Journal of Supramolecular Structure 6:389–398 (1977)
Molecular Aspects of Membrane Transport 251–260

Energetics of Galactose, Proline, and Glutamine Transport in a Cytochrome-Deficient Mutant of Salmonella typhimurium

A. P. Singh and P. D. Bragg
Department of Biochemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

The effect of inhibitors and uncouplers on the osmotic shock-sensitive transport systems for glutamine and galactose (by the β-methyl galactoside permease) was compared to their effect on the osmotic shock-resistant proline and galactose permease systems in cytochrome-deficient cells of Salmonella typhimurium SASY28. Both osmotic shock-sensitive and -resistant systems were sensitive to uncouplers and to inhibitors of the membrane-bound Ca^{2+}, Mg^{2+}-activated adenosine triphosphatase. This suggests that uptake by both types of systems is energized in these cells by an electrochemical gradient of protons formed by ATP hydrolysis through the ATPase.

Key words: amino acid transport, transport energetics, cytochrome-deficient mutant, shock-sensitive transport, shock-resistant transport, Salmonella typhimurium

We have previously utilized a strain of Escherichia coli not able to form cytochromes in the absence of 5-aminolevulinic acid in order to study the energization of membrane-dependent processes under conditions in which the contribution of substrate oxidation through the respiratory chain is minimized (1–3). Moreover, these cells have low levels of endogenous substrates so that it is not necessary to pretreat the cells with uncouplers to deplete these energy reserves. In the present paper we have used a similar strain of Salmonella typhimurium to investigate the energization of the transport of galactose, proline, and glutamine.

The energization of the transport of proline in E. coli is believed to be fundamentally different from that of glutamine. Thus, transport of proline is driven by an electrochemical gradient of protons across the membrane ("energized state") generated by substrate oxidation through the respiratory chain or by hydrolysis of ATP by the membrane-bound Ca^{2+}, Mg^{2+}-activated ATPase[1] (4), whereas transport of glutamine does not involve the energized state but appears to require the direct involvement of ATP by a mechanism not utilizing the ATPase (5). Berger and Heppel (6) have extended this hypothesis to include certain other transport systems present in E. coli. They suggest that all transport systems which

[1] Abbreviations: ATPase – adenosine triphosphatase; CCCP – carbonylcyanide-m-chlorophenylhydrazone; DCCD – N, N′-dicyclohexylcarbodiimide.

Received May 4, 1977; accepted May 4, 1977

like glutamine have a binding-protein which can be released by osmotic shock treatment of intact cells are energized directly by ATP. Shock-resistant systems, like that of proline, are driven by the energized state.

S. typhimurium possesses both shock-sensitive and shock-resistant transport systems for D-galactose (7). In the cells used in our experiments the methyl β-thio-D-galactoside systems I and II are absent and galactose is transported by the binding-protein dependent β-methyl galactoside permease or by the binding-protein independent galactose permease system. Although Wilson (8, 9) has suggested that the β-methyl galactoside and galactose permease systems are both driven by ATP directly, the results of Henderson et al. (10) which show that the uptake of galactose through the galactose permease, but not that of β-methyl galactoside, is accompanied by symport of protons suggest that the galactose permease system may be driven by the energized state.

In this paper we have examined the action of compounds which effect the energization of transport in cytochrome-deficient cells of S. typhimurium. We have found that there is essentially no difference between the response to these compounds of the binding-protein-dependent and -independent transport systems. Moreover, the sensitivity of glutamine and β-methyl galactoside permease systems to uncouplers and to inhibitors of the Ca^{2+}, Mg^{2+}-dependent ATPase suggests that their uptake, like that of proline and of galactose through the galactose permease system, is driven by the energized state generated on ATP hydrolysis by the ATPase.

METHODS

Growth of Organism

S. typhimurium SASY28 (SA1889) (hem A^-, pro^-, pur E^-), originally isolated by Dr. A Sasarman (University of Montreal, Canada), was obtained from Dr. K. E. Sanderson (University of Calgary, Canada).

