CHARACTERIZATION OF THE RECEPTOR PROTEIN FOR PHAGE T5 AND COLICIN M IN THE OUTER MEMBRANE OF *E. COLI* B

V. BRAUN and H. WOLFF

Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, W. Germany

Received 11 May 1973

1. Introduction

We have isolated the receptor protein for phage T5 from cells of *E. coli* B and shown that it is also the receptor for colicin M [1]. Earlier genetic evidence for a common receptor for phage T5 and colicin M was based on the simultaneous loss of the sensitivity of cells towards both agents by mutation [2, 3]. In other cases, however, a closer biochemical study of common receptors as they have been suggested from genetic experiments revealed clear differences in the structural requirements for adsorption, e.g. for the phages T2, T6 and colicin K [4] and for the phages T3, T4 and T7 [5]. The first isolation of a pure receptor protein was also the biochemical proof that proteins can serve as phage receptors. Earlier indirect evidence such as sensitivity towards heat, proteases or protein-specific chemicals have shown that proteins are involved in binding of the phages T2, T5 and T6 [4, 6]. Most recently it has been shown that a glycoprotein with a mol. wt. of 60 000 most probably binds colicins E2 and E3 [7].

In this paper we describe a first characterization of the T5, colicin M receptor protein. It is shown that the receptor protein consists of a single polypeptide chain with a mol. wt. of 85 000 and that it is localized in the outer membrane of the cell.

2. Methods

Isolation of the receptor by extraction of *E. coli* B cells with sodium hydroxide and purification by differential centrifugation, chromatography on Biogel A-50 and DEAE—cellulose in the presence of 2% Triton X-100 has been described [1]. For electrophoresis in SDS-(sodium dodecyl sulfate) gels the Triton X-100 was removed by precipitating the protein with ethanol. Gel electrophoreses: system 1: lyophilized receptor protein was dissolved in sample buffer (6 M urea, 1% SDS, 1% mercaptoethanol, 0.01 M EDTA, 0.01 M sodium borate, pH 8). The same buffer but with reduced amounts of SDS (0.1%) and without mercaptoethanol was used in the gels and in the electrode buffer. In the electrode buffer also urea was omitted [1]. Gels were prepared with 6% or 8.5% acrylamide, 0.12% methylenebisacrylamide, 0.14% *N,N,N′,N′*-tetramethyleneamine and 0.045% ammonium persulfate. Electrophoresis was performed at a constant current of 3 mA/gel until the tracking dye bromophenol blue reached the end of the gel. The procedure followed otherwise largely that of Weber and Osborn [8]; system 2: the sample buffer consisting of 0.1 M Tris–HCl, 0.1 M EDTA, 1% SDS pH 7.9 was diluted ten times for use as gel and electrode buffer.

3. Results

3.1. *The receptor protein*

The receptor-containing cell extract after differential centrifugation and chromatography on Biogel A-50 did not contain all the proteins of the outer membrane in relative amounts as they occur in the membrane. In fact, the composition of the receptor fraction was rather simple. In fig. 1 the protein pattern of the outer membrane (left gel) is shown after removal

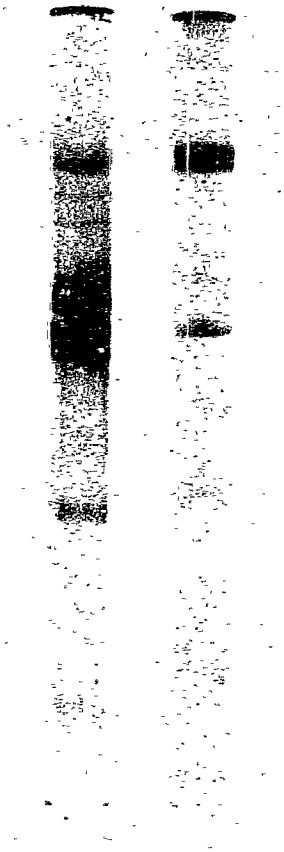

Fig. 1. Polyacrylamide gel electrophoresis of outer membrane and receptor. Enriched outer membrane (left gel) was obtained by preferential extraction of the inner cytoplasmic membrane with Triton X-100 [11]. Receptor was extracted from 5 g freshly grown cells with sodium hydroxide and purified by differential centrifugation and column chromatography on Biogel A-50 [1]. Outer membrane and receptor were dissolved in the sample buffer of system 1 (see Methods) and run on gels with 8.5% acrylamide.

