THE LOSS OF THE phoS PERIPLASMIC PROTEIN LEADS TO A CHANGE IN THE SPECIFICITY OF A CONSTITUTIVE INORGANIC PHOSPHATE TRANSPORT SYSTEM IN ESCHERICHIA COLI.

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SUMMARY

The phoS periplasmic protein, implicated in alkaline phosphatase regulation, is shown to be involved in inorganic phosphate (Pi) transport in E. coli. Although phoS$^-$ cells dependent upon the PST system for Pi transport can grow in minimal medium with 1 mM Pi as source of phosphorus, the affinity of these cells for Pi is greatly reduced; Km = 18 μM compared with Km = 0.4 μM for phoS$^+$ cells. phoS$^-$ cells dependent upon the PST Pi transport system acquire the ability to accumulate Asi from the medium in contrast to phoS$^+$ cells which exclude this toxic anion. It would appear that the periplasmic phoS protein is not essential for Pi accumulation but is involved in maintaining the specificity of the PST Pi transport system.

INTRODUCTION

Inorganic phosphate (Pi) transport in Escherichia coli can involve at least four genetically separable transport systems. Two of the systems are inducible and accept both Pi and arsenate ion (Asi) as secondary substrates (14, R. L. Bennett, Ph.D. thesis, Tufts University). These are the L-α-glycerophosphate permease system, coded for by the glpT gene (7), and the hexosephosphate permease system coded for by the uhp gene (15). The two other Pi transport systems are synthesized constitutively. These are the

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Abbreviations: Asi, arsenate ion; PST, Pi specific transport system; PIT, Pi transport system.

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PST system which has been mapped by Pl transduction near minute 74 on the chromosome (14), and the PIT system which has been shown by conjugation studies to map in the xyl-mal region of the chromosome (R. L. Bennett, Ph.D. thesis, Tufts University).

Alkaline phosphatase is a periplasmic protein (9) whose synthesis is repressed by high concentrations of Pi in the growth medium (6, 13). phoS and phoT, two genes implicated in alkaline phosphatase regulation, are also located near minute 74 on the E. coli chromosome (1, 3). Although mutations at both phoS and phoT lead to constitutive alkaline phosphatase production, we have shown that the primary function of the phoT gene is to code for at least a part of the PST Pi transport system (14). The phoS gene codes for a periplasmic protein (5, 12, unpublished results of this laboratory), the lack of which renders the cells sensitive to the growth inhibitory effects of Asi (14).

In this communication we report on the interaction of the phoS periplasmic protein with the PST Pi transport systems in strains constructed to retain only the PST constitutive Pi transport system (14). We have found that in cells dependent upon the PST system for all Pi transport, Asi is not transported into the cell if the strain is phoS$^+$, while it is transported if the cell is phoS$^-$. The sensitivity of phoS$^-$ strains dependent on the PST Pi transport system to growth inhibition by Asi results from a change in the specificity of the PST system so that Asi becomes a substrate for transport.

MATERIALS AND METHODS

Bacterial Strains - For these studies, strains dependent on the PST constitutive Pi transport system were used. All strains contain the PIT$^-$ allele present in a derivative of U7, introduced by bacterial conjugation (14). GR5172, the phoS$^+$ strain, contains the PST system derived from strain 13.6;

GR5158, the phoS$^-$ strain, contains the PST system derived from AB2277 (14). These PST systems are identical with respect to Pi transport (unpublished observations). GR5158 contains the phoS$^-$ mutation C72 originally described by Echols et. al. (3). The construction of GR5158 has been described in Table 4 of Willsky et. al. (14).

Media - All cells were grown in standard WT (2) medium containing 0.6% glucose, 1 mM Pi, and 2 μg/ml of thiamine.

Transport Assays - The procedure for Pi transport assays using (^{32}P) Pi has been previously described (14). The initial velocities of (^{32}P) Pi accumulation were calculated from the values obtained during the first 1 to 1.5 minutes of the transport assay. Km and Vmax values were determined using linear regression analysis. (^{74}As)Asi was purchased from Amersham-Searle as the sodium salt in isocitrate buffer. (^{74}As)Asi uptake studies were performed in the same manner as the (^{32}P) Pi uptake studies (14). Asi concentrations were determined by modifications of the Pi assay (2) and (^{74}As)Asi radioactivity on dried filters was determined in a Beckman LS235 Scintillation Counter using Liquifluor scintillation fluid (New England Nuclear). The specific activity of both the Pi and Asi solutions was between 10^3 and 10^4. counts per min/nmole.

