SOLUBILIZATION OF THE MEMBRANE BOUND LACTOSE SPECIFIC COMPONENT OF THE STAPHYLOCOCCAL PEP DEPENDANT PHOSPHOTRANSFERASE SYSTEM

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The membrane bound lactose specific component of the PEP dependant phosphotransferase system of *Staphylococcus aureus* has been solubilized using the non ionic detergent Triton X-100. Some properties of the crude soluble enzyme are reported.

1. Introduction

The PEP dependent phosphotransferase system plays a major role in the transport of carbohydrates in bacteria [1]. Evidence has been obtained that entry and accumulation of several carbohydrates into the bacterial cell are probably mediated by one component which is associated with the bacterial membrane together with the soluble components of the system [2]. Mutants of the various components of the phosphotransferase system of *Staphylococcus aureus* are not able to accumulate, among other sugars, glycoside lactose or β-D-galactoside derivatives [3].

The membrane bound component of the PEP-system (Enzyme II = EII) is probably involved in translocation of galactoside from the outside of the cell to the inside by simultaneous phosphorylation of the sugar [2]. We are therefore interested to obtain information about properties of this membrane bound component.

Since it is difficult to study the enzyme while it is still attached to the bacterial membrane, we have attempted to release the enzyme II specific for lactose from the membrane without destroying its activity. We have achieved a certain amount of success by using non ionic detergents such as Triton X-100. It is known that ionic detergents such as sodium dodecylsulfate have a strong denaturating effect on membrane proteins and proteins in general.

2. Materials and methods

Triton X-100, Tween 80 and Brij 58 were purchased from Serva Research Laboratories, Heidelberg, West Germany. Triton was purified prior to use [4]. All experiments were conducted at 4° unless otherwise stated.

2.1. *Preparation of the membrane fraction*

2 g of wet cell paste (*S. aureus* S 305 A [3]) washed once with standard buffer was suspended in 2 ml of standard buffer (0.1 M tris-HCl pH 7.5, 0.1 M NaCl, 0.01 M $MgCl_2$, 10^{-4} M EGTA, 0.1% mercaptoethanol) containing 100 mg DNAase (Worthington) and sonicated together with 2.5 g of glass-beads (0.1–0.2 mm diameter) with the Branson Sonifier model JT 7V reostat position 50 for 4 min. The temperature was kept below 20° by cooling with a solid CO_2-acetone mixture. Cell debris and whole cells were sedimented at 36,000 *g* for 30 min. Membrane fragements were sedimented at 220,000 *g* for 1 hr and washed by resuspension in standard buffer followed by high speed centrifugation.

2.2. *Solubilization of EII*

Washed membranes were resuspended in 2.6 ml buffer containing 2.5% Triton X-100 and centrifuged for 1 hr at 144,000 *g*. The supernatant was used for various experiments and contained about 3 mg of

proteins per ml (Lowry protein, BSA as standard) [5].

2.3. *Assay of EII*

EII was assayed as described earlier [3] using a cell free extract of strain S 174 B, a mutant without EII activity. EII activity was expressed as OD at 405 nm per 10 min at 37°.

2.4. *Sucrose gradient centrifugation*

Gradients (11 ml of 5–20% sucrose in standard buffer + 2% Triton X-100) were formed with a conventional gradient mixer equipped with a vibrating blade in the mixing chamber. Samples of 0.5 ml of solubilized EII were layered on top of the gradients. The centrifugations were performed in the Spinco preparative ultracentrifuge using the SW 41 rotor at 39,000 rpm, for 15 hr. Tubes were punctured at the bottom and fractions of 0.5 ml were collected.

3. Results and discussion

The effect of various non ionic detergents on the solubility of enzyme II specific for lactose was studied.

Table 1

Detergent	Concentration	Activity in the supernatant*
Tween 80	2.3%	24%
Brij 58	1.5%	27%
None		
Triton X-100	2.5%	70–90%

* After centrifugation at 144,000 *g* for one hour.