For studies of proline and glutamine transport the cells were grown aerobically at 37°C in the absence of 5-aminolevulinic acid in trypticase soy broth (Difco) containing 10 mg/liter adenine. In studies of galactose transport the cells were grown in a minimal salts medium (2) lacking sodium citrate and containing 0.5% (wt/vol) D-fructose, 0.5% (wt/vol) bactotryptone (Difco), 50 mg/liter proline and 10 mg/liter adenine. Galactose and β-methyl galactoside transport was induced by inclusion of 10 mM D-galactose or 2 mM D-fucose, respectively. The cells were harvested in the mid-exponential phase of growth, washed twice at 22–23°C with minimal salts medium or 50 mM Tris-HCl buffer, pH 7.3, containing 5 mM $MgCl_2$, and resuspended in the respective medium at a cell density giving 2.8 mg protein/ml.

Measurement of Transport

Uptake of [^{14}C] proline and [^{14}C] glutamine was measured as previously described (2). The incubation mixture to measure the uptake of [^{14}C] galactose and [^{14}C] β-methyl galactoside contained in a total volume of 0.1 ml, 0.01 ml cell suspension, 0.08 ml of either minimal salts medium or 50 mM Tris-HCl buffer, pH 7.3, containing 5 mM $MgCl_2$, and 100 μg/ml chloramphenicol. These buffers were used interchangeably since the rate of uptake was not noticeably affected by the nature of the buffer used. The energy source was added at the concentration indicated. The cells were preincubated without the energy source for 10 min at 23°C. The energy source was then added followed after 5 min by 10 μl of the radioactive transport solute diluted with unlabeled solute to give a final con-

centration of 150 μM (31 μM with β-methyl galactoside). In some experiments designed to measure the β-methyl galactoside permease system [^{14}C] galactose was added at a final concentration of 0.6 μM. Uptake was terminated by the addition of 3 ml 150 mM NaCl immediately followed by filtration of the mixture through a Millipore membrane filter (25 mm diameter; pore size, 0.45 μm). The cells were washed twice with 3 ml 150 mM NaCl. After drying, the filters were dissolved in Bray's scintillation fluid and the radioactivity measured with a Packard Tri-Carb liquid scintillation spectrophotometer, model 2425. L-[U-^{14}C] proline (290 Ci/mol) and D-[1-^{14}C] galactose (95 Ci/mol) were obtained from Amersham-Searle Corporation. L-[U-^{14}C] glutamine (235 Ci/mol) and [^{14}C] methyl β-D-galactopyranoside (8.12 Ci/mol) were purchased from New England Nuclear Corporation.

For inhibition studies the cells were preincubated at 23°C for 10 min in the minimal salts or Tris-$MgCl_2$ buffer with the indicated concentrations of inhibitor prior to the addition of the energy source. The 50 mM Tris-HCl buffer, pH 7.3, containing 5 mM $MgCl_2$ was always used for experiments with arsenate.

Determination of Intracellular ATP Concentration

These experiments were based on the conditions used in the transport assays except that the experiments were scaled up 10-fold. To a final volume of 1 ml of cells suspended at 0.24–0.34 mg protein/ml of the buffer various concentrations of the inhibitors were added, and the samples preincubated at 23°C for 10 min prior to the addition of the energy source (glucose or fructose). After 5 min incubation at 23°C the ATP was extracted from the cell suspension by the addition of 0.5 ml 1.4 M perchloric acid. In the experiments with 2,4-dinitrophenol, the cells were pelleted by centrifugation and the supernatant discarded. The cells were resuspended in 1 ml buffer and 0.5 ml perchloric acid was added as above. The mixture was agitated for 10 sec with a Vortex mixer and then kept at 0°C for 15 min. The cells were sedimented by centrifugation at 8,000 × g for 5 min and 1.0 ml of the supernatant removed for neutralization with 0.5 ml 0.72 M KOH in 0.15 M $KHCO_3$. The mixture was kept at 0°C for 30 min and then centrifuged to remove the precipitate of potassium perchlorate. From the supernatant 1.0 ml was removed and frozen in an ethanol-dry ice bath. The sample was allowed to thaw at room temperature and the freezing-thawing process repeated. This process resulted in the precipitation of further potassium perchlorate which was sedimented by centrifugation. Samples (10 μl) of the supernatant were assayed for ATP by the luciferin-luciferase method (11).

