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Energy Coupling of the Hexose Phosphate Transport System in *Escherichia coli*

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The active transport of hexose phosphates in *Escherichia coli* was inhibited by many uncouplers or inhibitors of oxidative metabolism. Fluoride and the lipid soluble cation, triphenylmethylphosphonium, had little effect. The uninduced level of transport was sensitive to fluoride, but not to azide. After energy uncoupling of active transport, the cells could equilibrate their intracellular water with the glucose-6-phosphate in the medium and displayed exit counterflow suggesting the existence of carrier-mediated transport in the energy-uncoupled cells. The uncoupled transport of glucose-6-phosphate was inhibited by fructose-6-phosphate; the uninduced level of glucose-6-phosphate transport was not inhibited by fructose-6-phosphate. After energy uncoupling, the influx had a low affinity suggesting that, unlike the transport of β-galactosides, the energy coupling for the active transport of hexose phosphate involved a change in the affinity of influx.

Glucose-6-phosphate (G6P) is transported in *Escherichia coli* by the inducible hexose phosphate active transport system (3, 8, 14, 17). Fructose-6-phosphate, mannose-6-phosphate, glucose-1-phosphate, and 2-deoxyglucose-6-phosphate are also substrates of this system. The unusual nature of the inducer is perhaps this transport system's most remarkable facet. Only "exogenous" G6P will induce; neither the presence of the other substrates nor the presence of intracellular G6P is sufficient for induction to occur (4, 9, 18, 19). Mutants, both negative and regulatory, have been obtained, and the corresponding genetic loci have been mapped (5, 12, 13).

Work from the laboratories of Dietz (2) and of Kaback (10) have shown that the uptake of hexose phosphate in membrane vesicles could be energized by D-lactate or α-glycerophosphate. The energy coupling of the transport of G6P in membrane vesicles is thus similar to that of the β-galactosides (10, 11) and dissimilar to that of glucose and other phosphotransferase-mediated systems (11, 15). Hexose phosphates and β-galactosides are actively transported rather than group translocated. A comparison of the energy coupling of G6P with that previously described for β-galactosides is thus appropriate and is the subject of this investigation.

The active transport of β-galactosides in *E. coli* can be viewed kinetically as an asymmetrical carrier-mediated system in which the affinity of the carrier for the sugar is high for influx and low for efflux (20). This leads to a high ratio of intracellular to extracellular sugar concentration when a steady state, in which influx and efflux are equal, is obtained. Energy uncoupling, either with metabolic poisons or genetic lesions, converts active transport to facilitated diffusion, a carrier-mediated system in which the affinities of influx and efflux are equal and, hence, only equilibration of the intracellular concentration with that in the medium can be achieved. Significantly, energy-uncoupling changes the affinity for efflux from a low to a high value. Thus, the energy-uncoupled cell has a high-affinity-facilitated diffusion system in which the influx of sugar remains as rapid as in the cell with an intact active transport system (20, 21).

How broadly applicable this model is to other transport systems or even to other "D-lactate dehydrogenase-energized" sugar transport systems (11) is not known. Studies, similar to those with β-galactosides, have not been reported. We have, herein, investigated the transport of G6P to determine whether after metabolic poisoning the energy-uncoupled cell still has carrier-mediated transport and whether the uncoupled cell has a high-affinity or low-affinity system, which would indicate whether the coupling had been to efflux or influx, respectively.

MATERIALS AND METHODS

Bacterial strains and media. Experiments were performed with *E. coli* DF2000, a mutant of K-10 provided by D. Fraenkel, which lacks phosphoglucose isomerase (*pgi*$^-$) and zwischenferment (*zwf*$^-$) (6, 7). This strain has no metabolism of G6P other than the biosynthesis of polysaccharides via phosphoglucomutase. Cells were grown on glycerol (0.4%) and Difco Casamino Acids (1%) in a basal medium which consisted of Davis medium A (1) supplemented with thiamine (1 μg/ml) and $FeSO_4 \cdot 7H_2O$ (0.0005%). Induction was accomplished by the addition of 1 mM G6P to the growth medium. ^{14}C-G6P (U) was obtained from Amersham/Searle Corp. Unlabeled hexose phosphates were Sigma Chemical Co. products.