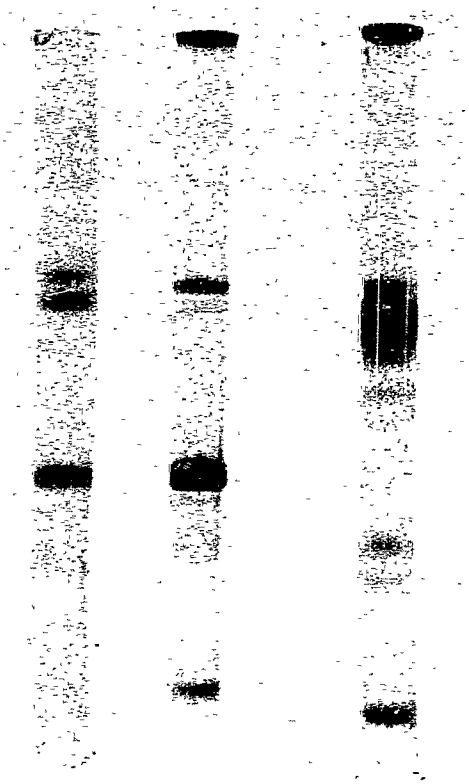

Fig. 2. Polyacrylamide gel electrophoresis of active receptor from T5-sensitive *E. coli* B and inactive receptor from a T5-resistant mutant. Active receptor (left gel) and inactive receptor (middle gel) were boiled for 5 min in the SDS-electrophoresis buffer II (see Methods) and run with the system 2 on gels with 6% acrylamide. The right gel shows the inactive receptor without boiling in this system. This appearance is also typical for the active receptor.

of the cytoplasmic membrane from cell envelopes with Triton X-100 [11]. In the receptor fraction (right gel) two slow moving protein bands were seen and in addition only one of the three major protein bands of the outer membrane (approx. mol. wt. 40 000) was observed in very reduced amounts. It has been shown previously that the lower of the major slow moving protein bands is the receptor protein [1] (see also fig. 3, right gel). This very simple protein pattern from a receptor preparation which started from 5 g cells became more complex when larger amounts (50–100 g cells) had been extracted (fig. 2, left gel). The '40 000 protein' appeared stronger. When the urea-free SDS-buffer II was used for gel electrophoresis, the sample had to be heated, otherwise a large portion of the '40 000 protein' moved like a protein with a mol. wt. of 65 000–80 000 (note the diffuse band of the right gel of fig. 2). This unusual behavior of some membrane proteins of *E. coli* was already observed by several investigators [7, 9, 10]. However, heating in SDS did not affect the position of the receptor protein. Also addition of mercaptoethanol did not reduce the molecular weight which would be expected in the case of disulfide-linked polypeptide chains.

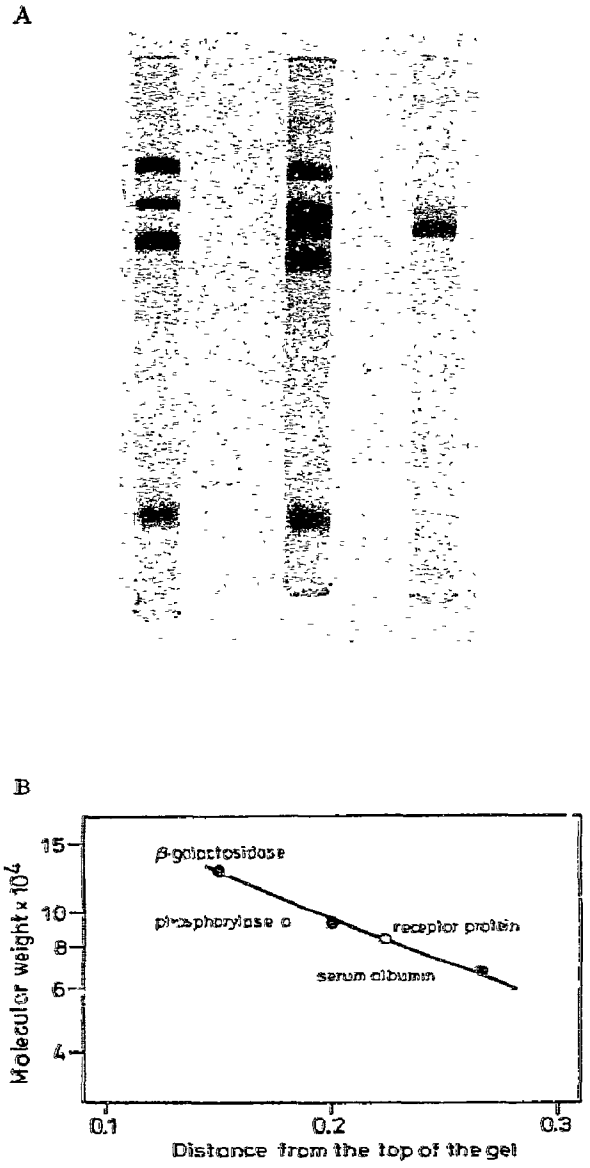

Fig. 3. Molecular weight estimation of receptor protein. A) Bands starting from the top of the gel: left gel *E. coli* β-galactosidase (mol. wt 130 000); rabbit muscle phosphorylase a (mol. wt. 94 000); bovine serum albumin (mol. wt. 68 000); horse heart cytochrome *c* (mol. wt. 12 400); middle gel: the same proteins as in the left gel but with added receptor protein; right gel: receptor alone. The conditions of electrophoresis are described in Methods under 1. B) The distances of the protein bands from the top of the gel are plotted against the logarithm of the molecular weight.