Measurement of Proton Translocation

The cells were harvested in the mid-exponential phase of growth, washed twice with 2 mM glycylglycine buffer, pH 6.8, containing 100 mM KCl and 5 mM $MgCl_2$, and suspended in the same buffer at a cell concentration of 2.3 mg protein/ml. Proton translocation was measured using 5 ml samples of the cell suspension as described before (3).

RESULTS

Transport in Cytochrome-Deficient Cells of S. Typhimurium SASY28

As shown on Fig. 1 cytochromes were not formed in S. typhimurium SASY28 unless the medium was supplemented with 5-aminolevulinic acid. The rate of oxidation of NADH and D-lactate by membrane particles prepared from cytochrome-deficient cells was negligible. Thus, in contrast to the results with cytochrome-containing cells, the uptake of D-galactose and of β-methyl D-galactoside (data not shown) could not be energized by

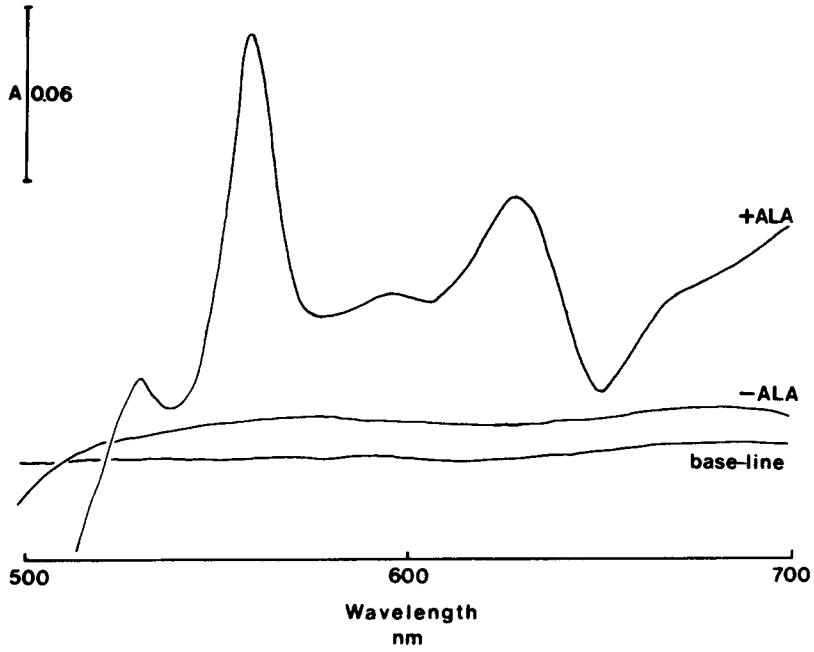
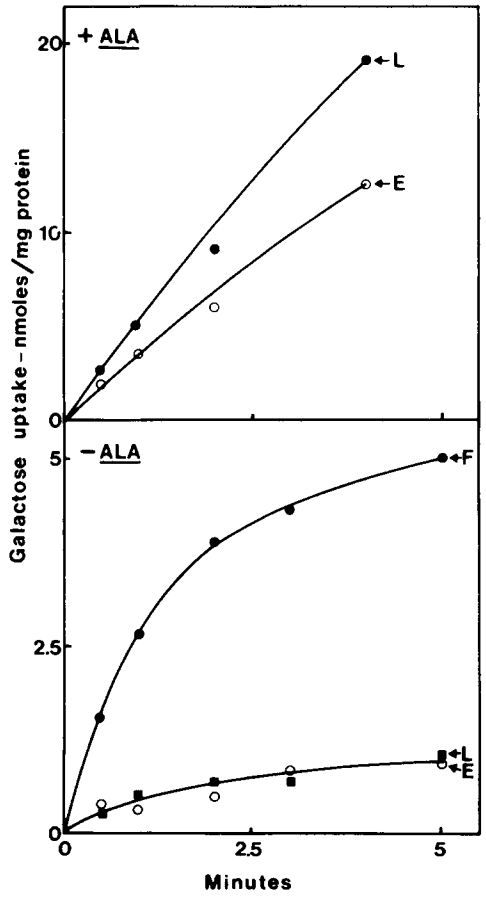


Fig. 1. Dithionite reduced minus oxidized difference spectra of intact cells of S. typhimurium SASY28 grown in the presence and absence of 5-aminolevulinic acid (ALA) (50 mg/liter). Concentration of cells, 0.15 g wet weight/ml.



