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ATP-binding sites in the membrane components of histidine permease, a periplasmic transport system

(energy-coupling/photoaffinity labeling/*hisP* and *hisM* proteins)

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ABSTRACT Two components of the histidine permease in *Salmonella typhimurium*, the membrane-bound P and M proteins, react with the photoaffinity labeling reagent 8-azido-ATP in isolated membranes. The extent of labeling is decreased by the addition of ATP and somewhat less by addition of GTP, CTP, UTP, and ADP. Cyclic AMP, NAD, FAD, and *S*-adenosylmethionine have little effect. We propose that one or both of these proteins have a site capable of binding an adenine nucleotide and that, therefore, they may be involved in the energy-coupling step in active transport.

One of the basic problems of active transport concerns the mechanism of energy coupling. In bacterial cells, active transport systems can be subdivided into various categories on the basis of several characteristics, one of which is their mode of energy-coupling. Thus, some systems utilize the proton-motive force—e.g., the lactose permease (1). Another category has been postulated to utilize energy sources derived from substrate-level phosphorylation [variously suggested in the past to be ATP (2, 3) or acetylphosphate or a compound derived from them] (4) and/or the proton-motive force (5). This category is represented by the periplasmic systems, all of which contain a periplasmic binding protein and at least two or three membrane-bound proteins. The high-affinity histidine transport system of *Salmonella typhimurium* and *Escherichia coli* is a periplasmic system that has been characterized extensively (6). It is composed of a periplasmic histidine-binding protein, J, and three membrane-bound proteins, Q, M, and P. The P protein has been postulated on the basis of genetic evidence to interact directly with the J protein (7). The Q and the M proteins are very rich in hydrophobic amino acids and are presumed to be embedded in the membrane. The four transport proteins are encoded by four genes constituting an operon, the entire nucleotide sequence of which has been obtained (6).

Recently an extensive sequence homology was uncovered between the P protein and the MalK protein (8), a membrane-bound component of an unrelated periplasmic system transporting maltose. This homology led us to postulate that the MalK protein of the maltose system and the P protein of the histidine system are performing similar functions (9). The similarity extends to their remarkable overall hydrophilicity, which contrasts with the fact that they are membrane-bound proteins (8). Gilson *et al.* recently discovered that the MalK protein is also homologous to the NADH dehydrogenase of *E. coli* (*ndh*, ref. 10), which suggests the possibility that the MalK protein has a similar nucleotide-binding site. Therefore, we decided to investigate whether the P protein has a nucleotide-binding site. This report presents data demonstrating that in fact this is true. In addition, we determined that the M protein also might contain such a site.

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MATERIALS AND METHODS

Bacterial Strains. TA3662 and TA3663 carry plasmids pFA17 and pFA18, respectively, in *E. coli* strain K-12 ΔH1Δ*trp*[M72 Sm^R *lacZ*amΔ(*bio uvrB*) Δ(*trpEA*)2 ΔNam7 *N*am*53 cI857* ΔH1] obtained from Walter Fiers (11). Plasmid pFA17 carries a 2.8-kilobase fragment of DNA that contains the *hisQ*, *hisM*, and *hisP* genes (in that order) under the temperature-sensitive control of the λ P_L promoter. The DNA fragment was inserted in the *Hpa* I site of pKC30 (unpublished results). Plasmid pFA18 contains the same 2.8-kilobase DNA fragment as pFA17 in the reverse orientation. TA1889 and TA3874 were constructed by introducing into TA3662 and TA3663, respectively, an *unc* deletion mutation from LM1084 [*unc*(=*atp*)Δ*702*(*IBEFHAGD*) *asnA*::Tn*10* *asnB relA spoT*] (12), by selecting for the Tn*10* insertion in the closely linked gene *asnA* and scoring for inability to grow on succinate.

