Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8257–8261, November 1989
Biochemistry

Energy coupling to periplasmic binding protein-dependent transport systems: Stoichiometry of ATP hydrolysis during transport *in vivo*

(maltose transport/glycine betaine/peptide transport)

M. L. MIMMACK*, M. P. GALLAGHER*, S. R. PEARCE*, S. C. HYDE*, I. R. BOOTH†, AND C. F. HIGGINS*‡

*Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland; United Kingdom; and †Department of Microbiology and Genetics, Marischal College, University of Aberdeen, Aberdeen AB9 1AS, Scotland, United Kingdom

Communicated by Hans Kornberg, July 24, 1989

ABSTRACT **Periplasmic binding protein-dependent transport systems mediate the accumulation of many diverse substrates in prokaryotic cells. Similar transport systems, including the P-glycoprotein responsible for multidrug resistance in human tumors, are also found in eukaryotes. The mechanism by which energy is coupled to the accumulation of substrate by these transport systems has been controversial. In this paper we demonstrate that ATP hydrolysis occurs *in vivo* concomitantly with transport. These data strongly suggest that ATP hydrolysis directly energizes substrate accumulation by these transport systems. The apparent stoichiometry is one to two molecules of ATP hydrolyzed per molecule of substrate transported.**

Active transport systems in bacteria can conveniently be classified according to their sensitivity to osmotic shock (2). This distinction reflects real differences in organization, mechanisms, and mode of energy coupling between transport systems. Unlike osmotic shock-resistant transport systems, the shock-sensitive or binding protein-dependent transport systems each require several protein components. The proteins from different systems are generally related to one another, regardless of the specific substrates to be transported (for reviews, see refs. 4 and 5). Each system requires a specific substrate-binding protein, which is located in the periplasm and serves as the primary receptor for transport (6). For some transport systems (e.g., for maltose) the periplasmic component also serves as a chemoreceptor. In addition, each system normally requires one or two highly hydrophobic integral membrane proteins, which facilitate transport of the substrate across the cytoplasmic membrane, and one or two hydrophilic proteins associated with the cytoplasmic face of the inner membrane (7). These latter proteins are believed to play a role in coupling energy to the transport process. Homologous proteins have also been identified in plants, insects, and mammals, including the P-glycoprotein responsible for multidrug resistance in human tumors (for reviews, see refs. 1, 10, and 11).

Early experiments with metabolic inhibitors demonstrated that energy coupling to binding protein-dependent transport systems was distinct from that of shock-insensitive transport systems, which are energized directly by electrochemical gradients of ions (2). These studies led to the suggestion that binding protein-dependent transport systems were energized directly by ATP hydrolysis. However, data derived solely from inhibitor studies can only be indicative, and many other potential energy sources for this class of transport system have subsequently been proposed, including acetyl phosphate (12), NADPH (13), lipoic acid (14), and succinate (16). Arguments against a direct role for ATP have included the demonstration that, under certain conditions, a reduction in ATP pools does not necessarily lead to a corresponding reduction in transport (17–19). Further confusion has arisen from findings that perturbation of the electrochemical gradient can inhibit binding protein-dependent transport without significantly reducing ATP pools (16, 17, 20, 21).

The identification of a consensus ATP-binding site on the peripheral membrane components of several transport systems (22, 23) strongly implicated a role for ATP in these transport systems. Furthermore, the OppD, HisP, and MalK proteins of the oligopeptide, histidine, and maltose transport systems, respectively, have been shown to bind ATP analogues (22, 24). Recently ATP has been demonstrated to be required for transport by binding protein-dependent transport systems in vesicle systems (25, 26), implying that ATP is the energy source for transport. However, ATP hydrolysis by these proteins has not been shown, and the possibility that ATP binding plays a purely structural or regulatory role cannot be eliminated. In this paper we have measured ATP hydrolysis during transport by the maltose and the glycine-betaine (*N,N,N*-trimethyl glycine) transport systems of *Escherichia coli*. A stoichiometry of close to two ATP molecules hydrolyzed per molecule of substrate transported was determined. These data provide direct evidence that hydrolysis of ATP provides the driving force for substrate accumulation by binding protein-dependent transport systems.

METHODS

Bacterial Strains and Growth. The genotypes of bacterial strains used in this study are given in Table 1. Cells were grown with shaking in Luria broth (LB; ref. 33) at 37°C unless otherwise stated. Minimal medium was low-osmolarity medium (LOM; ref. 34) containing 0.4% Casamino acids and 0.4% glycerol as carbon sources. Where appropriate, tetracycline at 15 μg per ml, ampicillin at 25 μg per ml, and kanamycin at 25 μg per ml were added. Transductions using phage $P1_{vir}$ were done as described by Silhavy *et al.* (35). The Mal^- phenotype was screened on MacConkey-maltose (35) plates. The Unc^- phenotype was characterized by slow growth on LB, restored to normal by adding 1% glucose.