2

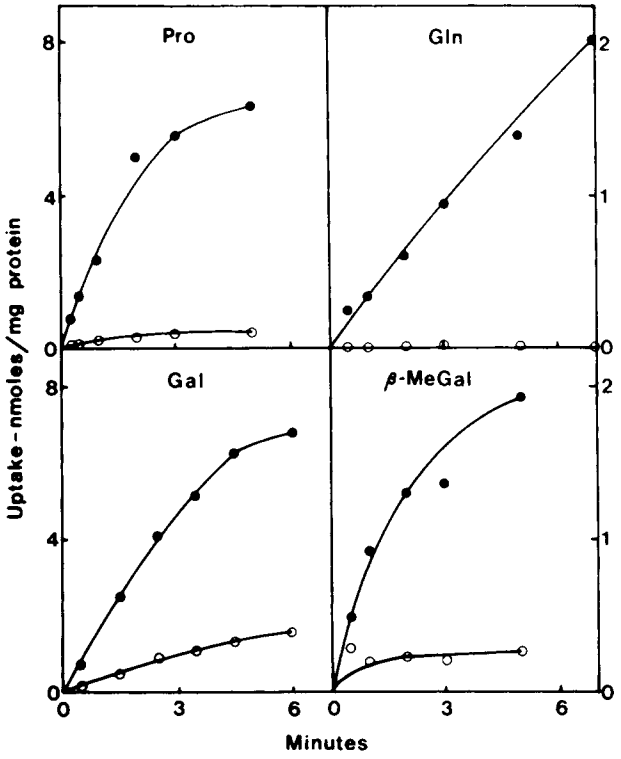


Fig. 3. Uptake of [^{14}C] proline (Pro), glutamine (Gln), galactose (Gal) and β-methyl galactoside (β-MeGal) by cytochrome-deficient cells in the presence of 10 mM glucose (Pro, Gln) or 20 mM fructose (Gal, β-MeGal) (•) or in the absence of exogenous substrates (○). Galactose and β-methyl galactoside transport was induced by growth in the presence of 2 mM D-fucose.

D-lactate in cytochrome-deficient cells (Fig. 2). However, ATP generated by glycolysis of glucose and fructose could support the uptake of proline, glutamine, galactose, and β-methyl D-galactoside in cytochrome-deficient cells (Figs. 2 and 3). Uptake of these solutes was linear with time for the first minute following addition to the cell suspension.

Effect of Inhibitors and Uncouplers on Uptake of Proline, Glutamine, and Galactose

The effect of inhibitors and uncouplers on the uptake of proline, glutamine, and galactose by cytochrome-deficient cells is shown on Figs. 4–6. Uptake of galactose was measured at 2 concentrations (0.6 μM and 150 μM). Kinetic studies with these cells showed that 2 transport systems for galactose with K_m values of 0.6 μM and 27 μM were present. These values can be compared to the K_m values of 1 μM and 50 μM determined for galactose uptake by the β-methyl galactoside and galactose permease systems, respectively, in transport mutants of S. typhimurium (7).

Fig. 2. Uptake of [^{14}C] galactose in cytochrome-containing (+ALA) and cytochrome-deficient cells (−ALA). Galactose transport was induced by growth in the presence of 10 mM D-galactose. The concentration of 5-aminolevulinic acid, where present, was 50 mg/liter. Uptake was energized by endogenous substrate (E), 20 mM D-lactate (L) or 20 mM fructose (F). Concentration of galactose, 150 μM.