Membrane Preparation. Bacteria were grown to an A_{650} of 0.4–0.6, in 100 ml of LB medium containing ampicillin, at 30°C (13) and then shifted to 42°C for an additional 50 min. Control cultures were maintained at 30°C. The bacteria were harvested by centrifugation at 17,000 × *g* for 10 min, resuspended in 4 ml of 10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.25 M sucrose at 0°C, and sonicated (4 × 15 sec) with a Branson Sonifier fitted with a microtip. Unbroken cells were removed by centrifugation at 3000 × *g* for 15 min and the supernatant was centrifuged at 166,000 × *g* for 1 hr. The membranes were resuspended in 0.22 ml of the same buffer to give a protein concentration (as assayed by the Lowry method) of ≈5 mg/ml and stored on ice until use.

Photoaffinity Labeling. Membrane samples (100 μg of protein) were added to cold 10 mM Tris·HCl, pH 7.4/5 mM $CaCl_2$ containing 0.02–2 μM 8-azido-[γ-^{32}P]ATP (N_3[^{32}P]-ATP) (≈1.5 μCi; 1 Ci = 37 GBq) in a final volume of 100 μl and placed in the wells of a porcelain spot plate that was maintained on ice during the irradiation. After 30 sec the reaction mixture was irradiated with UV light from a lamp emitting at 366 nm and at lower wavelengths, or with a short-wavelength UV lamp (254 nm), at a distance of 8–10 cm for 2–4 min. The samples were then centrifuged for 10 min in an Eppendorf centrifuge (which caused large losses of material but had the advantage of rapidity) or for 20 min in a Beckman Airfuge at 132,000 × *g*, resuspended without boiling in Laemmli sample buffer (14) with the omission of 2-mercaptoethanol, and subjected to electrophoresis on a sodium dodecyl sulfate/polyacrylamide gel.

ATPase Assay. The reaction mixture containing 100 mM Tris·HCl (pH 9.0), 0.5 or 5 mM ATP, 5 mM $CaCl_2$, and membrane samples in a volume of 0.5 ml (10–50 μg of protein) was incubated for 30 min at 37°C. The reaction was stopped by the addition of 50 μl of 100% (wt/vol) trichloroacetic acid. Samples were centrifuged for 10 min at 15,000 × *g* and

Abbreviation: N_3ATP, 8-azido-ATP.

the supernatant fractions were assayed for the presence of either inorganic phosphate (15) or ADP (by HPLC using a C_{18} 5-μm column with 10 mM tetrabutylammonium phosphate, pH 7.2/8.75% acetonitrile as the mobile phase). Alternatively, the release of ^{32}P from 10 mM [γ-^{32}P]ATP in 50 mM Tris·HCl (pH 8.0) with 10 mM $MgCl_2$ was determined by chromatography on a PEI-cellulose TLC sheet in saturated ammonium sulfate/4% EDTA.

Assay of the NADH Dehydrogenase. Membrane samples (100 μg of protein) were added to 3 ml of 10 mM Tris·HCl, pH 7.4/5 mM $CaCl_2$ at room temperature. The change in oxygen concentration in response to the addition of NADH to 10 mM was measured by using an oxygen electrode (MSE Spectro-Plus). Alternatively, the disappearance of NADH was followed by the absorbance decrease at 340 nm in the presence of 2 mM $MgCl_2$.

Chromatography on Blue Agarose. Bacteria, grown and harvested as for membrane preparations, were resuspended in 4.5 ml of 10 mM Tris·HCl (pH 7.5), disrupted by two passages through a French pressure cell at 18,000 psi (1 psi = 6.89 kPa), and centrifuged at 257,000 × *g* for 1 hr. One milliliter of the supernatant fraction (≈1.25 mg of protein) was applied to a 0.6 × 3 cm column of Affi-Gel blue 100–200 mesh (Bio-Rad) equilibrated with 10 mM Tris·HCl (pH 7.5). Proteins were eluted successively with this buffer alone, the same buffer containing first 10 mM or 100 mM ATP, then 1 M NaCl, and then 5 M NaCl. One-milliliter fractions were collected, the proteins were precipitated by the addition of 100 μl of 100% (wt/vol) trichloroacetic acid and then dissolved in 65 μl of Laemmli sample buffer (14), and aliquots were subjected to sodium dodecyl sulfate gel electrophoresis.

