# Implication of proteases in the respiration dependent inactivation of the lactose permease of *E. coli*

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The lactose permease of *E. coli* becomes irreversibly inactivated during lactose transport under conditions of high respiratory activity. This inactivation is characterized by a decrease in the steady state of lactose accumulation, a decrease in the influx rate of lactose, and a decrease in the transmembrane electrical potential. We report here that inhibitors of serine proteases (phenylmethylsulfonyl fluoride and *N*-α-*P*-tosyl-L-lysine chloromethyl ketone) prevent this inactivation, thus implicating proteases in this process.

E. coli *Lactose permease* *Protein inactivation* *Serine protease* *Respiratory chain*

## 1. INTRODUCTION

The transport of β-galactosides across the cytoplasmic membrane of *E. coli* is mediated by the lactose permease, an integral membrane protein, which catalyzes a proton/β-galactoside coupled transport (symport) (review [1]).

We have shown that in the presence of an added energy source, the transport of lactose via the lactose permease of E. coli leads to an irreversible inactivation of the carrier resulting in a low final steady-state level of lactose accumulation, a low influx rate of lactose, and a dramatic decrease in the transmembrane electrical potential [2]. This inactivation is suppressed by the addition of inhibitors of the respiratory chain (cyanide, HQNO) [2,3]. In addition, the inactivation is stimulated by the presence of one class of β-galactosides (lactose, TMG) and inhibited by the presence of a second class of β-galactosides (TDG, α-NPG) [3]. We concluded that the lactose permease may exist in different conformational states depending on the nature of the bound substrate and that one form is more susceptible to inactivation by the respiratory activity.

Here, we report that some inhibitors of serine proteases suppress the inactivation of the lactose permease, thus implicating proteases in this process. We discuss these results in terms of either a direct activation of proteases or an enhanced susceptibility of the lactose permease to proteolytic attack, both as a consequence of the respiratory activity.

*Abbreviations:* HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; TMG, methyl-1-thio-β-D-galactoside; TDG, β-D-galactosyl-1-thio-β-D-galactopyranoside; α-NPG, *p*-nitrophenyl-α-D-galactoside; PMSF, phenylmethylsulfonyl fluoride; TLCK, *N*-α-p-tosyl-L-lysine chloromethyl ketone; TPCK, L-1-tosylamide-2-phenylethyl chloromethyl ketone; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone

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## 2. MATERIALS AND METHODS

### 2.1. *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 path length). To render the membrane permeable to the membrane potential probe, the cells were systematically treated with EDTA [4]. EDTA treated cells were



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resuspended in 10 mM Tris-HCl, 150 mM choline chloride, 1 mM KCl, pH 7.6, at an absorbance of 40 (20 mg dry w/ml), kept on ice and used within 5 h.

### 2.2. *Lactose uptake*

Cells were resuspended in the above medium at a final concentration of 1 mg dry w/ml and incubated with agitation for 2 min at 25°C in the presence of appropriate additions. [$^3$H]Lactose (10 mCi/mmol, final concentration 0.5 mM) was then added and the time course of lactose uptake was determined as described [2].

### 2.3. *Determination of the influx rate of lactose during its accumulation*

Cells were resuspended and incubated as describe above. At time zero, non-radioactive lactose was added at 0.5 mM final concentration. Aliquots (100 $\mu$L) were withdrawn. To these, trace amounts of [$^3$H]lactose (266 mCi/mmol, final concentration 4 $\mu$M) were added at various time intervals. Uptake of radioactive lactose was allowed to proceed for 15 s. The amount of radioactive lactose taken up during the 15 s incubation was determined as described in [2].

### 2.4. *Determination of the transmembrane electrical potential ($\Delta\psi$)*

$\Delta\psi$ was determined by the accumulation of [$^3$H]tetraphenylphosphonium bromide in EDTA-treated cells [4].

### 2.5. *Rate of respiration*

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

### 2.6. *Materials*

[$^3$H]Tetraphenylphosphonium bromide and [$^3$H]lactose were obtained from CEA, Saclay, France. All other materials were of reagent grade and obtained from commercial sources.

## 3. RESULTS

Fig.1 show the inhibition of lactose uptake in *E. coli* cells upon addition of an external energy source, and the protective effect of protease inhibitors on the accumulation of lactose. In the absence of added energy source (resting cells) the internal concentration of lactose increased monotonically to a steady-state level; in contrast, in the presence of added glycerol (or D-lactate, or succinate, or ascorbate/phenazine methosulfate) (energized cells), the internal lactose concentration increased initially, then decreased and stabilized at a steady-state level significantly lower than that of resting cells [2]. This inhibition of lactose transport is the result of an irreversible inactivation of the lactose carrier [2]. In the presence of PMSF (8 mM) or TLCK (2.5 mM) the decrease in the steady-state level of lactose accumulation in energized cells did not take place (fig.1). In table 1 the effect of various concentrations of PMSF and TLCK on the steady-state level of lactose accumulation in energized and resting cells is shown. It is apparent that the protective effect of PMSF and TLCK against inhibition of lactose accumulation in energized cells took place despite the slight inhibitory effect of these compounds on lactose transport itself as judged by their effect in resting cells (~ 5–20% inhibition of transport).