Transport assays. The activity of the hexose phosphate transport system was determined by the membrane filter technique as previously described (18). To determine initial rates, 0.2 ml of the washed cell suspension (500 Klett units) was added to 0.1 ml of ^{14}C-G6P at the appropriate substrate concentration, and a 0.2-ml sample was filtered after incubation for 12 s. The assays were performed in triplicate or quadruplicate. To establish time courses, nine volumes of cells were added to one volume of ^{14}C-G6P, and samples were filtered at the indicated times. An isotopic ^{14}C-G6P concentration of 0.1 μCi/ml was used when active transport was measured, and 1 μCi/ml was used when uncoupled or uninduced transport was measured. The chemical concentration of substrate is indicated in the text. A cell water value of 0.5 μliters per 0.5 ml of cells at 100 Klett units at 420 nm has been determined for this strain (19, 20). Metabolic inhibitor was added to the cell suspension, usually 15 min prior to substrate addition.

RESULTS

Effects of metabolic poisons on active transport. A variety of metabolic poisons were tested for their effect on the active transport of G6P by mutant DF2000. Figure 1 illustrates the effect of preincubating cells for 15 min with the indicated concentration of inhibitor on the subsequent uptake of G6P. Azide (N_3), carbonylcyanide-*p*-trifluoromethoxy phenylhydrozone (FCCP), carbonylcyanide *m*-chlorophenylhydrazone (CCCP), or 2,4-dinitrophenol (DNP) at appropriate concentrations abolished the transport of G6P against a concentration gradient. An uncoupled transport system capable of equilibrating the intracellular pool with the extracellular pool remains. Fluoride had little effect on the active transport of G6P and triphenylmethylphosphonium (TPMP) (10^{-4} or 10^{-3} M) had no effect. Figure 2 shows the response of G6P uptake in uninduced cells to metabolic poisons. The pattern in these cells is entirely different: the uninduced cells have a rapid, relatively inhibitor-insensitive, initial phase followed by a secondary phase sensitive to fluoride, but not to azide.

The fluoride sensitivity of the second phase

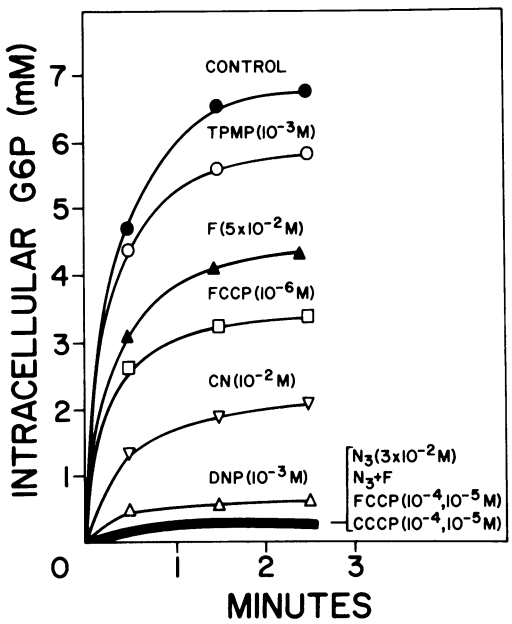

FIG. 1. *Effect of various metabolic inhibitors on the active transport of G6P. Induced cells were incubated for 15 min with the indicated concentration of inhibitor. A portion of these treated cells was then incubated with G6P (0.3 mM, 1 μCi/ml) and sampled at the indicated times. The heavy line contains the experimental points (0.5, 1.5 and 2.5 min) for the inhibitors in brackets.*

of uninduced transport of G6P suggests that the level of uptake may be due to the hydrolysis of the sugar phosphate to glucose and the subsequent uptake of the free hexose. To test this hypothesis, the effect of 1 mM α-methyl glucoside (αMG) or β-glycerophosphate (βGP) as inhibitors of glucose transport and phosphatase, respectively, on induced and uninduced uptake was examined. No effect of either αMG or βGP could be observed. Therefore, at this time the nature of the uninduced uptake is not known.