Polyacrylamide Gel Electrophoresis. The unidimensional system was as described by Laemmli (14), except that the pH of the resolving gel buffer had been adjusted to pH 8.65 (for photoaffinity labeling experiments) or pH 8.50 (for the analysis of fractions from blue agarose chromatography). The concentration of acrylamide was 11%. The two-dimensional gel system was as described for the identification of the P protein (16, 17); the concentration of acrylamide in the second dimension was 12%.

Materials. The N_3[^{32}P]ATP (16 mCi/μmol) was obtained by the generous gift of Boyd Haley (University of Wyoming) or synthesized (796 mCi/μmol) from 8-azido-ADP (Sigma) essentially as described (18).

RESULTS

Labeling of Histidine Transport Proteins with N_3[^{32}P]ATP. Photoaffinity labeling of nucleotide-binding sites on proteins has been successfully performed utilizing, among other reagents, arylazide derivatives of ATP (19). We chose N_3ATP, which can be labeled to high specific activity with ^{32}P. This allows it to be used at very low concentrations, thus maximizing the specificity of labeling. The azide position of the probe is activated by light of the appropriate wavelength to a very reactive, short-lived nitrene group, which then reacts with nearby amino acid residues at or near the binding site. Since the N_3ATP photoaffinity reagent is known to react only with a small percentage of the total protein available to it, we employed a strain, TA3662, that overproduces the P protein, which is normally present in very small amounts. Upon an increase in temperature, TA3662 produces large amounts of the P protein, which is tightly membrane-bound and can be easily visualized by Coomassie blue staining as a major protein band by unidimensional gel electrophoresis of membrane preparations. The Q and M proteins, which would also be expected to be induced by the heat treatment, are also visible on the gel.

Membrane proteins labeled with N_3[^{32}P]ATP are shown in Fig. 1. Several new Coomassie blue bands appear upon heat induction of TA3662 (Fig. 1*a*, lanes 2 and 3 compared to lane 1). The major band with an apparent M_r of 24,000 is the P protein, which overlaps another abundant protein not resolved from P in this particular gel system (lane 1). By lowering the pH in the resolving gel buffer from 8.65 to 8.5 (see *Materials and Methods*) we were able to resolve the heat-induced P protein band from this unknown protein, which was not affected by heat induction (data not shown). Two bands with a M_r of ≈20,000 are the Q and the M proteins. Fig. 1*b* (lane 2) shows the results of photolabeling the same membrane preparation with N_3[^{32}P]ATP. Two strong radioactive bands appear: band A has an M_r just above 24,000 and band B has an M_r of 20,000. A control culture that had not been shifted to 42°C (lane 1) does not show bands A and B. Both preparations also show a few comparatively minor radioactive bands, which presumably are due to the presence of several other adenine nucleotide-binding proteins in these preparations. The location of the radioactive band A in the 42°C culture strongly suggested that the P protein was labeled by N_3[^{32}P]ATP. The location of band B suggested that either the Q or the M protein was labeled. Since a slight change in mobility might occur upon reaction of a protein with N_3ATP, a thorough resolution of these two proteins was necessary. This was achieved by two-dimensional gel electrophoresis, which also yielded a complete separation of the photolabeled protein corresponding to band A from the overlapping contaminating band.

