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PHOSPHATE TRANSPORT IN *ESCHERICHIA COLI*

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SUMMARY

1. *Escherichia coli* accumulates phosphate against a concentration gradient by an energy-dependent process with an activation energy of over 12 kcal.

2. When cells grown in ample phosphate are starved for phosphorus, or alternately, when cells are grown in phosphate-limiting medium, a specific phosphate pool of the cells is depleted. When phosphate is again provided, this pool is rapidly filled. From then on, phosphate is taken up at a slower rate, sufficient to replenish pool phosphate used up predominantly for nucleic acid synthesis. Isotope displacement experiments show that all phosphate taken up passes through the pool.

3. The uptake of phosphate occurs by two kinetically distinct systems, a high-affinity component and a low-affinity component. The two components function simultaneously during the filling of the pool, but thereafter uptake takes place only by the low-affinity component. The phosphate-binding protein appears to function within the high affinity component. Either of the two components can be selectively abolished by a variety of treatments.

4. A number of arsenate-resistant mutants were shown to be deficient in phosphate uptake. One of these mutants, however, took up and metabolized phosphate at an abnormally high rate, but only over a period of time which did not exceed division time.

INTRODUCTION

Phosphorus is an essential nutrient of all cells and one of its most readily available sources is inorganic orthophosphate (P_i). The properties of a number of P_i transport systems have been described[1–12]. P_i is taken up by cells under a variety of conditions and the fate of the P_i immediately following entry also differs widely.

In a previous study of P_i transport in *Bacillus cereus*[13,14], we showed that P_i was taken up by phosphorus-deprived cells in a biphasic manner. In an energy-requiring process, P_i was accumulated against a steep concentration gradient. The anions phosphite, arsenate and pyrophosphate, appear to be taken up by the P_i transport system.

Recently, we described the isolation from *Escherichia coli* of a phosphate-

Abbreviation: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

binding protein and its participation in the transport of phosphate[15]. The present paper describes the general properties of the phosphate transport system in *E. coli*.

MATERIALS AND METHODS

Chemicals

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was a product of K and K Laboratories, Tris (Sigma 7–9 grade) was obtained from Sigma Chemicals and imidazole, from Fluka. All other chemicals used were of the highest purity available.

Radioactive compounds

Radioactive orthophosphate ($^{32}P_i$) was purchased from the Australian Atomic Energy Commission. The solutions, when received, were adjusted to contain 1 mM P_i to avoid the formation of particle-adsorbed $^{32}P_i$ which we, and other workers[16], have encountered. Labelled arsenate ([^{74}As]arsenate) and pyrophosphate ([^{32}P]pyrophosphate) were from The Radiochemical Centre, Amersham. They were adjusted to the desired concentration and specific radioactivity before use. Labelled orthophosphite([^{32}P]phosphite) was prepared, according to YAGI *et al.*[17], by neutron irradiation of orthophosphorous acid, performed at the facility of the Australian Atomic Energy Commission, Lucas Heights.

Bacterial culture

E. coli AB3311 (Reeves *met*$^-$), used throughout this study, was grown in TSYG medium (50 mM Tris–HCl buffer (pH 7.2), 10 mM $(NH_4)_2SO_4$, 15 mM KCl, 400 μM $MgSO_4 \cdot 7H_2O$, 20 mM glucose, 2 mM methionine and 1 mg/ml yeast extract) in a New Brunswick gyrotatory water bath at 37°.

Uptake studies

The labelled substrate was added to the desired concentration to pre-warmed (37°) cells at the required density in TSG medium (50 mM Tris–HCl buffer (pH 7.2), 10 mM $(NH_4)_2SO_4$, 15 mM KCl, 400 μM $MgSO_4 \cdot 7H_2O$, 20 mM glucose, 2 mM methionine and 70 nM vitamin B_1) or, in later work, in ISG medium (100 mM imidazole–HCl buffer pH 7.0), 10 mM $(NH_4)_2SO_4$, 15 mM KCl, 400 μM $MgSO_4 \cdot 7H_2O$, 20 mM glucose, 2 mM methionine and 70 nM vitamin B_1)and samples (0.5 ml) were withdrawn at intervals. They were filtered immediately on membrane filters (Gelman Metricel, 0.20 μm) and washed twice with 2 ml 140 mM NaCl solution containing 7 mM KH_2PO_4. The membranes were soaked in this solution before use to minimize the adsorption of the label.

Paper electrophoresis

High-voltage paper electrophoresis was performed on Whatman 3-MM paper in a cooled-plate apparatus using formate–acetate buffer (pH 2) (43.5 ml glacial acetic acid and 12.5 ml formic acid made up to 1 l with distilled water . The current (5000 V, 150 mA) was applied for 20 min. Radioautographs were prepared by exposing the dried papers for a suitable interval to Kodak 'Blue Brand' film. Phosphates were detected on the paper as described previously[18].

