PHAGE LAMBDA RECEPTOR PROTEIN FROM *ESCHERICHIA COLI*

Solubilization and purification in an aprotic solvent

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Received 10 August 1978
Revised version received 5 September 1978

1. Introduction

The phage lambda receptor protein of *Escherichia coli* has two major functions: it is the initial recognition site for phage lambda, and it catalyzes the facilitated diffusion of maltose, and possibly other sugars as well [1,2]. After induction by growth of *E. coli* on maltose, the lambda receptor protein becomes a major component of the outer membrane. The protein has been partially purified by the use of cholate detergent [3]. More recently, it has been highly purified after pronase digestion of the total envelope fraction, and extraction with sodium dodecyl sulphate (SDS) [4]. It was stated, however, that the yield of receptor protein was rather poor [4].

Organic solvents rather than detergents have been successfully employed in the purification of a number of tightly membrane-bound proteins [5,6]. Aprotic solvents like hexamethylphosphoric triamide (HMPT) have been particularly effective [7]. This methodology has now been applied to lambda receptor protein. The specific solubilization by 90% HMPT in the presence of divalent cations, and the gel chromatographic purification are reported here.

2. Experimental

2.1. *Materials*

Cells of *E. coli* were grown on Luria broth containing maltose (8 mg/ml), and outer membrane fractions were prepared as in [8]. Strain AB2847 was a K12 derivative, containing proteins Ia and Ib but not lambda receptor in the outer membrane [9]. Strain T19 (identical with the transductant c of fig.2 in [9]) was a derivative of AB2847 and produced lambda receptor but not Ia or Ib. Strain CM7-T1 was a derivative of *E. coli* B/r, and produced lambda receptor but not Ia or Ib [2]. A pure grade of HMPT was purchased from Serva, Heidelberg. A less pure material (Aldrich Co.) was used for column chromatography. Sephacryl S-200 was obtained from Pharmacia.

2.2. *General procedures*

Protein was determined by the Lowry assay [10]. Discontinuous SDS–polyacrylamide slab gel electrophoresis was performed as in [11]. Under the present conditions, the presence of up to 20% (v/v) of HMPT in the sample did not affect the gel electrophoretic separation.

3. Results and discussion

3.1. *Solubilization of the lambda receptor protein*

Preliminary extraction experiments with HMPT were performed with outer membranes from various strains of *E. coli* which differed in the relative amounts of the outer membranes proteins. In the presence of Mg^{2+} or Ca^{2+}, neither protein Ia or Ib, nor II*, or its modified form, was appreciably extracted, whereas large amounts of the lambda receptor protein were solubilized (fig.1). (Here we

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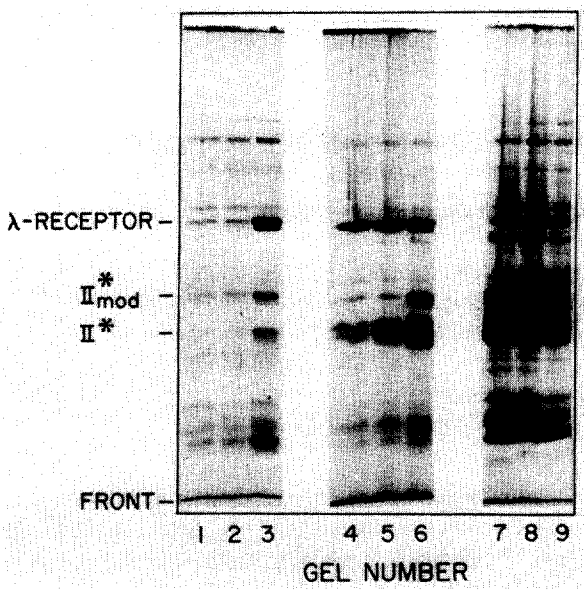

Fig.1. Effect of divalent cations on the solubilization of the lambda receptor protein by 90% HMPT. An outer membrane fraction was prepared from cells of *E. coli* T19. A 40 μl portion (42 mg protein/ml in 10 mM Hepes–NaOH, pH 7.4) was supplemented with 5 μl 100 mM $CaCl_2$ (gels no. 1, 4, 7), 100 mM $MgSO_4$ (gels no. 2, 5, 8), or sodium EDTA (gels no. 3, 6, 9). After 5 min at 25°C, the membranes were reisolated in an Eppendorf 3200 centrifuge (6 min, 25°C). The supernatants were discarded. Neat HMPT (Serva, 50 μl) was added, and the membrane pellets were suspended by means of a Vortex shaker. After 40 min at 25°C with occasional vortexing, the membranes were sedimented by centrifugation in the Eppendorf centrifuge (6 min, 25°C). The supernatants were analyzed by SDS–polyacrylamide slab gel electrophoresis (gels no. 1–3). The pellets were resuspended in 50 μl solvent mixture consisting of 9 parts HMPT (by vol.) and 1 part 50 mM Tris–SO_4, 100 mM LiCl (pH 7.5). After 60 min at 25°C with occasional vortexing, the undissolved material was again sedimented in the Eppendorf centrifuge (6 min). The supernatant and pellet fractions were then analyzed by SDS–polyacrylamide slab gel electrophoresis (gels no. 4–6 and 6–9, respectively). To obtain the gel patterns shown, 5 μl portions of the 90% HMPT extracts were added to 50 μl SDS–β-mercaptoethanol sample buffer [11], and 20 μl aliquots thereof were applied to the gel after heating for 15 min at 100°C. The final pellets were solubilized by the addition of 100 μl SDS–β-mercaptoethanol sample buffer [11], and 4 μl thereof were applied to the gel after the heating step. II^*_{mod} refers to the slower running form of protein II* [13].