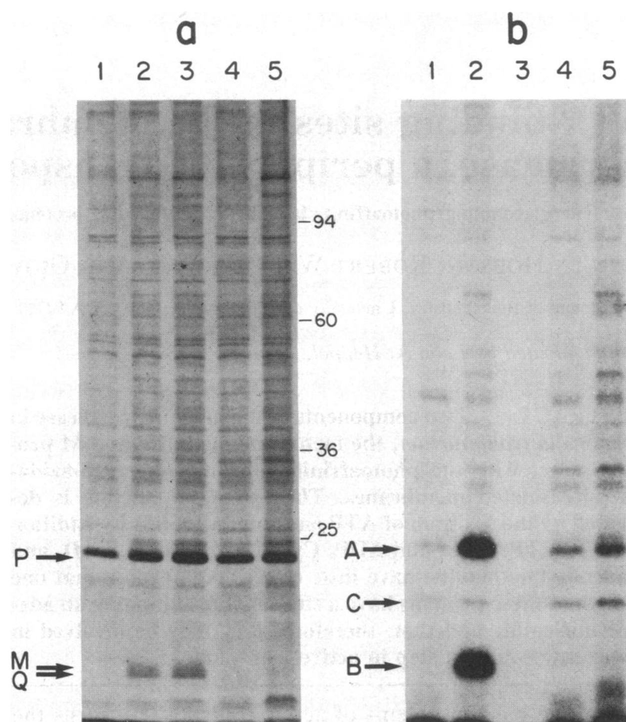

FIG. 1. Overproduction and labeling of transport proteins. (*a*) Dried polyacrylamide gel stained with Coomassie blue. Membrane preparations from TA3662 (lanes 1–3) and from TA3663 (lanes 4 and 5) were treated with N_3[^{32}P]ATP. Lanes 1 and 4, cells were grown at 30°C; all other lanes, cells were shifted to 42°C. Lane 3, the UV irradiation step was omitted. The numbers refer to molecular weight standards (shown as $M_r \times 10^{-3}$). (*b*) Autoradiogram of the same gel. The labeled band marked C is unrelated to heat induction and appears unreproducibly as do other bands. It is not unexpected that the P protein and its modified derivative have different mobilities.

The results of a two-dimensional electrophoretic resolution comparing a culture maintained at 30°C (control) with a culture exposed to 42°C are shown in Fig. 2. The radioactive spot A is located slightly to the right (more acidic) of the unlabeled P protein (16), but quite far from the position of

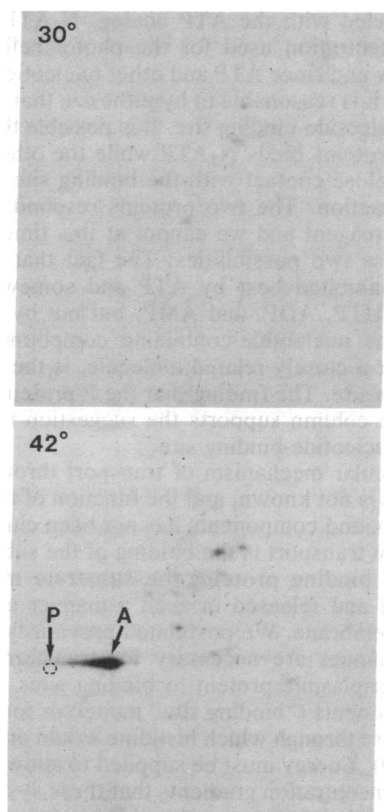

FIG. 2. Autoradiogram of two-dimensional gel electrophoresis of photolabeled membranes. TA3662 membranes were labeled and the proteins were resolved by non-equilibrium two-dimensional gel electrophoresis (18). Cells were grown either at 30°C (*Upper*) or 42°C (*Lower*). The arrow marked P points to the P protein location (dotted circle) as determined from the Coomassie blue staining of the 42°C membranes. No P protein is visible in 30°C membranes. Basic proteins are at left end.

the interfering protein, which is quite acidic. Both proteins were identified by Coomassie blue staining (not shown). A shift to a more acidic location would be expected for a P protein that had reacted with N_3ATP and thus carried its negatively charged phosphate groups. Cells maintained at 30°C completely lack this labeled spot, while they still display the interfering protein. This evidence supports our conclusion that spot A is due to photolabeling of the P protein by $N_3[^{32}P]ATP$. Spot B is absent from the gels shown in Fig. 2. This suggests that it corresponds to labeled M protein, since the very basic M protein (as known from its sequence; ref. 6) would be electrophoresed off the gel under these conditions, while the Q protein is slightly more acidic than P. This possibility was confirmed (see below).

The absence of both spots A and B in 30°C cultures is in agreement with the notion that these proteins are under the temperature-sensitive regulation of the P_L promoter. The possibility that the labeled spots are due to heat-shock proteins that have electrophoretic properties similar to P and M was excluded by heat-inducing and photolabeling an isogeneic strain (TA3663) that carries pFA18 containing the same DNA fragment as pFA17, but in inverted orientation. Fig. 1*b* (lane 5) shows that the labeling pattern at 42°C lacks bands A and B when TA3663 is utilized and is identical to the 30°C pattern (lane 4). This confirms that the two labeled proteins are not heat-shock proteins and is in agreement with their being coded for by the histidine transport genes.