Selection of mutants defective in phosphate transport

Two classes of such mutants have been isolated as described previously[15]. The first type, arsenate-resistant mutants, were selected by plating MNNG-treated cells on meat-infusion agar plates containing 10 mM sodium arsenate. The bacteria from arsenate-resistant colonies were examined for their ability to transport P_i, and selected clones were maintained on arsenate-containing medium to prevent the growth of revertants. The other class of mutants lacked functional phosphate-binding protein. These were selected by plating MNNG-treated cells on a minimal salts medium containing P_i (40 mM KH_2PO_4, 20 mM $Na_2HPO_4 \cdot 2H_2O$, 10 mM $(NH_4)_2SO_4$, 400 μM $MgCl_2$, 40 μM $Ca(NO_3)_2 \cdot 4H_2O$, 40 μM $FeSO_4 \cdot 7H_2O$, 20 mM glucose, 2 mM methionine, 70 nM vitamin B_1 and 20 mg/ml agar). Minute colonies were picked from these plates and the mutants were tested for their ability to grow on minimal plates (50 mM Tris–HCl buffer (pH 7.2), 10 mM $(NH_4)_2SO_4$, 400 μM $MgCl_2$, 40 μM $Ca(NO_3)_2 \cdot 4H_2O$, 40 μM $FeSO_4 \cdot 7H_2O$, 20 mM glucose, 2 mM methionine and 70 nM vitamin B_1) supplemented with either yeast extract (1 mg/ml) or P_i (5 mM) as the source of phosphorus. The colonies that grew with the yeast extract, but not with P_i, were selected and the bacteria tested for the presence of the phosphate-binding protein and for their ability to transport P_i. All mutants described in the text have been isolated from distinct clones.

RESULTS

The effect of phosphorus deprivation on the rate of P_i uptake

Cells were grown to the stationary phase in TSYG medium, washed twice with TSG medium and resuspended in TSG. These cells accumulated P_i in a biphasic manner: The initial rapid phase (which will be referred to as 'primary phase') occupied about 2 min during which time 10–12 nmoles of P_i was taken up by 10^9 cells. The rate of P_i uptake abruptly decreased at this stage to a slower ('secondary') rate. Both uptake rates were further increased if the 50 mM Tris buffer in the medium was substituted by 100 mM imidazole–HCl buffer (pH 7.0) (ISG medium). Cell division did not occur during the uptake experiments. If cells at 10^9/ml were pre-incubated with 12 μM unlabelled P_i for 4 min, $^{32}P_i$ added subsequently was taken up at the secondary rate only (Fig. 1).

The pattern of uptake was similar in cells which had been grown to the log phase. If, on the other hand, the cells were grown in the presence of P_i, then the biphasic uptake was no longer observed, but could be induced by phosphorus deprivation for 2 h (Fig. 1).

Requirements for the rapid initial uptake of P_i

Cells lost the capacity for the primary uptake of P_i if K^+ was omitted from the uptake medium. The K^+ requirement could be satisfied by Rb^+ (Fig. 2). Mg^{2+} is necessary for maximal secondary uptake (Fig. 2).

Glucose grown cells, when placed in a glucose-free medium took up P_i at a markedly decreased rate. The rapid initial uptake of P_i could be immediately and completely recovered by the addition of either glucose or galactose. Mannitol or fructose were less efficient in this respect. Addition of succinate, glycerol or acetate to cells in glucose-free medium did not elicit the biphasic uptake pattern. Acetate-

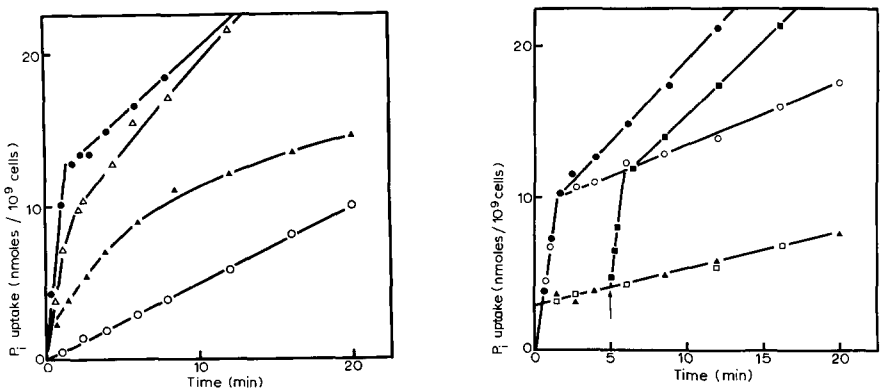


Fig. 1. The reversible suppression of the fast primary uptake of P_i in *E. coli* by abundant P_i. Phosphate uptake was measured at 50 μM $^{32}P_i$ and at a cell density of 10^9/ml. (1) ●—●, cells grown with yeast extract as source of phosphorus; (2) ○—○, as (1), but pre-treated with 12 μM P_i for 4 min before the addition of $^{32}P_i$; (3) ▲—▲, cells grown in media supplemented with 50 mM P_i; (4) △—△, as (3), but incubated in phosphorus-free medium for 2 h before the introduction of $^{32}P_i$.