RESULTS

The presence of a phoS$^-$ mutation in a strain dependent upon the PST system for Pi transport does not affect the ability of the strain to grow in a glucose minimal medium with 1 mM Pi as sole source of phosphorus (Figure 1). The generation time for both phoS$^+$ and phoS$^-$ strains is 90 min. However, when a tenfold excess of Asi is added to the cultures during the exponential growth phase, a striking difference between the two strains is observed. Growth of the phoS$^-$ cells ceases immediately and never resumes, whereas

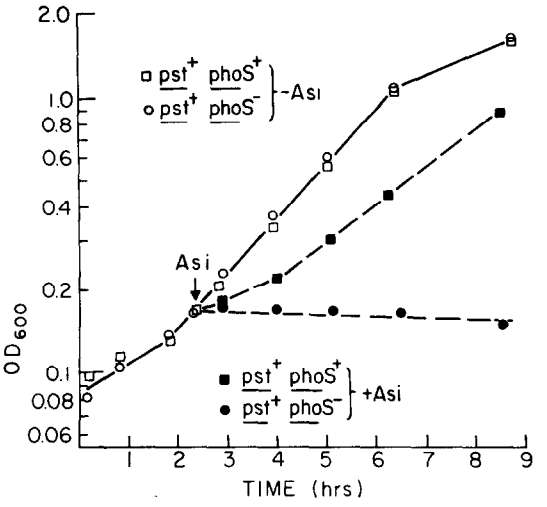

Figure 1. Growth of phoS$^+$ and phoS$^-$ cells in the presence or absence of Asi. Stationary phase cultures in standard WT medium were washed and resuspended in fresh media. When exponential growth resumed (at the arrow), the cultures were divided into two portions, one of which received 10 mM Asi. Growth was followed spectrophotometrically at 600 nm.

the phoS$^+$ strain resumes growth after a short but variable lag period. The new growth rate for phoS$^+$ strains is less than that observed in the absence of Asi (generation time = 130 min.). [The altered characteristics of PST mediated Pi transport in the phoS$^+$ cells growing in the presence of Asi will be the subject of a separate communication (Willsky and Malamy, in preparation)].

The kinetic parameters of Pi transport in the phoS$^+$ and phoS$^-$ strains were determined from (^{32}P) Pi uptake studies. Figure 2 presents Lineweaver-Burk plots of the data obtained. The Km and Vmax for Pi transport for the phoS$^+$ strain are 0.4 μM and 5.3 nmoles Pi/O.D.$_{600}$/min respectively (Figure 2A). Although the phoS$^-$ strain is capable of transporting and accumulating Pi, these cells have a much lower affinity for Pi, Km = 18 μM, while exhibiting a lower Vmax, 2.3 nmoles Pi/O.D.$_{600}$/min (Figure 2B). Thus, E. coli cells are able to accumulate Pi through the PST Pi transport

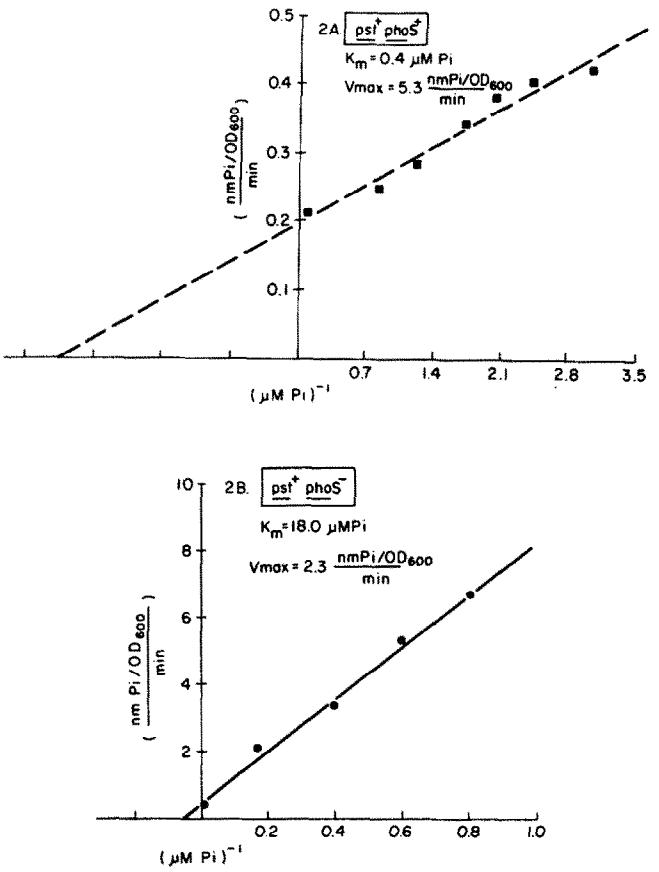

Figure 2. Kinetics of Pi transport in phoS$^+$ and phoS$^-$ cells. The Km and Vmax values were determined as described in Materials and Methods and in reference 14. Cells were maintained at a constant O.D.$_{600}$ of 0.4 by frequent dilution with fresh media.

