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Dependence of *Streptococcus lactis* Phosphate Transport on Internal Phosphate Concentration and Internal pH

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Uptake of phosphate by *Streptococcus lactis* ML3 proceeds in the absence of a proton motive force, but requires the synthesis of ATP by either arginine or lactose metabolism. The appearance of free P_i internally in arginine-metabolizing cells corresponded quantitatively with the disappearance of extracellular phosphate. Phosphate transport was essentially unidirectional, and phosphate concentration gradients of up to 10^5 could be established. Substrate specificity studies of the transport system indicated no preference for either mono- or divalent phosphate anion. The activity of the phosphate transport system was affected by the intracellular P_i concentration by a feedback inhibition mechanism. Uncouplers and ionophores which dissipate the pH gradient across the cytoplasmic membrane inhibited phosphate transport at acidic but not at alkaline pH values, indicating that transport activity is regulated by the internal proton concentration. Phosphate uptake driven by arginine metabolism increased with the intracellular pH with a pK_a of 7.3. Differences in transport activity with arginine and lactose as energy sources are discussed.

The mechanisms of energy coupling and regulation of phosphate transport have been studied extensively in *Escherichia coli* (10, 23, 24). Phosphate transport in this organism is mediated predominantly by two systems, (i) Pit, a constitutive phosphate transport system which is driven by the proton motive force (10, 23, 24), and (ii) Pst, an inducible binding-protein-dependent transport system which is driven by phosphate-bond energy (23, 25). Recently, the transport systems for *sn*-glycerol 3-phosphate and hexose phosphate, encoded by the *glpT* and *uhpT* genes, respectively, have also been implicated in phosphate transport in *E. coli* (1, 7). Transport of these anions has been shown to occur in symport with protons as well as by exchange with P_i (1, 7). Moreover, these transport systems catalyze a reversible $^{32}P_i$-P_i exchange. A similar anion antiport system has been described for *Streptococcus lactis* 7962 (14). This system, studied both in intact cells and in membrane vesicles, catalyzes the homologous exchange of phosphate and the heterologous exchange of phosphate and sugar 6-phosphates (2, 3). With the exception of *Streptococcus cremoris* E8, the anion exchange activity has not been found in several other related streptococci, including *Streptococcus faecalis* (13, 14). In *Streptococcus pyogenes*, however, a transport system has been described which catalyzes homologous phosphate exchange but not heterologous exchange between phosphate and organic phosphate esters (21). Some evidence was presented indicating that phosphate exchange in *S. pyogenes* is regulated by the intracellular concentrations of ATP and phosphate. Experiments performed with *S. lactis* 7962 cells suggested the presence of a phosphate transport system, in addition to the phosphate/sugar 6-phosphate antiporter, which facilitates phosphate uptake with a rate similar to that of the exchange reaction (14). This system has not yet been studied. P_i transport has been analyzed in *S. faecalis* (8). In this organism, phosphate translocation is an electroneutral process which is coupled not directly to the proton motive force but rather to ATP or another energy-rich phosphorylated intermediate. In the present investigation, phosphate transport was studied in the "starter" organism *S. lactis* ML3, a well-characterized strain with several properties different from those of *S. lactis* 7962 (26). The studies were prompted by the lack of data about phosphate transport and the regulation of the cytoplasmic concentration of P_i in the starter streptococci used in milk fermentations. Information about these processes is important in view of the role of P_i in regulating (at various levels) carbohydrate metabolism in these organisms (20, 27). The diversity of phosphate transport mechanisms in the streptococci investigated thus far does not, at first glance, allow a prediction about the corresponding transport mechanism(s) in the starter streptococci. The results indicate that uptake of phosphate by *S. lactis* ML3 is driven directly by phosphate-bond energy, as has been proposed for *S. faecalis* (10) and also for transport of glutamate-glutamine by *S. lactis* and *S. cremoris* (17, 18). The activity of the phosphate transport system appears to be under the control of the internal phosphate and proton concentration.