Fig. 2. The effect of metal ions on P_i uptake in *E. coli*. The basal uptake medium contained: 100 mM imidazole–HCl buffer (pH 7.0), 10 mM $(NH_4)_2SO_4$, 20 mM glucose, 2 mM methionine and 70 nM vitamin B_1. It was complemented with monovalent cations (10 mM) and Mg^{2+} (1 mM) in the following combinations: ▲—▲, no supplement; ●—●, K^+ and Mg^{2+}; ○—○, K^+ or Rb^+; □—□, Na^+ or Cs^+ or Li^+; ■—■, K^+ added to basal medium, containing Mg^{2+}, at 5 min (arrow). P_i uptake was measured at 50 μM $^{32}P_i$ and a cell density of 10^9/ml.

TABLE I

INITIAL ('PRIMARY') RATES OF P_i UPTAKE AS A FUNCTION OF THE CARBON SOURCE PRESENT IN THE GROWTH AND UPTAKE MEDIA

Cells were washed twice with, and resuspended in basal medium: 100 mM imidazole–HCl buffer (pH 7.2), 10 mM $(NH_4)_2SO_4$, 15 mM KCl, 400 μM $MgCl_2$, 2 mM methionine and 70 nM vitamin B_1. Carbohydrates were added after the $^{32}P_i$ (see Fig. 2) to final concentrations of: hexoses, 0.2%; succinate, 0.3%; glycerol, 0.4% and acetate, 0.6%. Phosphate uptake was measured at 100 μM $^{32}P_i$ and 10^9 cells per ml, and initial rates were measured over the first 20 sec.

Condition	*Initial rate of P_i uptake (nmoles P_i per 10^9 cells per min)*	*Size of primary pool (nmoles P_i per 10^9 cells)*
(a) Glucose-grown cells		
uptake in glucose-free medium	1.5	—
+ glucose	14.8	10
+ galactose	14.8	10
+ mannitol	5.4	8
+ fructose	5.4	8
+ 2-deoxyglucose	1.5	—
+ succinate	6.14	—
+ glycerol	6.8	—
+ acetate	1.2	—
(b) Acetate-grown cells		
uptake in glucose-free medium	0.02	—
+ glucose	0.18	—
+ succinate	0.02	—
+ glycerol	0.39	—
+ acetate	0.03	—

grown cells exhibited lower rates of P_i uptake, regardless of the carbon source in the uptake medium (Table I).

The uptake of phosphate shows a strong temperature dependence. From the Arrhenius plot of uptake rate *versus* temperature, the activation energy of the uptake was calculated to be 12.5 kcal.

The effect of inhibitors on P_i uptake

The uptake of P_i in *E. coli* was found to be sensitive to a variety of inhibitors, the pattern of inhibition depending on the type of compound used (Fig. 3). Thus, biphasic uptake was abolished in the presence of glycolytic inhibitors such as *N*-ethylmaleimide, azide–iodoacetamide and cyanide. 2,4-Dinitrophenol produced a similar effect. Arsenate, an inhibitor of many reactions involving phosphate, is a potent inhibitor of the primary uptake.

Ni^{2+} had no effect on the primary uptake, but completely abolished P_i uptake from then on (Fig. 3).

The distribution of the $^{32}P_i$ label within the cells

The distribution of the label during P_i uptake was examined by direct electrophoresis of the whole cells after collection on membrane filters. Details of this method have already been described[14]. Quantitative measurement of the radioactivity in the various fractions (Fig. 4) showed that P_i was the major radioactive fraction, and that,

