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Inactivation of the lactose permease of *Escherichia coli* by the respiratory activity

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We had shown previously that the lactose permease of *Escherichia coli* ML 308225 becomes irreversibly inactivated during lactose transport when the cells are energized by addition of an external energy source; this inactivation is concomitant with an irreversible decrease of the electrochemical potential gradient of protons (Ghazi, A., Thérisod, H. and Shechter, E. (1983) J. Bacteriol. 154, 92–103). Addition to energized cells of 2-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO), an inhibitor of the respiratory chain, suppresses these phenomena. Also, the inactivation of the lactose permease does not take place in energized *E. coli* K 207 cells, a mutant devoid of a functional respiratory chain. The inactivation of the lactose permease may take place in nonenergized cells and in the absence of lactose, but at a much slower rate. Addition of lactose or methyl-1-thio-β-D-galactoside (TMG), a competitive analogue of lactose, enhances the inactivation. On the other hand, addition of β-D-galactosyl-1-thio-β-D-galactoside (TDG), or *p*-nitrophenyl-α-D-galactoside (α-NPG), or *o*-nitrophenyl-β-D-galactoside (ONPG), other competitive analogues of lactose, strongly inhibits the inactivation. From these data, it is concluded that the respiratory activity of the cell in itself leads to an inactivation of the lactose permease. The presence of one class of galactosides enhances the susceptibility of the permease towards inactivation, probably by immobilizing the protein in a conformation more susceptible to the inactivating agent. In contrast, the presence of another class of galactosides can protect the protein against inactivation.

Introduction

The transport of β-galactosides across the cytoplasmic membrane of *Escherichia coli* is mediated by the *lac* permease, product of the *y* gene of the *lac* operon. It catalyzes a proton/β-galactoside coupled transport (symport) [1].

We have recently shown that in energized *E. coli* cells (i.e., cells in the presence of an exogenous energy source), the lactose permease becomes irreversibly inactivated during lactose transport [2]. While in resting cells the uptake of lactose is monotonous, in energized cells (whatever the added energy source: glycerol, D-lactate, succinate, ascorbate/phenazine methosulfate), for an external lactose concentration near or above the K_T, the internal lactose concentration reaches a maximum and then declines to a steady-state level 2–10-times lower than in resting cells.

This low steady-state level of lactose accumula-

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Abbreviations: Ph_4P^+, tetraphenylphosphonium ion; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; PCMBS, *p*-chloromercuriphenylsulfonic acid; TDG, β-D-galactosyl-1-thio-β-D-galactopyranoside; TMG, methyl-1-thio-β-D-galactoside; α-NPG, *p*-nitrophenyl-α-D-galactoside; ONPG, *o*-nitrophenyl-β-D-galactoside; HQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide.

tion is due to an irreversible large decrease in the rate of influx and to an increase in the rate constant of efflux. It is accompanied by a large and irreversible decrease in the transmembrane potential. These phenomena are not observed in resting cells, or at high external K^+ or Na^+ concentrations. EDTA treatment enhances these phenomena. We suggested that the inactivation is related to an irreversible modification of the lactose carrier leading to a proton entry via the lactose permease no longer coupled to lactose uptake.

Since the addition of cyanide to energized cells suppresses these phenomena, we proposed that the high rates of respiration observed upon energization are responsible for an irreversible inactivation of the lactose permease, which is, in turn, responsible for the decrease of $\Delta\psi$ [2].

In the present paper, we present additional evidences which confirms that the inactivation of the lactose carrier is due to the activity of the respiratory chain. Moreover, we show that while a high respiratory activity and the presence of lactose accelerate the specific inactivation of the carrier and the decrease of $\Delta\psi$, these phenomena also take place at low respiratory activity and in the absence of lactose, but at a slower rate.

In addition, we show that while the presence of lactose and TMG enhances the susceptibility of the permease towards inactivation, the presence of TDG, or α-NPG, or ONPG (competitive analogues of lactose) protects the carrier against inactivation. We interpret these results in terms of different conformations of the permease in the presence of the various classes of substrates.