Table 1
Localization of the phage T5—colicin M receptor in the outer membrane of *E. coli* B.

Number of phages							Protein concentration (μg/ml) in the 1:20 dilution
Membrane dilution							
1:10			1:20				
179	174	168	217	171	164	157	1220
165	134	127	222	181	165	181	780
25	66	41	97	116	145	148	1980
13	14	12	47	41	44	34	4000

Membranes from 300 ml of *E. coli* B grown in M3 medium (Difco) to A_{578} = 0.72 were prepared and separated by sucrose density gradient centrifugation according to Osborn's procedure [12]. The membranes were washed with 0.01 M sodium phosphate, pH 7.0 and finally suspended in 1 ml of this buffer. Dilutions were made with the phage adsorption buffer (0.01 M sodium phosphate, pH 7.0, 0.01 M $MgSO_4$). To test for receptor activity 0.1 ml of phage (about 2×10^3 plaque forming units) suspended in adsorption buffer were mixed with 0.9 ml of the various membrane fractions. After incubation for 1 hr at 37°C, 0.1 ml were withdrawn and mixed with 0.2 ml *E. coli* B (2×10^8 cells) in 2.5 ml molten nutrient soft agar and poured over nutrient plates. After incubation overnight at 37°C plaques were counted. For protein determination membrane aliquots were hydrolysed with 2.5 N NaOH for 2.5 hr at 100°C, neutralized with 30% acetic acid and then stained with ninhydrin. Bovine serum albumin served as standard protein.

Number of T5 plaque-forming units without membrane added, 4 controls: 191, 183, 187, 228.

The molecular weight of the receptor protein was estimated by SDS-gel electrophoresis. It has been shown [8] that the electrophoretic mobility of polypeptide chains in polyacrylamide gels in the presence of SDS is correlated with their molecular weight. A receptor protein highly purified by column chromatography on DEAE—cellulose in the presence of 2% Triton X-100 (fig. 3A, right gel) was run together with three standard proteins of known molecular weight (fig. 3A, left gel, and middle gel). The electrophoretic mobilities of the standard proteins were plotted against the logarithm of their molecular weights (fig. 3B). The electrophoretic mobility of the receptor protein fits into this curve at a mol. wt. of approx. 85 000.

3.2. *Localization of the receptor in the outer membrane*

The cytoplasmic and outer membrane of *E. coli* B were separated according to Osborn's procedure and we asked where the receptor activity resides (table 1). Most activity was found in the outer membrane (H-band), some in the mixture of outer and cytoplasmic membrane (M-band) and only little in the cytoplasmic membrane (L_1 + L_2 bands). Although the membrane fractions contain different amounts of protein, the 1:10 and 1:20 dilution of the H, M and L_1 band can be compared. It is concluded that the receptor isolated originates from the outer membrane where it is probably localized at the surface. The table also shows the degree of reliability of T5 titration with increasing amounts of receptor and demonstrates that double the amount of protein does not inactivate twice as many T5 particles.

4. Discussion

Already after differential centrifugation and chromatography on Biogel A-50, the receptor extract contains only few protein bands from which the receptor protein is the most prominent (fig. 1). Compared to the other membrane proteins it is largely enriched. The greatest 'contamination' is one of the major proteins of the outer membrane and the question arises whether this is also localized *in vivo* near the receptor protein. The fastest moving protein band (fig. 2, middle and right gel) is probably the murein—lipoprotein [13, 14, 15] which is covalently bound to the murein but which also exists in free form [16]. This is a major protein of the outer membrane [17]. Further studies are needed to show whether these proteins, together with lipopolysaccharide and phospholipids, form a defined membrane area or whether their joint appearance is an artefact of the receptor preparation. Since the receptor activity was localized in the outer membrane, it is likely that it is situated near the surface where it can be approached by the phage and the colicin. Fig. 2 also documents that in a T5 and colicin M resistant mutant of *E. coli* B [1] the receptor protein is present and shows the same molecular weight. However, it neither binds phage T5 nor colicin M. The reduced amount present in this preparation does not account for the inactivity [1]. Although it has been shown that periodate-sensitive sugars are not essential for receptor activity [1], the receptor could still be a glycoprotein. In this case one has to consider the molecular weight determination with caution, since the electrophoretic mobility of glycoproteins in polyacrylamide gels in the presence of SDS does not always correspond to their molecular weight [18].

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