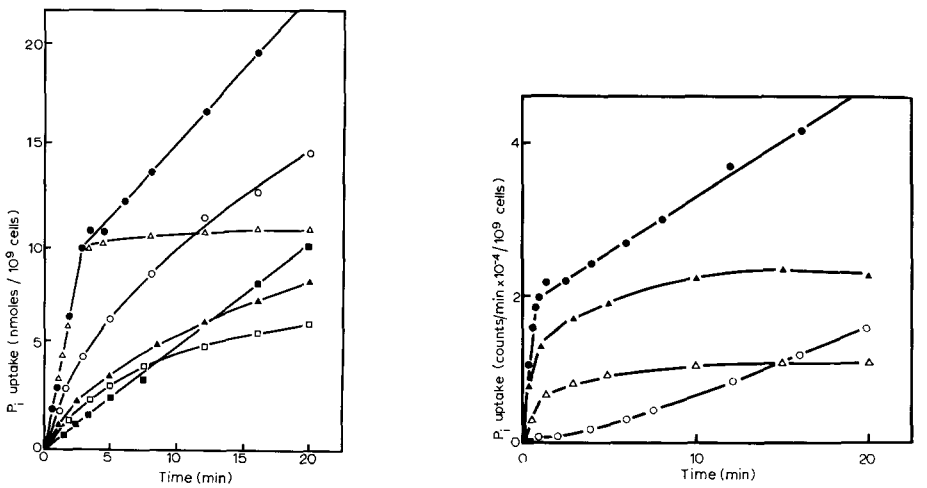


Fig. 3. The effect of inhibitors on P_i uptake in *E. coli*. Cells (10^9/ml) were pre-incubated with the inhibitor before the addition of 100 μM $^{32}P_i$. ●—●, uptake in control cells; cells treated with: ○—○, 10 mM azide with 1 mM iodoacetamide; △—△, 10 mM Ni^{2+}; ■—■, 5 μM (or 1 mM) arsenate; □—□, 1 mM 2,4-dinitrophenol; and ▲—▲, 2.5 mM *N*-ethylmaleimide. P_i uptake in presence of 1 mM cyanide (not shown) was intermediate between that in the presence of 2,4-dinitrophenol and *N*-ethylmaleimide.

Fig. 4. The distribution of radioactivity from $^{32}P_i$ taken up by *E. coli*. The data were obtained after electrophoresis of whole cell contents using a procedure described in detail elsewhere[14]. The relevant areas of label were localized with the aid of the autoradiogram, excised and the radioactivity determined: ●—●, total $^{32}P_i$ uptake; ▲—▲, intracellular P_i; △—△, organophosphate esters; ○—○, insoluble residue remaining at the origin.

initially, the rate of intracellular P_i accumulation paralleled the initial rate of P_i uptake. Examination of the acid-soluble organophosphates which were separated from P_i on electrophoresis showed that, in the initial 90 sec of uptake, the radioactivities of the three detectable compounds were well below that of P_i (Table II).

TABLE II

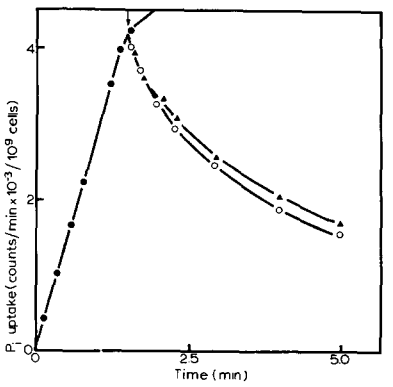
TOTAL ^{32}P RADIOACTIVITY IN INTRACELLULAR P_i AND IN THE UNIDENTIFIED ACID-SOLUBLE ORGANOPHOSPHATES IN THE EARLY STAGES OF UPTAKE

Data were obtained as for Fig. 4.

Time (*sec*)	*Total counts/min per* 10^9 *cells*			
	P_i	*Compound 1*	*Compound 2*	*Compound 3*
10	4 500	1250	Not detectable	Not detectable
20	6 500	2000	Not detectable	Not detectable
30	9 200	2700	1800	Not detectable
90	10 000	3700	3200	500

The exchange and displacement of the primary pool

When a cell suspension was allowed to take up $^{32}P_i$ for 2 min and was then supplemented with a large excess of unlabelled P_i or arsenate, part of the accumulated label was lost at a rate initially equal to the uptake rate (Fig. 5). If the amount of $^{32}P_i$ supplied was just sufficient to fill the P_i pool, it was taken up and totally retained. If at that stage excess unlabelled P_i was added to the medium, part of the incorporated label was lost by exchange, and all the remaining intracellular label was cleared from the pool into the macromolecular fraction (Fig. 6). This fraction, which is the acid-



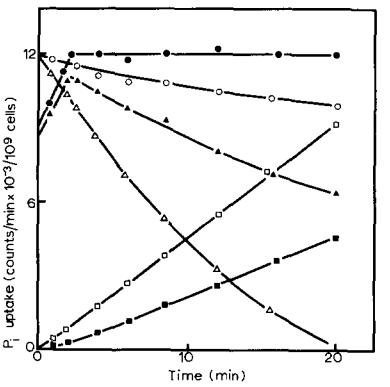


Fig. 5. The exchange of $^{32}P_i$ in *E. coli* with excess P_i or arsenate. 100 μM $^{32}P_i$ was added to 10^9 cells per ml and, after 2 min (arrow), a 100-fold excess of non-labelled P_i was added to one part, a 100-fold excess of arsenate to another. ●—●, control uptake; ▲—▲, P_i chase; ○—○, arsenate chase.