Kinetics of uptake. The K_t (K_m of transport) and V_{max} of the uptake of G6P under various conditions were determined by measuring influx at 0.2 min in triplicate or quadruplicate at several substrate concentrations. Figure 3 shows graphically influx as a function of G6P concentration in cells with active, uncoupled, or uninduced transport. Table 1 lists the constants, K_t and V_{max}, determined in these systems by using the Wilkinson linear regression analysis program (16). The kinetics of active transport are similar to those we have reported (19): a K_t of 0.2 mM and a V_{max} of 23 nmol per μliter of cell water per min. The constants for the uninduced cells were also determined and were found to range from 10 to 33 mM. However, because

substrate concentrations greater than 10 mM were not employed, the accuracy of these determinations was not as great as those determinations in the low K_t systems. The kinetics constants for the uninduced cells were not significantly altered by any poisoning regimen. In contrast, in the induced cells, poisoning these cells with azide or azide plus fluoride increased the K_t of influx as much as 40-fold. The V_{max} remained approximately the same. Similar results were obtained with DNP (1 mM) in these studies. The effects of DNP and azide on the kinetics of β-galactoside transport are also similar (20). These results are in contrast to those of Winkler and Wilson (20) for β-galactoside transport where K_t of influx was not altered by energy uncoupling.

The ratio of the K_t of influx to the K_t of efflux will predict the maximum accumulation ratio (intracellular G6P-extracellular G6P) obtainable by the cell, assuming that the V_{max} of all fluxes are equal (20). In the energy-uncoupled system, the maximum ratio is 1, i.e., equilibration, since the K_t of efflux equals the K_t of influx. We have been unable to measure the K_t of efflux directly because of the large intracellular pool necessary to observe saturation. The maximum accumulation ratio observed after reaching steady state at room temperature was about 80-fold (Fig. 4). At high extracellular concentrations of G6P, the ratio is underestimated because of diffusional and secondary transport

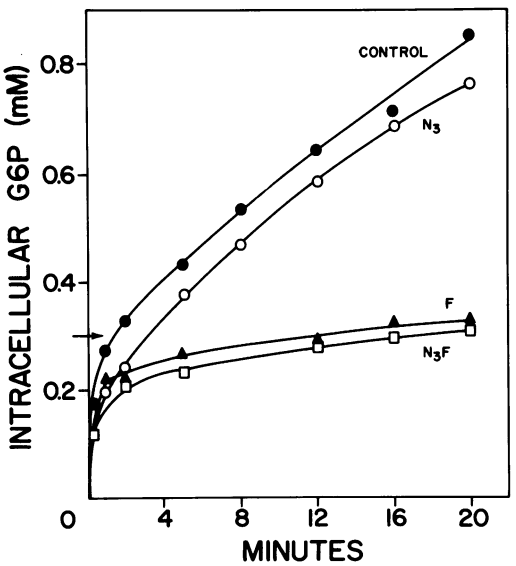

FIG. 2. *Effect of azide and fluoride on the uninduced uptake of glucose-6-phosphate. The experimental conditions were as described in the legend to Fig. 1.*

system components, and at low extracellular G6P concentrations, the ratio is probably overestimated, because some of the radioactivity in the cell is not free G6P, but rather bound or products of metabolism. In light of these considerations, an estimate of a maximum accumulation ratio of 60-fold would predict a K_t of efflux 60 times that of influx, about 18 mM. The measured K_t of influx in the poisoned cells, which should equal the K_t of efflux in these cells, is in good agreement with this value.

Counterflow. The phenomenon of counterflow can demonstrate the existence of carrier-mediated facilitated diffusion after energy uncoupling. Both "entrance counterflow," where preloading the cell with unlabeled substrate saturates efflux without affecting the influx of subsequently added labeled substrate and "exit counterflow," where influx of labeled substrate is saturated by the addition of unlabeled substrate without affecting the efflux of labeled substrate, have been accomplished and described in detail with the energy-uncoupled β-galactoside transport system (20, 22). In contrast to the β-galactoside system, the G6P transport system, as was shown above, has low affinity fluxes after energy uncoupling. The low affinity efflux precludes both the demonstration of entrance counterflow and direct measurements of the K_t of efflux. Despite many attempts with a variety of methods, we have never been able to preload the cell with enough G6P to saturate the efflux system. Exit counterflow, however, may readily be seen with energy-uncoupled G6P transport (Fig. 5). In these experiments, the poisoned cells are allowed to equilibrate with the extracellular radioactive G6P so that the rate of influx and efflux are the same. Once equilibrated, any decrease in intracellular radioactivity would represent a movement against a concentration gradient and would not be possible by simple passive diffusion. However, in a carrier-mediated system behavior, such as that shown in Fig. 5, it can be observed upon addition of saturating concentrations of nonradioactive G6P to the medium. The influx of radioactive G6P is prevented, whereas the efflux continues, resulting in a lowering of the isotopic pool within the cell. The large concentrations of G6P needed to saturate the influx, 20 mM or more, emphasizes the difficulties involved in attempting to saturate the efflux mechanism. The addition of α-glycerophosphate (50 mM) to the medium had little effect and served as a control for the increased organic phosphate concentration and osmotic strength of the medium after the addition of G6P.