Strain Construction. Strain CH1750 (Δ*uncIBEF malT*^{c} Δ*malPQ*) was the parental strain used for these studies and was constructed as follows. Strain CH1762 (*malT*^{c}-*1 malF-lacZ*) was constructed by transducing MM335 to Kan^r with a phage P1 lysate of strain CH1431. The *malT*^{c} mutation allows constitutive expression of the *malF–lacZ* fusion (blue on

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LOM, low osmolarity medium; PEP, phospho*enol*-pyruvate; Opp, oligopeptide permease.

‡To whom reprint requests should be addressed at: ICRF Laboratories, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, U.K.

Table 1. Bacterial strains

Strain	Genotype	Source or ref.
CH1431	*malF*::MudI1681 (*lac* Kan^r)	27
Strain 7	HFrPO2A *relA1 tonA22 phoA8*(Δ10) T2^r *glp*^{c}	28
CGL103	Strain 7 Δ*uncIBEF*	I.R.B.
CH1663	CGL103Δ*uncIBEF malPQ*::Tn*10*	I.R.B.
CH1748	CGL103Δ*uncIBEF malPQ*::Tn*10* *malT*^{c}-1	This study
CH1750	CGL103 Δ*uncIBEF* Δ*malPQ* (Tet^s) *malT*^{c}-1	This study
CH1751	CH1750Δ*malE444 zjb-729*::Tn*10*	This study
CH1752	CH1750*malF*::Tn*10*	This study
CH1753	CH1750Δ*tar-5201 zec*::Tn*5-14*	This study
CH1758	CH1750*putPA proP*	This study
CH1761	MC4100 *malT*^{c}-1 *malF–lacZ* *malPQ*::Tn10	
CH1762	MC4100 *malT*^{c}-1 *malF–lacZ*	
DF621	*araD lacA169 strA thi relA* *malF*::Tn*10*	D. Fraenkel*
JB200	Δ*malB*(*malEFGK lamB*)112 Δ*tar-5201* *malT*^{c}-1	29, 30
MC4100	*araD139* Δ(*argF-lac*)*U196 rpsL150* *relA1 deoC1 ptsF25 rbsR flbB5301*	31
MM109	Δ*malE444 zjb-729*::Tn*10*	30, 32
MM335	MC4100 *malT*^{c}-1	30
RP5107	*zec*::Tn*5-14 thr*$_{am}$*1 leuB6 his-4* *metF*$_{am}$*159 supD rpsL136 thi-1* (*gal-attL*)Δ*99 ara-14 lacY1 mtl-1* *xyl-5 tonA31 tsx78*	J. S. Parkinson†

*, Harvard University, Boston.
†, University of Utah, Salt Lake City.

minimal glycerol plates containing 40 μg of 5-bromo-4-chloro-3-indolyl β-D-galactoside; X-gal). A *malPQ*::Tn*10* derivative of strain CH1762 (designated CH1761) was then constructed by transducing CH1762 to Tet^r with a phage P1 lysate of strain CH1663. As *malPQ* and *malT* are tightly linked, the small proportion of Tet^r transductants that retained the *malT*^{c} mutation (2–3%) were identified as blue colonies on minimal glycerol plates containing X-gal. The closely linked *malPQ*::Tn*10* and *malT*^{c}-*1* mutations were then transduced together from strain CH1761 into the *unc* deletion strain CGL103 selecting for Tet^r. The *malT*^{c} mutation is >90% linked to *malPQ*::Tn*10*. As the strains are Mal$^-$, due to the *malPQ* lesion, the *malT*^{c} lesion was shown to be present in one transductant (CH1748) by the presence of the periplasmic maltose-binding protein during growth in the absence of maltose [detected by SDS/PAGE, as described (27); data not shown]. Finally, CH1750 was derived by imprecise excision of the Tn*10* from CH1748, selected by the fusaric acid method (36). CH1750 was phenotypically Mal$^-$ (i.e., imprecise deletion of the Tn*10* resulted in a *malPQ* deletion) but was shown to have retained the *malT*^{c} lesion, as maltose-binding protein was still synthesized and maltose was transported (Fig. 1*B*) by cells grown in minimal glycerol medium.