Fig. 6. Unlabelled chase of the P_i pool into the acid-insoluble residue. Cells were incubated at 10^9/ml in uptake medium containing 10 μM $^{32}P_i$. After 2 min unlabelled P_i was added to 100 μM to one-half of the cells. The data were collected as described for Fig. 4. (a) Cells given $^{32}P_i$ alone: ●—●, total uptake; ▲—▲, P_i pool; ■—■, acid-insoluble residue. (b) Cells given unlabelled chase: ○—○, total uptake; △—△, P_i pool; □—□, acid-insoluble residue.

insoluble residue remaining on the origin after electrophoresis of the whole cells, behaves predominantly as nucleic acids. Thus, about 10 % of the label in this residue was removed by extraction with chloroform–methanol (2:1 v/v). The bulk of the label (> 85 %) was extracted after 20 min in 5 % trichloroacetic acid at 90°. Only trace amounts (< 5 %) of radioactivity were left after the hot trichloroacetic acid extraction.

Properties of mutants defective in P_i transport

The two types of mutants (see MATERIALS AND METHODS) were examined for their ability to transport P_i. Most representatives of the first class (resistant to arsenate) were found to accumulate phosphate at a considerably lower rate than the parent cells and failed to display biphasic uptake. These mutants were unable to incorporate $^{32}P_i$ into the trichloroacetic acid-insoluble fraction (Fig. 7).

However, one arsenate-resistant mutant, 20-1, took up P_i to a much greater extent than the parent cells, and at a rate similar to that of the primary uptake of the parent cells. The size of the intracellular P_i pool was constant and the same as in the wild type, but the label accumulated in the acid-insoluble fraction at an abnormally high rate (Fig. 7).

The other class of mutants were selected for the deficiency of the phosphate-binding protein. Mutants of this type were found to be unable to grow on media containing low concentrations of P_i and could not transport P_i as efficiently as the parent strain (Fig. 8).

The kinetic parameters of P_i uptake

Kinetic plots of initial uptake velocities (over initial 30 sec) against P_i concentration revealed that the transport system is composed of two parts (Fig. 9). Only

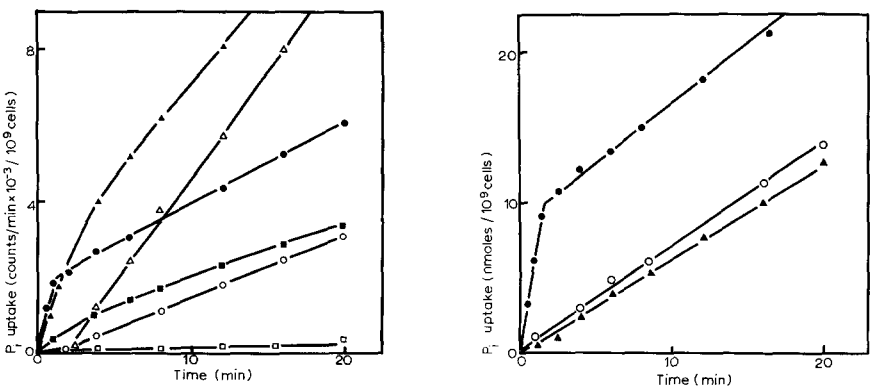


Fig. 7. P_i uptake in some arsenate-resistant mutants of *E. coli*. 50 μM $^{32}P_i$ was added to 10^9 cells/ml. (a) P_i uptake in parent cells: ●—●, total uptake; ○—○, acid-insoluble label. (b) P_i uptake in 20-1 mutant: ▲—▲, total uptake; △—△, acid-insoluble label (radioactivity remaining in the cells after 20 min extraction at 0° in 10% aqueous trichloroacetic acid). (c) P_i uptake in R-6 (or C-11, R-10) mutant: ■—■, total uptake; □—□, acid-insoluble label.

Fig. 8. P_i uptake by mutants of *E. coli* deficient in the phosphate-binding protein. 50 μM $^{32}P_i$ was added to 10^9 cells per ml. P_i uptake: ●—●, in parent cells; ○—○, in 20-2 mutant; ▲—▲, in 10-1 mutant.

one of these components (high-affinity component) appeared to function at low concentration of P_i; and both the high-affinity component and the second (low-affinity) component operated simultaneously at the higher concentrations. The kinetic constants were calculated from plots according to HOFSTEE[19] drawn over the respective concentration ranges for the high-affinity component (Fig. 9) and the low-affinity components (Fig. 10). The data are presented in Table III.

