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ISOLATION OF A THERMOLABILE ENTEROTOXIN FROM Escherichia coli AND THE STUDY OF ITS BIOLOGICAL PROPERTIES

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An enterotoxin was isolated from strain Escherichia coli 015 by salt precipitation and gel chromatography. In the process of isolation and purification the toxic activity of the preparation increased: by 60 times according to the ligated segment of rabbit intestine method and 66-100 times according to the skin test. The plateau and second fraction obtained by gel chromatography were inactive according to the ligated segment of intestine method but possessed permeability factor (PF) activity in the skin test. Two hypotheses were put forward: The vascular permeability factor and the diarrheagenic factor are possibly two different substances (molecules) and the skin test is more sensitive as a method of determining toxicity than the ligated segment of rabbit intestine method.
KEY WORDS: enterotoxin of E. coli; vascular permeability factor; diarrheagenic factor.

Recent work has shown that certain species of escherichias which produce thermolabile and thermostable enterotoxins are the agents of acute cholera-like diseases in man and domestic animals [3]. In its biological properties, including immunological specificity, the thermolabile enterotoxin is similar to the cholerogen of the cholera vibrio and, in particular, it reacts with anticholera serum [4, 5].

The biological properties of the thermolabile colienterotoxin have been inadequately studied, since it has not been isolated in a purified form. The question of identity of the vascular permeability factor (PF activity) and the diarrheagenic factor, both of which are found in preparations of enterotoxin, still remains open.

The object of this investigation was to isolate and purify a thermolabile colienterotoxin and to study its diarrheagenic effect and PF activity.

EXPERIMENTAL METHOD

Strain E. coli O15, generously provided by Dr. Gorbach (USA), was used. The strain was grown on nutrient medium consisting of 2% casaminic acid (Difco), 0.6% yeast extract (Difco), and inorganic salts for 24 h at 37°C with aeration. The culture was then centrifuged (18,000g, 30 min, 4°C) and the residue (microbial cells) discarded.

The supernatant was filtered through millipore membranes with a pore diameter of 0.45 μ. The filtrate was lyophilized and designated the original preparation of enterotoxin. Part of the cultural filtrate was treated with ammonium sulfate (to 90% saturation) and the residue separated by centrifugation (6000 g, 30 min, 4°C), redissolved in distilled water and lyophilized. This preparation was described as the residue. Purification was then carried out by gel chromatography on a Sephadex G-150 column. The original preparation or residue was applied in an amount of 150 mg to the column (3 × 90 cm). Elution was carried out in distilled water (40-

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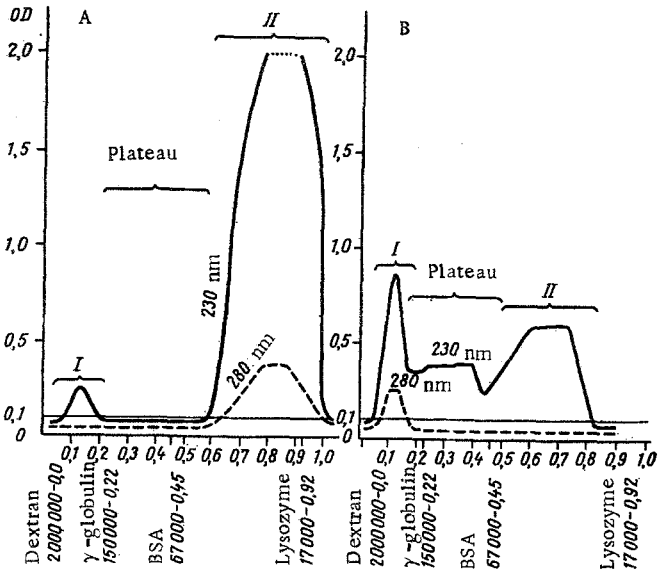

Fig. 1. Elution curves of original preparation (A) and residue (B) obtain by gel-filtration on Sephadex G-150 column. Abscissa, volume of eluate (in ml); ordinate, optical density (OD). I) Fraction 1; II) fraction 2.

