and exchange was measured in proteoliposomes equilibrated in the presence of 150 μM P_i and 10 mM $MgSO_4$. Metal phosphate efflux and exchange occurred with pseudo-first-order kinetics (Figure 4). The metal phosphate efflux rate increased as a function of pH [half-times ($t_{1/2}$) of 9.4, 4.3, and 1.9 min at pH 6.3, 7.0, and 7.7, respectively] whereas the metal phosphate exchange rate was essentially pH independent ($t_{1/2}$ = 1.8 min). At pH values below 7.7, metal phosphate efflux was slower than metal phosphate exchange. Apparently, the release of a proton at the outer surface of the membrane is rate-limiting for metal phosphate efflux whereas in the exchange process no release of a proton is needed. In an analogous series of experiments, the effects of artificially imposed ion gradients on metal phosphate efflux and exchange were monitored. Imposition of a $\Delta\psi$ of −120 mV retarded the efflux rate at pH 7.0 by a factor of 3 ($t_{1/2}$ increased from 3.4 to 9.6 min) (Figure 5A). Imposition of a $-Z\Delta$pH of −120 mV resulted in a 2-fold inhibition of the rate of metal phosphate release ($t_{1/2}$ = 6.7 min). The effects of a $\Delta\psi$ and ΔpH on metal phosphate efflux were additive. Imposition of a Δp of −240 mV retarded metal phosphate efflux more than 6-fold ($t_{1/2}$ = 23.2 min). The inhibition of metal phosphate efflux by a Δp is consistent with an electrogenic proton symport mechanism. Metal phosphate exchange was not affected by a ΔpH (Figure 5B) but was retarded by the presence of a $\Delta\psi$

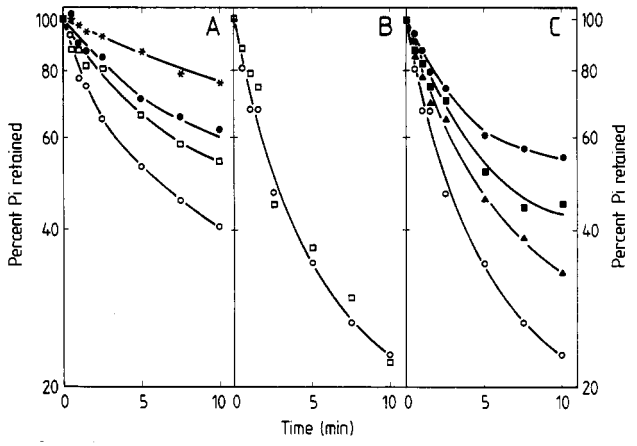

FIGURE 5: Effect of Δp on metal phosphate efflux and exchange. Proteoliposomes were equilibrated in PPA buffer containing 150 μM [^{32}P]P_i and 10 mM Mg^{2+} and diluted 100-fold into the appropriate buffers to study efflux (A) and exchange (B, C) in the presence of a $\Delta\psi$ of −40 mV (▲), −80 mV (■), or −120 mV (●), a $-Z\Delta pH$ of −120 mV (□), or a Δp of −240 mV (*), or in the absence of imposed diffusion gradients in the presence of 20 μM CCCP (○).

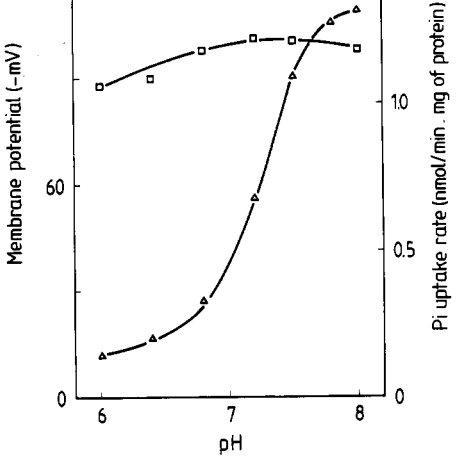

FIGURE 6: Effect of pH on Δp-driven uptake of metal phosphate. Membrane vesicles in MPH buffer were energized by glucose oxidation in the presence of 0.1 nmol of nigericin/mg of protein [$\Delta p = \Delta\psi$ (□)]. The metal phosphate uptake rate (△) was determined at P_i and Mg^{2+} concentrations of 120 μM and 10 mM, respectively.

(Figure 5C) ($t_{1/2}$ increased from 1.8 min in the absence of a $\Delta\psi$ to 3.0, 4.0, and 4.9 min in the presence of a $\Delta\psi$ of −40, −80, and −120 mV, respectively).

Effect of pH on Δp-Driven Uptake of Metal Phosphate. Deprotonation of the carrier on the outside of the membrane is affected by the external pH and was found to limit the rate of metal phosphate efflux (Figure 4). Under uptake conditions, the release of metal phosphate and proton occurs on the inside of the membrane. Internal pH effects on metal phosphate uptake may be anticipated. Δp-driven uptake of metal phosphate was studied as a function of the pH in membrane vesicles in which the ΔpH was dissipated by the addition of nigericin. The $\Delta\psi$ remained fairly constant when the pH was lowered from 8.0 to 6.0 while the metal phosphate uptake rate fell at least 7-fold with an apparent pK of 7.2 (Figure 6).