In order to determine the physiological role of the two components, the uptake

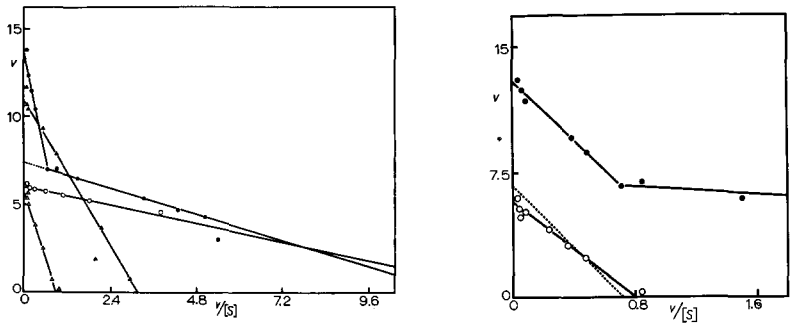


Fig. 9. The effect of substrate concentration on P_i uptake in *E. coli*. Initial velocities (over first 30 sec), expressed as v = nmoles P_i per 10^9 cells per min, were plotted according to HOFSTEE[19] against substrate concentration in the range 0.4–400 μM ([S] = [P_i] in μM). ●—●, control cells; ○—○, cells treated with 10 mM Ni^{2+}; △—△, cells treated with 10 μM arsenate and washed; ▲—▲, 10-1 mutants. The kinetic constants derived from these graphs are listed in Table III.

Fig. 10. The effect of substrate concentration on P_i uptake in cells pre-treated with P_i. Data were obtained and plotted as described for Fig. 9. ●—●, control uptake; ○—○, uptake in cells pre-treated with 12 μM P_i per 10^9 cells and washed; – – – –, contribution of low-affinity component to uptake in control cells (calculated by differential plot).

TABLE III

THE KINETIC CONSTANTS OF THE UPTAKE OF P_i IN *E. coli* AB3311 AND IN MUTANTS DEFECTIVE IN P_i TRANSPORT

Data were calculated from plots according to HOFSTEE[19] (shown in Figs. 9 and 10).

Strain	K_m (μM)	v_{max} (*nmoles* P_i *per* 10^9 *cells per min*)
AB3311		
high-affinity system	0.7	7.4
low-affinity system	9.2	6.7
Ni^{2+}-treated cells *	0.4	5.7
arsenate-treated cells **	9.0	6.0
cells pre-treated with P_i ***	7.3	5.8
10-1 mutant	3.3	10.8
20-2 mutant	7.0	10.0
20-1 mutant	0.8	6.8

* P_i uptake analysed in the presence of 10 mM $NiSO_4$.

** Cells were pre-treated with 10 μM arsenate per 10^9 cells for 4 min, then washed twice with cold 0.14 M NaCl before P_i uptake.

*** Cells were pre-treated with 12 μM P_i per 10^9 cells for 2 min, then washed twice with cold 0.14 M NaCl before P_i uptake.

of P_i in cells treated in various ways was analysed kinetically. As shown in Fig. 1, cells which have been pre-treated with 12 μM unlabelled P_i per 10^9 cells took up P_i only at the secondary rate. It was also known (Fig. 6) that P_i did not leak out of this pool unless a large excess of P_i was added to the medium. Therefore, with the low concentration of external P_i used in the kinetic experiments, valid results on the secondary phase could be obtained with cells carefully 'pre-loaded' with 12 nmoles unlabelled P_i per 10^9 cells. Such cells appeared to take up P_i by only one mechanism, similar to the low-affinity component (Fig. 10). Similar results were obtained when the uptake of P_i was kinetically analysed in cells grown up in the presence of ample P_i.

In the presence of Ni^{2+} the secondary uptake is abolished (Fig. 3). Only one uptake mechanism, resembling the high-affinity component in its kinetic properties can be demonstrated in such cells (Fig. 9). Arsenate was shown to be a powerful inhibitor of the primary uptake (Fig. 3), and only one mechanism, resembling the low-affinity component kinetically, could be demonstrated in arsenate-treated cells (Fig. 9).

Similarly, kinetic studies on the mutants 10-1 (Fig. 9) and 20-2 showed that these mutants take up P_i by one mechanism, which resembles, in its kinetic properties, the low-affinity component of the parent cells. The arsenate-resistant mutant, 20-1, also takes up P_i by one mechanism, which resembles the high-affinity component (Table III).