MATERIALS AND METHODS

Growth conditions. *S. lactis* ML3 was grown to stationary phase in a complex medium (MRS; pH 6.4) (5), containing 1.0% (wt/vol) galactose and 10 mM arginine (18). *S. cremoris* Wg2 was grown on MRS medium (pH 6.4) containing 1.0% (wt/vol) lactose. Cells were harvested by centrifugation, washed, and incubated for 30 min at room temperature with 1 mM methyl-1-thio-β-D-galactopyranoside (TMG). Organisms were treated with TMG to deplete the cells completely of endogenous energy sources (18). Subsequently, the cells were washed twice and resuspended in a buffer as described in the Figure legends or text. All experiments were performed at 30°C.

Transport assays. Washed cells were diluted to 0.5 to 1.0 mg of protein per ml in buffer containing either 10 mM lactose or 5 mM arginine. After 5 min of preenergization, $^{32}P_i$ was added to the desired concentration, after which the cells were separated from the medium by filtration using 0.45-μm-pore-size cellulose nitrate filters (Millipore) (18). Initial rates of phosphate uptake were determined in duplicate samples after 10 s of incubation. To remove contaminating P_i, the glassware used for $^{32}P_i$ transport assays and P_i determination

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(see below) was kept in chromic acid and rinsed several times with distilled water before use.

Silicone oil centrifugation. The silicone oil centrifugation method was used to measure the intracellular pH, volume, and phosphate concentrations (19). For silicon oil centrifugation, 0.8 to 1.0 ml of a cell suspension was placed on top of 200 μl of 14% (wt/wt) perchloric acid plus 9 mM EDTA and 800 μl of silicon oil (1.03 g/ml). The mixture was centrifuged for 5 min, after which samples were taken from the cell-free fluid and the perchloric acid extract for further determinations.

Determination of P_i. The intracellular P_i concentration was determined in cell extracts which were obtained after silicon oil centrifugation. The assay mixture consisted of 50 μl of a neutralized extract and 400 μl of an ammoniumheptamolybdate, Malachite green color reagent (16). The reaction was carried out in borosilicate test tubes (6 by 50 mm), in which the absorbance at 660 nm could be measured directly.

Other analytical procedures. The intracellular pH was determined from the distribution of [U-^{14}C]benzoic acid (50 mCi/mmol) and [U-^{14}C]methylamine (56 mCi/mmol), using the silicon oil centrifugation method (17). ATP concentrations were determined with the firefly luciferase assay as described (16). A specific internal volume of 2.9 μl/mg of protein (18) was used for the calculation of intracellular concentrations, i.e., phosphate, protons, or ATP. Protein was measured by the method of Lowry et al. (11), using bovine serum albumin as a standard.

Materials. $NaH_2{}^{32}PO_4$ (9,090 mCi/mmol), ^3H_2O (1 mCi/ml), [U-^{14}C]benzoate (50 mCi/mmol), and [U-^{14}C]methylamine (56 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, U.K. All other chemicals were reagent grade, obtained from commercial sources.

RESULTS

Fate of intracellular phosphate. Starved (TMG-treated) cells of *S. lactis* maintained a large, outwardly directed P_i gradient for several hours. Upon addition of a glycolytic substrate, the internal phosphate concentration decreased from about 80 mM to 10 to 20 mM due to the formation of phosphorylated (glycolytic) intermediates. In contrast, the P_i pool was reduced by less than 5 mM when arginine was used as the source of energy for ATP synthesis. To minimize possible interference of phosphate metabolism with the transport process, *S. lactis* cells were energized by arginine in most experiments. Moreover, phosphate was used instead of the nonmetabolizable phosphate analog arsenate since this latter compound may interfere with energy coupling to the phosphate transport system (see below) by affecting ATP production in the cell (18). Upon the addition of arginine, the external phosphate concentration decreased concomitantly with an increase in internal phosphate concentration (Fig. 1). External phosphate consisted of the phosphate added to the cell suspension plus a small fraction (about 20 μM) that had leaked from the cells prior to energization. A small but significant decrease in intracellular phosphate concentration at zero time in the presence of arginine most probably reflects the synthesis of organic phosphate compounds (e.g., carbamoyl phosphate, ATP) by the arginine deiminase pathway. (With lactose as the source of energy, more than 80% of the accumulated phosphate was incorporated into organic phosphate compounds [data not shown].) At a protein concentration of 0.64 mg/ml and at steady state, the extracellular phosphate was completely taken up at external phosphate concentrations of 120 μM or below. A residual external phosphate concentration of about 150 μM was observed when the initial phosphate concentration was brought to 270 μM. At all concentrations tested, the decrease in external phosphate concentration corresponded quantitatively with the increase of the internal phosphate concentration. The intracellular phosphate pool saturated at approximately 130 mM.