Specificity of uninduced and uncoupled

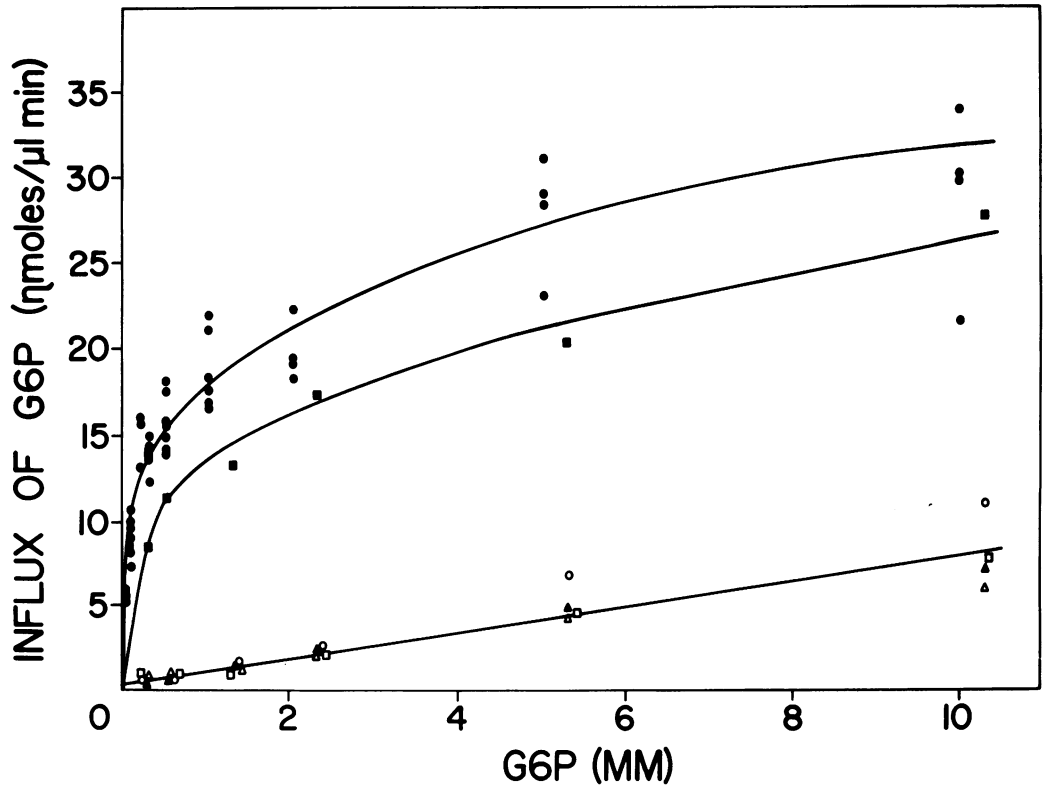

FIG. 3. *Influx of glucose-6-phosphate. Cells were preincubated with inhibitors, azide (30 mM) or fluoride (50 mM), for 15 min. Influx at each point shown was measured in triplicate at 12s. The suspensions were: induced cells (●); induced cells with fluoride (■); induced cells with azide plus fluoride (▲); uninduced cells (○); uninduced cells with fluoride (□) and uninduced cells with azide plus fluoride (△).*