The uptake of phosphite, pyrophosphate and arsenate

The uptakes of [^{32}P]phosphite, [^{74}As]arsenate and [^{32}P]pyrophosphate were measured in cells grown in TSYG, washed twice and resuspended in ISG medium. These cells took up only minute amounts of phosphite, and this was not affected by a pre-treatment of the cells with phosphite or P_i; phosphite did not have any effect on the uptake of P_i. The pattern of pyrophosphate uptake was similar to that of P_i uptake, and the extent of the primary uptake (on a phosphorus basis) was identical to that found with P_i. However, pyrophosphate was completely hydrolysed to P_i within 1 min of entry, and no intracellular pyrophosphate could be detected. Arsenate was taken up by cells within the first 2 min of exposure, to the extent of about 3 nmoles per 10^9 cells. The arsenate was then gradually lost. The arsenate-resistant mutant, 20-1, although possessing an increased capacity for P_i uptake, took up the same quantity of arsenate as did the parent cell; and the R-10 mutant, which had a severely impaired P_i uptake, took up slightly less arsenate than the parent. A 20-fold excess of either arsenate or P_i added to cells at the point of maximal [^{74}As]arsenate uptake, caused rapid loss of the incorporated label (Fig. 11). The arsenate was taken up with a K_m of 50 μM and a v_{max} of 5 nmoles arsenate per 10^9 cells per min.

DISCUSSION

E. coli grown in media devoid of orthophosphate deplete a pool which is rapidly refilled when cells are presented with P_i. The capacity of this pool is 10–12 nmoles of P_i per 10^9 cells. The pool can be 'pre-loaded' by adding P_i to the exact capacity, when the rapid $^{32}P_i$ entry into the pool can no longer be demonstrated. Cells grown in the presence of P_i have a fully loaded pool at harvesting. This pool can be depleted again if these cells are deprived of phosphorus for 2 h.

Although the esterification of orthophosphate commences soon after it enters the cell, the P_i is taken up as such, against a steep concentration gradient. The initial rate of accumulation of intracellular P_i parallels that of the total uptake. Chase data indicate that the P_i passes through the pool and is esterified, the passage of label into the acid insoluble fraction commencing after the pool has been filled.

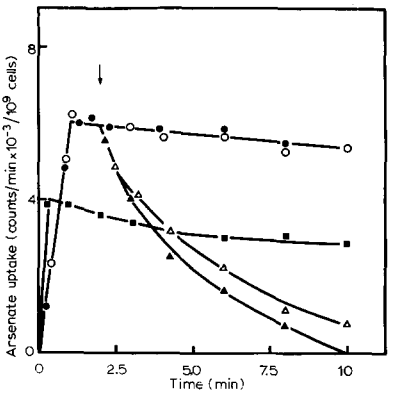


Fig. 11. Arsenate uptake in *E. coli*. 100 μM [^{74}As]arsenate was added to 10^9 cells per ml. A 100-fold excess of P_i or arsenate was added at 2 min (arrow). (a) Arsenate added alone: ●—●, uptake in parent cells; ○—○, 20-1 mutant; ■—■, uptake in R-10 mutant. (b) Followed by unlabelled chase: ▲—▲, of P_i; △—△, or of arsenate.

The system is reminiscent of P_i transport of *B. cereus* which we described previously[14]. However, the *E. coli* primary pool is filled much more rapidly, and the kinetic parameters differ. The pattern of phosphate transport in both organisms is in direct contrast to that in yeast[20] or the red blood cell[21,22] where P_i is esterified upon entry.

A fast, initial uptake of P_i has also been reported in P_i-deficient algae[12] and biphasic uptake curves have been obtained for sulphate uptake in sulphate-deprived Chlorella[23]. This phenomenon therefore is not unique, and may be widespread.

Stimulation of P_i uptake by monovalent metal ions has been demonstrated in yeast[3], in *Streptococcus faecalis*[6], in a marine fungus[24] and in other systems. The rapid initial P_i uptake in our strain also requires the presence of K^+; Mg^{2+} is required for the maintenance of maximal rates of P_i uptake from then on. However, some uptake does take place without added Mg^{2+} and may be due to the presence of residual Mg^{2+}. A K^+-stimulated rapid P_i uptake in K^+-deprived *E. coli* has been described by WEIDEN *et al.*[25]. These workers found that the addition of K^+ caused an immediate stimulation of P_i uptake. This observation was confirmed in our system, where the specific area of K^+ stimulation of P_i uptake was shown to be in the restoration of the primary uptake phase in cells which had been transporting phosphate at the lower rate, from media lacking K^+.

Both phases of P_i uptake require an external energy source. Glucose-grown cells do not show the rapid P_i uptake in a glucose-free medium, but the complete biphasic P_i uptake system returns immediately upon the addition of glucose. This immediate enhancement of P_i uptake by glucose or galactose (and to a lesser extent, by mannitol or fructose) suggests either that these hexoses can provide the necessary energy very rapidly, or that they may be co-transported with P_i. No enhancement of P_i uptake could be elicited by 2-deoxyglucose which is unable to support the growth

of these cells. These findings and the results of inhibitor studies, suggest that the metabolism of glucose is necessary for the efficient uptake of P_i.

