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# Requirement of acetyl phosphate for the binding protein-dependent transport systems in *Escherichia coli*

[shock-sensitive transport system/phosphate acetyltransferase (phosphotransacetylase)/acetate kinase/*ecf* mutant/acetyl coenzyme A]

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**ABSTRACT** **In *Escherichia coli*, acetyl phosphate can be formed from acetyl-CoA via the phosphotransacetylase (phosphate acetyltransferase; acetyl-CoA:orthophosphate acetyltransferase, EC 2.3.1.8) reaction and from acetate (plus ATP) via the acetate kinase (ATP:acetate phosphotransferase, EC 2.7.2.1) reaction. By restricting acetyl phosphate formation to the phosphotransacetylase reaction alone, through the use of metabolic inhibitors, we were able to show that, with pyruvate as a source of energy, mutants defective in phosphotransacetylase are unable to transport glutamine, histidine, and methionine. However, with the same energy source, mutants defective in acetate kinase are normal in the transport of these amino acids. The inability of the phosphotransacetylase mutants to transport is due to their presumed inability to form acetyl phosphate, because pyruvate is found to be metabolized to acetyl-CoA in these mutants. Thus acetyl phosphate has been implicated in active transport. Evidence is also presented that neither the protonmotive force nor the *ecf* gene product is required for the shock-sensitive transport systems.**

Many active transport systems in bacteria are coupled to the circulation of protons across the cytoplasmic membrane (refs. 1–3; for review, see ref. 4). However, it has been demonstrated by Berger (5) and Berger and Heppel (6) that, in *Escherichia coli*, ATP or some form of phosphate bond energy, not the proton gradient, may be used to drive osmotic shock-sensitive active transport systems, permeases that require the periplasmic binding proteins for activity.

Berger and Heppel reached this conclusion on the basis of the following observations: (*i*) arsenate almost completely abolishes shock-sensitive but not shock-resistant transport activity; (*ii*) mutants with a nonfunctional Ca,Mg-ATPase, which are thus defective in oxidative phosphorylation, cannot drive shock-sensitive transport with the respiratory substrate D-lactate or the artificial electron donor ascorbate/phenazine methosulfate as a source of energy, whereas shock-resistant transport is active with these electron donors; and (*iii*) glucose, owing to substrate-level phosphorylation, stimulates shock-sensitive transport in the same mutants, and this effect is not strongly inhibited by anaerobiosis or the uncoupler 2,4-dinitrophenol.

At variance with this conclusion are the findings that ATP or phosphate bond energy alone cannot be the direct energy donor for these transport systems as observed with *E. coli* cells defective in the energy coupling factor (ECF) protein (7) and that the electrochemical proton gradient is required in *E. coli* cells treated with colicin K (8) or in *Salmonella* cells deficient in cytochromes (9).

In this paper, we show that acetyl phosphate is required for shock-sensitive transport, and that neither the electrochemical proton gradient nor the *ecf* gene product is required.

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## MATERIALS AND METHODS

**Bacterial Strains.** All strains used were *E. coli* K-12. *ecf* mutants JSH270 and MAL312 have been described (10, 11).

**Media.** Minimal salts medium E (12) containing 0.5% carbon source and 40 μM vitamin B1 was used. When required, amino acids were added to 0.4 mM. Solid media contained 1.5% agar.

**Isolation of Mutants.** Mutants deficient in acetate kinase (AcK; ATP:acetate phosphotransferase, EC 2.7.2.1) or phosphotransacetylase (PTA; phosphate acetyltransferase; acetyl-CoA:orthophosphate acetyltransferase, EC 2.3.1.8) were isolated from JSH210 ($F^-$ *thi, metC*) on minimal glucose or minimal succinate plates containing 50 mM sodium monofluoroacetate, as described (13). Temperature-sensitive (ts) mutants were isolated as fluoroacetate-resistant colonies at 42°C. Those colonies that exhibited ts growth on acetate as the sole carbon and energy source were considered candidates and checked for PTA activity. One such *pta* mutant, JSH285, was derived through nitrosoguanidine mutagenesis. This mutant has less than 1% of wild-type PTA activity at 42°C, and 2% at 30°C. The other ts *pta* mutant, AGH18, and the two *ack* mutants, AGH11 and AGH12, were isolated as spontaneous mutants. AGH18 has 0.5% of wild-type PTA activity at 42°C and 2% at 30°C. The two *ack* mutants have less than 3% of wild-type AcK activity.