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system both in the presence and absence of the phoS protein.

To determine whether the sensitivity of phoS$^-$ cells to growth inhibition by Asi was a result of Asi transport into the cells, (^{74}As)Asi uptake studies were performed. Figure 3 demonstrates that the phoS$^+$ cells are unable to transport (^{74}As)Asi above a very low level which is reached within the first 15 seconds of the assay. This level of (^{74}As)Asi associated with the cells is probably a result of Asi binding to the surface of the cell and not a result of Asi transport into the cells. By contrast, the phoS$^-$ cells accumulate significantly higher levels of (^{74}As)Asi in an energy dependent process. The

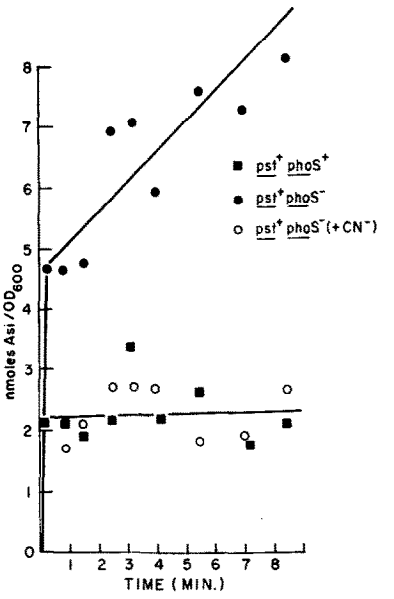

Figure 3. Asi accumulation in phoS$^+$ and phoS$^-$ cells. The procedure for (^{74}As)Asi uptake is described in Materials and Methods. Cells were grown to O.D.$_{600}$ = 0.4. (^{74}As)Asi to give a final concentration of 660 μM was added at zero time. The lines drawn on the figure were obtained by linear regression analysis.

addition of cyanide eliminates (^{74}As)Asi accumulation in the phoS$^-$ cells and lowers (^{74}As)Asi levels to that found in the phoS$^+$ cells.

DISCUSSION

E. coli cells dependent upon the PST transport system for Pi accumulation are normally able to discriminate between Asi and Pi in the medium and will not transport significant levels of Asi into the cells. However, in phoS$^-$ mutants lacking the periplasmic phoS protein, the PST Pi transport systems allows the transport of larger amounts of Asi into the cells. This explains the inability of phoS$^-$ cells to grow in the presence of Asi in the growth medium. In a phoS$^+$ cell, the presence of the phoS protein confers a high degree of specificity to the PST Pi transport system and allows the exclusion of Asi, a toxic analog of Pi, from the cell.

Previous studies have emphasized that the phoS protein, initially characterized as the R2 "regulatory protein" (4), was different from a periplasmic phosphate-binding protein thought to be involved in Pi transport (10, 11). However, more recent studies from the same laboratory have established that these two proteins are identical (5). If the phoS protein is indeed the only phosphate binding protein, then our studies demonstrate that this protein is not essential for PST mediated Pi transport. The affinity of the PST system is decreased in the absence of the phoS protein but accumulation still occurs in the presence of low substrate concentrations. It would appear that the PST Pi transport system is not as dependent upon the phoS Pi binding protein for accumulation as other transport systems in E. coli with associated binding proteins (8). It is possible that additional phosphate binding proteins or other components located in the outer layers of the cell envelope can substitute for the phoS protein, albeit with a loss in the specificity of the system.

In contrast to our results with phoS$^-$ strains, Medveczky and Rosenberg (10) have reported that a phosphate binding protein mutant had greatly impaired Pi transport. However, since the mutant strain was selected to grow poorly with Pi as the sole source of phosphorus, the strain must contain mutations in other Pi transport systems, in addition to any alteration in the phosphate binding protein.

The observation that the introduction of a phoS mutation alters the specificity of the PST transport system supports our earlier hypothesis (14) that the phoS gene, like the phoT gene, plays a primary role in Pi transport in E. coli. It is only as a result of this function that the phoS and phoT genes are involved in the regulation of alkaline phosphatase synthesis.

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