Regulation of phosphate uptake by intracellular phosphate. Time courses of P_i uptake driven by arginine at various external phosphate concentrations are shown in Fig. 2. Almost all the medium phosphate was taken up by the cells (at a protein concentration of 0.68 mg/ml) up to a phosphate concentration of 100 μM, and concentration gradients were achieved that exceeded 10^5. At higher initial phosphate concentrations, the internal phosphate pool saturated at about 130 mM. This concentration was calculated from the amount of P_i taken up and an endogenous phosphate pool of 68 mM. The rate of P_i uptake decreased as the internal phosphate pool reached its maximum, even when the external phosphate concentration remained above the K_T for uptake (see below). The decrease in the net uptake rate appeared not to be due to an increased rate of efflux (passive, carrier mediated, or both) at high intracellular phosphate concentrations. The rate of phosphate exit (calculated on the basis of $^{32}P_i$ exit and the total free intracellular phosphate concentration) from cells accumulating P_i varied between 0.3

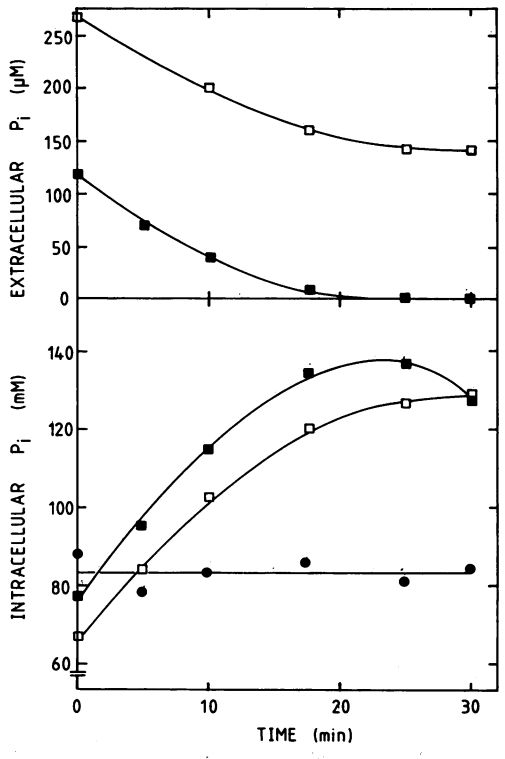

FIG. 1. Changes in intracellular and extracellular phosphate upon the addition of arginine to *S. lactis* ML3 cells. Cells were suspended to 0.64 mg of protein per ml in 100 mM K-HEPES (potassium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.5)–5 mM $MgSO_4$ with (■, □) or without (●) 10 mM arginine. After 1 min of preenergization (zero time), phosphate was added to a final concentration of 0 (●), 100 (■), or 250 (□) μM. At the times indicated, 800-μl portions of cells were centrifuged through silicon oil as described in the text. P_i was determined in the cell-free fluid and the neutralized extract.

and 1.0 nmol/min × mg of protein when a 100-fold excess of unlabeled phosphate (exchange) was added (Fig. 2, inset). Similar rates of phosphate exit were observed at higher internal phosphate concentrations and under conditions of phosphate efflux (data not shown), indicating that the phosphate transport system operates essentially unidirectionally. The decrease of the rate of P_i uptake with increasing internal phosphate concentrations can be explained by a negative feedback control on phosphate uptake by the intracellular phosphate pool.