follow the nomenclature for the proteins in [12].) When the solubilization procedure was carried out with water instead of HMPT, no membrane protein was extracted (data not shown). When divalent cations were complexed by addition of ethylenediaminetetraacetate (EDTA), the 90% HMPT reagent was much less specific. Now protein II* was also extracted as shown in fig.1 for *E. coli* strain T19. In parallel studies on outer membranes from strain AB2847 it was shown that protein Ia and Ib likewise were solubilized with HMPT only when EDTA was added (data not shown).

The controlled use of HMPT under various conditions may reveal details of the different ionic interactions which appeared to be involved in the binding of the major outer membrane proteins to the membrane matrix.

Gross electrostatic interactions appeared to be unlikely, at least in the case of the lambda receptor protein, since isoelectric focusing in the presence of Triton X-100 indicated that the isoelectric point of the protein was near pH 5.3 (data not shown).

3.2. *Purification of the lambda receptor protein*

A partially purified outer membrane preparation was isolated from *E. coli* B/r strain CM7-T1. The membrane suspension (4 mg protein in 200 μl water) was supplemented with 5 μl 1 M $MgSO_4$. After 5 min at 25°C, the membrane was pelleted in an Eppendorf 3200 centrifuge (4 min). The solubilizing mixture was freshly prepared by adding 30 μl aqueous solution containing Tris–SO_4 (500 mM), LiCl (1 M), and $MgCl_2$ (100 mM) pH 7.5, to 500 μl HMPT. The solvent mixture was added to the membrane pellet, with thorough mixing with a Vortex mixer. The suspension was held at 25°C for 5.5 h, with occasional vortexing. Undissolved material was removed by first centrifuging in the Eppendorf 3200 centrifuge for 4 min, followed by ultracentrifugation in a Beckman L5-50 centrifuge (25°C, rotor 65 Ti, 40 000 rev./min, 90 min). On the basis of SDS–gel electrophoresis, the lambda receptor protein was eluted in a yield of 60–70%. The supernatant was used for chromatography, after the addition of blue dextran and [1-^{14}C]lactose as markers.

Gel chromatography in 90% HMPT was performed on a column of Sephacryl S-200 (fig.2). It was possible to calibrate this column by the use of marker proteins, as well as by running SDS–polyacrylamide gels of