Derivatives of strain CH1750 were constructed as follows. The nonpolar *malE444* deletion (32) was introduced by cotransduction with the linked Tn*10* of strain MM109. A Tet^r derivative, which had acquired the *malE444* deletion (strain CH1751) was identified by the absence of maltose-binding protein from periplasmic shock fluids. A *malF*::Tn*10* derivative of strain CH1750 (designated strain CH1752) was constructed by transduction from strain DF621. A derivative deleted for the *tar* gene was constructed by transducing the linked Tn*5* from strain RP5107 into the Δ*tar* strain JB200 and then into strain CH1750; the Tar phenotype was screened on

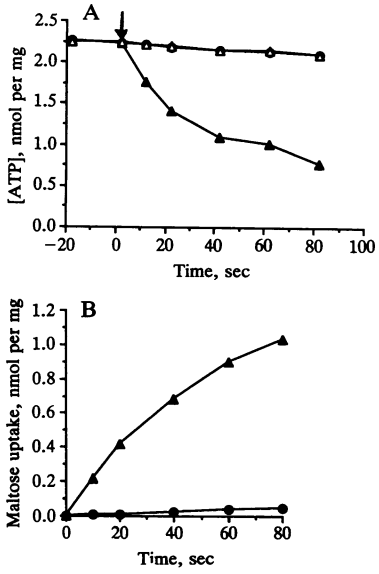

FIG. 1. ATP utilization and maltose transport. Cells were treated with iodoacetate. At t_0 (arrow) maltose was added, and the intracellular ATP pools and maltose uptake were monitored. Each point is an average of at least three separate determinations; variation between samples was no more than 15%. (*A*) ▲, ATP pools of the parental strain CH1750 with maltose; ●, strain CH1750 without maltose; △, strain CH1751 (Δ*malE444*) with maltose added. (*B*) ▲, Maltose uptake by the parental strain CH1750; ●, Δ*malE444* derivative strain CH1751.

tryptone swarm plates (37). A *putPA proP* derivative, which was used for betaine transport experiments (strain CH1758), was isolated by selecting sequentially for resistance of strain CH1750 to azetidine carboxylic acid and dehydroproline, respectively, as described elsewhere (39).

Transport Assays. One-hundred microliters of an overnight culture of cells was inoculated into a 250-ml conical flask containing 30 ml of LOM/glycerol/Casamino acids and the cells were grown to an OD_{600} of 0.4. The cells were washed twice, centrifuged, and resuspended in 200 mM Na–Hepes buffer, pH 7.5 (41), and finally resuspended to a final volume of 6 ml (OD_{600} = 2.0) in Hepes buffer. A 2-ml aliquot of washed cells was equilibrated to 25°C with constant stirring, and iodoacetate was added (final concentration, 5 mM). After 3-min incubation radiolabeled substrate (Amersham) was added to a final concentration of 7 μM for [^{14}C]maltose [30.3 mCi per mmol; 1 Ci = 37 GBq; purified free of contaminating glucose as described by Brass and Manson (30)] or 30 μM for [*methyl*-^{14}C]glycine betaine (6.6 mCi per mmol) and [U-^{14}C]proline (20 mCi per mmol). Aliquots (180 μl) were taken at t_0 and appropriate time intervals thereafter, and the cells were rapidly collected on a Millipore 0.45-μm filter and washed twice with 10 ml of Hepes buffer. The filter discs were subsequently dried and the accumulated substrate was measured by scintillation counting. Each point is an average of two independent determinations repeated on at least three independent occasions.

ATP Assays. The measurement of intracellular ATP pools was based on the luciferase enzyme assay system as adapted by Stewart *et al.* (41). Cells were grown and washed as described above. After appropriate treatments (see *Results*) 180-μl aliquots were taken and added to 180 μl of 12% perchloric acid/5 mM phosphoric acid at 4°C. After 30-min incubation 180 μl of 2 M KOH/0.3 M Mops was added to neutralize each sample; the samples were then centrifuged to remove perchlorate and lysed by freeze-thawing. A 50-μl aliquot was added to 925 μl of assay buffer (20 mM diglycine/NaOH, pH 8.0/5 mM sodium arsenate/4 mM $MgSO_4$), and 25

μl of crude firefly lantern extract (Sigma) was added. After 10-sec delay the luminescence was measured in an LKB luminometer. The ATP pools that were determined were slightly lower than those estimated for growing cells (42), a consequence of the extensive washing, the absence of a carbon source during the assay, and the iodoacetate treatment. However, when growth and washing conditions were varied to give higher internal ATP pools, the ATP hydrolysis observed upon substrate addition was unchanged.

