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(STUDY ON THE β-GALACTOSIDE PERMEASE OF ESCHERICHIA COLI) SOLUBILIZATION OF A THIODIGALACTOSIDE-BINDING PROTEIN FROM E.COLI MEMBRANE CONTAINING β-GALACTOSIDE PERMEASE

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SUMMARY

A Thiodigalactoside binding protein is solubilized from membrane vesicles of Escherichia Coli containing the M protein by use of the detergents Triton X-100 or Emulfogen BC 720. Thiodigalactoside binding affinity of the soluble protein is the same as the membrane embedded β-galactoside permease whereas the residual particulate fraction is free of affinity for this substrate.

INTRODUCTION

Several publications have appeared during the last five years on the binding of the β and α galactosides to the lactose permease M protein of Escherichia Coli embedded in the membrane (1,2,3,4,5). Recently we reported a thermodynamic study on the binding of thiodigalactoside and lactose by E.Coli membrane vesicles containing the β-galactoside permease. Both substrates were bound without added D-lactate. The respective K_D values determined from equilibrium dialysis were 5.10^{-5} and 10^{-4} M; about 600 pmoles of substrate were bound per mg of membrane protein. The enthalpic and entropic contributions of the specific binding reaction were measured (6,7).

We have now solubilized a thiodigalactoside binding protein from E.Coli membrane vesicles containing the β-galactoside permease in order to study the thermodynamic properties of the protein freed from membranous surrounding.

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MATERIALS AND METHODS

Cell strains used in this study were: E.Coli ML 30 (i^+ z^+ y^+) and E.Coli ML 308,225 (i^- z^- y^+ a^+)Thia$^-$. The latter was a generous gift of Dr. T.H.Wilson. The cells were grown in minimal medium with succinate (5 gr/liter) as carbon and energy source, supplemented with thiamin where needed; the bacteria were harvested during the logarithmic growth phase. Membrane vesicles were prepared by the lyzozyme/ethylenediamine tetraacetate(EDTA) method of Kaback (8) with 10 mM β-mercaptoethanol added throughout the preparation. Membrane vesicles were resuspended in 0.1 M phosphate buffer pH 6.6 containing the β-mercaptoethanol and 50 µg/ml chloramphenicol. Protein concentration was measured by the method of Lowry et al.,(9) using bovine serum albumin as a standard. Membrane preparations were stored for 2 or 3 days in ice, but were not frozen. Chemicals were analytical grade purchased from Sigma and GAF France (Emulfogen BC 720). Tritiated thiodigalactoside was manufactured by the CEA (France) according to the method of Kennedy(1). The binding of TDG was measured by dialysis equilibrium as previously described (6), but with the solubilized protein a Sartorius 11739 membrane was employed.

RESULTS AND DISCUSSION

Preliminary experiments based on detergent action with various non-ionic detergents (Triton X-100, Brij 35, Brij 36 T, Emulfogen BC 720, Tween 80) showed Triton X-100 and Emulfogen BC 720 to be most effective in releasing thiodigalactoside binding activity. Since the stability was greater in Triton X-100 and, as shown in Fig.1, below 1% the Triton X-100 does not significantly inhibit the TDG binding by whole vesicles, this detergent was used routinely; maximum solubilization was observed at pH 7.4. A membrane vesicle suspension (5ml) at a protein concentration of 13 mg/ml was adjusted to pH 7.4 with potassium hydroxyde, mixed with 250 µl of 20% Triton X-100 at room temperature and stirred continuously for 15 min. The mixture was cooled to 5°C and centrifuged at 220,000 g for 1 hour. The supernatant was removed carefully by pipette, the pellet suspended in 5 ml of 0.1 M phosphate buffer pH 7.4 containing 10 mM β-mercaptoethanol

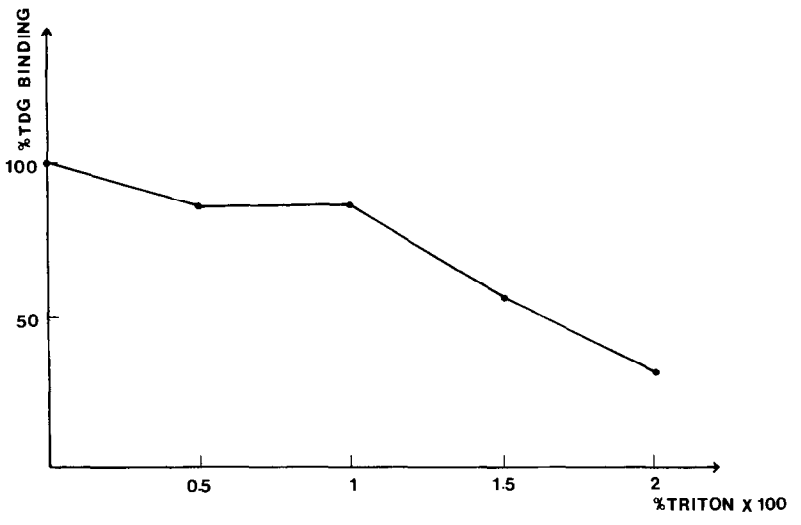

Figure 1
Effect of Triton X-100 concentration on Thiodigalactoside binding to E.Coli ML 308,225 vesicles.

and 1% Triton X-100, and the binding activities of both measured by dialysis equilibrium against (^{3}H)TDG solutions in the same buffer. As shown on Table I, about 30% of the binding activity and of the protein was recovered in the supernatant; the pellet was free of binding activity.

