CHARACTERISTICS OF THE B SUBUNIT OF THE THERMOLABILE ENTEROTOXIN PRODUCED BY *Escherichia coli* STRAIN A^-B^+

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Many bacteria of the enteric group produce what are called cholera-like enterotoxins, which cause diarrhea in man and animals [5]. They are proteins, similar in structure and antigenic properties to cholera enterotoxin. The cholera-like enterotoxin which has been studied the most is the thermolabile (LT) enterotoxin of *Escherichia coli* which, like cholerogen, has a bifunctional structure and consists of acceptor and activator subunits [6, 11, 15]. The antigenic kinship of cholerogen and the thermolabile enterotoxin is due to their common antigenic determinants, which are found in both the A and B subunits [10].

Antibodies to the B subunit prevent recognition and binding of the whole toxin with the attacked cell and, consequently, they do not perimit all the remaining events which, in the absence of antibodies, lead to the development of diarrhea, to be realized [9, 13, 14]. By molecular cloning it is possible to create strains that produce only one subunit to the toxin. Strain KS 1654 [2], carrying the part of the LT operon that determines synthesis of the B subunit of the thermolabile enterotoxin has been constructed and it produces a protein immunologically related to whole toxin.

The aim of this investigation was to obtain and identify the B subunit of the thermolabile enterotoxin of *E. coli* produced by a clone containing the A^-B^+ gene.

EXPERIMENTAL METHOD

The following strains were used as producers: to obtain cholerogen (CT), strain 569B of *Vibrio cholerae* serotype Inaba; to obtain the thermolabile enterotoxin of *E. coli* (LT), *E. coli* KS 1651; and to obtain its B subunit, *E. coli* strain KS 1654. Strains KS 1651 and KS 1654 of *E. coli* contain recombinant plasmids carrying genes for the biosynthesis of whole LT and of its B subunit from the plasmid of the parent strain *E. coli* H74-114,respectively [2]. The strains were obtained from the Laboratory of Molecular Genetics of Episomes, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

Culture of *V. cholerae* and purification of the cholera enterotoxin were carried out by the method described previously [1]. The methods in [3, 4] were used to grow *E. coli* and purified LT.

To obtain subunits of CT and LT, the method used was based on that in [8], described for separation of the CT subunit. Subunits of the two toxins were isolated by gel-filtration under denaturing conditions in 5% formic acid. The concentrated preparation of the toxin was transferred into formic acid by addition of 85% formic acid, and applied to a column (1.5 × 200 cm) with Sephadex G-75, equilibrated with 5% formic acid. The same solution was used for elution, at the rate of 10 ml/h. Fractions containing the separated subunits were pooled and lyophilized. In the case of the B subunit from LT, urea was added to the preparation before application to the column up to a final concentration of 6M. By this modification it was possible to use the method [8] to separate the LT subunits. The lyophilized preparations of the subunits, after being dissolved in 8 M urea, were renatured, not by dialysis as described in [8], but by gel-filtration on a column with Sephadex G-25, equilibrated with buffer containing 0.05 M Tris-HCl, 0.2 M NaCl, 0.001 M EDTA, pH 7.5.

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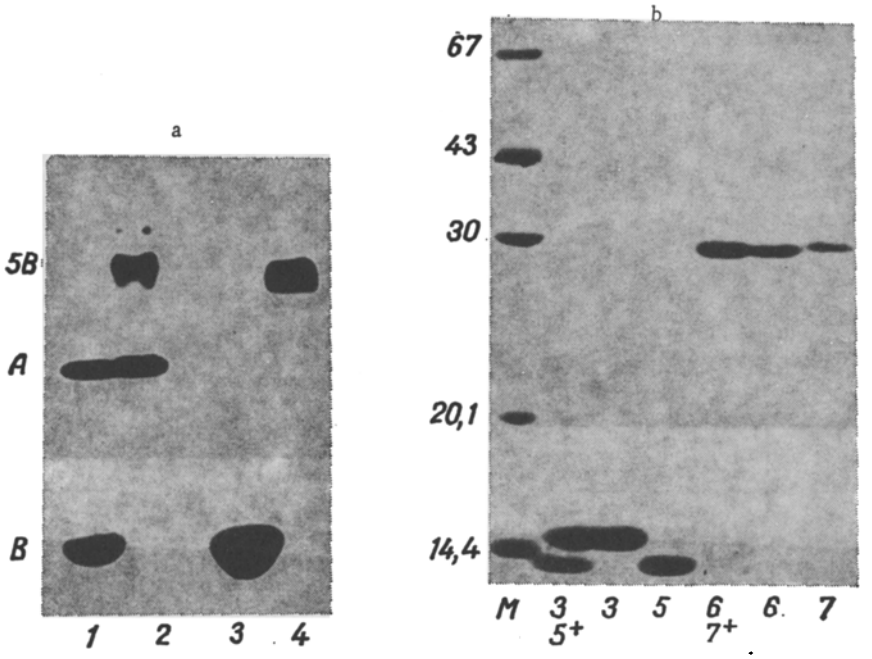