The possibility that spot B is the labeled M protein was further investigated. Since the M protein is retained on a gel during isoelectric focusing only for a very short time, a reaction mixture was resolved by a two-dimensional gel in which the isoelectric focusing time was shortened to 1.5 hr (from its usual length of 5.5 hr) and the voltage was reduced to 300 V (from the normal 500 V). Under these conditions spot B appears in a location compatible with it being labeled M, having traveled further than Q and less than M (Fig. 3). The location of the M protein under these electrophoretic conditions has been established by using *in vitro*-synthesized M protein (unpublished results). It should be noted that the level of M protein formed and entering a two-dimensional gel is such that it is not visible by Coomassie blue staining. The highly hydrophobic and basic nature of the M protein is possibly responsible for its unorthodox behavior on two-dimensional gels.

Specificity of Labeling. N_3ATP is known to mimic ATP in the phosphoryl transfer reaction and it may, therefore, phosphorylate proteins independently of photolysis (19). Fig. 1*b* (lane 3) shows that in the absence of UV light there is no formation of the A and B bands, thus demonstrating that the observed labeling is dependent upon UV irradiation and that it is therefore due to the creation of photolabeled derivatives.

The specificity of the site at which photolabeling occurs was explored by adding competing nucleotides. Table 1 shows that ATP, ADP, or GTP resulted in inhibition of labeling of the P protein in a dose-dependent manner. CTP, UTP, and AMP also exhibited an inhibition comparable to GTP, whereas NAD, NADH, adenine, adenosine, *S*-adenosylmethionine, FAD, and cyclic AMP had essentially no effect (data not shown). The M protein showed parallel changes but was not quantitated.

Enzymatic Activity of the P Protein. Since the results indicate clearly that the P protein contains a nucleotide-binding

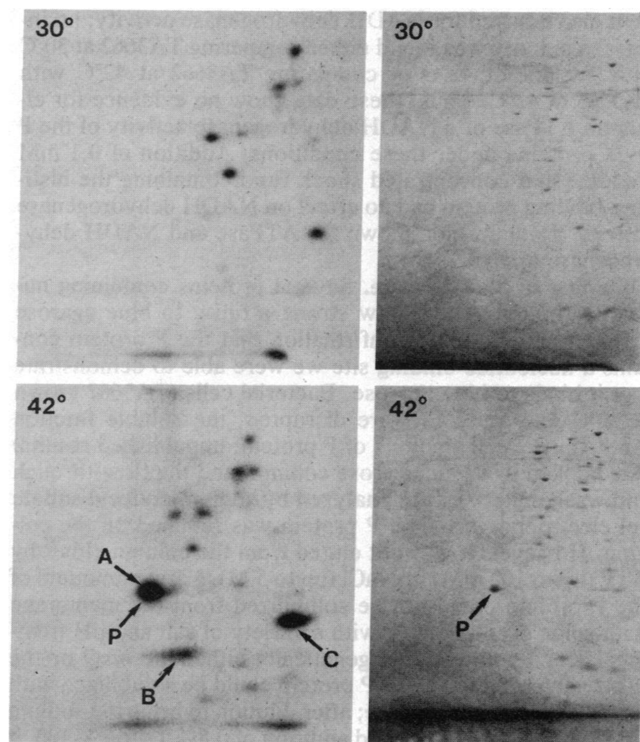

FIG. 3. Abbreviated two-dimensional gel electrophoresis of photolabeled membranes. TA3662 membranes from bacteria grown at 30°C (*Upper*) and 42°C (*Lower*) were resolved as in Fig. 2, except that isoelectric focusing was for 1.5 hr at 300 V. (*Right*) Dried and stained gels. (*Left*) Autoradiogram of the same. As expected for such a short focusing time, the position of the unlabeled P protein (from Coomassie blue) coincides with that of the photolabeled P protein. For labeled spot marked C, see legend to Fig. 1.