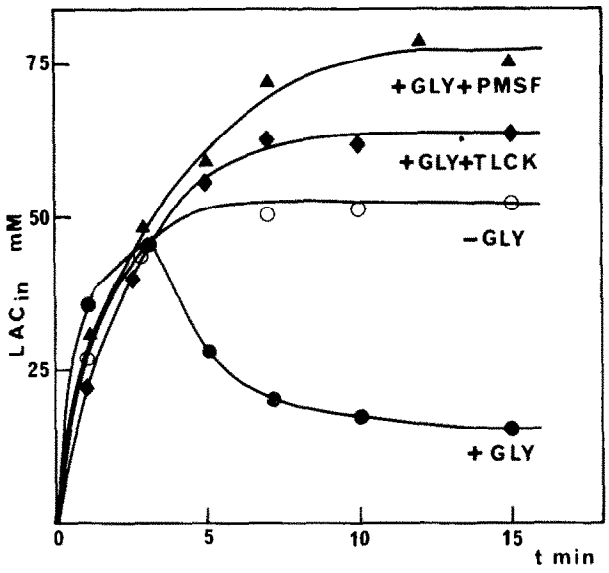


Fig.1. Time course of lactose uptake (0.5 mM external concentration) by *E. coli* cells. (○) In the absence of an external energy source; in the presence of: (●) an external energy source (0.4% glycerol), (◆) glycerol and 2.5 mM TLCK, (▲) glycerol and 8 mM PMSF.

A putative protective effect of TPCK, another

inhibitor of serine proteases, could not be assayed since it strongly inhibits lactose transport in resting cells, i.e., in the absence of inactivation (not shown).

We have shown that inhibitors of the respiratory chain (cyanide, HQNO) prevent the inactivation of the lactose permease induced by the addition of an external energy source [2,3]. We have verified that PMSF and TLCK do not act in this way: up to a concentration of 10 mM, both compounds had no effect on the rate of respiration (not shown).

In energized cells, the decrease in lactose accumulation was mainly a result of a decrease in the influx rate of lactose as it accumulates [2]. This is shown in fig.2 where we present the influx rate of lactose as a function of time: in resting cells, the influx rate remained constant during the time course of lactose accumulation; in energized cells, it decreased from a value slightly higher than that of resting cells to a value some 4-times lower. We show in the same figure the effect of PMSF (8 mM) and TLCK (5 mM) on this influx rate in energized cells. The presence of PMSF and TLCK prevented the decrease in the influx rate due to the addition of an external energy source. The slight inhibitory effect of these compounds on lactose transport itself was again observed (decrease by some 20–30% of the influx rate in resting cells).

Lactose uptake in energized cells results in a marked decrease in $\Delta\psi$, which at pH 7.6 is the sole component of the proton motive force [4], while in resting cells the decrease in $\Delta\psi$ is modest or absent [2]. Typically, in energized cells $\Delta\psi$ decreased from 205 mV to a value around 40 mV during the time course of lactose uptake (15 min); on the other hand in resting cells $\Delta 2$ remained relatively constant (155–150 mV) (see table 1). Although addition of PMSF or TLCK to energized cells resulted in a slight depolarization, which may be responsible for the observed slight inhibition of these compounds on lactose transport, it reduced markedly the extent of depolarization during the uptake of lactose in energized cells (table 1).

We have shown that while a high respiratory activity and the presence of lactose result in a rapid inactivation of the lactose carrier, inactivation can also take place at low respiratory activity and in the absence of lactose, but at a much slower rate [3]. Thus, when cells were first incubated in the absence of lactose and external energy source at room temperature for 2 h, and then assayed for lactose transport (in the absence of external energy source), the final steady-state level of lactose accumulation was only 40% that of the control (transport performed without prior incubation for 2 h) (see fig.3). We have shown that this inhibition of transport is a result of a specific inactivation of the lactose permease: indeed, 2 h incubation of the