Fig. 1. Disk electrophoresis of LT and CT subunits in polyacrylamide gel with sodium dodecylsulfate: a) disk electrophoresis of LTB subunit from *E. coli* strain A^-B^+; b) coelectrophoresis of LT and CT subunits. A) LTA subunit; B) LTB subunit in monomeric state; 5B) LTB subunit in oligomeric state. 1) LT after heat treatment; 2) untreated LT; 3) LTB from *E. coli* strain A^-B^+ after heat treatment; 4) LTB from *E. coli* strain A^-B^+ untreated; 5) CTB; 6) LTA; 7) CTA. M) Marker proteins: bovine serum albumin — 67 kilodaltons (kD), ovalbumin — 43 kD, carbonic anhydrase — 30 kD, trypsin inhibitor — 20 kD, lactalbumin — 14.4 kD.

The B subunit from LT (LTB) from *E. coli* strain KS 1654 (the A^-B^+ strain), producing only this component of the toxin, was obtained by the method described for obtaining whole LT toxin [4].

Disk electrophoresis was carried out in 15% polyacrylamide gel in the presence of sodium dodecylsulfate [7].

The immunochemical properties of the enterotoxins and their subunits were compared by the double immunodiffusion in agar gel test [12].

Monospecific antitoxic sera to the whole toxin and to their B subunits were obtained by immunization of rabbits with purified preparations, by the scheme described previously [1].

EXPERIMENTAL RESULTS

As was shown previously [2], the cloned strain *E. coli* KS 1654, containing the A^-B^+ gene, synthesized a protein which showed antigenic stimilarity with whole LT. The purified protein of the B subunit has not hitherto been obtained from the cloned (A^-B^+) strain, although complete identification of the product requires investigations to be carried out on homogeneous preparations. To isolate and purify the B subunit the same method was used as to obtain whole LT of *E. coli* [4]. The method included affinity chromatography on agarose gels, and gave a virtually 100% yield of the LTB subunit (6 mg from 1 liter of culture fluid).

The isolated protein was characterized with respect to its molecular properties and antigenic specificity. Besides the B subunit from the (A^-B^+) strain, the B subunits obtained from whole LT and CT, and also the A subunits of the above-mentioned toxic proteins (LTB, CTB, LTA, and CTA, respectively) also were used.

Electrophoresis of native preparations of the B subunit from the A^-B^+ strain and whole LT showed that LTB from the A^-B^+ strain behaved as an oligomer with the mobility of a protein

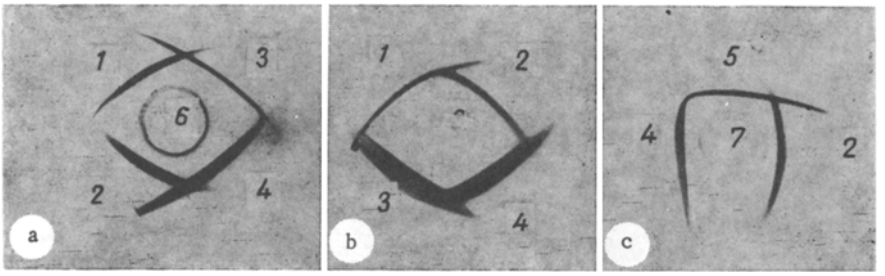

Fig. 2. Double immunodiffusion test on LTB obtained from *E. coli* strain A^-B^+ and LT and CT subunits obtained by the traditional methods of purification, with antitoxic sera. a) Absence of immunologic kinship between the A and B subunits; b) partial immunologic kinship between homologous subunits of two toxins; c) complete immunologic identity of LTB from the A^-B^+ strain and of LTB obtained from whole toxin. 1) CTA; 2) LTA; 3) CTB; 4) LTB; 5) LTB from *E. coli* strain A^-B^+; 6) antitoxic serum to CT; 7) antitoxic serum on LT.

with mol. wt. of 48,000 daltons, like the B component of the whole toxin. During heat treatment this subunit changed from the oligomeric to the monomeric state, with mobility identical with that of the monomeric B subunits from whole LT toxin (Fig. 1). We know that LTB has lower electrophoretic mobility than CTB [10]. The present results confirmed these data and showed that monomeric LTB subunits obtained from the A^-B^+ strain as a molecular weight 1000 daltons greater than that of the corresponding CT subunits (Fig. 1). Meanwhile the results are evidence of the molecular identity of B subunits of the thermolabile enterotoxin of *E. coli*, obtained from different sources. Electrophoretic investigation revealed no contamination of the LTB preparation obtained from the A^-B^+ strain by the LTA subunit.