RESULTS

Rationale. *unc* mutants of *E. coli* are unable to synthesize ATP via the F_1–F_0 translocating ATPase and, hence, can only generate ATP by substrate level phosphorylation. The addition of iodoacetate inhibits glyceraldehyde-3-phosphate dehydrogenase and prevents *de novo* ATP synthesis. Fig. 1*A* shows that, as expected, the intracellular ATP concentration declines slowly after adding iodacetate to *unc* cells (CH1750). If a transportable substrate is then added, any increase in the rate at which the ATP pool declines can be attributed to ATP consumed by the transport process, assuming, of course, that the substrate cannot be further metabolized (41). Maltose uptake provides a suitable model system for a number of reasons. (*i*) There is only a single pathway for maltose uptake into the cell, the maltose binding protein-dependent transport system (for review, see ref. 43). (*ii*) This transport system, encoded by the *malEFGK* genes, is well characterized genetically and biochemically. (*iii*) In *malPQ* mutants, no further metabolism of maltose can occur once it has entered the cell. (*iv*) The rate of maltose uptake is greater than that of many similar transport systems [e.g., 3.2 nmol per min per mg of protein (25)]. (*v*) The maltose transport system can be expressed constitutively, without the need for preaddition of exogenous inducer, in a *malT*^{c} strain (45).

Maltose-Dependent ATP Utilization. Strain CH1750 (*malT*^{c} Δ*unc malPQ*) was grown to midexponential phase in minimal glycerol medium and washed and resuspended in Hepes buffer; iodoacetate was then added, and the cells were incubated for 3 min. This preincubation with iodoacetate was found essential to completely inhibit glyceraldehyde-3-phosphate dehydrogenase activity. At t_0 maltose was added (7 μM). Fig. 1*A* shows a rapid decrease in the intracellular ATP pool after maltose addition (maltose was purified free of any possible glucose contamination; see *Methods*). To demonstrate that the ATP consumption was from maltose uptake (and not, for example, a contaminant in the maltose) the *malE444* deletion was introduced into strain CH1750. The resulting strain (CH1751) was, as expected, completely deficient in maltose uptake (Fig. 1*B*) and, in this strain, maltose did not cause increased utilization of ATP (Fig. 1*A*). However, the periplasmic maltose-binding protein MalE is required for maltose chemotaxis as well as transport, interacting with the Tar receptor (methyl-accepting chemotaxis protein II) (6). As chemotaxis is an energy-dependent process and involves ATP-dependent protein phosphorylation (46), the possibility that MalE-dependent chemotaxis contributed substantially to maltose-dependent ATP consumption was eliminated in two ways. First, a *malF*::Tn*10* insertion was introduced into strain CH1750; the resulting strain (CH1752) synthesized MalE protein but was maltose-transport deficient. Secondly, a *tar* deletion derivative of strain CH1750 (CH1753) was constructed. Strain CH1752 showed no significant increase in ATP consumption on maltose addition, while strain CH1753 showed the same rate of ATP consumption as the Tar$^+$ parent (data not shown). These results unambiguously demonstrate that the ATP consumed after maltose addition is a consequence of *malEFGK*-dependent transport into the cell.

Stoichiometry of ATP Utilization. To determine the stoichiometric relationship between maltose uptake and ATP hydrolysis, [^{14}C]maltose uptake was measured in parallel with measurements of ATP pool sizes. Extrapolation from the data in Fig. 1*B* shows that the initial rate of maltose uptake was 1.3 nmol per min per mg of dry weight. This uptake is rather slower than has been described for metabolizing cells (32), a consequence of the specific growth conditions and cell treatment. Calculations reveal a stoichiometry of 1.9 ATP molecules hydrolyzed per molecule of maltose transported during the first 10 sec of assay. Slight differences in cell preparations meant that absolute values for ATP pool size and rates of maltose transport varied from day to day (although these parameters were very reproducible for a single cell preparation). However, the initial stoichiometry remained relatively constant between preparations, varying between 1.5 and 2.1 molecules of ATP hydrolyzed per molecule of maltose transported for five independent preparations. As the assay proceeded, this ratio fell to ≈1.0, possibly due to adenylate kinase activity regenerating ATP from the ADP released as transport proceeded. Maltose uptake rates did not fall substantially as the ATP pools fell—presumably because the transport system has a relatively high affinity for ATP. A K_m of ≈100 μM for ATP binding has been estimated for the related histidine transport system (47).