To analyze the dependence of the initial rate of phosphate uptake on the intracellular phosphate concentration, cells were allowed to accumulate (unlabeled) phosphate to different levels, after which $^{32}P_i$ was added to monitor the unidirectional rate of influx (Fig. 3). The final intracellular phosphate concentrations consisted of the endogenous phosphate pool and the various amounts of $^{32}P_i$ accumulated as determined in parallel samples (see legend to Fig. 3). The unidirectional rate of P_i uptake decreased with increasing intracellular phosphate concentration (Fig. 3). A threefold decrease in the rate of P_i transport was observed upon an increase of the internal phosphate pool of about 100 nmol/mg of protein, i.e., approximately 35 mM. For technical reasons (retention of the phosphate pool in starved cells [see above]), it was not possible to vary the intracellular phosphate concentration over a wider range. The decrease in the rate of P_i uptake with increasing intracellular P_i concentration (and consequently decreasing external P_i concentration) is unlikely to be caused by the increase in concentration gradient,

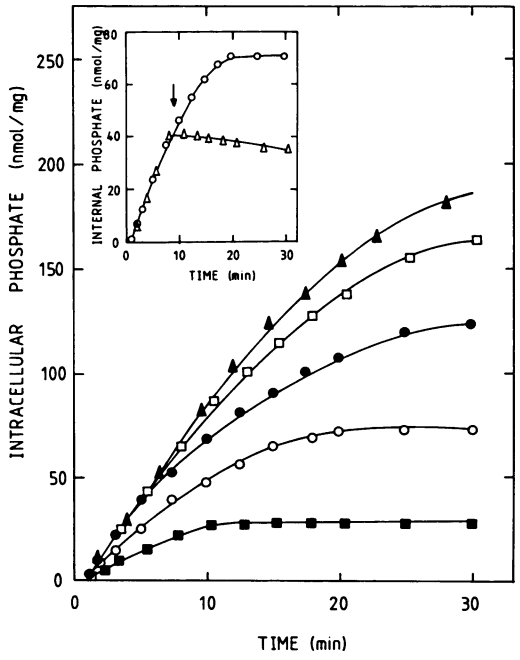

FIG. 2. Time courses of phosphate uptake (nanomoles per milligram of protein) at various initial phosphate concentrations in *S. lactis* ML3. Cells were suspended to a final protein concentration of 0.68 mg/ml in 100 mM K-HEPES (pH 7.5)–5 mM $MgSO_4$ containing various concentrations of $^{32}P_i$. Arginine was added to a final concentration of 10 mM at zero time. Samples (100 μl) were withdrawn at various time intervals, after which the cells were separated from the medium by filtration. The initial $^{32}P_i$ concentrations were 20 (■), 50 (○), 100 (●), 200 (□), and 500 (▲) μM. Inset, Effect of the addition of 5 mM unlabeled phosphate (at t = 9 min) (△) on transport of $^{32}P_i$ (50 μM, initial concentration).