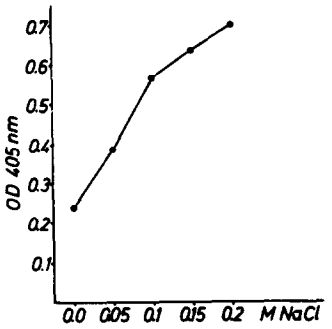

Fig. 1. Dependance of the solubilization of EII activity upon the salt concentration. Equal samples of membranes were suspended in 1.3 ml of 0.01 M tris HCl pH 7.5, 0.01 M $MgCl_2$, 10^{-4} M EGTA, 0.1% mercaptoethanol, 3% Triton containing varying amounts of salt and centrifuged at 144,000 *g* for 1 hr.

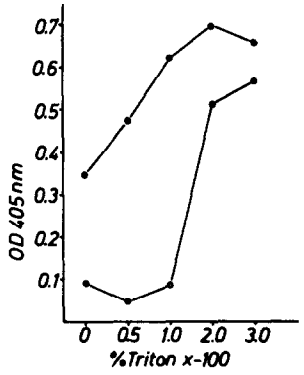

Fig. 2. Dependance of the EII activity upon Triton concentration. Standard buffer was used containing the indicated concentrations of Triton X-100. EII activity in the suspension was estimated before (●—●) and after centrifugation (○—○) at 144,000 *g* for 1 hr.

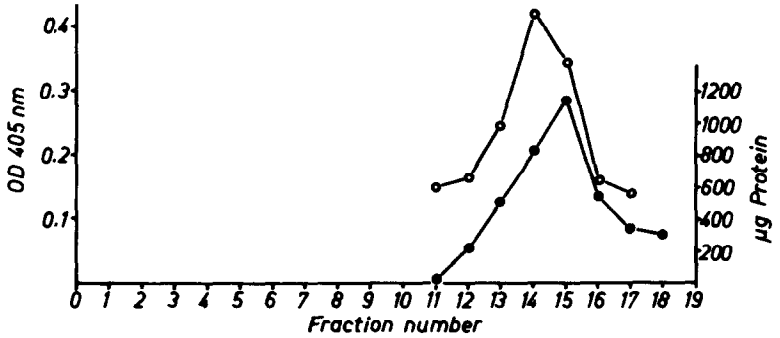

Fig. 3. Sedimentation of EII in a sucrose gradient containing Triton X-100. A sample of solubilized EII was separated on a sucrose gradient. 0.1 ml of the fractions were analyzed for E11 activity (○—○) and protein (●—●). The EII peak contained 70% of the activity layered on top of the gradient.

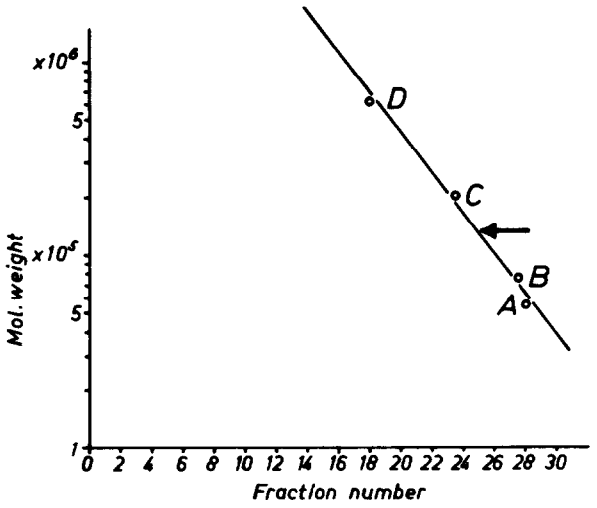

Fig. 4. Estimation of the apparent molecular weight of EII on a column of Sepharose 4B. For the calibration of the Sepharose column (1.5 X 27 cm) the following markers were used: β-galactosidase M.W. 540,000 (A), Ferritin M.W. 750,000 (B), Dextran blue 2000 M.W. 2×10^6 (C), ΦX 174 ^{32}P labeled 6.2×10^6 (D) [8]. The proteins were eluted with standard buffer containing 2% Triton. One fraction = 1.4 ml. 85% of the EII activity applied on top of the column was recovered in the eluate. The position of EII activity is indicated by the arrow.