Regeneration of ATP Restores Transport. When cells treated with iodoacetate and maltose were incubated for 15 min, the intracellular ATP pools fell to subnanomolar levels. As expected, when radiolabeled maltose was then added, no maltose transport was detected (Fig. 2*B*). However, ATP could be regenerated in these iodoacetate-treated cells from exogenously added phospho*enol*pyruvate (PEP). To enable PEP to enter the cell, plasmid pBR322-pgt2, which carries the cloned phosphoglycerate transporter (48), was transformed into strain CH1750. When PEP was added, the ATP pool increased rapidly to ≈9 mM (Fig. 2*A*). Immediately after ATP regeneration, rapid maltose transport was restored (Fig. 2*B*).

ATP Consumption During Glycine-Betaine Transport. Glycine betaine is an osmoprotectant that is not metabolized once it has been transported into *E. coli* (for review, see ref. 49). At low extracellular concentrations of glycine betaine there are only two routes for glycine-betaine uptake, the ion-linked proline permease system (ProP) and the periplasmic binding protein-dependent transport system (ProU) (39, 50, 51). A derivative of CH1750 (designated CH1758) was constructed which lacked ProP activity to measure ATP consumption during transport through the binding protein-dependent ProU system. As ProU is only synthesized in cells grown at high osmolarity, the cells were grown in LOM to an OD_{600} of 0.4, NaCl was added to a final concentration of 0.3 M to induce *proU* expression (50), and the cells were grown for another hour before harvesting. Fig. 3*A* shows that adding glycine betaine to the cells rapidly decreased intracellular ATP pools. The ATP hydrolysis depended on glycine-betaine uptake and did not occur in a *proU* mutant (data not shown). Fig. 3*B* shows glycine-betaine uptake during the same period. The calculated stoichiometry was initially (over the first 10 sec) ≈1.85 molecules of ATP used per molecule of substrate transported at initial times, declining to ≈1.0 as the assay proceeded and similar to the stoichiometry obtained for maltose uptake.

The oligopeptide permease (Opp) is also a periplasmic binding protein-dependent transport system (52). Addition of 100 μM of L-prolylglycylglycine (an Opp-specific substrate) to iodoacetate-treated cells of strain CH1750 also resulted in ATP hydrolysis (data not shown). This hydrolysis was essentially eliminated in an *opp* mutant. For this transport system, however, the stoichiometry of ATP transport could not be accurately determined because the multiple intracel-

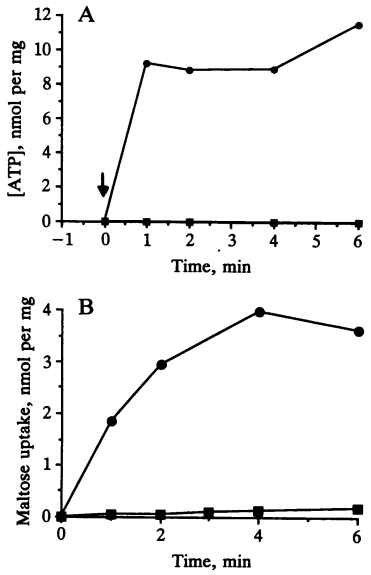

FIG. 2. Maltose uptake after regeneration of ATP from PEP. (*A*) Cells of CH1750 pBR322-pgt2 were incubated for 15 min with iodoacetate and maltose as described. At t_0 (arrow) intracellular ATP pools had declined to very low levels. Intracellular ATP pools were then monitored with (●) or without (■) 5 mM PEP. (*B*) Maltose uptake was measured simultaneously with (●) or without (■) added PEP. Each value is an average of at least three separate determinations; variation between samples was no more than 15%.

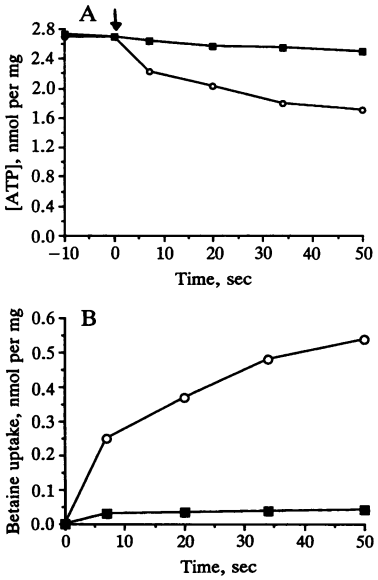

FIG. 3. ATP utilization and glycine-betaine uptake. (*A*) Cells of the *proP putP* Δ*unc* strain CH1758 were grown, as described in the text, and treated with iodoacetate. The 200 mM Hepes buffer, in which the cells were resuspended, was of high enough osmolarity to activate the osmotically sensitive ProU transport system. At t_0 (arrow) glycine betaine was added, and intracellular ATP pools and glycine-betaine uptake were monitored. (*A*) Changes in intracellular ATP with (○) and without (■) glycine-betaine addition. (*B*) Uptake of glycine betaine by strain CH1758 (○) and its *proU* derivative (■) monitored simultaneously with the changes in ATP pools. Each value is an average of at least two determinations; variation between samples did not exceed 15%.

lular peptidases preclude the elimination of peptide metabolism after uptake.