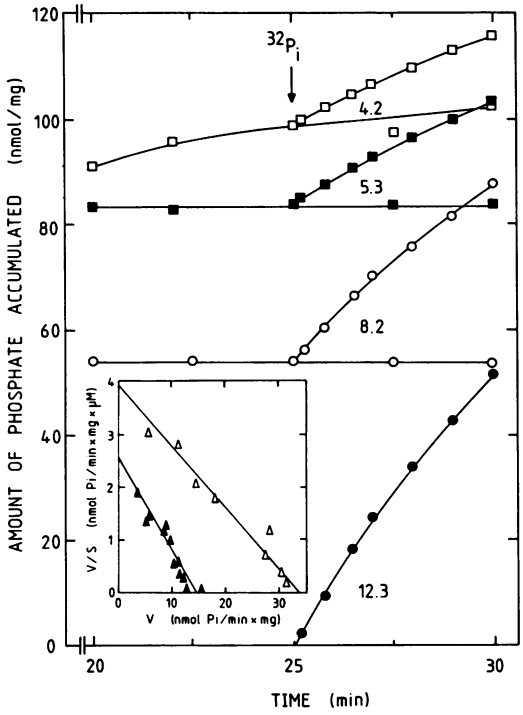

FIG. 3. Effect of the intracellular phosphate concentration on the unidirectional rate of phosphate uptake in *S. lactis* ML3 cells. Cells were suspended to a final protein concentration of 0.89 mg/ml in 100 mM K-HEPES (pH 7.5)–5 mM $MgSO_4$ containing 10 mM arginine. Subsequently, cells were allowed to accumulate P_i to various levels after the addition of 0 (●), 50 (○), 80 (■), or 100 (□) μM phosphate at zero time. After 25 min of uptake, $^{32}P_i$ was added to a final concentration of 100 μM and the unidirectional rate of uptake was measured. The numbers in the figure represent the unidirectional rates of phosphate uptake in nanomoles per minute times milligrams of protein. The amount of P_i accumulated after 20 to 30 min of incubation was determined in parallel samples by adding the appropriate concentration of $^{32}P_i$ at time zero. These measurements indicated that up to an (initial) external concentration of 80 μM, phosphate was completely taken up by the cells within 25 min of incubation. At an initial phosphate concentration of 100 μM, approximately 8 μM was left after 25 min of incubation. Inset, Kinetics of $^{32}P_i$ uptake as a function of external phosphate concentration in the presence of arginine (▲) or lactose (△) as an energy source. *S. lactis* ML3 cells were suspended to final protein concentrations of 0.53 and 0.22 mg/ml in 50 mM K-HEPES (pH 7.0)–5 mM $MgSO_4$ in the presence of 5 mM arginine or 10 mM lactose, respectively. Initial rates were determined in duplicate after 10 s of uptake. The data are reported as Eadie-Hofstee plots.

since the initial rates of P_i uptake (at saturating substrate concentrations) were constant over a wide range of external concentrations and at a fixed internal concentration (data not shown). The dependence of the initial rate of phosphate uptake on the intracellular phosphate concentration may offer an explanation for the difference in the maximal rate of uptake (V_{max}) with arginine and lactose as sources of energy (Fig. 3, inset). The V_{max} values for phosphate uptake were 15 and 33 nmol/min × mg of protein with arginine and lactose, respectively. Under the conditions employed (i.e., at an external pH of 7.5), other parameters, like the internal ATP concentration (data not shown) and the internal pH (Fig. 4), which could affect P_i transport (see below) were very similar with both sources of metabolic energy. A major difference, however, was found in the size of the phosphate pool in cells metabolizing arginine as compared to lactose, e.g., 80 to 90

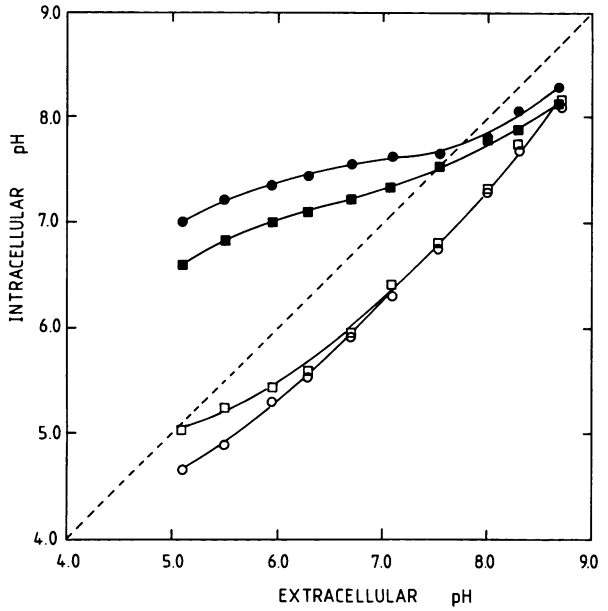

FIG. 4. Relationship between the intracellular and the extracellular pH in *S. lactis* ML3 cells metabolizing arginine (■, □) and lactose (●, ○) in the presence (open symbols) and absence (closed symbols) of nigericin. Cells were suspended to a final protein concentration of 1.25 mg/ml in 30 mM K-MES (potassium morpholineethanesulfonic acid), 30 mM K-PIPES [potassium piperazine-*N*,*N*'-bis(2-ethanesulfonic acid)], 30 mM HEPES, 30 mM K-Tricine, and 5 mM $MgSO_4$ buffers, at the pH values indicated, containing either [U-^{14}C]benzoic acid (20 μM) or [U-^{14}C]methylamine (17.8 μM). Arginine and lactose were added to final concentrations of 5 and 10 mM, respectively. After 5 min of energization in the presence or absence of nigericin (1.0 μM), 1.0-ml portions of cells were separated from the medium by silicone oil centrifugation as described in the text.

mM versus 10 to 20 mM. The affinity constants (K_T) for phosphate uptake were 6.4 and 8.4 μM with arginine and lactose, respectively.

