TWO LOCATIONS OF THE LAC PERMEASE SULPHYDRYL IN THE MEMBRANE OF E. COLI

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1. Introduction

lac Permease is a protein involved in the active transport of lactose, and is located in the membrane of *E. coli* [1, 2]. The *lac* permease molecule has a sulphydryl group which is essential for transport [3, 4]. The mechanism by which permease facilitates the transport of lactose into the cell is not known. One of the possibilities is that permease serves as a vehicle which physically moves the substrate through a permeability barrier. If this is so, permease must exist in the membrane in at least two states: one in which the binding site is on one side of a permeability barrier, and another in which the site is on the other side of the barrier.

In this work we have used mercurials (PMBS and and P-Hg)** to inactivate *lac* permease and sulphydryl compounds (mercaptoethanol and P-SH) to reactivate it. Permease inactivated either by PMBS or by P-Hg can be fully reactivated by mercaptoethanol. A difference between PMBS inactivated and P-Hg inactivated permease is observed when reactivation by P-SH is attempted: P-Hg inactivated permease is fully reactivated whereas PMBS inactivated permease is only partially reactivated.

These findings strongly suggest the existence of two interconvertible states of *lac* permease, which are separated by a permeability barrier and thus support the mobile-carrier model for transport of galactosides by the permease.

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** Abbreviations used: PMBS, *p*-chloromercuribenzene sulphonic acid; P-Hg, *N*-(3-mercuri-2-methoxy propyl) poly-DL-alanyl amide; P-SH, poly-DL-alanyl cysteine; IPTG, isopropyl-1-thio-β-D-galactopyranoside.

2. Materials and methods

2.1. *Materials*

Melibiose was a product of Pfanstiehl Laboratoies, Inc., Waukegan. C-14-lactose, 10.3 mC/mmole, was a product of The Radiochemical Centre, Amersham. IPTG was a product of Calbiochem, Los Angeles. 2-Mercaptoethanol was a product of Eastman Kodak, Rochester. PMBS was a product of Sigma Chemical Company, St. Louis.

Preparations of *N*-(3-mercuri-2-methoxy propyl) poly-DL-alanyl amide (P-Hg) and of poly-DL-alanyl cysteine (P-SH) will be described in detail elsewhere. P-Hg was obtained by inititating the polymerization of *N*-carboxy-DL-alanine anhydride with allylamine and stirring a suspension of the allylamine derivative with Hg-203 mercuric acetate in methanol. The water soluble fraction of the polymer, corresponding to approx. 30% of product, was chromatographed on a Sephadex G-25 column and fractions containing 12–55 μmoles mercury per 100 mg of polymer were combined. The polymer solution, 22 mg/ml, was stored in liquid air. Comparison of total *N* (Kjeldahl) with amino *N* (Van Slyke) gave a number average mol wt of 1400. The ratio of mercury to amino-nitrogen was 1 : 5. The concentration of P-Hg as given in the experimental section refers to the

Table 1
Inactivation of permease by PMBS.

Concentration of PMBS (M)	Activity (μmoles lactose/g cells)		
	Cells treated with PMBS	Cells treated in presence of 10^{-2} M melibiose	Cells reactivated with 10^{-3} M mercaptoethanol
0	27	22	32
0.5×10^{-4}	14	37	27
1.0×10^{-4}	5	27	32
3.0×10^{-4}	2	4	21
9.0×10^{-4}	2	2	28

Table 2
Inactivation of permease by P-Hg

Concentration of P-Hg (M)	Activity (μmoles lactose/g cells)		
	Cells treated with P-Hg	Cells treated in presence of 10^{-2} M melibiose	Cells reactivated with 10^{-3} M mercaptoethanol
0	21	22	25
1×10^{-4}	19	37	-
2×10^{-4}	2	25	25
3×10^{-4}	3	9	21

mercury concentration. P-SH was obtained by initiating the polymerization of *N*-carboxy-DL-alanine anhydride with mono-DNP-cystine. The water soluble fraction, corresponding to approx. 50% of product, was treated with mercaptoethanol and was chromatographed on a Sephadex G-25 column. In the elution, poly-DL-alanyl cysteine was clearly separated from mercaptoethanol and DNP-cysteine. Fractions containing 20–40 μmoles per 100 mg of polymer were combined and stored, 41 mg polymer/ml, in liquid air. Comparison of total N (Kjeldahl) with amino N (Van Slyke) gave a number average mol wt of 580. The ratio of sulphydryl to amino-nitrogen was 1 : 10. Concentration of P-SH, as given in the experimental section, refers to the sulphydryl concentration. This was determined by Ellman's reagent [5].

