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The protein receptor for cholerabacteriophage φ149

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Choleraphage φ149 receptor activity was found in the outer membrane (OM) protein of *Vibrio cholerae* 154. Receptor protein for phage φ149 was separated from trypsin-treated OM on a Sephadex G-100 column. Of the three peaks obtained, phage receptor activity was noted only in peak II. SDS-PAGE showed that the M_r of the protein was 35000. The protein was heat-labile and protease-sensitive. The specificity of this protein as choleraphage φ149 receptor was investigated by carrying out a protection experiment by anti-protein (peak II) rabbit sera.

Choleraphage φ149; Receptor; (*Vibrio cholerae* 154)

1. INTRODUCTION

Choleraphage φ149 is capable of lysing all strains of classical *Vibrio cholerae* irrespective of their serotypes [1]. However, little information is available on the mechanism of adsorption of choleraphage φ149 into the host cells. It is known that LPS and protein of gram-negative bacteria [2] such as *E. coli* and *Salmonella* can act as receptors for the respective phages. Our preliminary studies [3] indicated that choleraphage φ149 does not use LPS as a receptor for adsorption. Here, an attempt has been made to identify the outer membrane (OM) protein as a phage receptor for choleraphage φ149.

2. MATERIALS AND METHODS

Classical *V. cholerae* 154 and choleraphage φ149 were used in this study. Phages were routinely concentrated and purified by using polyethylene glycol [4].

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2.1. *Media*

0.8% nutrient broth (Difco) containing 0.5% NaCl (pH 7.6) was used for growth of bacteria and for phage propagation.

2.2. *Preparation of OM and purification of receptor protein for phage φ149*

OM was prepared according to Kelly and Parker [5]. 5 mg OM protein was mixed with 200 μg trypsin suspended in 20 mM Tris-HCl buffer (pH 8.3) and left at room temperature (27°C) for 24 h. An equal amount of trypsin inhibitor was added to the mixture and centrifugation performed at 30600 × *g* for 30 min. The pellet was washed 3 times with 20 mM Tris-HCl (pH 8.3) and once with double-distilled water. The sediment was then suspended in 2 ml of 10 mM Tris-HCl (pH 7.5) containing 1.5% SDS and 5 mM EDTA and boiled for 5 min in a boiling water bath. The insoluble material was removed by centrifugation at 40000 × *g* for 60 min. The clear supernatant was used for the separation of protein on a Sephadex G-100 column. The column (40 × 1.6 cm) was equilibrated with elution buffer (10 mM Tris-HCl containing 5 mM EDTA and 1.5% SDS, pH 7.5) before adding the sample to the column. Sample was

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eluted with buffer in 1.2 ml fractions in each tube and protein was measured at 280 nm using a Shimadzu spectrophotometer. Different peaks were pooled separately and precipitated by adding 90% acetone. Precipitate was collected after centrifugation at 40500 × *g* for 30 min. The process was repeated once with supernatant. The two precipitates were pooled and washed with 10 mM Tris-HCl buffer (pH 7.5). The protein precipitate was then tested for its capacity to inactivate choleraphage ϕ149. The carbohydrate content of the void volume and 3 peaks was measured [6].

2.3. *Phage inactivation*

To demonstrate the inactivation of bacteriophage, 0.1 ml phage ϕ149 (1.5×10^3 p.f.u./ml) was incubated with different concentration of protein fractions at 37°C for 5 min. The free phage was assayed by a standard procedure [7].

2.4. *SDS-polyacrylamide gel electrophoresis (SDS-PAGE)*

SDS-PAGE was performed according to Segrest and Jackson [8] adopting a suitable modification.

2.5. *Preparation of antibody against receptor protein*

Receptor protein was emulsified with an equal volume of complete Freunds adjuvant (Difco, USA) and injected intramuscularly into the hindlegs of rabbits. Antigen was administered on days 0, 7, 10 with 100, 125 and 250 μg receptor protein, respectively. Rabbits were bled 10 days after the last immunizing dose and sera were stored at −70°C. An Ouchterlony double-diffusion experiment showed a single sharp immunological precipitin band between anti-protein serum and pure receptor protein (fig.1).