Materials and Methods

Growth conditions and cell treatment. Cells of *E. coli* ML 308225 (i^-, z^-, y^+, a^+) were grown in minimum medium M9 containing 0.4% glycerol as the sole carbon source and harvested at an absorbance of 0.4 at 650 nm (1 cm pathlength). Cells of *E. coli* K 207, a mutant of ML 308225 defective in 5-aminolevulinic acid synthesis, were grown according to Ref. 3. Cells of *E. coli* T 206, which carries the *lac y* gene in a recombinant DNA plasmid [4], and cells of *E. coli* G 148, which carries the *lac y* gene modified at cys-148 in a recombinant DNA plasmid [5], were grown and induced with isopropyl-1-thio-β-D-galactopyranoside as described in [4]. In order to render the membrane permeable to the membrane potential probe, the cells were systematically treated with EDTA according to Ref. 6. EDTA-treated cells were resuspended in 10 mM Tris-HCl/150 mM choline chloride/1 mM KCl (pH 7.6) at an absorbance of 40 (20 mg dry wt./ml), kept on ice and used within 5 h.

Lactose uptake. Cells were resuspended in the above medium at a final concentration of 1 mg dry wt./ml and incubated with agitation for 2 min at 25°C in the presence of appropriate additions. [^{3}H]Lactose (10 mCi/mmol) was then added at the desired concentration. At given intervals, aliquots (100 μl) were removed, immediately diluted with 4 ml buffer and filtered on Whatman glass microfiber filters (GF/F). The filters were washed twice with 4 ml buffer and counted for radioactivity in a liquid scintillation counter. Uptakes were corrected by subtracting blanks obtained in identical conditions, except that the cells were pretreated with 25 μM CCCP and 200 μM PCMBS. The internal lactose concentration was calculated assuming an internal cytoplasmic volume of 1 μl/mg dry wt. [6].

Determination of the influx rate of lactose during its accumulation. Cells were resuspended and incubated as described above. At time zero, nonradioactive lactose was added(500 μM final concentration). Aliquots (100-μl) were withdrawn. To these, trace amounts of [^{3}H]lactose (266 mCi/mmol, final concentration 4 μM) were added at various time intervals. Uptake of radioactive lactose was allowed to proceed for 15 s before dilution and filtration as described above.

Determination of the transmembrane electrical potential ($\Delta\psi$). $\Delta\psi$ was determined by the accumulation of [^{3}H]Ph_4P^+. EDTA-treated cells(1 mg dry wt./ml) were incubated at 25°C in the suspension buffer as above. [^{3}H]Ph_4P^+ (75 mCi/mmol) was then added (final concentration: 10 μM). Aliquots (100-μl) were withdrawn as a function of time, diluted with 4 ml buffer and filtered on 0.45 μm EH millipores. The filters were washed once with 4 ml buffer and counted for radioactivity. The uptake of Ph_4P^+ was corrected by subtracting blanks obtained in identical conditions except that the cells were pretreated with 25-μM CCCP and the

aliquots filtered immediately after addition of the radioactive probe. The internal Ph_4P^+ concentration was calculated assuming a cytoplasmic value of 1μl/mg dry wt. The accumulation of Ph_4P^+ reaches a plateau within 3 to 5 min. $\Delta\psi$ was calculated from this plateau value using the Nernst equation.

Rate of respiration. Oxygen consumption was determined polarographically with a Gilson oxygraph using a Clark oxygen electrode. Cell concentration was 1 mg dry wt./ml.

Material. [^{3}H]Ph_4P^+ and [^{3}H]lactose were obtained from CEA, Saclay, France. Strain K 207 was a generous gift from Dr. P. Overath (Tübingen, F.R.G.). Strains T 206 and G 148 were kindly provided by Dr. H.R. Kaback (Nutley, U.S.A.). All other materials were of reagent grade and obtained from commercial sources.

Results

Respiratory activity is responsible for the inactivation of the lac carrier

The typical effect of an externally added energy source on lactose uptake in *E. coli* cells, fully described in Ref. 2, is presented in Fig. 1. While in the absence of energy source (resting cells) the internal concentration of lactose increases monotonously to a steady-state level, in the presence of glycerol (or D-lactate, or succinate, or ascorbate/phenazine methosulfate) (energized cells), after an initial increase, it decreases and stabilizes at a steady-state level significantly lower than that of resting cells.

When energized cells were treated with HQNO, which, at the concentration used (0.25 mM) blocked 70% of the respiration (data not shown), the steady-state level of lactose accumulation was several-times higher than in energized cells and similar or even higher, depending on the cell preparation, than in resting cells. Fig. 1 shows the result obtained with one cell preparation, with glycerol as the energy source.