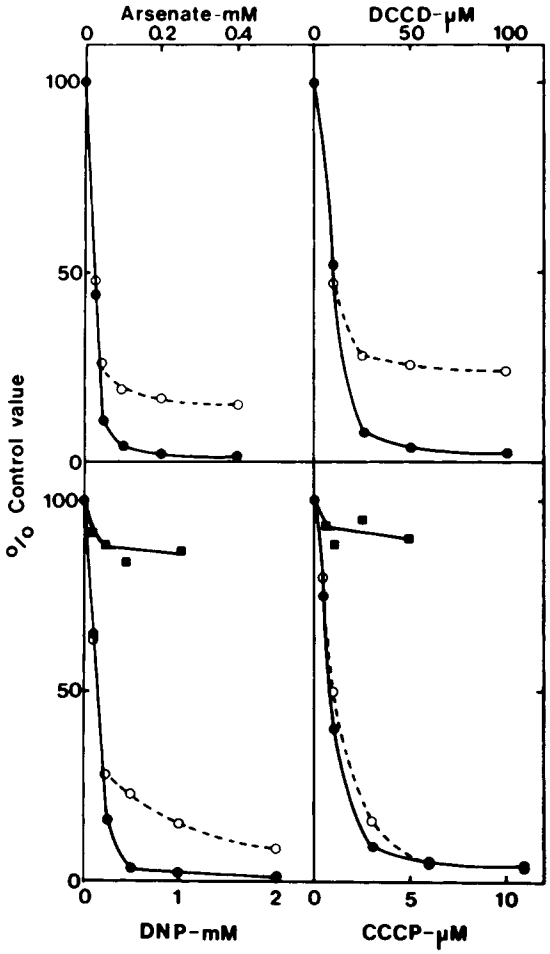
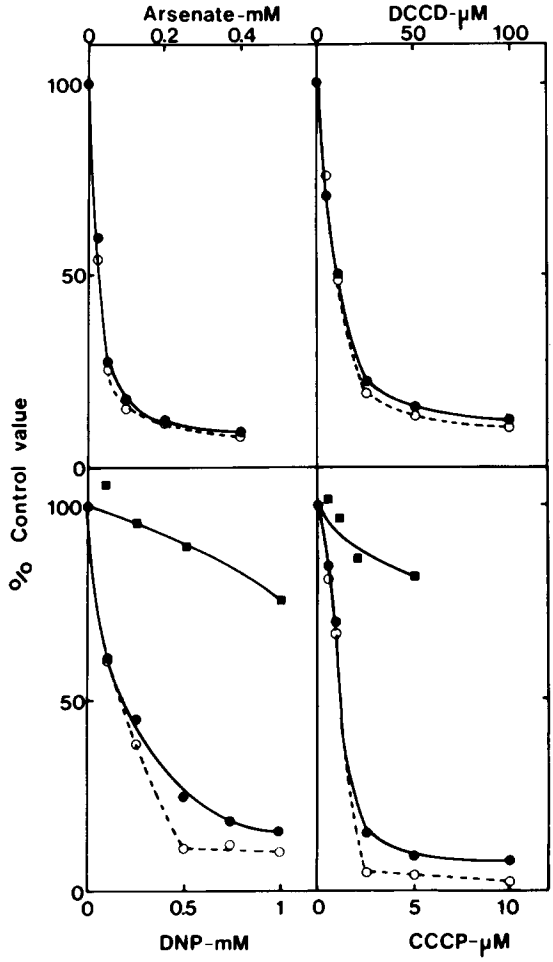