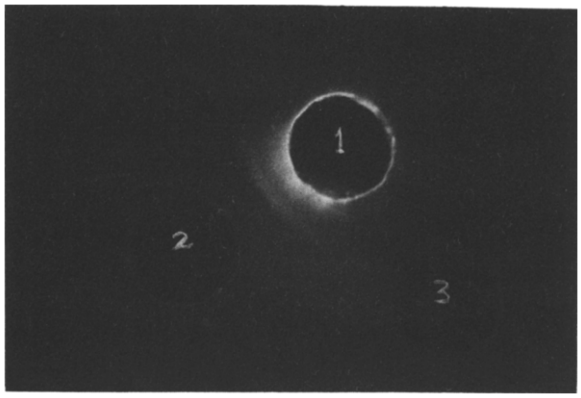

Fig.1. Ouchterlony double-diffusion test. (1) 2.5 μg receptor protein. (2) Antibody raised against the purified receptor protein. (3) Preimmune rabbit sera.

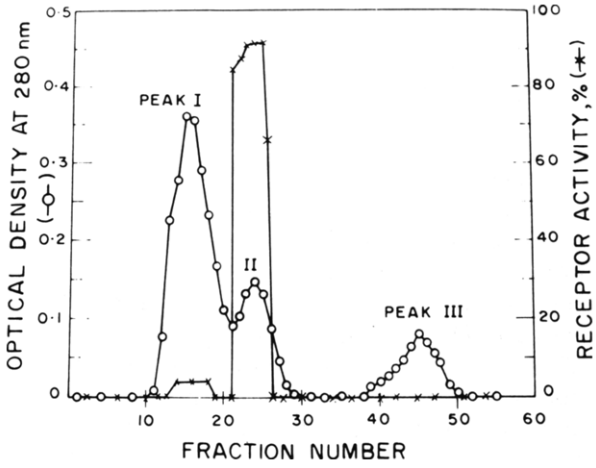

Fig.2. Receptor activity in the eluate of a Sephadex G-100 column. Receptor activity was measured in different fractions as described in section 2.

3. RESULTS

3.1. *Separation of receptor protein from trypsin-treated OM by column chromatography*

Trypsin-treated OM was used for the separation of proteins by a Sephadex G-100 column because of its higher phage inactivation capacity as compared to the untreated OM. An elution profile showing three peaks (fig.2) was recorded. A large amount of carbohydrate was present in the void volume but no receptor activity. Peaks I–III contained only protein and it was found that 98% of the receptor activity was present in peak II. Peaks I and III had little or no receptor activity. Further work was not done with these two protein fractions.

Table 1 lists the yield and purification of phage receptor protein. Complete inactivation of phage ϕ149 was obtained with 7 μg peak II protein as compared to 45 μg OM protein. The phage receptor activity was increased 6.5-times. It should be mentioned here that almost 50% of the OM protein was lost during trypsin treatment. However,

Table 1

Yield and purification of phage receptor protein

	Protein (mg)	Protein used (μg) for 100% phage inactivation	% adsorbed phage	% of yield
OM	5.10	45	96	1
Trypsin-treated OM	2.77	15	94	3
Sephadex				
Peak I	1.95	–	–	
Peak II	0.47	7	95	6.5
Peak III	0.20	–	–	

the phage inactivation capacity of trypsin-treated OM was amplified 3-times. Fig.3 shows the percentage of unadsorbed phage in presence of different concentration of peak II protein.

3.2. *Analysis of OM protein pattern by SDS-PAGE*

Analysis of the OM protein by SDS-PAGE (fig.4) showed that the protein patterns of OM (lane 2) and trypsinized OM (lane 3) were significantly different. Several protein bands found in OM were absent in trypsin-treated OM. The most prominent protein bands of OM had an approximate M_r ranging from 82000 to 30000. In trypsin-treated membranes several major protein bands were either missing or reduced in quantity. SDS-PAGE of peak II protein revealed a single protein band of M_r ~35000 (lane 4).