ATP Is Not Consumed During Proline Transport. At low substrate concentrations the primary route for proline uptake is the PutP protein transport system; ProP provides a minor route for uptake (38, 39). Both these transport systems are binding protein-independent and energized by Na^+ (PutP) or H^+-symport (ProP) mechanisms (3, 38, 39). Unlike substrates of binding protein-dependent transport systems, proline addition to strain CH1750 had no effect on ATP pool levels (Fig. 4*A*). This was not due to resynthesis of ATP after proline uptake and metabolism, as the strain was a *putA* (proline oxidase) mutant. However, transport rates were sufficiently high (Fig. 4*B*) that ATP hydrolysis would have been easily detected if it had occurred at similar stoichiometries to those determined for the binding protein-dependent systems above. Thus, proline transport, in contrast to maltose, betaine, and peptides, does not increase ATP consumption by the cell.

DISCUSSION

The data presented in this paper show unambiguously that ATP is consumed during transport through three independent periplasmic binding protein-dependent transport systems. In contrast, ATP consumption was not an immediate consequence of transport by means of binding protein-independent transport systems.

The source of energy for binding protein-dependent transport systems has been controversial (for review, see ref. 4). For several years components of these transport systems have been known to possess ATP-binding sites (22, 24). Furthermore, recent studies with vesicles have shown that ATP is absolutely required for transport by means of this class of transport system (8, 25, 47). However, ATP hydrolysis has not been demonstrated. The purified transport proteins also do not appear to hydrolyze ATP. Presumably hydrolysis requires the entire transport complex, which cannot yet be reconstituted, although hydrolysis of ATP by the related UvrA protein has been shown (9). Thus, it has not previously been possible to exclude models in which ATP binding plays a regulatory or structural role, as for example in the F_1-ATPase (15). Our data show, beyond doubt, that ATP hydrolysis occurs concomitantly with transport. Furthermore, the stoichiometry and rapidity with which ATP hydrolysis occurs suggest a direct relationship between ATP hydrolysis and transport. Although the formal possibility remains that the high energy phosphate of ATP is transferred to an intermediate compound which then interacts with the transport proteins, this seems highly improbable, as the OppD, HisP, and MalK proteins from different transport systems specifically bind ATP. Even if an intermediate compound were involved, our results clearly show that direct ATP hydrolysis provides the primary source of energization for binding protein-dependent transport systems.

Our data suggest an initial stoichiometry of almost two molecules of hydrolyzed ATP consumed per molecule of substrate transported for both maltose and glycine betaine. Although the stoichiometry declines to ≈1.0 as the assay proceeds, the initial values are probably correct. A stoichiometry of between 1.0 and 1.2 was suggested previously from growth yields on different substrates (40). The many potential experimental variables mean we would not exclude the possibility that actual stoichiometry is 1, although our many independent measurements all gave values >1.5. A stoichiometry of 2:1 may appear relatively inefficient but may be the penalty paid for the ability of such high-affinity transport systems to accumulate substrate against substantial concentration gradients. Further, a ratio of 2:1 is not unreasonable. Several binding protein-dependent transport systems (e.g., Opp) require two ATP-binding components, and in others (such as ribose from *E. coli*) these two are fused into a single polypeptide possessing two ATP-binding sites (for review, see refs. 5 and 11). At least for Opp, both ATP-binding

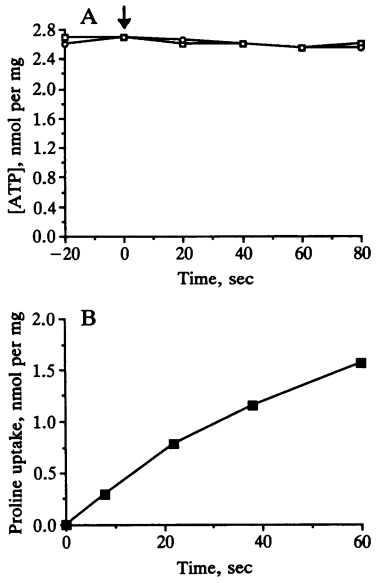

FIG. 4. Proline uptake. Cells of strain CH1750 were grown and treated as described in the text except that K-Hepes was used instead of Na-Hepes to preclude sodium effects on the sodium-linked PutP transporter (38). (*A*) Intracellular ATP pools were measured with (□) or without (○) proline. Proline was added at t_0 (arrow). (*B*) Proline uptake by strain CH1750 was monitored simultaneously with the ATP pools. Each value is an average of at least two samples; variation did not exceed 15%.