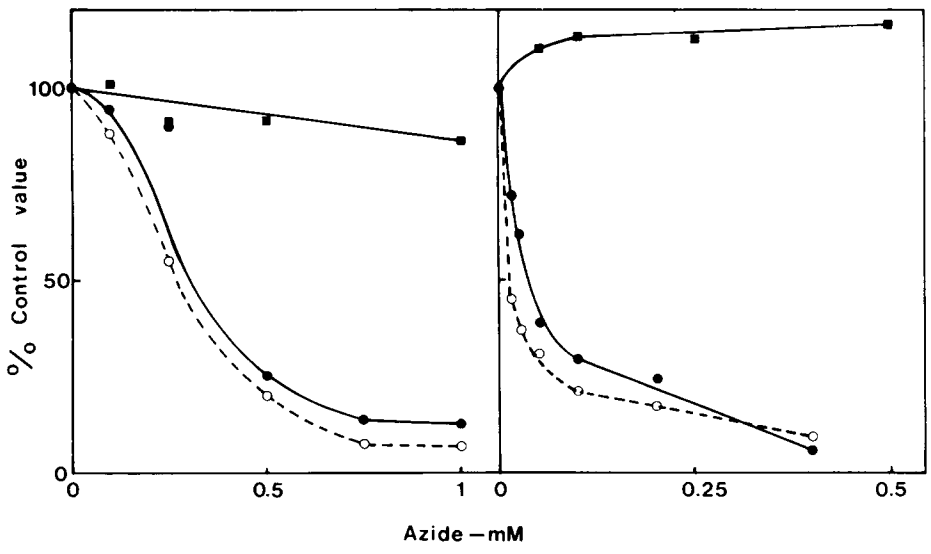
Fig. 4. Inhibition of the uptake of proline (•) and glutamine (○) in cytochrome-deficient cells by arsenate, DCCD, 2,4-dinitrophenol (DNP)and CCCP, and the effect of inhibitors on the level of cellular ATP (▪). Uptake was energized by 10 mM glucose. The 100% control values for glutamine and proline uptake were 0.24 and 1.1 nmol/min/mg protein, respectively. The 100% ATP level was 2.63 nmol/mg protein.

Fig. 5. Inhibition of the uptake of 0.6 μM (○) and 150 μM (•) D-galactose in cytochrome-deficient cells by arsenate, DCCD, 2,4-dinitrophenol (DNP), and CCCP, and the effect of inhibitors on the level of cellular ATP (▪). Uptake was energized by 20 mM fructose. The 100% control values at 0.6 μM and 150 μM galactose were 1.3–2.0 and 160 nmol/min/mg protein, respectively. The 100% ATP level was 5.2–5.6 nmol/mg protein.

Fig. 6. Effect of azide on the uptake of D-galactose (left) (○, 0.6 μM; •, 150 μM), proline (•) and glutamine (○) (right), and on the levels of cellular ATP (▪) in cytochrome-deficient cells. The uptake of galactose was energized by 20 mM fructose and that of proline and glutamine by 10 mM glucose. The 100% control values for 0.6 μM galactose, 150 μM galactose, proline, and glutamine were 1.4, 156, 2.7, and 0.61 nmol/min/mg protein, respectively. The 100% ATP levels were 6.3 (left) and 3.9 nmol/mg protein (right), respectively.


Arsenate-mM
0
0.2
0.4
DCCD-μM
0
50
100
100
50
0
% Control value
100
50
0
0
0.5
1
DNP-mM
0
5
10
CCCP-μM

5


100
50
0
% Control value
0
0.5
1
0
0.25
0.5
Azide — mM

6

Arsenate inhibits the formation of ATP by glycolysis. Since the energy source for transport in cytochrome-deficient cells is ATP generated by glycolysis, the inhibition of proline, glutamine, and galactose uptake by arsenate confirmed the dependency of the uptake of these solutes on the availability of ATP. To distinguish between the use of ATP through the ATPase or its use by an as yet unknown mechanism independent of the ATPase, the effects of azide and DCCD were examined (Figs. 4–6). These compounds inhibit the membrane-bound Ca^{2+}, Mg^{2+}-activated ATPase (12). Uptake of galactose, proline, and glutamine was inhibited by azide and DCCD suggesting that the ATPase was involved in the transport of these solutes. Azide had little effect on the cellular level of ATP in the cytochrome-deficient cells with fructose and glucose as energy sources (Fig. 6). The small increase in the level of ATP found with the latter substrate is consistent with the blocking by azide of the hydrolysis of ATP by the ATPase.