TABLE 1. Specific Activity of Enterotoxin of E. coli (data obtained by ligated segment of rabbit intestine method)

Preparation	Stage of purification	Yield per ml culture fluid		TD_{50}, ng	Protein, ng	Specific activity	Yield of specific activity, %
		mg	%				
Original	—	63	100	3 140	800	—	—
Residue	1-	2,4	3,8	150	700	16,6	63
Fraction 1	2-	0,7	1,1	12	190	56,5	62
Plateau	2-	3,5	13,5	25 000	800	—	—
Fraction 2	2-	48,4	77,0	25 000	400	—	—

Legend. TD_{50}) Dose of preparation causing 50% dilatation of intestine, calculated by method of De and Chatterjee [2]. Specific activity) reciprocal of ratio of TD_{50} to protein content in preparation.

45 ml/h). The column was first calibrated with Dextran blue (mol. wt. $2 \cdot 10^6$ daltons), lyophilized γ-globulin (mol. wt. $1.5 \cdot 10^5$ daltons), crystalline bovine serum albumin (mol. wt. $6.7 \cdot 10^4$ daltons), and crystalline lysozyme (mol. wt. $1.7 \cdot 10^4$ daltons). Protein in the original preparation and fractions was determined by Lowry's method and carbohydrates with anthrone reagent.

Activity of the preparations was determined by the ligated segment of rabbit intestine method [2] and the skin test [1].

EXPERIMENTAL RESULTS

Elution profiles of the original preparation (A) and residue (B) are illustrated in Fig. 1.

As Fig. 1 shows, the preparations were separated into two fractions: 1) with high molecular weight (over $1.5 \cdot 10^5$ daltons) and 2) of low molecular weight (under $5 \cdot 10^4$ daltons). A more or less well defined plateau was present between the fractions. On gel-filtration of the residue an increase in the yield of function 1 was observed compared with the corresponding fraction after gel filtration of the original preparation.

Combined results for the toxicity of the preparation relative to protein are given in Table 1 (obtained by the ligated segment of rabbit intestine method). A diarrheagenic effect was found to be given by the original preparation, the residue, and fraction 1, whereas the plateau and fraction 2 were inactive. Fraction 1 was a partially purified preparation of enterotoxin containing a small quantity of carbohydrates (hexoses), indicating contamination evidently with a small amount of O-antigen (cell wall endotoxin). In the course of purification of the original preparation an increase in its diarrheagenic activity was observed: by 20.9 times in the first stage of purification and by 262 times in the second stage. On calculation of the activity of the preparation allowing for the yield and the protein concentration, it was shown that the specific activity at the first stage of purification was increased by 16.6 times, and at the second stage by 56.5 times. The yield of the active preparation at the first stage was 63% and at the second stage about 100%, or 62% compared with the yield of the original preparation.

The diarrheagenic effect of the preparations obtained in these experiments appeared after 18-20 h, a characteristic feature of the thermolabile enterotoxin of E. coli [3].

The vascular permeability factor (PF activity) was studied in rabbits weighing 1.5-2 kg. The diameter of the blue patch and its intensity, and also induration of the skin were taken into consideration. An increase in PF activity was found in the course of purification: A zone of deep blue coloration 8 mm in diameter was produced by injection of 3000 ng of the original preparation, 1000 ng of residue, and 30 ng of fraction 1. Induration of the same extent was produced by injection of 400 ng of the original preparation, 50 ng of residue, and 6 ng of fraction 1. However, unlike the results of the experiments with a ligated segment of rabbit intestine, fraction 2 and the plateau reacted in the skin test. Their activity was much less than that of fraction 1 and was roughly equal to the activity of the residue. After heating of the enterotoxin preparations to 100°C for 45 min their toxic activity was considerably reduced.

A thermolabile enterotoxin was thus isolated from the culture medium of E. coli O15 by salt fractionation and gel-chromatography. In the process of purification the toxic activity of the preparation was increased: according to results obtained by the ligated segment of rabbit intestine method by about 60 times, and according to the skin test by 66-100 times. Fractions obtained by gel chromatography (fraction 2 and the plateau) which had no diarrheagenic effect were found to be active in the skin test, i.e., they contained vascular permeability factor.

There are two possible explanations of this result: 1) Vascular permeability factor and diarrheagenic factor are possibly two different substances (or protein molecules); 2) the possibility likewise cannot be ruled out that the skin test is a more sensitive method of detecting toxicity of the enterotoxin than the ligated segment of rabbit intestine method.

The production of a purified colienterotoxin can be followed by a study of the pathogenesis of coli diarrhea and an investigation of antigenic relationships between colienterotoxin and the enterotoxins of other enterobacteria.

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