2.2. *Bacterial strain*

A β-galactosidase-less strain of *E. coli* K12 in which *lac* permease is inducible was used. The strain W2244 ($i^+ z^- y^+$) was obtained by courtesy of Dr. Y.S.Halpern. It was grown in a salts medium containing per litre 13.6 g KH_2PO_4; 2.0 g $(NH_4)_2SO_4$; 0.2 g $MgSO_4$ and 0.5 mg $FeSO_4 \cdot 7H_2O$. The pH of the medium was adjusted to 7 with KOH. Glycerol, 4g/ℓ, served as carbon source. Areated cultures were grown at 37°C in the presence of 0.5×10^{-4} M ITPG and were harvested in the exponential phase at densities ranging from 0.9 to 1.2×10^9 cells/ml.

Density of bacterial suspensions was calculated from absorption at 650 mμ. $E_{1\ cm}$ 650 mμ of a suspension containing 10^9 cells /ml was taken as 1.0.

2.3. *Inactivation and reactivation of permease*

Inactivation of permease by mercurials and reactivation by sulphydryls was done on bacterial suspensions (approx. 5×10^9 cells/ml) in the salts medium (containing 50 μl chloramphenicol/ml) in a water bath at 37°C.

For inactivation, 0.5 ml of a cell suspension in a 12 ml, thick walled, glass centrifuge tube was incubated for 5 min. The reagent was added, in a volume not exceeding 45 μl, to give the required concentration. After an additional 15 min the tube was transferred to ice and 5 ml of ice-cold salts medium were added. It was centrifuged, the supernatant decanted and the tube drained by inversion. In experiments where melibiose was included it was routinely added to the bacterial suspension (5 μl of a 1.0 M solution) at the beginning of incubation. Cells were suspended in 5 ml of salts medium, sedimented and resuspended in 5 ml of salts medium and assayed as described below.

For reactivation, the cells obtained in the first washing were resuspended in 0.5 ml salts medium. After 5 min incubation at 37°C the sulphydryl reagent used for reactivation was added in a volume not exceeding 120 μl. After an additional 15 min of incubation the tube was transferred to ice and the contents diluted with 5 ml of salts medium. It was centrifuged and drained as before. The cells were suspended in 5 ml of salts medium for the assay.

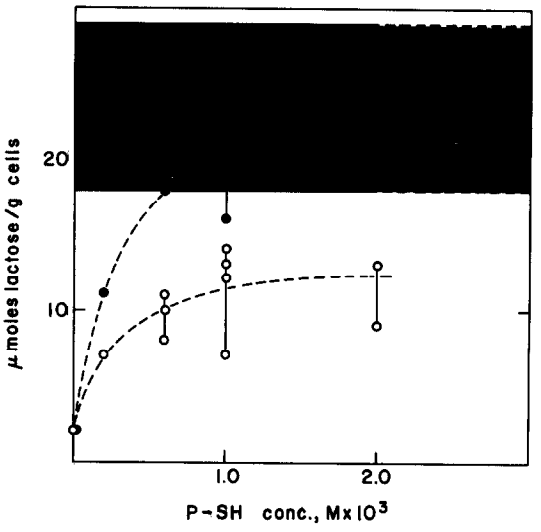

Fig. 1. Reactivation of permease by P-SH. The horizontal lines at the top of the figure give the extreme values of controls in this experiment. Full circles designate P-Hg inactivated cells; open circles PMBS inactivated cells.