Lactose uptake in energized cells results in a marked decrease in $\Delta\psi$, which at pH 7.6 is the sole component of the protonmotive force [6], while in resting cells, the decrease in $\Delta\psi$ is modest or absent (Table I). Although this phenomenon, already reported in Ref. 2, was qualitatively highly

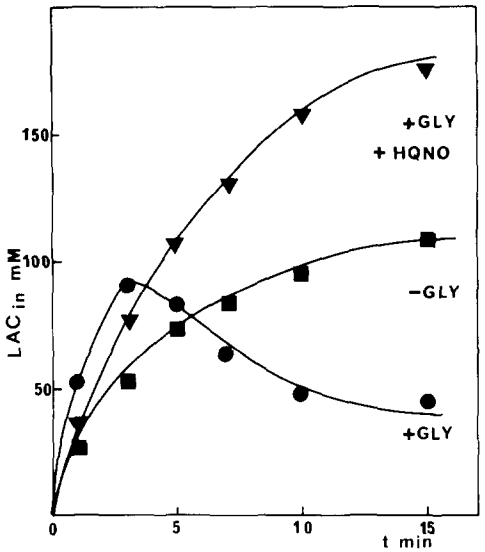

Fig. 1. Effect of HQNO on the time course of lactose (LAC) uptake (0.5 mM, lactose external concentration) by *E. coli* ML 308225 cells (EDTA-treated, 1 mg dry wt/ml). Uptake in the presence of glycerol (GLY) (0.4%) (●), in the presence of glycerol and of HQNO (0.25 mM) (▼) and in the absence of glycerol (■).

TABLE I

EFFECT OF LACTOSE UPTAKE ON THE TRANSMEMBRANE ELECTRICAL POTENTIAL

In all cases, $\Delta\psi$ was determined before addition of lactose ($t = 0$) and 15 min after addition of lactose ($t = 15$ min) (final external lactose concentration, 0.54 mM).

Experimental conditions	$\Delta\psi$ (mV)	
	$t = 0$	$t = 15$ min
ML 308225		
Resting cells [a]	145	135
Energized cells [b]	170	55
Resting cells [a] + HQNO (250 μM)	148	140
Energized cells [b] + HQNO (250 μM)	176	134
Respiring K 207 cells [c]		
Resting cells [a]	130	100
Energized cells [b]	150	90
Nonrespiring K 207 cells [d]		
Resting cells [a]	156	160
Energized cells [b]	147	142

[a] No added energy source.
[b] Cells incubated with 0.4% glycerol.
[c] K 207 cells grown in the presence of 5-aminolevulinic acid.
[d] K 207 cells grown in the absence of 5-aminolevulinic acid.

reproducible, the extent of the depolarization was found to vary with the cell preparation. Accordingly, the data presented in Table I are the mean values of 3–5 independent experiments. Strikingly, in energized cells pretreated with HQNO, the extent of the depolarization induced by lactose uptake was significantly smaller (from 176 to 134 mV) than in energized cells in the absence of HQNO (from 170 to 55 mV) (see Table I).

These results prompted us to examine lactose transport in an *E. coli* strain deprived of its respiratory chain. A mutant (K 207) of *E. coli* ML 308225 which is defective in 5-aminolevulinic acid synthesis has been isolated by Devor et al. [3]. When grown in the absence of 5-aminolevulinic acid, this strain is unable to synthesize hemes and has no detectable cytochromes [3]. As reported by Devor et al. [3], we found that K 207 cells grown in the absence of 5-aminolevulinic acid showed respiration rates at least 10-times lower than the rates exhibited by ML 308225 cells. In contrast, K 207 cells grown in the presence of 5-aminolevulinic acid show normal respiration rates.

Fig. 2 shows the time-course of lactose uptake in respiring (i.e., grown in the presence of 5-aminolevulinic acid) and nonrespiring (i.e., grown in the absence of 5-aminolevulinic acid) K 207 cells. The energy source used was maltose, the one provided for growth, since in the absence of respiratory chain, cells are unable to metabolize glycerol. Glucose has not been used, to avoid the phenomenon of inducer exclusion which would have obscured the results. In K 207 respiring cells, the steady-state level of lactose uptake in the presence of maltose was 2-times lower than in the absence of maltose. In contrast, in nonrespiring K 207 cells, the steady-state level of lactose accumulation was 50% higher in the presence of maltose than in its absence. In addition, the transport of lactose in K 207 energized respiring cells led to a large decrease of $\Delta\psi$ from 150 to 90 mV, not observed in nonrespiring K 207 energized cells (Table I).