Fig. 2 shows the TDG saturation of the supernatant and the absence of binding in a control supernatant of the ML 30 strain uninduced for the Lac operon. The double reciprocal plot (insert Fig.2) indicates a K_D of 3.10^{-5} M with 950 pmoles of binding sites per mg of soluble protein at saturation. This K_D is the same as for TDG with the M protein embedded in the membrane. A polyacrylamide gel electrophoresis shows about 15 protein bands in this preparation. We are thus encouraged that the method will provide a purified M protein for thermodynamic analysis of the purified soluble protein.

Fox and Kennedy (10) in 1965 first solubilized the M protein

TABLE I

Solubilization of a TDG-binding activity by Triton X-100

Sample	Protein concentration mg/ml	Total protein mg	Total activity pmol	Specific activity pmol / mg prot
Vesicle suspension	12.7	63.5	57.15	900
Supernatant	5.2	23.4	22.33	950
Pellet	7.1	35.5	0	0

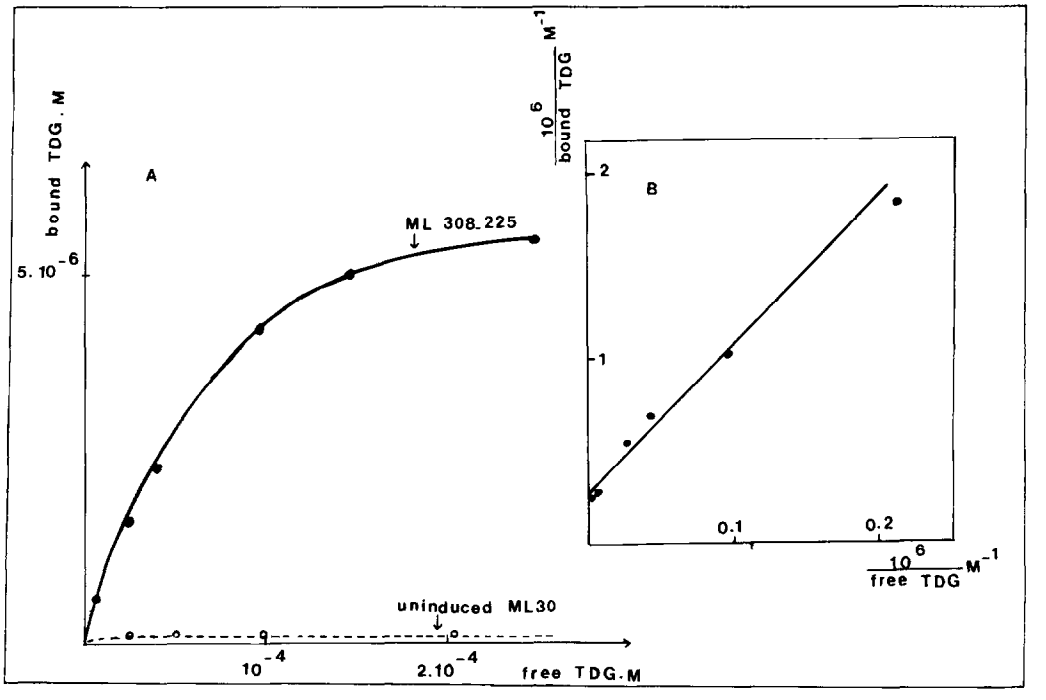

Figure 2

A- Saturation of Thiodigalactoside binding sites in Triton X-100 extract of membrane vesicles

● : soluble fraction obtained from ML 308,225 membrane vesicles

o : soluble fraction obtained from ML 30 uninduced membrane vesicles

B- Double reciprocal plot of 2 A

labeled with radioactive N-ethylmaleimide. They used 2% Triton X-100 and after centrifugation at 40,000 g for 1 hour found all the labeled molecules in the soluble fraction; the pellet showed no significant label. Jones and Kennedy (11) in 1969 repeated the M protein extraction with vesicles of E.Coli ML 308 and sodium dodecyl sulfate as detergent. The protein, always covalently linked with (^{3}H)NEM and consequently denatured was partially purified.

More recently (12), Altendorf et al., were able to solubilize the membrane proteins from E.Coli ML 308,225 membrane vesicles using aprotic solvent (hexamethylphosphoric triamide) and to reconstituate β-galactoside transport in E.Coli ML 35 vesicles (i^- z^+ y^- a^-) in presence of lithium D-lactate.

Since we are primilary interested in a thermodynamic study of the binding of substrate by soluble M protein we have tried to extract the M protein in the least denaturing conditions. We have thus chosen to use non-ionic detergents at the lowest concentration giving a good extraction. That the protein so extracted is the M protein is indicated by the fact that the solubilized binding activity was found only in the vesicles of E.Coli containing the M protein. No TDG binding activity was found in supernatants coming from vesicles devoided of β-galactoside permease. Experiments are undertaken for the total purification and consequently the thermodynamic study of this protein.

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