**Preparation of Cells and Transport Assay.** Cells growing exponentially on minimal succinate medium at 27°C were washed three times with 25 mM Tris·HCl, pH 7.3, and resuspended in the same buffer. For transport assays at 30°C, cells (0.2 ml) in Tris buffer at $1.0 \times 10^{10}$ cells per ml were first starved by treatment with 25 mM potassium arsenate (pH 7) for 18 min at 30°C, followed by dilution with 0.8 ml of 0.12 M potassium phosphate, pH 7.0, containing 1 mM $MgCl_2$, and by addition of KCN (5 mM) and NaF (30 mM). Aliquots (50 μl) were then distributed to test tubes, incubated for 5 min in the presence of these inhibitors, and further incubated for an additional 10 min in the presence or absence of 20 mM potassium pyruvate. Their ability to transport was then assayed as described previously (14). For transport assays at 42°C, cells were processed identically at 42°C as at 30°C except that the starvation with arsenate was initially done at 30°C (10 min) followed by an additional 8 min at 42°C and that prewarmed (42°C) potassium phosphate buffer was used. Unless otherwise indicated, the specific activities and final concentrations of the radioactive compounds used were as follows: L-[*U*-$^{14}$C]glutamine (122 Ci/mol), 8.2 μM; L-[*methyl*-$^3$H]methionine (954

Abbreviations: ECF, energy coupling factor; PTA, phosphotransacetylase; AcK, acetate kinase; PMF, protonmotive force; ts, temperature-sensitive.

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Ci/mol), 1.4 μM; L-[3-$^3$H]histidine (600 Ci/mol), 0.83 μM (1 Ci = 3.7 × $10^{10}$ becquerels).

**Intracellular Acetyl-CoA Levels.** For the measurement of intracellular acetyl-CoA levels, Tris·HCl-washed cells (1 ml) at 3.3 × $10^{10}$ cells per ml were processed identically as those used for transport assays through the treatment with KCN and NaF. At the end of the 5-min incubation with these inhibitors, pyruvate was added to 20 mM and at various intervals aliquots (1 ml) were removed to test tubes containing 0.25 ml of 10 M perchloric acid. After 20 min at 0°C, precipitates were removed by centrifugation, and the pH of these extracts was adjusted to 6.8–7.2 with concentrated KOH. Precipitates formed were removed by centrifugation. Acetyl-CoA was measured as described (15), using a Perkin–Elmer double wavelength double beam spectrophotometer.

**Enzyme Assays.** AcK was measured by following the formation of ADP from acetate and ATP in a coupled enzymatic assay, as described (16). The reaction mixture, in 3 ml, contained 210 μmol of Tris·HCl at pH 7.3, 1 mmol of sodium acetate, 10 μmol of ATP, 3.2 μmol of phospho*enol*pyruvate, 0.96 μmol of NADH, 0.1 mg of pyruvate kinase, 0.05 mg of lactate dehydrogenase, and 5–150 μg of protein of cell-free extract. PTA was measured by following the formation of acetyl-CoA at 232 nm. The reaction mixture in 1 ml contained 50 μmol of Tris·HCl at pH 7.3, 2 μmol of 2-mercaptoethanol, 0.2 μmol of CoA, 10 μmol of acetyl phosphate, and 10–100 μg of protein of cell-free extract.

**Protein Determinations.** Protein was determined by the method of Lowry *et al.* (17), with bovine serum albumin as the standard.

**Materials.** All radioactive materials were obtained from New England Nuclear. All other materials were reagent grade and were purchased from commercial sources. Citrate synthase and malate dehydrogenase were from Boehringer Mannheim, pyruvate kinase was from Worthington, and lactate dehydrogenase was from Sigma.