In *E. coli*, P_i transport occurs by two kinetically distinct systems, reminiscent of the histidine permease system of *Salmonella typhimurium*[26]. The high-affinity P_i transport component has a K_m of 0.7 μM. The phosphate-binding protein has been shown to be involved in P_i transport both by reconstitution of P_i uptake[15], and by the selection of mutants which lack this protein and are defective in P_i transport.

The mutants which lack phosphate-binding protein, also have a reduced ability to take up P_i from media containing P_i at a low concentration. The primary uptake appears to be missing, and kinetic studies show that the high-affinity component is missing as well. Because the kinetic constant of the phosphate-binding protein, $K_D = 0.8$ μM, is very similar to that of the high-affinity component, and since this component is absent from mutants which lack this protein, we believe that the phosphate-binding protein is involved in the high-affinity transport component. In this respect the high-affinity P_i-transport component resembles the high-affinity histidine component described by AMES AND LEVER[27].

P_i uptake by the high-affinity component is not demonstrable in cells in which the pool has been preloaded with 12 μM P_i per 10^9 cells. The high-affinity component appears thus to be regulated by the amount of P_i available to the cell: Once the P_i pool is filled, this component is effectively shut down. Up to that stage, the flux of P_i is unidirectional, exchange being demonstrable only after the pool has been filled. Thus the primary uptake cannot involve exchange-equilibration with the P_i pool. It is possible that this pool is, in fact, a membrane-associated pool. Similar pools have been postulated by PASSOW[28] and DEUTICKE[29] for anion uptake into the red blood cell. The low-affinity P_i-transport component ($K_m = 9$ μM) appears to operate simultaneously with the high-affinity system in the primary rapid uptake while the pool fills (Fig. 9) although the high-affinity component alone can load the pool. This is evident in the Ni^{2+}-treated cells or in the 20-1 mutant, both of which exhibit only the high-affinity transport component on kinetic analysis.

The 20-1 mutant represents a special case of transport derepression. This mutant not only took up P_i to a greater extent, but also incorporated it into nucleic acids at a higher rate than did the parent cells. The process was of a limited duration since these cells clotted in the ISG medium 10 min after incubation with P_i. The enhanced uptake of P_i could only be observed under conditions where the cells eventually clotted. When harvested from media containing ample P_i, 20-1 cells transported P_i less efficiently.

Incorporation of the label into nucleic acids in 20-1 cells was inhibited by Ni^{2+}, and Ni^{2+}-treated cells took up P_i to the same extent and at the same rate as the Ni^{2+}-treated parent cells. All the accumulated label was extractable by cold trichloroacetic acid. When the incorporation of label into nucleic acids was partially inhibited by acridine orange, 5-bromodeoxyuridine or low concentrations of Ni^{2+}, the total uptake of P_i was depressed to the same extent, the pool size being unaffected. This suggests that the capacity of the P_i pool is well defined and is about the same in the parent strain and the 20-1 mutant. The filling of this pool is rapid and, in the parent strain, its rate declines only when the pool is filled and uptake becomes limited to replenishing the P_i removed at a slower rate from the pool for intracellular use, particularly for the synthesis of nucleic acids. In the 20-1 mutant, this latter synthesis

appears to be derepressed and is no longer the rate-limiting step (at least in the first 10–20 min of incubation). Here the primary uptake rate may well have become the rate-limiting process and the uptake of P_i continues at this high rate.

Arsenate is an efficient inhibitor of the primary uptake (Fig. 3) and appears to inhibit only the high-affinity component, as it has relatively slight effect on the secondary uptake. This was also confirmed by kinetic studies. Furthermore the inhibition of total P_i uptake by arsenate is not as great as would be expected if it were transported by both components. This is in accord also with the finding of BENNETT AND MALAMY[30] who, on the basis of differential sensitivity of their parent strain and arsenate-resistant mutants to cyanide, postulated that P_i is taken up by at least two mechanisms. We have demonstrated by kinetic techniques that there are two P_i transport systems in *E. coli*, one being extremely sensitive to arsenate. Our parent strains and mutants are both sensitive to cyanide.

The present results suggest that phosphate uptake in *E. coli* may involve the following steps: (i) The rapid filling of an intracellular (possibly intramembrane) pool of defined capacity by both the high-affinity and low-affinity P_i transport components functioning simultaneously. The phosphate-binding protein is involved in the high-affinity component. P_i is accumulated against a concentration gradient in an energy-dependent process in this step. (ii) A slower passage of P_i into the pool by the low-affinity component to replace phosphate removed from it for esterification in the proces of oxidative and other phosphorylation, the removal being the rate-limiting step. This is also sensitive to a variety of inhibitors and depends on the metabolic activity of the cell.

This system, and especially the high-affinity component, allows this organism to accumulate an essential ion from highly dilute solution, at a rate exceeding the rate of its utilization. Such a system must confer an obvious advantage on the organism.

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