Immunochemical analysis, using antiserum to whole LT, revealed that LTB obtained from whole LT was identical with that obtained from the LTB producer (Fig. 2). A similar picture was observed when antiserum to LTB obtained from the A^-B^+ strain was used in the gel diffusion test, confirming identity of the LTB subunits obtained from the different sources. Meanwhile both LTB subunits showed partial identity with the B subunit from cholera enterotoxin, when antisera both to whole LT and CT and to LTB and CTB were used (Figs. 2 and 3). This means that the homonymous LT and CT subunits have both common and individual antigenic determinants. Immunochemical analysis also confirmed the absence of the LTA subunit as an impurity in the LTB preparation obtained from the A^-B^+ strain.

These investigations thus showed that LTB produced by the A^-B^+ strain is completely identical with LTB of the whole thermolabile enterotoxin of *E. coli* and that it possesses partial

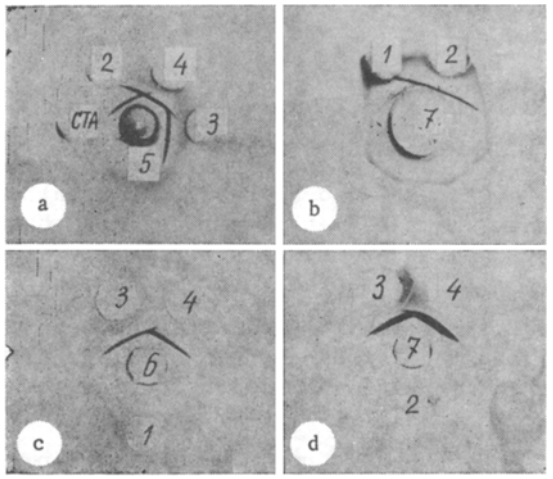

Fig. 3. Double immunodiffusion test on LT and CT subunits with antitoxic serum and with sera to B subunits of both toxins. a) Absence of immunologic kinship between A and B subunits, partial immunologic kinship between LTB and CTB subunits; b) partial immunologic kinship of LTA and CTA subunits; ç, d) discovery of individual antigenic determinants in CTB and LTB, respectively. 1) CTA; 2) LTA; 3) CTB; 4) LTB from *E. coli* strain A^-B^+; 5) antitoxic serum to LT; 6) serum to CTB; 7) serum to LTB.

antigenic kinship with the B subunit of colerogen. When prospects for the use of the B subunit for construction of a vaccine preparation are discussed, it must be noted that difficulties connected with the obtaining of these subunits from the whole toxic protein can be resolved only by the use of a strain that produces only the B subunit. This approach not only will facilitate the process of obtaining the subunit, but will also significantly increase its yield in the form of the purified product, by the use of affinity chromatography. Under these circumstances the purified product will be completely identical in molecular weight and immunochemical properties with LTB obtained by the traditional method of purification. The use of the B subunit as a component of a vaccine against diarrheal diseases will be the subject of our forthcoming research.

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EFFECT OF REGULATORY CELLS INDUCED BY INFLUENZA VIRUS DURING ADOPTIVE TRANSFER

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The differential role of various factors of immunity in protection of the organism has been demonstrated on a model of influenza infection in mice, protected by blood sera for cells of the lymphoid-macrophagal system, obtained from immunized syngeneic mice [4, 5].

The model of adoptive transfer is widely used in immunology to assess the protective role of different cells of the lymphoid-macrophagal series. By means of this method the antiviral protective effect of cytotoxic T lymphocytes has been confirmed [9] and the role of macrophages in modification of the virus antigen in the inductive phase of the immune response in virus infections has been established [2]. However, during induction of the immune response to a test antigen complex mechanisms of intracellular relationships have been found in recipients [10]. Under these circumstances, the resultant effect of cellular cooperation is not always equal in value. Not only the absolute numbers of interacting cells, but also the ratio between them are decisive factors [8]. Recording of the immune response in the recipient also depends on the time of observation [14].

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