## RESULTS

### Protonmotive force (PMF) and ECF are not required

Following the discovery of Berger (5) that ATP or some form of phosphate bond energy might serve as the energy donor for the binding protein-dependent transport systems, we examined these transport systems in an *E. coli ecf* mutant, which is temperature-sensitive in the coupling of metabolic energy to active transport (7). We found that although the intracellular ATP levels increased 2-fold at the nonpermissive temperature (42°C), transport of shock-sensitive solutes was totally defective. This result demonstrates that ATP alone cannot be the energy requirement. In this mutant, both shock-resistant transport (18) and shock-sensitive transport (M. A. Lieberman, unpublished) can be stimulated by glucose, and the extent of stimulation is proportional to the quantity of glucose added. If the glucose present is low and limiting (≤1 mM), the stimulation is only transient.† For shock-resistant transport, the transient accumulation of solutes in the *ecf* mutant has been shown (7) to be due to the transient establishment of the membrane potential, the major component of the PMF.

Typical data for transient amino acid accumulation in the *ecf* mutant MAL312 are shown in Fig. 1. Although both shock-sensitive and shock-resistant transport systems behaved

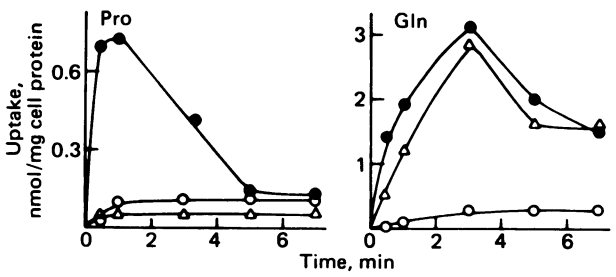


FIG. 1. Uptake of proline and glutamine by the *ecf* mutant MAL312 at 42°C: stimulation by glucose and effect of cyanide. Cells were grown in minimal glucose at 26°C, harvested in exponential growth, washed two times with carbon-free salts medium (19), and resuspended in the same medium, containing chloramphenicol at 100 μg/ml, to an $OD_{660}$ of 3.0. Transport was assayed as described (14). Glucose (0.4 mM) was added 15 sec before the addition of radioactive substrate. Cyanide-treated cells were prepared by adding KCN to 5 mM and incubating at 25°C for 5 min. The cells were stored on ice until assayed. ○, Nontreated cells; ●, nontreated cells + 0.4 mM glucose; △, cyanide-treated cells + 0.4 mM glucose.

similarly under the conditions used, a clear distinction in the energy requirement can be made between them: proline transport, which is shock-resistant and is driven by the PMF, is completely inhibited by cyanide (5 mM), whereas glutamine transport, which is shock-sensitive, is relatively insensitive to cyanide. Uptake of glutamine transport in the *ecf* mutant in the presence of glucose and cyanide must, therefore, indicate that (*i*) neither the PMF nor the *ecf* gene product is required for shock-sensitive transport and (*ii*) some metabolite of glucose (or glucose itself) other than the Krebs cycle intermediates is the energy requirement for shock-sensitive transport. Moreover, this metabolite must be one that, in the absence of an exogenous energy source, is not present in a sufficient quantity to effect transport and is generated only transiently when glucose provided is low and limiting (Fig. 1).

To eliminate some glycolytic intermediates as candidates for the energy source for shock-sensitive transport, enolase was inhibited by sodium fluoride. Enolase catalyzes the interconversion of phospho*enol*pyruvate and 2-phosphoglycerate. Glycolytic intermediates produced prior to the enolase reaction should accumulate in the presence of NaF. If any of these intermediates were the energy source, addition of glucose to NaF-treated cells should stimulate glutamine transport. Experiments with NaF were carried out with the *ecf* mutant JSH270 (10) which is ts in amino acid transport. It was found that 30 mM NaF totally suppresses the glucose stimulation of glutamine transport at 42°C (Fig. 2). If acetyl phosphate, acetyl-CoA, or phospho*enol*pyruvate were the energy source, addition of pyruvate to NaF-treated cells should stimulate glutamine transport. Pyruvate, which shows absolutely no effect on glutamine transport by itself, significantly stimulates uptake of glutamine in the presence of 30 mM NaF (Fig. 2). In the absence of NaF, acetyl phosphate, acetyl-CoA, and phospho*enol*pyruvate made from pyruvate might be dissipated through the gluconeogenic pathway. In the presence of NaF, however, these metabolites probably accumulate to some extent and are available for shock-sensitive transport.