Dependence of phosphate transport on the external pH. The (maximal) rates of phosphate uptake, determined at a final $^{32}P_i$ concentration of 100 μM, appeared to be largely independent of the extracellular pH between pH 5.5 and 8.0 with both arginine and lactose as energy sources (data not shown). In agreement (see below), the intracellular pH was relatively constant at external pH values between pH 5.5 and 8.0, but was 0.3 to 0.4 pH units lower in cells metabolizing arginine than in lactose-fermenting cells (Fig. 4).

The kinetics of phosphate uptake in the presence of arginine was analyzed further at pH 6.2, 7.2, and 8.2, i.e., 1 pH unit below, at, and 1 pH unit above the pK_a of P_i, respectively. The K_T values were 6.2 ± 0.5 μM irrespective of the extracellular pH (data not shown), indicating that the phosphate transport system has no preference for either the mono- or the divalent phosphate anion.

Driving force for phosphate transport. The ability of *S. lactis* cells to accumulate phosphate to concentration gradients (in/out) exceeding 10^5 and the unidirectional nature of the transport process suggested that phosphate uptake is not catalyzed by a secondary transport system (9). To examine the energy requirements of the transport system, the components of the proton motive force (Δp) were selectively dissipated by using classical uncouplers and ionophores. The potassium ionophore valinomycin, which collapses the membrane potential ($\Delta\psi$), had no effect on phosphate transport between pH 5.0 and 8.0 and at external potassium concentrations of at least 50 mM, suggesting an electroneutral translocation process. The ionophore nigericin, which collapses the pH gradient (ΔpH) by exchanging potassium ions for protons, inhibited phosphate uptake to various extents depending on the pH of the medium (see below). Similar results were obtained with protonophores such as carbonyl cyanide *m*-chlorophenylhydrazone and the combination of valinomycin plus nigericin, which dissipate the $\Delta\psi$ and ΔpH, except that some (up to 40%) inhibition of the initial rate of $^{32}P_i$ uptake could be observed (also) at alkaline pH values. When the total Δp was dissipated by valinomycin plus nigericin, the intracellular ATP concentrations were reduced (approximately 60%) to about 0.6 mM. The F0F1 ATPase inhibitor dicyclohexylcarbodiimide, which prevents the formation of a Δp without having a significant effect on intracellular ATP levels, also inhibited transport at acidic but not at alkaline pH values (data not shown). These results suggested that the components of the Δp were not involved in the energization of the phosphate transport system, but that Δp formation was required under certain conditions to maintain an alkaline cytoplasm.

Since transport of phosphate is not driven by a Δp, but requires the synthesis of ATP derived from the catabolism of either arginine or lactose (glycolysis), it is likely that ATP or a related metabolite provides the energy for the transport process directly.

Dependence of phosphate transport on the internal pH. Regulation of phosphate uptake by the intracellular pH was examined further by studying the initial rate of uptake at various external pH values in the presence of nigericin. Under those conditions, a saturating amount of nigericin (0.8 nmol/mg of protein) resulted in a reversed ΔpH, with the cytoplasm 0.7 to 0.8 pH units more acidic than the outside medium with both arginine and lactose as energy sources (Fig. 4). Using these data together with the transport measurements, the initial rates of phosphate uptake were plotted as a function of the intracellular pH (Fig. 5). The apparent pK value for the dependency of phosphate uptake on the internal pH was 7.3 with arginine as the energy source. Cells metabolizing lactose exhibited a similar dependence of phosphate transport on the internal pH, with a pK_a of about 7 (data not shown).