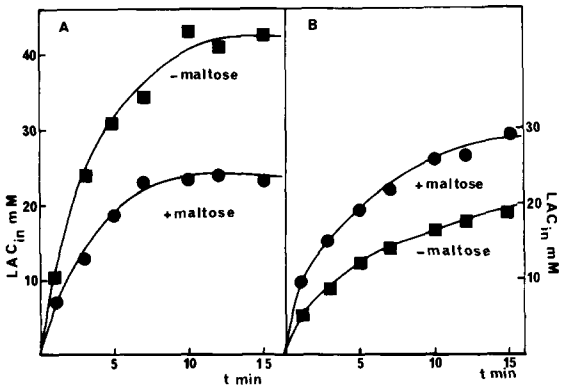

Fig. 2. Time course of lactose (LAC) uptake (0.5 mM, lactose external concentration) by *E. coli* K 207 cells (EDTA-treated, 1 mg dry wt/ml). (A) Respiring cells, grown in the presence of 5-aminolevulinic acid. Uptake in the presence (●) and absence (■) of maltose (0.4%). (B) Nonrespiring cells, grown in the absence of 5-aminolevulinic acid. Uptake in the presence (●) and absence (■) of maltose (0.4%).

Inactivation of the lac permease under prolonged incubation

The data presented above refer to the uptake of lactose followed over 15 min. When accumulation was followed for much longer times (2 h) it was observed that in energized cells the internal lactose concentration diminished to a negligeable level suggesting that inactivation of the permease can be complete (Fig. 3). In addition, even in the absence of an energy source, inactivation of the permease did occur, but at a much slower rate than in energized cells (Fig. 3). Importantly, addition of

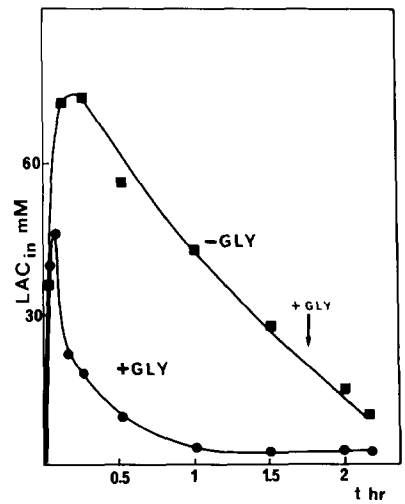

Fig. 3. Time course of lactose (LAC) uptake (0.5 mM, lactose external concentration) by *E. coli* ML 308225 cells (EDTA-treated, 1 mg dry wt./ml). Uptake in the presence (●) and absence (■) of glycerol (GLY) (0.4%). Arrow: addition of glycerol (0.4% to resting cells).

glycerol to resting cells which had been transporting lactose for 2 h had no effect on the internal lactose concentration, indicating that this phenomenon was not due to the depletion of endogenous energy source.

Effect of various substrates of the permease on the inactivation process under prolonged incubation

The fact that even in the absence of an energy source the internal lactose concentration decreases when transport is followed over a prolonged period of time (2 h) led us to examine lactose transport after the cells have been incubated for 2 h in the absence of energy source but under respiring conditions (room temperature, diluted cells). The results are shown in Table II.

When the cells were incubated 2 h at room temperature at the concentration at which the transport experiments are performed ($A_{650} = 2$, i.e., 1 mg dry wt/ml), in the absence of lactose and of energy sources, and then assayed for lactose transport in the absence of an energy source, the steady-state level of lactose accumulation was some 3-times lower than in the control (assay performed without prior incubation) (Table II). When the cells were incubated 2 h in the absence of lactose and of an energy source and then assayed for transport in the presence of glycerol, it was observed that the final steady-state level of lactose accumulation was similar to that in the absence of glycerol, indicating that the low level of internal lactose concentration observed when the incubation was performed in the absence of energy source was not the result of a depletion of endogeneous energy sources due to the long incubation time (data not shown). The data in Table II show that incubation of the cells for 2 h in the absence of lactose and glycerol results in a significant decrease of $\Delta\psi$ from 143 to 110 mV.