It was not possible to directly examine acetyl-CoA, acetyl phosphate, or phospho*enol*pyruvate for their ability to drive transport because *E. coli* cells are impermeable to these compounds (ref. 20; J-s. Hong, unpublished results). However, they can be indirectly tested by examining the effect of acetoacetate on transport. Acetoacetate is actively transported into *E. coli* cells as acetoacetyl-CoA by an inducible transport system, and

† The transitory stimulation of amino acid transport is probably unique to *ecf* mutants. It is not known whether such transitory stimulation of transport is observable with 2,4-dinitrophenol-starved Ca,Mg-ATPase$^-$ mutant or wild-type cells under the conditions used by Berger (5) and Berger and Heppel (6).

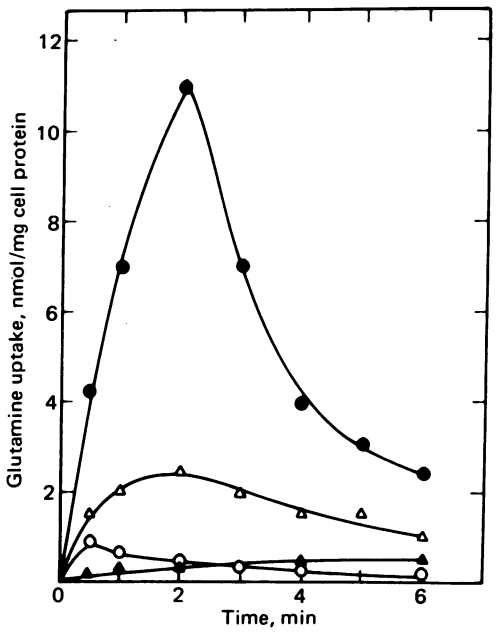


FIG. 2. Effect of NaF on glutamine uptake by JSH270. Cells were grown and processed for transport assay as described for Fig. 1. NaF-treated cells were prepared by adding NaF to 30 mM and incubating at 25°C for 8 min. The cells were stored on ice until assayed. Glucose (0.4 mM) or pyruvate (20 mM) was added 15 sec before the addition of radioactive glutamine. Assays were done at 42°C. ●, Nontreated cells + glucose; ○, treated cells + glucose; ▲, nontreated cells + pyruvate; △, treated cells + pyruvate.

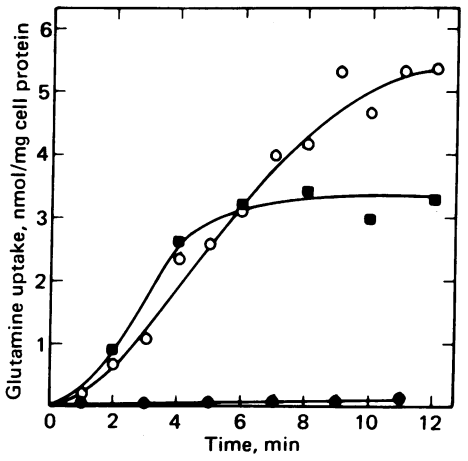


FIG. 3. Stimulation of glutamine transport by acetoacetate. Strain JSH210 was grown at 37°C on minimal salts medium with 0.1% acetoacetate (Li salt) as the sole carbon source and harvested in exponential growth. Cells were washed twice with 0.1 M $KP_i$, pH 7.0, containing 1 mM $MgCl_2$, resuspended in the same buffer, and then starved with 5 mM 2,4-dinitrophenol for 13 hr at 37°C. After the starvation, dinitrophenol was removed by washing the cells three times with the potassium phosphate buffer, and the cells were resuspended in the same buffer to an $OD_{660}$ of 3.0. Transport was assayed as described (14). Acetoacetate (Li salt) was added 15 sec before the addition of radioactive glutamine. Cyanide-treated cells were prepared by adding KCN to 5 mM and incubating at 37°C for 5 min. Assay temperature was 37°C. ●, No exogenous energy source present; ○, + acetoacetate (3.2 mM); ■, cyanide-treated cells + acetoacetate (3.2 mM).