Table 1

Effect of various concentrations of PMSF and TLCK on the steady-state level of lactose accumulation, and on the transmembrane electrical potential ($\Delta\psi$) in resting cells (-glycerol) and in energized cells (+ glycerol)

| | -glycerol | | | +glycerol | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No addition | PMSF (5 mM) | TLCK (2.5 mM) | No addition | PMSF | | | | TLCK | |
| | | | | | 2 mM | 5 mM | 8 mM | 1 mM | 2.5 mM | 5 mM |
| Accumulation of lactose (mM)[a] | 52 | 50 | 40 | 15 | 20 | 40 | 75 | 30 | 65 | 68 |
| $\Delta\psi$ (mV) ($t=0$ min)[b] | 155 | nd | nd | 205 | nd | 160 | 140 | nd | 204 | 185 |
| $\Delta\psi$ (mV) ($t=15$ min)[c] | 150 | nd | nd | 40 | nd | 145 | 125 | nd | 110 | 155 |

[a] Internal lactose concentration at steady state (15 min)
[b] Transmembrane electrical potential prior addition of lactose
[c] Transmembrane electrical potential at steady state of lactose accumulation
nd, not determined

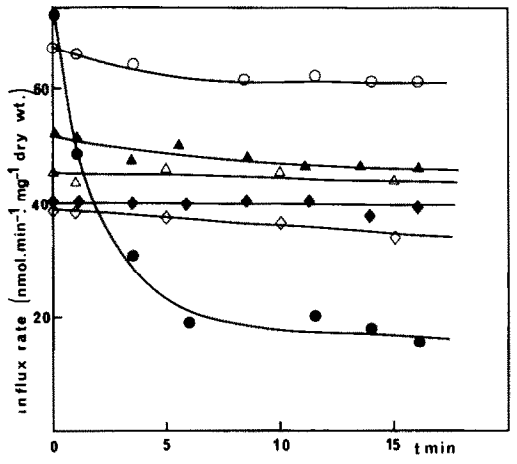


Fig.2. Influx rate of lactose as a function of time during lactose accumulation (0.5 mM final external concentration): (○) in the absence of glycerol and protease inhibitor, (△) 8 mM PMSF, (◇) 5 mM TLCK, (●) glycerol, (▲) glycerol and 8 mM PMSF, (◆) glycerol and 5 mM TLCK.

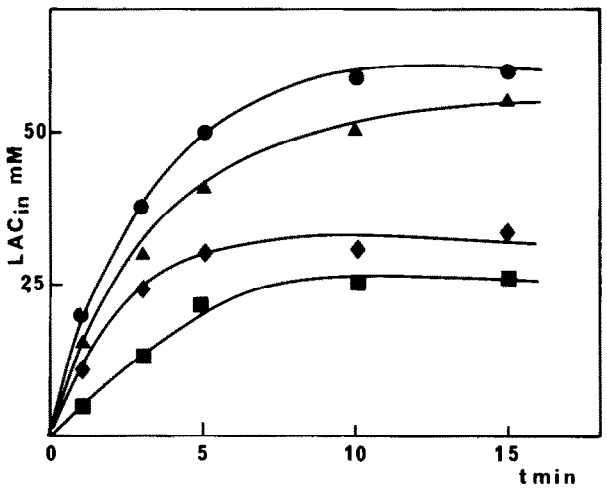


Fig.3. Time course of lactose uptake (0.5 mM external concentration) by *E.coli* cells in the absence of added energy source after a 2 h incubation in the absence of added energy source and lactose (■); after 2 h incubation in the absence of added energy source and lactose but in the presence of either 0.5 mM PMSF (◆), or 1 mM PMSF (▲). Control: lactose uptake in the absence of added energy source and protease inhibitor without prior 2 h incubation (●).

cells in the presence of one class of substrates (TDG, $\alpha$-NPG) protected against this inactivation while incubation in the presence of a second class of substrates (lactose, TMG) led to a complete inactivation of transport [3].

The data presented in fig.3 show the protective effect of PMSF against this type of inactivation. When the cells were incubated for 2 h in the presence of PMSF (respectively 0.5 and 1 mM) the final steady-state level of lactose accumulation was 55 and 90%, respectively, of that of the control instead of 40% when the incubation was performed in the absence of PMSF. TLCK had no protective effect against this type of inactivation. This could be due to its toxic effect which is greater than that of PMSF. Alternatively, the rapid inactivation observed during lactose transport in energized cells and the inactivation observed during the 2 h incubation in resting cells may be under the control of different proteases. This could also explain the fact that the PMSF concentrations needed for full protection against the long term inactivation and the rapid inactivation are different; 1 and 8 mM, respectively.