2.4. *Assay of permease*

One ml of a bacterial suspension (approx. 5×10^8 cells/ml) in salts medium containing 50 μg/ml was shaken in a water bath at 26°C for 5 min. C-14-lactose was added to give a concentration of 1×10^4 M. Incubation was continued for 20 min. A 0.5 ml sample was then diluted with 0.5 ml of ice-cold salts medium, filtered (HAWP 02500, HA 0.45 μ, 25 mm, Millipore Filter Corp., Bedford) and washed with five 0.5 ml portions of ice cold medium. The filters were then transferred to scintillation vials and dissolved in 15 ml Bray's solution [6]. Counting rates were measured in a liquid-scintillation spectrometer (Packard Tri-Carb).

3. Results

Measurements of accumulation of non-metabolized substrates by bacteria are subject to a high degree of scatter. Results recorded in the tables are of single point experiments and are representative values. The choice of strain, of inducer, and of substrate was directed to restrict the assay to *lac* permease, the TMG-I permease by the nomenclature of Rotman, Ganesan and Guzman [7].

Inactivation of *lac* permease by PMBS is inhibited by melibiose, a *lac* permease substrate (cf. Carter, Fox and Kennedy [8]), and is completely reversed by treatment with mercaptoethanol (table 1).

Inactivation of *lac* permease by P-Hg is similar to inactivation by PMBS in that the inactivation is inhibited by melibiose and reversed by treatment with mercaptoethanol (table 2). The residual activity after treatment with P-Hg is of the same magnitude as that remaining after treatment with PMBS (tables 1 and 2).

Reactivation by poly-DL-alanyl cysteine (P-SH) of cells inactivated by PMBS and by P-Hg is summarized in fig. 1. Cells inactivated by P-Hg are fully reactivated by treatment with P-SH at sulphydryl concentrations above 0.6×10^{-3} M. Cells inactivated by PMBS are only partially reactivated by treatment with P-SH, recoverable activity reaching 50% of that of controls.

4. Discussion

Inactivation of *lac* permease by P-Hg is inhibited by melibiose and this, by analogy with a previous study [4], defines the attacked sulphydryl as one that belongs to *lac* permease and resides in the membrane. Inactivation by these reagents is completely reversible by mercaptoethanol.

The difference between the mode of action of P-Hg and PMBS becomes apparent when reactivation by P-SH rather than by mercaptoethanol is attempted. P-Hg inactivation is completely reversed by P-SH whereas reversal of PMBS inactivation is only partial (fig. 1). This proves that there are two states of the permease: one which is available to poly-DL-alanyl reagents (state x_1) and another which is not (state x_2). The difference between the two mercurials is explained by the limited permeability of P-Hg which can modify the essential sulphydryl in state x_1 only, whereas PMBS inactivates both states. The properties of the two states of *lac* permease can be summarized as follows: (a) in native permease the two states are interconvertible, i.e. $x_1 \rightleftharpoons x_2$; (b) blocking the essential sulphydryl with one of the mercuri reagents (PMBS or P-Hg) in either state yields the corresponding mercuri derivatives of the permease which are not interconvertible, i.e.

x_1' and x_2'; (c) PMBS reacts with the two states of permease, x_1 and x_2, to give the corresponding derivatives x_1' and x_2'; (d) P-Hg reacts with only one of the states, x_1, and therefore permease is all converted into the corresponding derivative x_1'; (e) Mercaptoethanol reactivates both x_1' and x_2' to give x_1 and x_2; (f) P-SH reactivates only one of the states, x_1', to give x_1.

We propose that the two interconvertible states of *lac* permease correspond to two locations of the essential sulphydryl in the membrane, the outer and the inner locations in relation to a permability barrier. Indeed, a reagent will not distinguish between two states of a molecule if they are not separated by a barrier to diffusion, except in the case where the two forms have intrinsically different reactivities. There is no evidence of a difference in the reactivity of the essential sulphydryl in the two states.

Since transport of substrate by *lac* permease must include motion of the permease molecule in the membrane, the mechanism by which modification of the essential sulphydryl inactivates the permease could be a restriction of its motion in the membrane. Our results support such an explanation of *lac* permease inactivation by sulphydryl reagents.

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