the resulting acetoacetyl-CoA undergoes thiolytic cleavage to form two molecules of acetyl-CoA (21). Fig. 3 shows that in wild-type (JSH210) cells grown on acetoacetate as the sole carbon source and subsequently starved by 2,4-dinitrophenol treatment, glutamine uptake is stimulated by acetoacetate, and this stimulation is only slightly affected by cyanide. Because, in the presence of cyanide, pyruvate—and hence phospho*enol*pyruvate—is not expected to be derived from acetyl-CoA, uptake of glutamine in the presence of acetoacetate and cyanide indicates that pyruvate and phospho*enol*pyruvate are not required for shock-sensitive transport and suggests that acetyl phosphate or acetyl-CoA is required.

## Acetyl phosphate is required

To determine whether acetyl phosphate is required for the shock-sensitive transport systems, we isolated mutants deficient in PTA and AcK, two enzymes that are required for the activation of acetate to acetyl-CoA via acetyl phosphate (13), and we examined the effect of these mutations on transport. Using cells not treated with any inhibitor (such as 2,4-dinitrophenol, arsenate, or cyanide), we found no significant reduction in the transport ability in these mutants as compared to the wild-type strain (data not shown). This finding is not surprising, however, because, in the *pta* mutants, acetyl phosphate can also be derived from acetate via the AcK reaction and in the *ack* mutants from acetyl CoA via the PTA reaction (Fig. 4). Therefore, to investigate the involvement of acetyl phosphate in active transport, it was necessary to biochemically block either the AcK reaction in the *pta* mutants or the PTA reaction in the *ack* mutants. We chose to block the AcK reaction because it could be easily done by lowering the intracellular ATP levels through arsenate treatment of the cells. The strategy eventually adopted for transport assays, after the arsenate treatment, was to use pyruvate as the exogenous source of energy and to assay transport in the presence of cyanide. The use of pyruvate and cyanide prevents a net formation of ATP through glycolysis and the Krebs cycle. Also, pyruvate can serve in the reaction catalyzed by the cytoplasmic lactate dehydrogenase as the oxidant for the conversion of NADH to NAD that is necessary for the oxidation of pyruvate to acetyl-CoA. In addition, NaF was also used in the assays to inhibit enolase and thus prevent acetyl phosphate production from glycolytic intermediates that might still be present after the arsenate treatment.

Using these conditions, we expected the formation of acetyl phosphate from pyruvate in the wild-type and *ack* mutants, but not in the *pta* mutants. Thus, if acetyl phosphate is required for transport, only *pta* mutants and not *ack* mutants are expected to be defective in transport. This was found to be the case. Fig.

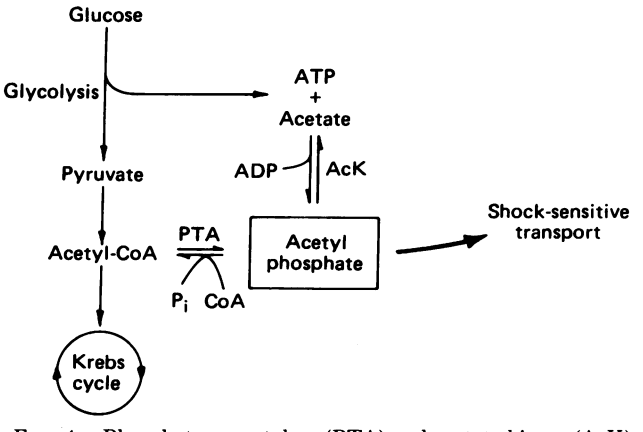


FIG. 4. Phosphotransacetylase (PTA) and acetate kinase (AcK) reactions and their metabolic positions relative to glycolysis and the Krebs cycle.