## 4. DISCUSSION

We show here that inhibitors of serine proteases (PMSF, TLCK) suppress the irreversible inactivation of the lactose permease characterized by the decrease in the steady-state level of lactose accumulation, the decrease in the influx rate, and the decrease in the transmembrane electrical potential. PMSF and TLCK have been shown in vivo to block protein breakdown in *E. coli* cells [5]. More recently, Goldberg et al. [6,7] isolated and purified 8 soluble endoproteases from E. coli cells, 6 of which are serine proteases as shown by their inhibitor sensitivity. We therefore conclude that proteolytic enzymes, and thus a protein degradation, are involved in the inactivation of the lactose carrier. The degradation does not result in the disappearance of the protein from the membrane since lactose efflux is still observed [2]. It is more limited and consists of an uncoupling of lactose and proton transport via the permease resulting in the observed decrease in both active transport and proton-motive force, the latter due to the influx of protons no longer coupled to lactose transport.

The addition of an exogenous energy source

which leads to the inactivation of the lactose carrier results in an increase in the rate of respiration, the proton-motive force and the ATP level. We have shown that inactivation still takes place in energized cells when the proton-motive force (and therefore the ATP level) is partially decreased by addition of an uncoupler (CCCP) at a level below that of resting cells for which no inactivation is observed [2]. Since on the other hand, partial blocking of the electron transfer by inhibitors of the respiratory chain suppresses the inactivation [2,3], we concluded that it is the enhanced electron transfer in itself (and not the enhanced ATP level and/or proton-motive force) which is the primary event leading to the inactivation. It now appears that the rapid inactivation of the lactose permease requires simultaneously active proteases, high respiratory activity and the presence of one class of permease substrate (lactose, TMG).

The proteolytic attack on the lactose permease could be the result of a direct activation of proteases by the respiratory activity. This is unlikely in view of the results of Goldberg et al. [8] demonstrating that, in vivo, the protelytic activity leading to protein degradation is under the control of the ATP level but not the proton motive force nor the rate of respiration. However, the Goldberg et al. data [8] refer to a global proteolytic degradation so that a minor respiration-dependent proteolytic degradation may have remained unnoticed.

Alternatively, the enhanced electron transfer may modify the state of the permease in a way which renders it more susceptible to a subsequent attack by proteolytic enzymes. This could occur either by a direct interaction of the permease with a component of the respiratory chain (e.g., a semiquinone) or by an interaction with free radicals generated by the respiratory activity. In this respect we note that a similar mechanism has been advanced to explain photoinhibition in thylakoids. It has been shown, that a 32 kDa protein from thylakoid membrane, a structural component of PS II, becomes prone to proteolytic attack under conditions of high light intensity. This degradation is not due to a change in the ATP level but results directly from the light-dependent enhanced electron flow [9,10].

Finally, one may speculate as to the physiological implication of the effect of an enhanced electron transfer on the degradation of the lactose permease which we report here. The degradation of the lactose permease may be part of the general process of membrane protein degradation. In addition, it may serve as a control of lactose influx into the cell under conditions of high rates of respiration, i.e., when the cells are fully energized and do not require additional sugars as energy sources. The experimental conditions which we have deliberately chosen (Tris-EDTA treatment of the cells, low external potassium concentration, external lactose concentration around or above the $K_T$ of transport) amplify the inactivation to extreme levels [2]. Similarily, the inhibition of growth of *E. coli* upon addition of relatively high external lactose concentration (lactose killing) may be a consequence of this type of inactivation [11].

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## REFERENCES

[1] Hengge, R. and Boos, W. (1983) Biochim. Biophys. Acta 737, 443–478.
[2] Ghazi, A., Thérisod, H., and Shechter, E. (1983) J. Bacteriol. 154, 92–103.
[3] Thérisod, H., Ghazi, A., Houssin, C. and Shechter, E. (1985) Biochim. Biophys. Acta 814, 68–76.
[4] Booth, I.R., Mitchell, W.J. and Hamilton, W.A. (1979) Biochem. J. 182, 687–696.
[5] Prouty, W.F. and Goldberg, A.L. (1972) J. Biol. Chem. 247, 3341–3352.
[6] Swamy, K.H.S. and Goldberg, A.L. (1981) Nature 292, 652–654.
[7] Goldberg, A.L., Swamy, K.H.S., Chung, C.H. and Larimore, F.S. (1981) Methods Enzymol. 80, 680–702.
[8] Olden, K. and Goldberg, A.L. (1978) Biochim. Biophys. Acta 542, 385–398.
[9] Mattoo, A.K., Hoffman-Falk, H., Marder, J.B. and Edelman, M. (1984) Proc. Natl. Acad. Sci. USA 81, 1380–1384.
[10] Kyle, D.J., Ohad, I. and Arntzen, C.J. (1984) Proc. Natl. Acad. Sci. USA 81, 4070–4074.
[11] Wilson, D.M., Putzrath, R.M. and Wilson, T.H. (1981) Biochim. Biophys. Acta 649, 377–384.