Table 1. Specificity of labeling of the P protein

Competing substrate	Competitor, μM	% labeling
	Experiment 1	
ATP	0	100
	10	45
	50	27
	200	18
GTP	0	100
	10	69
	50	47
	200	25
	Experiment 2	
ADP	0	100
	1	83
	5	117
	20	68
	100	30

The concentration of $N_3[^{32}P]ATP$ was 1 μM and 0.1 μM in experiments 1 and 2, respectively. Quantitation was as described (20).

site and since a possible homology has been pointed out between the P protein and the proton-translocating ATPase, we assayed for ATPase activity in membrane preparations containing large amounts of the P protein. The level of ATPase in TA3662 was unchanged with or without the heat induction. Since the activity of the proton-translocating ATPase might have masked a low level of P protein ATPase activity, the activity was assayed in *unc*-containing strains: TA1889 and TA3874. A low and similar ATPase activity can be measured both in TA1889 and TA3874, and it is essentially unchanged after heat induction. Membrane preparations were also assayed for NADH dehydrogenase activity: no increased activity was found either comparing TA3662 at 30°C with TA3662 at 42°C or comparing TA3662 at 42°C with TA3663 at 42°C. Thus, these data show no evidence for either an ATPase or a NADH dehydrogenase activity of the P or M proteins under these conditions. Addition of 0.1 mM histidine and concentrated shock fluid containing the histidine-binding protein had no effect on NADH dehydrogenase activity. Data are not shown for ATPase and NADH dehydrogenase assays.

Binding to Blue Agarose. Several proteins containing nucleotide-binding sites show strong affinity to blue agarose (21). As an additional confirmation that the P protein contains a nucleotide-binding site we were able to demonstrate that it binds to blue agarose. Bacterial cells (TA3662 grown at 30°C and at 42°C) were disrupted, the soluble fraction (containing small amounts of P protein; unpublished results) was applied to a blue agarose column, and the flow-through and wash material were analyzed by sodium dodecyl sulfate gel electrophoresis. The P protein was retained on the column. However, it was not eluted from the column either by ATP (up to 100 mM) or NaCl (up to 5 M). Larger amounts of the P protein could not be solubilized from the membrane with sonic oscillation, or with a variety of salt and pH treatments, or by eliminating genetically either the *hisQ* or the *hisM* gene products. The P protein could be solubilized with 1% sodium dodecyl sulfate; after dilution to bring the sodium dodecyl sulfate to 0.1% and addition of 0.8% Triton X-100, it bound to a blue agarose column in 0.1% Triton X-100. The bound P protein could be eluted with 0.1% sodium dodecyl sulfate along with other retained proteins, but not with ATP or NaCl (data not shown).

DISCUSSION

We have demonstrated that two of the membrane-bound components of the periplasmic histidine permease, P and M, are photolabeled with the ATP analog, N_3ATP. Since the N_3ATP concentration used for the photolabeling reaction was quite low and since ATP and other nucleotides inhibited the reaction, it is reasonable to hypothesize that the proteins contain a nucleotide-binding site. It is possible that only one of the two proteins binds N_3ATP while the other is labeled by being in close contact with the binding site by protein–protein interaction. The two proteins respond similarly to the labeling reagent and we cannot at this time distinguish between these two possibilities. The fact that the labeling reaction is inhibited best by ATP and somewhat less by GTP, CTP, UTP, ADP, and AMP, but not by a variety of other adenine nucleotide-containing compounds, suggests that ATP, or a closely related molecule, is the natural substrate for the site. The finding that the P protein binds to an agarose blue column supports the suggestion that this protein has a nucleotide-binding site.