proteins are required (52). Two ATP-binding sites are also found on other classes of carrier, such as the arsenate transporter (44) and may be a general requirement. Although the maltose and some other binding protein-dependent transport systems only have a single ATP-binding component, we have suggested previously that this protein may function as a homodimer (11, 23). This question remains to be resolved experimentally.

Published data that apparently contradicted the notion that direct hydrolysis of ATP provides the driving force for binding protein-dependent transport can be explained. Data showing that transport rates do not fall as ATP pools are reduced (17–19) presumably reflect a relatively high affinity of the transport components for their substrate (≈100 μM; ref. 47). Furthermore, Joshi *et al.* (8) recently presented evidence that the protonmotive force is neither sufficient nor essential for histidine transport in *Salmonella typhimurium*. It seems probable that uncouplers and/or inhibitors that reduce transport by means of binding protein-dependent transport systems do so by altering cytoplasmic pH; many transport system in *Streptococcus* are regulated by internal pH (53).

The ATP-binding proteins of periplasmic transport systems are closely related to each other and to a variety of other prokaryotic and eukaryotic proteins associated with functions other than transport—for example, the HlyB protein required for hemolysin secretion and the FtsE protein involved in cell division (23). The finding that ATP hydrolysis is an integral part of the transport process strongly suggests that these related proteins each couple ATP hydrolysis to their respective biological processes.

We are grateful to Giovanna Ferro-Luzzi Ames for communicating results before publication and J. S. Parkinson, J. M. Brass, and M. M. Manson for providing strains. This work was supported by grants from the Medical Research Council, the Cancer Research Campaign, and by the Imperial Cancer Research Fund to C.F.H. C.F.H. is a Lister Institute Research Fellow.