TABLE 1. *Kinetic constants for glucose-6-phosphate transport*

Additions[a]	Induced cells		Uninduced cells	
	K_t[b]	V_{max}[c]	K_t[b]	V_{max}[c]
None	0.28 ± 0.07	27 ± 2	33 ± 15	57 ± 16
	0.12 ± 0.02	19 ± 2	18 ± 5	25 ± 4
F	1.2 ± 0.3	31 ± 3	12 ± 3	15 ± 2
	0.6 ± 0.3	23 ± 3	22 ± 4	31 ± 4
N_3	4.6 ± 0.7	18 ± 1	20 ± 3	38 ± 4
	8.9 ± 2	21 ± 2	11 ± 2	21 ± 2
N_3 + F	7.6 ± 0.3	12 ± 0.2	10 ± 2	11 ± 1
	14 ± 14	20 ± 13	10 ± 2	16 ± 2

[a] NaF = 50mM; NaN_3 = 30 mM.
[b] Millimolar ± standard error.
[c] Nanomoles per microliter of cell water per minute ± standard error.

transport. The active transport system has a specificity that includes both G6P and fructose-6-phosphate as substrates. The affinity of the active transport system for both these sub-

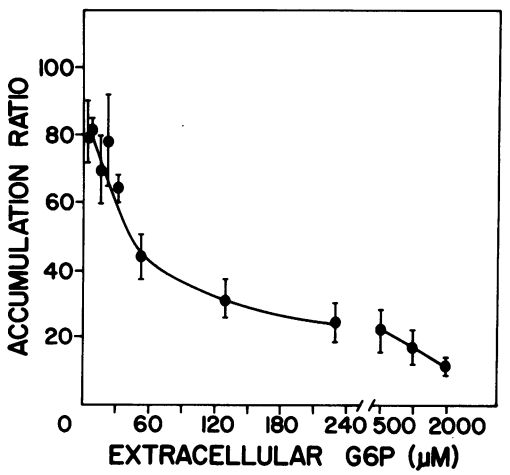

FIG. 4. *Maximal accumulation of G6P. Induced cells were incubated with the indicated concentration of G6P for 15 to 30 min and sampled. The mean ± standard error for three to six determinations is shown.*

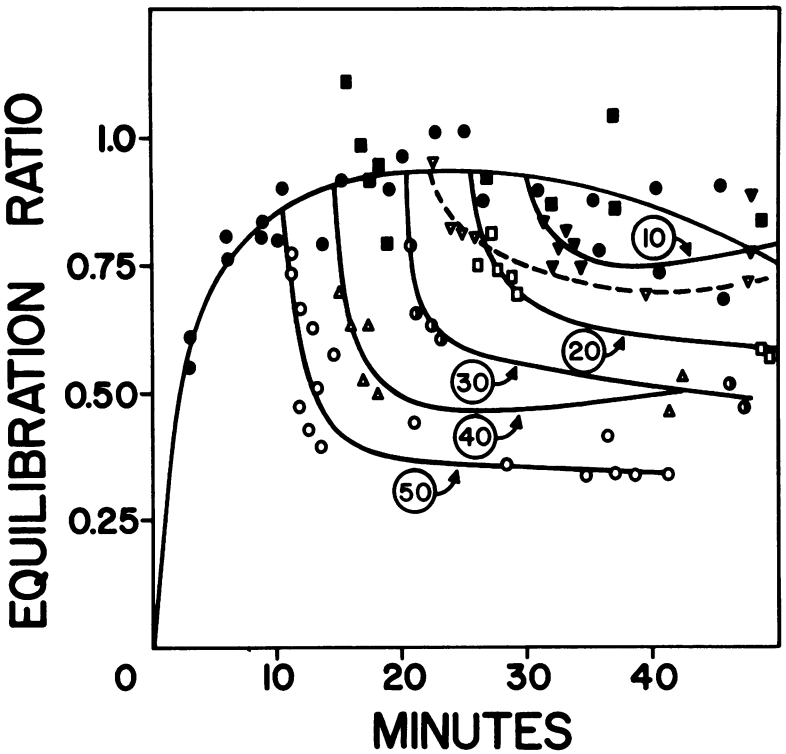

FIG. 5. *Exit counterflow of G6P. Induced cells were uncoupled by incubation with azide (30 mM) plus fluoride (50 mM) for 30 min. These cells were then incubated with G6P (0.3 mM, 1 μCi/ml) and sampled at the indicated times (●). At various times, 1.9 ml of the suspension was added to 0.1 ml of G6P to give the final concentration (mM) shown in the circle (also 5 mM [■]). These suspensions were sampled as above. The dashed line shows a specificity control with a final α-glycerophosphate concentration of 50 mM.*