When the cells were incubated in the absence of glycerol but in the presence of lactose for 2 h, washed twice to eliminate the accumulated lactose, resuspended at the same concentration and then assayed for lactose transport, a complete inactivation of the *lac* carrier was observed (Table II).

Strikingly, when the cells were incubated 2 h in the absence of lactose and glycerol but in the presence of 1 mM TDG, a competitive analogue of lactose for transport via the lactose permease [7], washed twice to eliminate the accumulated TDG, resuspended at the same concentration and then assayed for lactose transport in the absence of glycerol, the steady-state level of lactose accumulation was similar to that of the control (assay performed without prior incubation). A possible trivial explanation of this high level of lactose accumulation, namely that TDG does not prevent the inactivation of the permease but that, during

TABLE II

EFFECT OF PROLONGED INCUBATION (2 h) ON THE STEADY-STATE LEVEL OF LACTOSE UPTAKE AND ON THE TRANSMEMBRANE ELECTRICAL POTENTIAL

Except for the control (0 h, no incubation), ML 308225 *E. coli* cells were incubated 2 h at dilute concentration (1 mg dry wt./ml) and at room temperature, in the absence of an exogenous energy source and in the presence of the specified additions. Then, the cells, control included, were washed twice. Steady-state level of lactose accumulation (0.5 mM lactose final concentration) and the transmembrane electrical potential ($\Delta\psi$) were determined in the absence of exogenous energy source. n.d., not determined.

Incubation time (h): 0	2	2 [a]	2	2	2	2	2
Addition (mM): none	none	TDG (10)	TDG (1)	α-NPG (1)	ONPG (1)	TMG (8)	lactose (8)
Accumulation of lactose (mM) 75	30	24	72	72	60	11	6
$\Delta\psi$ (mV) 143	110	110	147	n.d.	n.d.	n.d.	n.d.

[a] Cells were first incubated 2 h without addition, then supplemented with 10 mM TDG and incubated an additional 15 min, washed twice and assayed as described above.

the lactose transport assay, residual internal TDG competes with lactose for exit via the permease, is ruled out by the following experiment: when the cells were incubated 2 h in the absence of TDG, then supplemented 15 min with TDG (10 mM external concentration), washed twice and assayed for lactose transport, lactose accumulation was similar to that observed when the cells were incubated throughout without TDG (Table II). Under these conditions, the capacity of the cells to accumulate TDG during incubation with this analogue is certainly diminished since the permease is partially inactivated, but this is compensated for by the high external TDG concentration used in this experiment (10 mM versus 1 mM in the protection experiment).

That the incubation of the cells with TDG protects the permease against inactivation is also corroborated by the absence of a decrease of the transmembrane potential (Table II).

The different behavior of lactose and TDG led us to investigate the effect of various galactosides on the inactivation process of the lactose permease. Cells were preincubated 2 h in the presence of various analogues of lactose and assayed for lactose transport in the absence of glycerol (Table II). Clearly these analogues fall into two classes: those which enhance the susceptibility of the permease towards inactivation (lactose and TMG); those which protect the permease (α-NPG, TDG and ONPG).

We have previously reported that inactivation of the *lac* carrier as a result of lactose transport in energized cells followed over 15 min was not accompanied by a cellular lysis [2]. When the cells were incubated 2 h as described above in the absence of galactosides, we observed that the absorbance of the cell suspension fell from 2 to 1.1. This decrease was even more pronounced when the incubation was performed in the presence of lactose or TMG (from 2 to 0.8/0.9). Strikingly, when the cells were incubated in the presence of TDG, or α-NPG, or ONPG, the decrease in absorbance was only from 2 to 1.7. This decrease in absorbance has been taken into account as a decrease of the total internal cellular volume for the calculation of the internal lactose concentrations and of the membrane potentials reported in Fig. 3 and in Table II. Without this correction, the phenomena reported here would be even more pronounced.

Inactivation of the lac carrier protein modified at cysteine 148 by site-specific mutagenesis

TDG and α-NPG have been shown to protect the *lac* permease against inactivation by thiol reactives in contrast to lactose and TMG [8,9]. It was shown later, that the inactivation by the thiol reactives was at the level of cysteine 148 [10]. In view of the above results, it was of interest to investigate the effect of an exogenous energy source on the uptake of lactose in a strain in which the *lac* permease is modified at cysteine 148. *E. coli* T 206 bears plasmids carrying the *lac y* gene allowing amplification of the *lac* carrier [4]. A glycine mutant (strain G 148) has been obtained which bears plasmids in which the *lac y* gene has been modified by oligonucleotide-directed site-specific mutagenesis, such that cysteine 148 is converted to a glycine residue [5]. The glycine mutant still transports lactose although at a lower rate than *E. coli* T 206 [5].