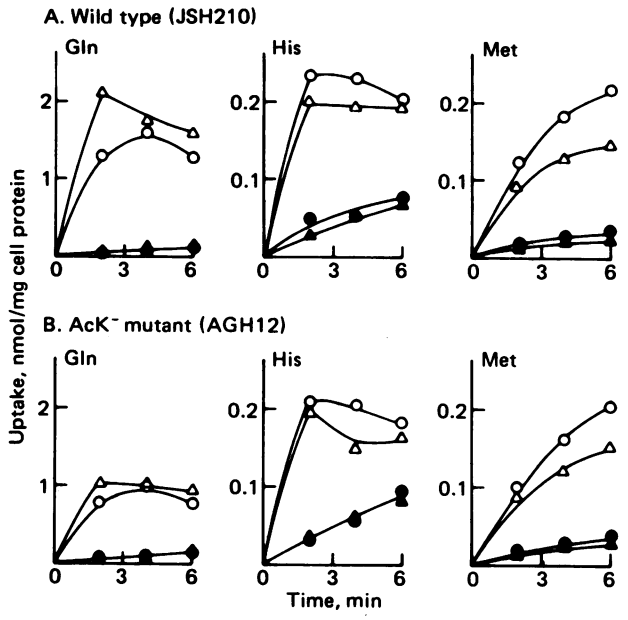


FIG. 5. Uptake of amino acids by wild-type JSH210 (*A*) and AcK mutant AGH12 (*B*). Cells were grown in minimal succinate at 27°C, harvested in exponential growth, and processed for transport assay as described in *Materials and Methods*. ●, 30°C; ○, 30°C and preincubated with 20 mM pyruvate; ▲, 42°C; △, 42°C and preincubated with 20 mM pyruvate.

5*A* shows that transport of glutamine, histidine, and methionine, substrates taken up by the shock-sensitive transport systems, by the wild-type JSH210 in the absence of an exogenous energy source, was insignificant and was greatly stimulated by pyruvate. A similar extent of stimulation was also observed with the *ack* mutants, AGH11 and AGH12. Typical data is shown for AGH12 (Fig. 5*B*). However, very little stimulation of uptake of these amino acids was observed with two ts *pta* mutants, AGH18 and JSH285, at the nonpermissive temperature, 42°C (Fig. 6). At the permissive temperature, 30°C, JSH285 takes up these amino acids at a rate about 50% that of the *ack* mutant AGH12, and the AGH18 uptake rate is about 30% that of the *ack* mutant.

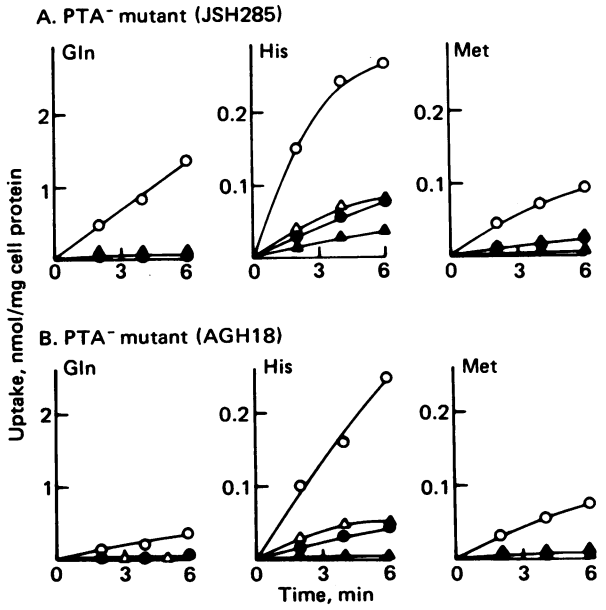


FIG. 6. Uptake of amino acids by PTA mutants JSH285 (*A*) and AGH18 (*B*). Cells were grown in minimal succinate at 27°C, harvested in exponential growth, and processed for transport assay as described in *Materials and Methods*. ●, 30°C; ○, 30°C and preincubated with 20 mM pyruvate; ▲, 42°C; △, 42°C and preincubated with 20 mM pyruvate. In the figure, ▲ and ● are superimposed in the case of glutamine transport and △ and ● are superimposed in the case of methionine transport.