The uncouplers 2,4-dinitrophenol and CCCP inhibited the uptake of proline, glutamine, and galactose (Figs. 4 and 5). These uncouplers are believed to inhibit transport by dissipating the electrochemical gradient of protons (energized state) (4). However, Wilson (8) and Curtis (13) in interpreting the inhibitory effect of these compounds on shock-sensitive transport systems in wild-type E. coli have suggested that they affect these systems by causing dissipation of the ATP pool of the cell and not because they cause breakdown of the proton gradient. As can be seen from Figs. 4 and 5 concentrations of uncoupler which cause maximal inhibition of the uptake of proline, glutamine, and galactose produced only small changes (5–15%) in the level of cellular ATP. The effect of 2,4-dibromophenol was similar to that of 2,4-dinitrophenol. These results are consistent with the findings of Pavlasova and Harold (14) on the effect of uncouplers on the ATP pools of wild-type E. coli.

Proton Symport During Galactose Uptake

According to the chemiosmotic hypothesis (4) uptake of neutral solutes, such as galactose, driven by an electrochemical gradient of protons should occur with symport of protons. A proton gradient was generated by cytochrome-deficient cells of S. typhimurium SASY28 metabolizing endogenous substrates or following addition of fructose. Addition of D-galactose or D-fucose, which is taken up by both the β-galactoside permease and galactose permease systems (7), resulted in uptake of protons (Fig. 7). The uncoupler CCCP abolished proton uptake following addition of galactose (data not shown) or fucose. There was no consumption of protons following addition of β-methyl D-thiogalactoside since the permease for this glycoside had not been induced. Although the β-methyl galactoside permease was present, uptake of protons did not occur on addition of this galactoside. A similar result was recently reported for E. coli (10).

DISCUSSION

The use of cytochrome-deficient cells of S. typhimurium has enabled us to investigate the energization by ATP of the uptake of certain solutes without interference from respiratory-chain mediated energization. Moreover, these cells have low endogenous activity so that it is not necessary to pretreat the cells with an uncoupler to deplete the endogenous energy reserves. This procedure, which has been used by some workers, is obviously undesirable if the effects of uncoupling agents on transport are to be studied subsequently.

Cytochrome-deficient cells of S. typhimurium SASY28 will take up proline, glutamine, galactose, and β-methyl galactoside in the presence of ATP generated by the glycoly-

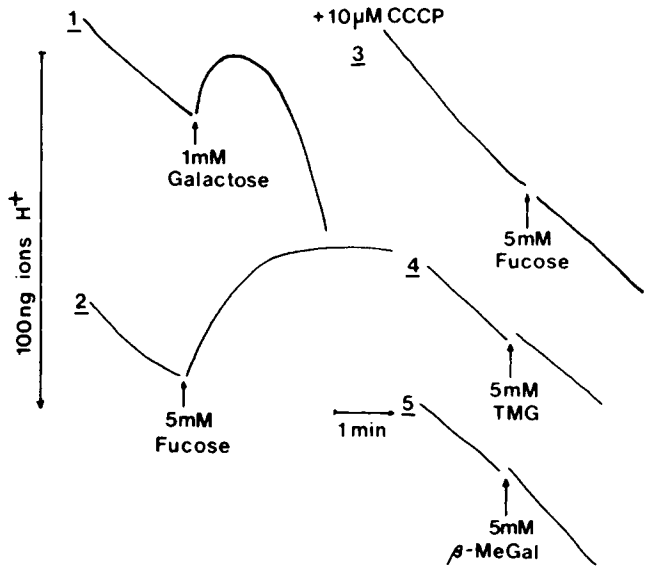


Fig. 7. Proton uptake following addition of solute (at the arrow) to cytochrome-deficient cells metabolizing endogenous substrates (1, 5) or 20 μM fructose (2–4). TMG: β-Methyl thiogalactoside; β-MeGal: β-methyl galactoside.

sis of glucose and fructose. Our principal finding is that the uptake of proline, glutamine, and of galactose used at 2 different concentrations to measure the β-methyl galactoside permease and the galactose permease system (8, 16), is inhibited by the uncouplers 2,4-dinitrophenol and CCCP, and by azide and DCCD, inhibitors of the Ca^{2+}, Mg^{2+}-activated ATPase. These results suggest that the uptake of all of these solutes in the cytochrome-deficient cells is driven by an electrochemical gradient of protons which is formed by hydrolysis of glycolytically generated ATP by the membrane-bound Ca^{2+}, Mg^{2+}-activated ATPase. Since the level of intracellular ATP is little affected by the inhibitors and uncouplers over a concentration range which drastically inhibits uptake, it is not likely that inhibition is caused by an effect on the ATP pool.