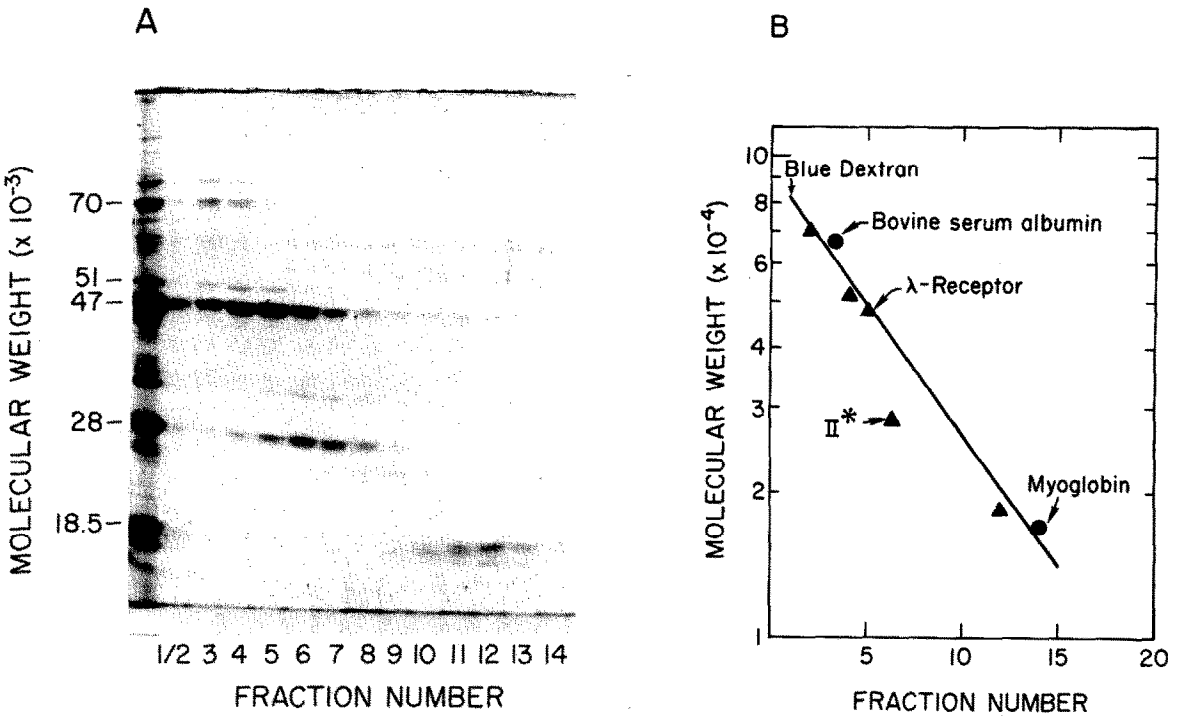

Fig.2. Gel chromatographic fractionation of HMPT-dissolved proteins on Sephacryl S-200. A column (59 × 1.5 cm) of Sephacryl S-200 was packed in a solvent mixture prepared by adding 500 mM Tris–SO_4, 1 M LiCl, pH 7.5 (1 part) to HMPT (9 parts). This solvent mixture deposited some solid LiCl upon aging, and was used only after storage for 2 days. The sample (450 μl, see text) was layered onto the column bed. Fractions of 0.95 ml were collected at 25°C at a flow rate of about 2 ml/h. Due to a malfunction of the fraction collector, fractions 1 and 2 of the experiment shown were collected in one tube. A portion of each fraction (usually 12 μl; for fraction 1/2 4 μl) was added to 50 μl SDS–β-mercaptoethanol sample buffer [11]. After heating (15 min, 100°C), 20 μl portions of these samples were analyzed by SDS–polyacrylamide slab gel electrophoresis (fig.2A). The protein profile of the sample applied to the column is shown in the left lane of fig.2A. The calibration of the SDS–gels with known marker proteins allowed us to assign molecular weights to the major polypeptide components of the HMPT extract. These molecular weights were then plotted on semi-logarithmic paper against the fraction number where maximal elution occurred (fig.2B, ▲). The elution positions (●) for bovine serum albumin and myoglobin were determined in independent experiments.

each of the fractions obtained from the outer membrane extract (fig.2B). The lambda receptor protein was clearly included in the column volume, and the contaminating polypeptides were eluted according to their molecular weights determined on SDS–polyacrylamide gels. The best fraction containing lambda receptor protein (fraction 5) still contained traces of contamination by overlapping polypeptides.

3.3. *Apparent molecular weight of the lambda receptor protein*

Gel chromatography in 90% HMPT (fig.2) as well as SDS–slab gel electrophoresis both gave mol. wt 47 000–48 000, which is in agreement with the values in [4,14]. However, it has also been reported that the smallest functional form of the lambda receptor protein is a dimer held together by disulfide bonds [15]. This possibility has been further examined by running SDS–polyacrylamide slab gels of outer membrane samples as well as HMPT solutions of the lambda receptor protein, without using β-mercaptoethanol in the solubilizing SDS-buffer [11]. In addition, duplicate samples were preincubated with a ferricyanide reagent which dimerizes thiol compounds [16]. The SDS–gels gave in no case any indication for dimer formation (data not shown). It is concluded that the lambda receptor protein is a single polypeptide chain of mol. wt 47 000–48 000.

3.4. *Apparent molecular weight of protein II**

Protein II* has an unusual property of showing two different rates of migration in SDS–polyacrylamide gels depending on the history of heat treatment of the sample. It has been shown [13] that modified II*, the form found after boiling the sample in SDS, has higher intrinsic viscosity than II*, and this result tends to favor the idea that the mobility of modified II*, rather than that of II*, correlates more with the true molecular weight of the protein. In contrast, amino acid analysis gave minimal mol. wt 27 000 [17], a figure similar to the apparent molecular weight indicated by the mobility of II* (28 000) rather than by that of the modified II* (33 000).

In the Sephacryl S-200 column (fig.2), both modified II* and II* were eluted at the same position, a finding indicating that the protein assumes only one conformation in 90% HMPT. The position of elution corresponded to mol. wt ~40 000.

Acknowledgements

This study was carried out while H. Sandermann was on leave of absence from University of Freiburg. It was supported in part by a grant from Deutsche Forschungs-Gemeinschaft (Sa 180/11) as well as by US Public Health Service research grant AI-09644 and an American Cancer Society grant BC-20.

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