Table 1. Acetyl-CoA levels in arsenate/cyanide/fluoride-treated cells

| Conditions | Acetyl-CoA, nmol/mg cell protein | | | |
|---|---|---|---|---|
| | JSH285 (PTA⁻) | AGH18 (PTA⁻) | AGH12 (AcK⁻) | JSH210 (wild type) |
| 30°C | ≤0.09 | ≤0.09 | ≤0.09 | ≤0.09 |
| 30°C + pyruvate | 0.90 | 1.22 | 0.75 | 0.60* |
| 42°C | ≤0.09 | ≤0.09 | ≤0.09 | ≤0.09 |
| 42°C + pyruvate | 1.21 | 1.07 | 1.07 | 1.20 |

Cultures were grown as described in the legend to Fig. 5 and processed for acetyl-CoA measurement as described in *Materials and Methods*.

* Acetyl-CoA level in wild-type cells not treated with the inhibitors was 0.31 nmol/mg of cell protein.

Reyertants of the *pta* mutants (acetate growers) regain the ability to transport glutamine, histidine, and methionine at 42°C (data not shown), indicating that the inability to transport is due to the *pta* mutations.

To eliminate the possibility that the inability of the *pta* mutants to transport in the presence of pyruvate might be due to the inability of the mutant to take up pyruvate or to convert pyruvate to acetyl-CoA, we measured acetyl-CoA levels in the cells treated identically as those used for transport assays. The results in Table 1 show that at both 30°C and 42°C acetyl-CoA levels in the treated *pta* mutants increased at least 10-fold upon addition of pyruvate. The same was true for the *ack* mutant AGH12 and wild-type JSH210. These results demonstrate that pyruvate is indeed metabolized in the *pta* mutants, and therefore imply that the inability of the *pta* mutants to transport is due to their presumed inability to form acetyl phosphate. It is to be noted that acetyl CoA levels were 2 to 3 times higher in the cells treated with inhibitors than in the untreated wild-type cells.

Under the conditions used for transport assay, the intracellular ATP levels in all strains in the absence of pyruvate were about 20–25% of that of untreated cells, and these levels did not increase significantly upon addition of pyruvate.

## DISCUSSION

We have presented evidence that implicates acetyl phosphate as the energy donor for the shock-sensitive transport systems in *E. coli*. To our knowledge, the involvement of acetyl phosphate in active transport has not been proposed previously. In our studies, an effort was made to directly measure acetyl phosphate, but the concentration of this compound in cells proved to be too small to be detected (K-i. Tomochika and J-s. Hong, unpublished results). Nevertheless, the evidence, although indirect, strongly argues for the existence of acetyl phosphate in the *ack* and wild-type strains and the lack of it in the *pta* mutants under the conditions used for transport assay.

Berger (5) and Berger and Heppel (6) have reported that ATP or some form of phosphate bond energy might be the energy donor for shock-sensitive transport systems. These authors found that ATPase mutants, which are unable to form ATP via oxidative phosphorylation, cannot use oxidative energy

sources, such as phenazine methosulfate/ascorbate or D-lactate, to drive shock-sensitive transport. However, the wild type, which is able to carry out oxidative phosphorylation, can use these energy sources to drive transport. In light of our discovery, the reported inability of the ATPase mutants to use these substrates to energize transport may now be attributed to the inability of the cells to synthesize acetyl phosphate under the conditions used. This inability could result from either a shortage of precursors of acetyl phosphate or an inactivation of enzymes involved.