The molecular mechanism of transport through periplasmic systems is not known, and the function of the individual membrane-bound components has not been elucidated. The initial step in transport is the binding of the substrate to the periplasmic binding protein; the substrate must then be translocated and released in such a manner as to make it cross the membrane. We postulated previously that conformational changes are necessary for transferring histidine from the periplasmic protein to binding sites on the membrane components ("binding site" model) or for opening and closing a pore through which histidine would diffuse ("pore" model) (6, 9). Energy must be supplied to allow formation of the large concentration gradients that these systems are able to achieve. The data presented in this paper suggest the possibility that the interaction between ATP and the P and/or M protein might be involved in the energy-coupling mechanism. We would like to propose specifically that the interaction of ATP with either the P or the M protein (or both) is involved in the postulated conformational changes. Fig. 4 is a schematic representation of this hypothesis (see legend to Fig. 4). Genetic evidence had suggested previously that a direct interaction exists between the periplasmic J protein and the membrane-bound P protein, since mutants in P could suppress defects in J (7, 22). Therefore, the P protein would have to protrude into the periplasmic space in order to interact with J. Since we have no evidence that ATP can reach the periplasm, we assume that at least that portion of P that interacts with ATP must be accessible to the cytoplasmic side of the membrane. We have obtained evidence that the P protein is accessible from the cytoplasmic side by the use of

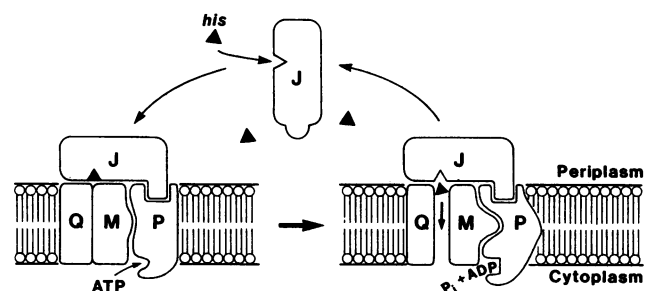

FIG. 4. Schematic representation of histidine transport. This model assumes that the system is involved in the formation of a pore in the membrane, rather than of histidine-binding sites (6). The distinction is irrelevant for this purpose. The interaction between J (with histidine bound to it) and P allows the binding of ATP to the cytoplasmic face of P. Hydrolysis of ATP allows a conformational change to occur in P, which is transmitted to the other transport proteins (M, as shown in this model), thus allowing the opening of a pore through which histidine can diffuse. The release of J and loss of ADP + P_i from P would complete the cycle, ending with closure of the pore.

impermeant membrane-labeling reagents (unpublished results). The extreme hydrophilicity of the P protein (8) need not be taken as evidence that the protein cannot cross the membrane, since an outer membrane component, *ompF* (23), also has a very hydrophilic composition, though it has been shown to be embedded in the membrane. Alternatively, the P protein may cross the membrane by forming a complex with the M and/or Q protein. On the other hand, it is possible that the genetic results have been misleading and that the P protein does not interact directly with J (though it must do so indirectly), thus removing the need for the P protein to be accessible on the periplasmic side.

We obtained no evidence that ATP is hydrolyzed by the P or M protein. However, we cannot exclude this possibility since hydrolysis might only occur during active transport. Our membrane preparations lack the J protein and have not been optimized for carrying on any form of transport. Thus, despite our negative results, it is entirely possible that ATP hydrolysis is the purpose of the ATP–P protein interaction. On the other hand, ATP may have only a regulatory function, dictating a conformational change without leading to hydrolysis. In this case, an additional energy-yielding reaction may be necessary.

The proposed requirement for an ATP interaction for active transport by a periplasmic system is in agreement with the data presented by Berger and Heppel, which implicated ATP as the source of energy for this class of systems (2, 3). On the other hand, conflicting data showing an effect of the level of the proton motive force on periplasmic transport have also been presented (5). It is entirely possible that both factors are involved in energy-coupling by these systems. For example, the magnitude of the proton gradient might affect the accessibility of the P protein on the cytoplasmic face of the membrane, and thus its accessibility to ATP. It is possible that the postulated interaction with ATP results in the phosphorylation of transport components. It is noteworthy that in the past we have been unable to demonstrate the existence of phosphorylated derivatives of the membrane components of the histidine permease (24). This suggests that binding of ATP does not result in the formation of long-lived phosphorylated derivatives. However, the possibility remains that such intermediates are very labile (even under the very mild electrophoretic conditions we developed for this purpose) (24) and/or that they are present in very small amounts. Additionally, the high degree of hydrophobicity and small amounts of the M and Q proteins may have been an obstacle to the resolution of their hypothetical phosphorylated derivatives. Thus, even though there is at present neither evidence for phosphorylation of the membrane-bound components nor a demonstrated need for it, the possibility remains that one or more of them is phosphorylated during transport.