Since ATP could be involved directly in the energization of phosphate transport, it was necessary to check whether the internal pH dependence of phosphate transport was affected by changes in the intracellular ATP levels. The internal ATP concentrations were relatively constant between internal pH values of 5.8 and 8.2 (Fig. 5, inset) and similar for the two energy sources. Below pH 5.8, the ATP levels increased almost twofold with arginine, whereas they dropped to zero with lactose. These results indicate that the dependence of phosphate transport activity on the intracellular pH is not due to varying ATP levels.

DISCUSSION

The properties of the phosphate transport system of *S. lactis* ML3 are probably best described by the experiments in which arginine has been used as energy source. Under those conditions the phosphate translocated enters quantitatively, i.e., without significant metabolism, into a free P_i pool (Fig. 1). The properties of the transport system are: (i) uptake proceeds in the absence of a proton motive force but requires the synthesis of ATP derived from either arginine or

lactose catabolism; (ii) transport is essentially unidirectional; (iii) the rate of uptake is feedback regulated by the internal phosphate concentration; (iv) transport activity exhibits a strong internal pH dependence; and (v) the transport system has no preference for either the mono- or the divalent phosphate anion. These properties are clearly distinct from those of the anion antiporter of *S. lactis* 7962 (14) and *S. pyogenes* (21), but resemble those of the phosphate transport system of *S. faecalis*. Preliminary experiments have indicated that a similar phosphate transport system is operative in *S. cremoris* Wg2. The requirement for phosphate-bond energy as driving force, the unidirectional nature, and the regulation by intracellular pH of the phosphate transport system are reminiscent of the glutamate-glutamine transport system in *S. lactis* ML3 and *S. cremoris* Wg2 (17, 18).

Regulation of solute transport in streptococci by the external and internal pH has recently been reviewed (B. Poolman, A. J. M. Driessen, and W. N. Konings, Microbiol. Rev., in press). At least two different external pH effects can be recognized, (i) a change in transport activity due to an affinity or activity change of the carrier protein and (ii) a change in the available substrate concentration as a result of changes in the relative concentration of the protonated species. Kinetic analysis of phosphate transport in *S. lactis* at various external pH values has revealed no significant external pH effects. Since both the mono- and divalent phosphate anions are accepted by the transport system, the overall electroneutrality of the transport process (as could be inferred from the failure of valinomycin to affect transport) suggests a variable number of (most probably) protons cotransported, depending on the valence of the P_i.

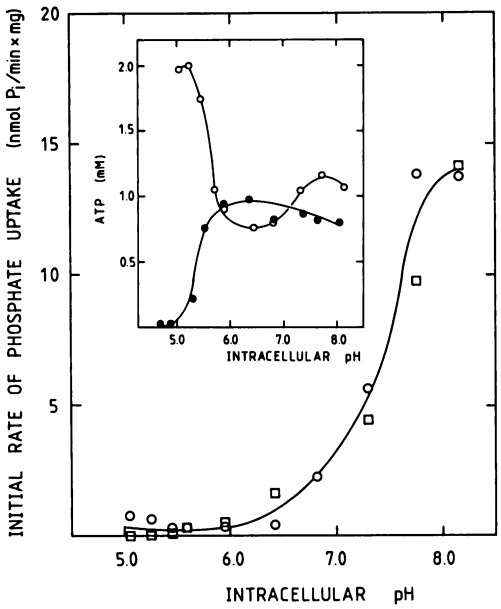

FIG. 5. Dependence of the initial rate of phosphate uptake on the intracellular pH in *S. lactis* ML3. Cells were suspended to a final protein concentration of 0.63 mg/ml in the buffers described in the legend to Fig. 4. Initial rates of $^{32}P_i$ (100 μM, final concentration) uptake (nanomoles of P_i per minute times milligrams of protein) were determined in the presence of 0.5 μM nigericin after 5 min of preenergization with 5 mM arginine. The intracellular pH was determined in parallel samples in experiments similar to that of Fig. 4. Data for two independent experiments are shown. Inset, Dependence of the intracellular ATP pool on the intracellular pH in *S. lactis* ML3 cells metabolizing arginine (○) or lactose (●). The experimental conditions were identical to those described above, except that cell metabolism was quenched after 5 min of energization by the addition of perchloric acid (5%, wt/wt, final concentration). ATP was measured in neutralized cell extracts as described in the text.