We have presented evidence (Fig. 1) that shows that neither the PMF nor the *ecf* gene product is required for the shock-sensitive transport systems. Although the present finding that the ECF is unnecessary contradicts our earlier conclusion (7), this discrepancy can be explained by an indirect effect of the *ecf* mutation, such as on the level of acetyl phosphate. The finding that the PMF is unnecessary confirms the earlier conclusion of Berger (5) and Berger and Heppel (6) but remains at variance with the finding of Plate *et al.* (8), who used colicin K as a probe of membrane transport capability and concluded that the PMF is required for the energization of glutamine transport. One plausible explanation for this contradiction may be that the inhibition of glutamine transport by colicin K is secondary to its primary effect, which is depolarization of membrane, or that it is a direct effect concomitant with depolarization. In light of our finding that acetyl phosphate is required for transport, it is possible that colicin K may inhibit acetyl phosphate-dependent transport systems by directly blocking reactions necessary for acetyl phosphate formation, by causing efflux of glycolytic intermediates that are precursors of acetyl phosphate, or by directly interfering with the functioning of the energization machinery. Colicin K-induced efflux of a number of glycolytic intermediates such as pyruvate, dihydroxyacetone phosphate, glucose 6-phosphate, fructose 1,6-bisphosphate, and 3-phosphoglycerate has been reported by Fields and Luria (22) and may be involved in the colicin K effect.

It is not possible at this time to determine whether acetyl phosphate is the sole required energy donor for the shock-sensitive transport systems or whether ATP is also required, either as an energy donor or as an effector. With acetyl phosphate as the energy donor, the most reasonable mechanism for the energization of transport is phosphorylation–dephosphorylation or acetylation–deacetylation of the carriers or other transport components. We hope that the answer to these questions will come from *in vitro* studies.

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1. Mitchell, P. (1973) *J. Bioenerg.* **4,** 63–91.
2. Altendorf, K., Hirata, H. & Harold, F. M. (1975) *J. Biol. Chem.* **250,** 1405–1412.
3. Ramos, S., Schuldiner, S. & Kaback, H. R. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1892–1896.
4. Simoni, R. D. & Postma, P. W. (1975) *Annu. Rev. Biochem.* **44,** 523–544.
5. Berger, E. A. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1514–1518.
6. Berger, E. A. & Heppel, L. A. (1974) *J. Biol. Chem.* **249,** 7747–7755.
7. Lieberman, M. A. & Hong, J-s. (1976) *Arch. Biochem. Biophys.* **172,** 312–315.
8. Plate, C. A., Suit, J. L., Jetten, A. M. & Luria, S. E. (1974) *J. Biol. Chem.* **249,** 6138–6143.
9. Singh, A. P. & Bragg, P. D. (1977) *J. Supramol. Struct.* **6,** 389–398.
10. Hong, J-s. (1977) *J. Biol. Chem.* **252,** 8582–8588.
11. Lieberman, M. A. & Hong, J-s. (1976) *J. Bacteriol.* **125,** 1024–1031.
12. Vogel, H. J. & Bonner, D. M. (1956) *J. Biol. Chem.* **218,** 97–106.
13. Brown, T. D. K., Jones-Mortimer, M. C. & Kornberg, H. L. (1977) *J. Gen. Microbiol.* **102,** 327–336.
14. Lieberman, M. A. & Hong, J-s. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4395–4399.
15. Pearson, D. J. (1965) *Biochem. J.* **95,** 23c–24c.
16. Janson, C. A. & Cleland, W. W. (1974) *J. Biol. Chem.* **249,** 2567–2571.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
18. Lieberman, M. A., Simon, M. & Hong, J-s. (1977) *J. Biol. Chem.* **252,** 4056–4067.
19. Gutnick, D., Calvo, J. M., Klopotowski, T. & Ames, B. N. (1969) *J. Bacteriol.* **100,** 215–219.
20. Saier, M. H., Wentzel, D. L., Feucht, B. U. & Judice, J. J. (1975) *J. Biol. Chem.* **250,** 5089–5096.
21. Pauli, G. & Overath, P. (1972) *Eur. J. Biochem.* **29,** 553–562.
22. Fields, K. L. & Luria, S. E. (1969) *J. Bacteriol.* **97,** 64–77.