TABLE 2. *Effect of glucose-6-phosphate and fructose-6-phosphate on the transport of glucose-6-phosphate*

Inhibitor	Uninduced (counts per min per 0.25 μliter of cell water)		Uncoupled (counts per min per 0.25 μliter of cell water)	
	Expt 1	Expt 2	Expt 1	Expt 2
None	183 ± 30[a]	180 ± 18	206 ± 17	196 ± 20
G6P (20 mM)	48 ± 10	55 ± 18	63 ± 2	86 ± 15
G6P (50 mM)	36 ± 14	66 ± 20	53 ± 7	84 ± 29
F6P (20 mM)	125 ± 12	144 ± 14	87 ± 8	72 ± 11
F6P (50 mM)	126 ± 12	184 ± 33	74 ± 19	52 ± 10

[a] Mean of sextuplicate determinations ± standard deviation.

strates is similar. Table 2 compares the specificity of uncoupled and uninduced transport. In these experiments, the uptake (0.2 min at 15 C) of labeled G6P at 1.3 mM was tested for its ability to be inhibited by G6P (self-inhibition) or F6P (competitive inhibition) at 20 and 50 mM. The inhibition observed by addition of unlabeled G6P was in both cases that expected with a K_t of the magnitude measured. However, only the induced cells respond to F6P. The low-affinity uptake system for G6P present in the uninduced cells has a very different specificity from that in the uncoupled cells. F6P is not a substrate in the uninduced cells, whereas F6P is a substrate for the induced transport system, either energy coupled or uncoupled.

DISCUSSION

The accumulation of G6P in *E. coli* is inhibited by those agents which uncouple or block oxidative processes or, alternatively, dissipate proton-motive gradients. Fluoride, which inhibits the generation of phospho-enol-pyruvate, and hence the operation of a phosphotransferase systems such as glucose transport, had little effect. Fluoride also has little effect on the accumulation of β-galactosides and amino acids. KCN has an intermediate effect, and kinetic analysis of this population of cells showed a biphasic substrate dependence sug-

gesting that a portion of the cells were completely unable to accumulate and a portion fully capable of active transport. TPMP, which is a lipid-soluble cation in *Streptococcus faecalis*, had no effect: an appreciable inhibition might have been expected if a potential difference across the cell membrane was important in the accumulation of G6P. However, a flux of protons or cations may still be important in the accumulation of G6P or, alternatively, TPMP may not be lipid soluble in *E. coli*.

After energy uncoupling with metabolic poisons, the cells retained the ability to equilibrate the intracellular water with the G6P in the medium by a carrier-mediated progress. Demonstration of competitive inhibition and counterflow provides evidence of a specific, saturable, mobile carrier in the uncoupled cell. The energy coupling in active transport had apparently been to the influx reaction, for the K_t of influx increased markedly upon metabolic poisoning. This is in contrast to the facilitated diffusion system resulting from metabolic poisoning of the β-galactoside transport system in which the K_t of influx was not altered. The low-affinity hexose phosphate system, in contrast to the high-affinity β-galactoside system, is difficult to characterize because tests for facilitated diffusion rely so heavily on the ability to demonstrate saturation. Furthermore, no selection procedure for an energy-uncoupled mutant, such as that devised with the lactose system (21), could be obtained in view of the limited uncoupled influx.

Studies in membrane vesicles have shown that the transport of both G6P and thiomethyl-β-galactoside are stimulated by the "D-lactate dehydrogenase system" (2, 10). The present investigation emphasizes that the mechanism by which energy is transduced to a form directly involved in the mechanics of active transport may differ from one transport system to another, even though both are stimulated by the same energy sources in the vesicle preparations. For example, in G6P transport, energy coupling serves to increase the affinity of the carrier for substrate (K_t influx) at the outer surface of the membrane, whereas energy is utilized in the β-galactosidase system to decrease the affinity of the carrier for substrate (K_t efflux) at the inner surface of membrane.

Finally, it should be pointed out that at neutral pH, G6P is an anion, and its uptake must be accompanied by equivalent charge movement (cation or proton influx or anion efflux). This interesting aspect of the transport of a charged carbohydrate has not been approached experimentally and may bear directly on energy coupling.

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