Most of the streptococcal transport systems studied thus far are regulated by the intracellular pH, with an apparent pK of approximately 7 (Poolman et al., submitted). With the exception of the alanine-glycine carrier of *S. cremoris* (6), most transport systems require an alkaline pH for maximal activity. The same holds true for the phosphate transport systems of *S. faecalis* (8) and *S. lactis* (this study). The relationship between the rate of transport and the internal pH depends on the source of energy for ATP synthesis. Differences in the fate of phosphate under the two conditions could affect the internal pH dependence of the transport system (assuming that further metabolism of phosphate in glycolyzing cells is pH sensitive). During lactose fermentation a considerable fraction (more than 80%) of the accumulated phosphate is incorporated into organic phosphate compounds, whereas with arginine, phosphate enters into a free P_i pool. Furthermore, as a consequence of phosphate metabolism the free intracellular phosphate pool, and thus the transport activity, changes (Fig. 3; see below). The energy status of the cells, as judged from the intracellular ATP levels, is the same with arginine and lactose in the pH range of interest. Therefore, it seems unlikely that the energy supply to the transport system differs significantly with the two energy sources. The differences in ATP concentrations below pH 5.8 can be attributed to the relative insensitivity of the arginine deiminase pathway to acid pH values, whereas the glycolytic pathway is rapidly inactivated below pH 6 (12, 15, 16a). The rise in ATP concentration in arginine-metabolizing cells below an internal pH of 5.8 is most likely explained by the stronger inhibition of ATP-consuming processes relative to the production of ATP by the arginine deiminase pathway.

An important parameter in resolving the internal pH dependency of the phosphate transport system has been the measurement of the cytoplasmic pH. It is noteworthy that the cytoplasmic pH becomes acid with respect to the pH of the outside medium when the internal pH is varied by the external pH in the presence of nigericin (Fig. 4). This reversed pH gradient is observed irrespective of the nature of the energy source and also in energy-starved (TMG-treated) cells in the absence of the ionophore at alkaline pH values or when the intracellular potassium concentration is low (unpublished results), suggesting the presence of a compensatory (Donnan) potential of opposite polarity. Since nigericin depletes the intracellular potassium pool to a large extent (B. Poolman, unpublished results) by exchange of potassium ions for protons, the reversed pH gradient can also be generated in energized cells.

Kinetic analysis of phosphate transport in *S. lactis* has revealed a marked difference in V_{max} with arginine and lactose as energy sources (Fig. 3; inset). This difference may be attributed entirely to *trans*-inhibition of the transport system. This type of regulation of transport activity, usually not found for secondary transport systems, has been described for the major potassium transport system of *S. faecalis* (4) and the *trkA* and *trkD* systems of *E. coli* (22). For Δp-driven transport systems, the steady-state levels of accumulation are determined by the magnitude of the Δp, by the stoichiometry of solute to coupling ion, and by the charges of solutes translocated and the passive leak processes (A. J. M. Driessen, K. J. Hellingwerf, and W. N.

Konings, J. Biol. Chem., in press). In contrast, phosphate-bond-driven transport systems operate essentially unidirectionally and can catalyze the uptake of solutes to very high accumulation levels as compared with Δp-driven transport systems. *trans*-Inhibition acts as a regulatory device in these transport systems, including the phosphate uptake system described here, to prevent accumulation to unacceptably high internal levels. The phosphate transport system of *S. lactis* does not exhibit significant exchange (and efflux) activity in the absence or presence of metabolic energy. In contrast, the potassium transport system of *S. faecalis* catalyzes energy-dependent potassium exchange (and efflux). Despite the use of the protocols described for the assay of anion antiport in *S. lactis* 7962 (2, 14), no evidence has been obtained for the presence of a similar phosphate transport system in *S. lactis* ML3 or *S. cremoris* Wg2 (unpublished data).

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