DISCUSSION

The results lead us to conclude that Pit mediates Δp-driven uptake of metal phosphate. This conclusion is supported by the demonstration of (i) extensive complexation of P_i and Me^{2+} into a neutral $MeHPO_4$ complex, (ii) equimolar cotransport of Me^{2+} and P_i, (iii) electrogenic proton/metal phosphate symport, and (iv) a metal phosphate/proton stoichiometry of 1. In addition, Pit mediates efflux and homologous exchange of metal phosphate but not heterologous exchange of metal phosphate and P_i, glucose-6P, or glycerol-3P. The absence of the heterologous exchange reactions is consistent with the specificity of Pit for metal phosphate and shows the lack of interference of anion-exchange reactions catalyzed by GlpT and UhpT in this study. In the kinetic experiments about 74% of the P_i was present as a $MgHPO_4$ complex (Sillén & Martell, 1964). A reevaluation of the data reveals a corrected K_t for $MgHPO_4$ of 8.8 μM. This K_t value corresponds well to that previously determined for the P_i carrier of *A. johnsonii* 210A (Van Veen et al., 1993b). Because of the almost identical pH dependence of $MeHPO_4$ and HPO_4^{2-} in aqueous solutions, it is now understandable why earlier studies had pointed out HPO_4^{2-} as the physiological substrate of Pit (Rosenberg et al., 1984; Rosenberg, 1987). With hindsight, we could say that the previously observed Mg^{2+} dependence of Pit function in cells (Medveczky & Rosenberg, 1971; Rae & Strickland, 1976) and membrane vesicles (Konings & Rosenberg, 1978) reflected the translocation of $MgHPO_4$ via this system.

Although solute exchange in the absence of a driving force has been shown for various solute/proton symporters [*e.g.*, LacY (Kaczorowski & Kaback, 1979; Kaczorowski et al., 1979)], it was the general perception in literature that Pit does not carry out exchange (Elvin et al., 1985; Ambudkar et al., 1986; Rosenberg, 1987; Sonna et al., 1988). However, this reaction may have remained unnoticed in previous studies because (i) the substrate is metal phosphate rather than P_i, (ii) metal phosphate exchange is inhibited by a $\Delta\psi$ and will therefore be difficult to detect in cells with a high $\Delta\psi$, and (iii) the maximal velocity of metal phosphate exchange in membrane vesicles (Van Veen et al., 1993b) is at least 2 orders of magnitude lower than that of homologous P_i exchange via the UhpT or GlpT system in fully induced cells or in its membrane vesicles (Maloney et al., 1990).

Metal phosphate transport via Pit can be considered as a cyclic process in which specific steps are affected by the pH and the Δp. During efflux, the process involves protonation of the carrier protein on the inner surface of the membrane, followed by binding of metal phosphate to generate the ternary carrier/proton/metal phosphate complex. A conformational transition leads to the exposure of binding sites to the outer surface of the membrane. Proton and metal phosphate release from the carrier protein is again sequential, but in the reverse order, the metal phosphate being released first. Finally, a conformational change of the empty carrier restores the initial orientation of the binding sites. During metal phosphate exchange the carrier recycles via the ternary complex without being deprotonated.

The deprotonation of the carrier protein on the outer surface of the membrane is rate-limiting for metal phosphate efflux and is affected by the external pH. In analogy with efflux, metal phosphate uptake appears to be limited by the rate of deprotonation of the carrier on the inside of the membrane. As a result, metal phosphate uptake is strongly inhibited by a low internal pH. For optimal function of Pit in cells it is therefore essential to maintain a constant alkaline pH in the cytosol. In bacteria, electrogenic uptake of K^+, accompanied by expulsion of H^+ from the cytoplasm, is an important mechanism for alkalinization of the cell's interior (Bakker, 1993). Evidence confirming the relevance of this process for phosphate transport via Pit came from the work of Russell and Rosenberg (1979, 1980), who demonstrated that although

potassium ions greatly stimulate Pit function in *E. coli* cells, the transport of K^+ and phosphate is linked indirectly via proton circulation.

The ordered binding model proposed for Pit shows analogies to those suggested for the LacY (Kaczorowski & Kaback, 1979; Kaczorowski et al., 1979; Kaback, 1990) and melibiose carrier of *E. coli* (Bassilana et al., 1987; Pourcher et al., 1990). Unlike exchange via LacY, metal phosphate exchange is inhibited by the $\Delta\psi$ (interior negative). This inhibition may result from a decrease of the translocation rate of a positively charged ternary complex across the membrane and/or an increase in the rate of dissociation of the cosubstrates from the carrier at the inner surface of the membrane as was proposed for the melibiose carrier.

The finding of metal phosphate/proton symport in *E. coli* (this work) and *A. johnsonii* 210A (Van Veen et al., 1993b) suggests that the transport of metal phosphates may be a general mechanism for the transport of divalent metal ions and P_i in bacteria. In *Micrococcus lysodeikticus* (Friedberg, 1977), *Acinetobacter lwoffi* (Yashphe et al., 1992), *Pseudomonas aeruginosa* (Lacoste et al., 1981), and *Bacillus cereus* (Rosenberg et al., 1969), P_i transport in general or via a Pit-like system in particular was reported to be stimulated by Mg^{2+}. In addition, in some studies a stimulation was observed of Me^{2+} transport by P_i, *e.g.*, the uptake of Mn^{2+} in *Lactobacillus plantarum* (Archibald & Dong, 1984), and of Mg^{2+}, Ca^{2+}, Mn^{2+}, and Co^{2+} via a general divalent cation transport system in *Bacillus subtilis* (Kay & Ghei, 1981). Interestingly, a Pit mutant of this latter organism was strongly impaired in the transport of Ca^{2+} and Co^{2+}. The mutant still elicited significant Mn^{2+} transport as a result of uptake via a second Mn^{2+}-specific high-affinity uptake system (Kay & Ghei, 1981). In many other studies on metal transport, the use of phosphate buffers may have masked P_i-dependent uptake of divalent cations. The mechanisms for Ca^{2+} entry in bacteria are unclear (Silver & Lusk, 1987; Lynn & Rosen, 1987). The transport of metal phosphate, including calcium phosphate, via Pit provides *E. coli* with such a mechanism.

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