Our results do not agree with those obtained with cytochrome-containing E. coli. Thus, Berger and Heppel (5, 6) found that although proline transport was driven by the energized state generated by ATP hydrolysis through the Ca^{2+}, Mg^{2+}-activated ATP, glutamine transport was energized by a different, as yet unknown, mechanism. In contrast to the findings of Kerwar et al. (15), Wilson has suggested that both the galactose permease and the β-methyl galactoside permease systems are driven by a mechanism similar to that used for glutamine transport. However, Parnes and Boos (16) have suggested that uptake by the β-methyl galactoside permease in E. coli is driven by the energized state. The recent results of Henderson et al. (10) suggest that the proton gradient is involved in the galactose permease system although uptake of protons was not found with the transport of β-methyl galactoside.

Other workers have obtained results which are difficult to reconcile with the Berger-Heppel hypothesis. Thus, Plate et al. (17) found that colicin K, which appears to act by deenergization of the energized state of the membrane, inhibited the transport of both proline and glutamine in an ATPase mutant of E. coli. This suggests that glutamine and proline transport systems share a common element sensitive to colicin K. Bradbeer and

Woodrow (18) found that the shock-sensitive transport of vitamin B_{12} in E. coli was driven by the energized state. Lieberman and Hong (19) have isolated a temperature-sensitive mutant in the common element of shock-sensitive and shock-resistant systems. They have suggested that ATP might function as a regulatory effector which could direct the use of the energized state to the transport of solutes belonging to the shock-sensitive systems. This would explain the apparent requirement of these systems for ATP. Recently, Rhoads and Epstein (20) have found that the Trk A system for potassium transport in E. coli shows a need for both the energized state and ATP. Thus, it can be concluded from the results presented in this paper, and from those of other workers, that there is no clear-cut distinction between shock-sensitive and shock-resistant transport systems in their mechanism of energization.

ACKNOWLEDGMENTS

We wish to thank Dr. K. E. Sanderson for the gift of the organism used in this work and the Medical Research Council of Canada for financial support.

REFERENCES

1. Singh AP, Bragg PD: Biochim Biophys Acta 396:229, 1975.
2. Singh AP, Bragg PD: Biochim Biophys Acta 423:450, 1976.
3. Singh AP, Bragg PD: Biochim Biophys Acta 464:562, 1977.
4. Simoni RD, Postma PW: Annu Rev Biochem 44:523, 1975.
5. Berger EA: Proc Natl Acad Sci USA 70:1514, 1973.
6. Berger EA, Heppel LA: J Biol Chem 249:7747, 1974.
7. Postma PW: J Bacteriol 129:630, 1977.
8. Wilson DB: J Bacteriol 126:1156, 1976.
10. Henderson PJF, Giddens RA, Jones-Mortimer MC: Biochem J 162:309, 1977.
11. Stanley PE, Williams SG: Anal Biochem 29:381, 1969.
12. Roisin MP, Kepes A: Biochim Biophys Acta 275:333, 1972.
13. Curtis SJ: J Bacteriol 120:295, 1974.
14. Pavlasova E, Harold FM: J Bacteriol 98:198, 1969.
15. Kerwar GK, Gordon AS, Kaback HR: J Biol Chem 247:291, 1972.
16. Parnes JR, Boos W: J Biol Chem 248:4429, 1973.
17. Plate CA, Suit JL, Jetten AM, Luria SE: J Biol Chem 249:6138, 1974.
18. Bradbeer C, Woodrow ML: J Bacteriol 128:99, 1976.
19. Lieberman MA, Hong J-S: Arch Biochem Biophys 172:312, 1976.
20. Rhoads DB, Epstein W: J Biol Chem 252:1394, 1977.