Of the three non ionic detergents (table 1), Triton X-100 gave excellent recoveries of soluble EII activity. These results may be compared with those of Dulaney et al. [6] who successfully solubilized esterases attached to rat liver plasma membranes by similar techniques.

The solubility of EII in solutions containing Triton is dependant upon the salt and the detergent concentration (figs. 1 and 2). The half life of EII in the soluble state is about three days.

The following criteria have been used to characterize the solubility of EII:

1) EII activity bands in a sucrose gradient containing Triton. The velocity of sedimentation is close to bovine serum albumin (fig. 3). The sedimentation constant of EII was estimated to be 5.5 S. In a gradient where Triton X-100 was omitted, EII sediments to the bottom of the tube after 4 hr at 39,000 rpm.

2) The EII activity can be eluted from a Sepharose 4 B column as a single symmetrical peak. The elution volume of the peak corresponds to an apparent molecular weight of about 1.3×10^6 (fig. 4).

3) In membranes not subjected to the treatment with Triton, EII is associated with the membrane

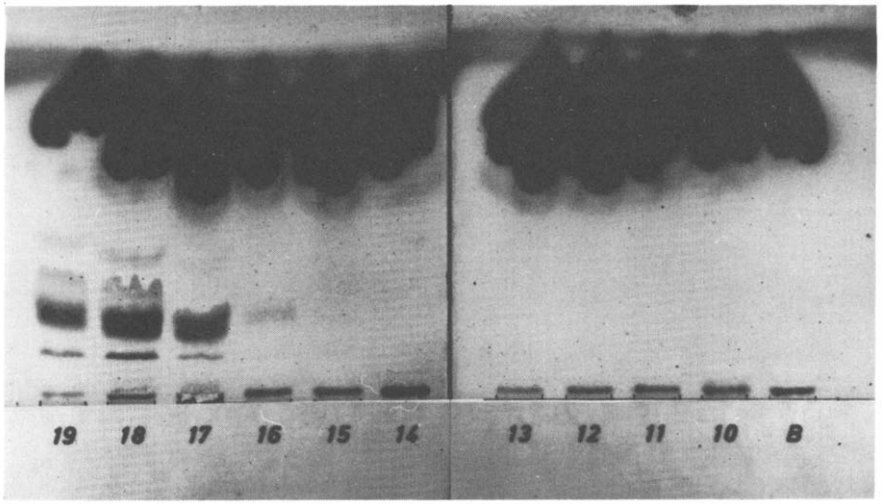

Fig. 5. Distribution of the phospholipids and the EII activity after sucrose gradient centrifugation. The fractions of the gradient were extracted with $CHCl_3$-MeOH (2:1) and treated further according to Folch et al. [7]. The $CHCl_3$ phase of each fraction was spotted on silica gel thin layer plates. The separation was performed with $CHCl_3$-MeOH-H_2O (65:25:4). The phospholipids were detected with the spray of Hanes et al. [9]. The large dark spots close to the solvent front are Triton X-100 (sample B represents the $CHCl_3$-MeOH extract of the gradient liquid). EII activity was found in fractions 14 and 15 (compare fig. 3).

lipids which cannot be removed by repeated washes with buffer. The solubilized EII was centrifuged on a sucrose gradient. The fractions were assayed for EII then for lipids by extraction with $CHCl_3$-MeOH [7] followed by thin layer chromatography.

The photograph (fig. 5) demonstrates that the phospholipid pattern usually associated with the particulate EII is clearly separated from EII after the gradient centrifugation. To confirm the complete absence of lipid associated with the EII activity requires further purification of EII and the use of larger quantities of material.

The large discrepancy between the molecular weight of EII roughly estimated from the S value (about 100,000) compared with the value obtained by gel permeation chromatography (1.3×10^6) may either reflect that the EII molecule is a complicated protein complex with extreme hydrodynamic properties or it may indicate that soluble EII binds large amounts of detergent. In order to distinguish between these two alternatives we shall try to further purify and characterize the EII.

The solvent conditions under which the purified enzyme would function properly without being attached to the membrane should be comparable to the natural environment of the membrane bound enzyme.

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