For both strains, *E. coli* T 206 and G 148, we found that the steady-state level of lactose accumulation was lower in the presence than in the absence of glycerol (data not shown). Further evidence for the inactivation of the *lac* carrier in the glycine mutant is given by the analysis of the rate of influx as a function of time during lactose accumulation. It was shown in Ref. 2 that the rate

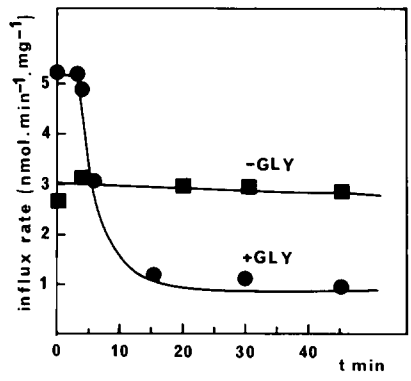

Fig. 4. Influx rate of lactose (nmol·min^{-1}·mg^{-1} dry wt.) as a function of time, during lactose accumulation (0.5 mM final external concentration) in EDTA-treated G 148 (glycine mutant) cells. Influx rate in the presence (●) and absence (■) of glycerol (GLY) (0.4%).

of influx decreases as the *lac* carrier inactivates. In the glycine mutant, in the presence of glycerol, the influx rate decreased drastically during lactose accumulation (Fig. 4); in contrast, no such decrease was observed in the absence of glycerol (Fig. 4). Thus, the presence of cysteine 148 is not necessary for the inactivation of the *lac* carrier.

Discussion

We have previously described the irreversible inactivation of the *lac* carrier of *E. coli* upon lactose transport in energized cells, mainly characterized by a dramatic decrease in the steady-state level of lactose accumulation and in the transmembrane potential [2]. This decrease in membrane potential is reflected in the inhibition of other protonmotive-force-dependent transport system such as the one catalyzed by the proline carrier [11].

The fact that cyanide can prevent these phenomena led us to propose that respiration, whose rate is much higher in energized cells than in resting cells, is responsible for the inactivation [2]. Nevertheless, cyanide effects are relatively nonspecific and it was thus desirable to test other inhibitors of the respiratory chain. Of all the inhibitors available to us (rotenone, antimycin, HQNO), only HQNO had an effect on the *E. coli* respiratory chain [12]. The results given here clearly shows that blocking the respiratory chain by HQNO inhibits the decrease of lactose accumulation and the depolarization in energized cells. The data obtained with the strain K 207 are in complete agreement with these findings: while in respiring K 207 cells lactose accumulation is lower in energized cells than in resting cells, the reverse is observed in K 207 cells deprived of a respiratory chain. Moreover, no depolarization is observed in K 207 nonrespiring energized cells upon lactose transport. The whole of these data thus demonstrate that it is the high activity of the respiratory chain in energized cells which is responsible for the inactivation of the carrier.

The results shown in Fig. 3 demonstrate that inactivation does occur in resting cells during lactose transport but that it is slower than in energized cells: this is readily explained by the fact that in resting cells the respiratory chain is active, although less than in energized cells.

Inactivation of the carrier can also occur during prolonged incubation in the absence of lactose and energy source but under conditions where the respiratory chain is still active (i.e., room temperature, relatively dilute concentration of bacteria). That this inactivation is specifically related to the permease is shown by the important protective effect of TDG, α-NPG and ONPG, specific substrates of the permease. This inactivation is more pronounced when lactose or TMG are present during the incubation. Thus, we conclude that the activity of the respiratory chain in itself leads to an inactivation of the *lac* carrier and that this inactivation is enhanced in the presence of one class of galactosides (lactose, TMG) while it is retarded in the presence of a second class of galactosides (TDG, α-NPG, ONPG).