1. Higgins, C. F. (1989) *Nature (London)* **340,** 342.
2. Berger, E. A. & Heppel, L. A. (1974) *J. Biol. Chem.* **249,** 7747–7755.
3. Milner, J. L., Grothe, S. & Wood, J. M. (1988) *J. Biol. Chem.* **263,** 14900–14905.
4. Ames, G. F.-L. (1986) *Annu. Rev. Biochem.* **55,** 397–425.
5. Higgins, C. F., Gallagher, M. P., Hyde, S. C., Mimmack, M. L. & Pearce, S. R. (1989) *Proc. R. Soc. London B,* in press.
6. Hazelbauer, G. L. (1975) *J. Bacteriol.* **122,** 206–214.
7. Gallagher, M. P., Pearce, S. R. & Higgins, C. F. (1989) *Eur. J. Biochem.* **180,** 133–141.
8. Joshi, A. K., Ahmed, S. & Ames, G. F.-L. (1989) *J. Biol. Chem.* **264,** 2126–2133.
9. Seeberg, E. & Steinum, A.-L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 988–992.
10. Ames, G. F.-L. (1987) *Cell* **47,** 323–324.
11. Higgins, C. F., Gallagher, M. P., Mimmack, M. L. & Pearce, S. R. (1988) *Bioessays* **8,** 111–116.
12. Hong, J.-S., Hunt, A. G., Masters, P. S. & Lieberman, M. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1213–1217.
13. Gilson, E., Nikaido, H. & Hofnung, M. (1982) *Nucleic Acids Res.* **10,** 7449–7458.
14. Richarme, G. (1985) *J. Bacteriol.* **162,** 286–293.
15. Cross, R. L. & Nalin, C. O. (1982) *J. Biol. Chem.* **257,** 2874–2881.
16. Hunt, A. G. & Hong, J.-S. (1983) *Biochemistry* **22,** 844–850.
17. Plate, C. A., Suit, J. L., Jetten, A. M. & Luria, S. E. (1974) *J. Biol. Chem.* **249,** 6138–6143.
18. Lieberman, M. A. & Hong, J.-S. (1976) *Arch. Biochem. Biophys.* **172,** 312–315.
19. Ferenci, T., Boos, W., Schwartz, M. & Szmelcman, S. (1977) *Eur. J. Biochem.* **75,** 187–193.
20. Plate, C. A. (1979) *J. Bacteriol.* **137,** 221–225.
21. Singh, A. P. & Bragg, P. D. (1979) *Can. J. Biochem.* **57,** 1376–1383.
22. Higgins, C. F., Hiles, I. D., Whalley, K. & Jamieson, D. J. (1985) *EMBO J.* **4,** 1033–1040.
23. Higgins, C. F., Hiles, I. D., Salmond, G. P. C., Gill, D. R., Downie, J. A., Evans, I. J., Holland, I. B., Gray, L., Buckel, S. D., Bell, A. W. & Hermodson, M. A. (1986) *Nature (London)* **323,** 448–450.
24. Hobson, A. C., Weatherwax, R. & Ames, G. F.-L. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7333–7337.
25. Dean, D. A., Fikes, J. D., Gehring, K., Bassford, P. J. & Nikaido, H. (1989) *J. Bacteriol.* **171,** 503–510.
26. Prossnitz, F., Gee, A. & Ames, G. F.-L. (1989) *J. Biol. Chem.* **264,** 5006–5014.
27. Newbury, S. F., Smith, N. H. & Higgins, C. F. (1987) *Cell* **51,** 1131–1143.
28. Hayashi, S., Cock, J. & Lin, E. C. C. (1964) *J. Biol. Chem.* **239,** 3098–3105.
29. Slocum, M. Y. & Parkinson, J. S. (1983) *J. Bacteriol.* **155,** 565–577.
30. Brass, J. M. & Manson, M. D. (1984) *J. Bacteriol.* **157,** 881–890.
31. Casadaban, M. J. (1976) *J. Mol. Biol.* **104,** 541–555.
32. Shuman, H. A. (1982) *J. Biol. Chem.* **257,** 5455–5461.
33. Roth, J. R. (1970) *Methods Enzymol.* **17,** 3–35.
34. Sutherland, L., Cairney, J., Elmore, M. J., Booth, I. R. & Higgins, C. F. (1986) *J. Bacteriol.* **168,** 805–814.
35. Silhavy, T. J., Berman, M. L. & Enquist, L. W. (1984) *Experiments with Gene Fusions* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
36. Bochner, B. R., Huang, H.-C., Schieven, G. L. & Ames, B. N. (1980) *J. Bacteriol.* **143,** 926–933.
37. Parkinson, J. S. (1978) *J. Bacteriol.* **142,** 953–961.
38. Cairney, J., Higgins, C. F. & Booth, I. R. (1984) *J. Bacteriol.* **160,** 22–27.
39. Cairney, J., Booth, I. R. & Higgins, C. F. (1985) *J. Bacteriol.* **164,** 1218–1223.
40. Muir, M., Williams, L. & Ferenci, T. (1985) *J. Bacteriol.* **163,** 1237–1242.
41. Stewart, L. M. D., Bakker, E. P. & Booth, I. R. (1985) *J. Gen. Microbiol.* **131,** 77–85.
42. Kashket, E. (1982) *Biochemistry* **21,** 5534–5538.
43. Shuman, H. A. (1986) *Annu. Rev. Genet.* **21,** 155–177.
44. Chen, C.-M., Misra, T. K., Silver, S. & Rosen, B. P. (1986) *J. Biol. Chem.* **261,** 15030–15038.
45. Débarbouillé, M., Shuman, H. A., Silhavy, T. J. & Schwartz, M. (1978) *J. Mol. Biol.* **124,** 359–371.
46. Hess, J. F., Oosawa, K., Kaplan, N. & Simon, M. I. (1988) *Cell* **53,** 79–87.
47. Ames, G. F.-L., Nikaido, K., Groarke, J. & Petithory, J. (1989) *J. Biol. Chem.* **264,** 3998–4002.
48. Hugenholtz, J., Hong, J.-S. & Kaback, H. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3446–3449.
49. Csonka, L. N. (1989) *Microbiol. Rev.* **53,** 121–147.4.
50. Cairney, J., Booth, I. R. & Higgins, C. F. (1985) *J. Bacteriol.* **164,** 1224–1232.
51. May, G., Faatz, E., Villarejo, M. & Bremer, E. (1986) *Mol. Gen. Genet.* **205,** 225–233.
52. Hiles, I. D., Gallagher, M. P., Jamieson, D. J. & Higgins, C. F. (1987) *J. Mol. Biol.* **195,** 125–142.
53. Poolman, B., Hellingwerf, K. J. & Konings, W. N. (1987) *J. Bacteriol.* **169,** 2272–2276.