Since the P protein bears strong sequence homology to the MalK protein it is possible that the latter protein similarly interacts with ATP. It has been suggested that the MalK protein is an energy-coupling component for the maltose transport system (25).

Since our labeling experiments have been obtained in membrane preparations that contained the transport proteins at an elevated level, it is important to investigate the possibility that these overproduced proteins are improperly located in the membrane, thus resulting in artifactual labeling. We have excluded this possibility because we have obtained labeling of the chromosomally encoded P protein from *S. typhimurium* (data not shown).

Note Added in Proof. We have recently obtained labeling of the MalK protein of *E. coli* with N_3ATP. In addition, C. F. Higgins has demonstrated that the oppD protein of the periplasmic oligopeptide permease of *E. coli* is labeled by another ATP analog, 5′-*p*-fluorosulfonylbenzoyladenosine (personal communication). These findings lead us to suggest that the existence of a nucleotide-binding site on one or more of the membrane-bound components is a general feature of periplasmic transport systems.

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1. Kaback, H. R. (1983) *J. Membr. Biol.* **76,** 95–112.
2. Berger, E. A. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 1514–1518.
3. Berger, E. A. & Heppel, L. A. (1974) *J. Biol. Chem.* **249,** 7747–7755.
4. Hong, J.-S., Hunt, A. G., Masters, P. S. & Lieberman, M. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1213–1217.
5. Plate, C. A. (1979) *J. Bacteriol.* **137,** 221–225.
6. Higgins, C. F., Haag, P. D., Nikaido, K., Ardeshir, F., Garcia, G. & Ames, G. F.-L. (1982) *Nature (London)* **298,** 723–727.
7. Ames, G. F.-L. & Spudich, E. N. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1877–1881.
8. Gilson, E., Higgins, C. F., Hofnung, M., Ames, G. F.-L. & Nikaido, H. (1982) *J. Biol. Chem.* **257,** 9915–9918.
9. Ames, G. F.-L. & Higgins, C. F. (1983) *Trends Biomed. Sci.* **8,** 97–100.
10. Gilson, E., Nikaido, H. & Hofnung, M. (1982) *Nucleic Acids Res.* **10,** 7449–7458.
11. Remaut, E., Stanssens, P. & Fiers, W. (1981) *Gene* **15,** 81–93.
12. Nielsen, J., Hansen, F. G., Hoppe, J., Friede, P. & Von Meyenburg, K. (1981) *Mol. Gen. Genet.* **184,** 33–39.
13. Miller, J. H. (1972) *Experiments in Molecular Genetics,* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 352–355.
14. Laemmli, L. (1970) *Nature (London)* **227,** 680–685.
15. Ames, B. N. (1966) *Methods Enzymol.* **8,** 352–355.
16. Ames, G. F.-L. & Nikaido, K. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5447–5451.
17. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12,** 1133–1142.
18. Johnson, R. A. & Walseth, T. F. (1979) *Adv. Cyclic Nucleotide Res.* **10,** 135–167.
19. Khatoon, S., Atherton, R., Al-Jumaily, W. & Haley, B. E. (1983) *Biol. Reprod.* **28,** 61–73.
20. Ames, G. F.-L. (1974) *J. Biol. Chem.* **249,** 634–644.
21. Thompson, S. T., Cass, K. H. & Stellwagen, E. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 669–672.
22. Kustu, S. G. & Ames, G. F.-L. (1974) *J. Biol. Chem.* **249,** 6976–6983.
23. Garavito, R. H. & Rosenbusch, J. P. (1980) *J. Cell Biol.* **86,** 327–329.
24. Ames, G. F.-L. & Nikaido, K. (1981) *Eur. J. Biochem.* **115,** 525–531.
25. Shuman, H. A. (1982) *J. Biol. Chem.* **257,** 5455–5461.