Kennedy and co-workers [8,9] distinguished two classes of substrates of the lactose permease. Type I substrates (lactose, TMG) could not protect the permease against inactivation by thiol reactives and could not displace carrier-bound TDG, while type II substrates (TDG, α-NPG) could. It was shown later by Wright et al. [13] that type I substrates correspond to compounds with a high K_D/K_T ratio while type II substrates have a K_D/K_T ratio close to 1. Because K_T is an overestimate of K_D for type I substrates, the concentrations of these compounds used in earlier experiments were subsaturating, which explains, according to Wright et al. [13], the observed lack of protection against thiol reactives. The data reported here exemplify the difference between type I and type II compounds. Type II substrates either directly protect the permease against the inactivating agent or cause the protein to adopt a conformation which renders it less sensitive to the inactivation by the respiratory activity. In contrast to what is observed in protection experiments against thiol reactives, type I compounds, at subsaturating concentrations, do not fail to protect the permease but enhance the susceptibility of the permease towards this inactivation, probably by immobilizing the protein in a conformation more accessible to the inactivating agent. The difference in the K_D/K_T ratios for the type I and type II substrates could be a reflection of the different conformations adopted by the permease in the

presence of the different classes of substrates.

As early as 1956 it had been noticed (without implicating the respiratory activity) that the lactose permease becomes inactivated when the cells are resuspended in a medium devoid of energy source (Ref. 14; see also Ref. 15). Later, Carter et al. [8] showed that this inactivation could be prevented by TDG. This led the authors to propose that the inactivation was at the level of the cysteine residue protected by TDG (known today to be cysteine 148 [10]). We have tested the possible involvement of cysteine 148 in the inactivation by the respiratory chain activity by analyzing the uptake of lactose in a strain that transports lactose but in which the *lac* carrier is modified such that cysteine 148 is converted to a glycine residue (glycine mutant, G 148 [5]). The absence of an inactivation would have indicated that cysteine 148 is the target of the inactivating agent. We show here that the glycine mutant is inactivated by the respiratory chain activity. The fact that an inactivation is observed does not allow us to conclude. Indeed, it has been reported in Ref. 5 that in the glycine mutant, the *lac* carrier is still inactivated by thiol reactives, although this inactivation is no longer prevented by TDG. This result clearly indicates that in the modified protein, other cysteine residue(s) becomes accessible to the thiol reactives. Nevertheless, the data reported here and in Ref. 2 show that the permease is much more sensible to the inactivation in the presence of lactose; yet, we could not observe any enhancement of susceptibility of the permease towards *N*-ethylmaleimide in the presence of lactose (data not shown). This is not in favor of the involvement of cysteine 148 in the inactivation which we report here.

Evidence has been recently reported by Konings and Robillard [16] that dithiol-disulfide interchanges affect the activity of different transport systems of *E. coli*: the phosphotransferase system, the lactose permease and the proline permease [16]. It has been hypothesized further by the same authors that, in these systems, two sets of dithiols are present, one located at the outer surface, the other at the inner surface of the membrane [17]. This latter point was experimentally demonstrated in the case of the proline permease [18]. It can be speculated that a dithiol could be the target of the inactivating agent. Nevertheless, we could see no reversal of the inactivation upon incubation with 10 mM dithiothreitol for 45 min, but this would be expected if inactivation was due to the formation of a disulfide at the inner surface of the membrane.

The lactose permease has been recently isolated, purified and reconstituted into *E. coli* phospholipid liposomes [19,20]. Upon imposition of artificial ion gradients, the carrier catalyzes active transport of lactose. In such preparations, the purified carrier shows a turnover number for active transport similar to that in right-side-out membrane vesicles, but some 15–20-times lower than in cells. The reason for this discrepancy has not been elucidated [21,22]. These differences in the turnover numbers could be understood, in the light of the above results, as an inactivation of the permease during the preparation of vesicles or the isolation of the protein. Indeed, these preparations include long-time incubations, under conditions where the respiratory chain is still active.

Finally, it can be expected that other permeation systems of *E. coli* are subject to the same kind of inactivation. Addition of respirable compounds to suspensions of *E. coli* which have accumulated α-methylglucoside via the phosphotransferase system, causes a decrease of the internal glucoside concentration. This is due to inhibition of the influx and acceleration of the efflux.

While the inhibition of the influx has been shown to be caused by the energization of the membrane [23], Hernandez-Asensio and Del Campo [24] have shown that the acceleration of glucoside exit is due neither to ATP synthesis nor to energization of the membrane, but to electron transfer along the respiratory chain. This could happen by a process very similar to the one described here in the case of the *lac* permease.

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