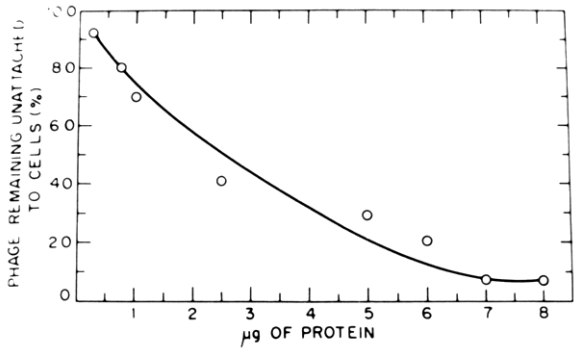

Fig.3. Percentage of unadsorbed phage in the presence of different concentrations of peak II protein.

Table 2

Protection of phage ϕ149 by anti-protein (peak II) sera from inactivation by peak II protein

Peak II protein (5 μg)	Preimmune serum (μl)	Anti-peak II protein sera (μl)	% of phage protected from inactivation
–	–	40	100
+	–	–	30
+	40	–	31
+	–	20	70
+	–	40	100

3.3. *Protection of bacteriophage ϕ149 by peak II protein sera*

To show unequivocally that peak II protein was responsible for the inactivation of phage ϕ149, a protection experiment was carried out with anti-peak II protein sera. 5 μg peak II protein and 40 μl anti-protein II serum were incubated at 37°C for 15 min and then mixed with 0.1 ml (10^3 p.f.u./ml) phage ϕ149 and incubated for a further 5 min. It

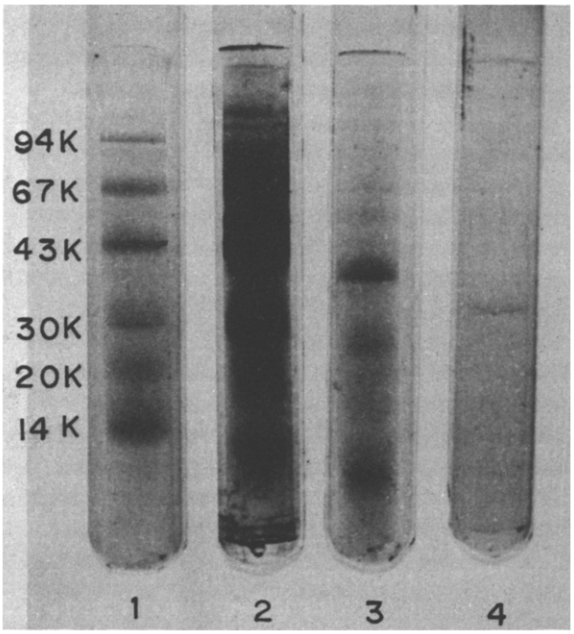

Fig.4. Protein pattern of OM (lane 2) trypsinized OM (lane 3) and purified protein (lane 4) in SDS-PAGE. Arrows point to low molecular mass marker.

was found that the bacteriophage titre was not reduced (table 2). However, under similar conditions when preimmune serum was used instead of anti-peak II protein sera, it failed to protect phage ϕ149 from inactivation by peak II protein. The result indicated that peak II protein was responsible for the inactivation of phage ϕ149. Thus, we refer to it as receptor protein for phage ϕ149. The receptor protein is heat-labile and protease-sensitive.

4. DISCUSSION

OM protein isolated from *V. cholerae* 154 can inactivate phage ϕ149. The limitation of expression of the phage receptor activity in untreated OM in comparison to trypsin-treated OM suggested that phage receptor molecules were embedded deeper in OM and were not affected by trypsin treatment. This phenomenon was also observed in Ton A protein activity which is the receptor for T_5 phage [9].

Although receptor protein was purified to homogeneity, its activity was increased only 6-fold. Perhaps some factors were lost during purification. We are currently engaged in looking for these factors.

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