Immunological and Physicochemical Characterization of Heat-Labile Enterotoxins Isolated from Two Strains of *Escherichia coli*

JOHN D. CLEMENTS,† DIANNE C. FLINT, and FREDERICK A. KLIPSTEIN

Departments of Medicine and Microbiology, University of Rochester Medical Center, Rochester, New York 14642

Received 11 June 1982/Accepted 16 July 1982

Heat-labile enterotoxins from *Escherichia coli* strains of human and porcine origins had identical subunit composition, arrangement, and size in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunological differences recently described by others were shown to reside exclusively in the B subunits.

The heat-labile toxin (LT) of *Escherichia coli* has recently been purified to homogeneity from both human (12) and porcine (3) isolates. A variety of techniques have been employed, and, in each case, LT had a subunit composition and biological activity remarkably similar to those of the enterotoxin of *Vibrio cholerae* (choleragen). Moreover, when human and porcine LTs were compared after cross-linking with dimethylpimelimidate, each had the same subunit number and arrangement (A5B) as did choleragen (6).

Recently, Honda et al. (8) have demonstrated immunological differences between human and porcine LTs. By immunodiffusion analysis of crude preparations, Honda et al. showed that the different LTs appeared to contain shared and nonshared antigenic determinants and that the nonshared regions were conserved along species lines. Working with purified holotoxin preparations, Geary et al. (5) and Takeda et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, B64, p. 28) also demonstrated antigenic and structural differences between human and porcine LTs. In all of these studies, the investigators compared LTs purified from different organisms grown in the presence of the antibiotic lincomycin. Although it is generally accepted that lincomycin increases the production of enterotoxin by *E. coli* and *V. cholerae*, the precise mechanism by which it does so is not known. Lincomycin is known to bind to the 50S ribosomal subunit and inhibit protein synthesis. Whether this influences the nature of those proteins that are produced is unclear, and whether these differences would be the same from strain to strain is unknown, but this factor does present a risk in attributing subtle differences in protein structure to gene function when the organisms are cultured in the presence of an antibiotic that affects protein synthesis. None of the previous studies have considered whether the observed immunological differences reside in either the A or B subunits or in both.

In the present study, we compared the subunit structures and immunological relatedness of human and porcine LTs that were produced in the absence of lincomycin. To eliminate any potential host-specific modifications, the LT plasmid of human strain H10407 was transferred to *E. coli* 711, which is the same strain into which the porcine LT genes had been inserted. This provided an isogenic background for the expression of both plasmids.

The bacterial strains employed were: (i) *E. coli* K12 (strain 711 phe trp pro his Str'R Nmr' lac'); (ii) *E. coli* 711 (FILT), a transformed derivative of 711 bearing LT genes of the Ent plasmid from porcine strain P307 (kindly provided by S. Falkow, Stanford University School of Medicine); (iii) *E. coli* HU735 strain C600, which carries a temperature-sensitive F'ts1144lac plasmid into which the kanamycin resistance transposon, Tn5 has been inserted (kindly provided by P. Sansonetti, Walter Reed Army Institute of Research, Washington, D.C.); and (iv) *E. coli* H10407, an enterotoxigenic human isolate (serotype O78:H11) which produces both LT and heat-stable enterotoxin. The parent H10407 was selected for resistance to nalidixic acid and inability to utilize lactose before being used as a recipient in the mating cross HU735 × H10407. This was done to facilitate selection after acquisition of the F'ts1144lac::Tn5 plasmid from F'ts1144lac::Tn5 plasmid from HU735.

The ability to make enterotoxin confers no character of selective value upon strains of *E. coli* which possess the Ent plasmid and produce enterotoxin. Thus, no phenotypic property exists which can be used to monitor the transfer of
FIG. 1. Agarose gel electrophoresis of partially purified lyases of *E. coli* 711 (A), H10407 (C), and the H10407 × 711 transconjugant (B). The transconjugant contained the two large plasmids (ca. 60 × 10⁶ daltons) from H10407. Plasmid DNA was prepared by the procedure of Bolivar and Backman (1) and examined by electrophoresis in 0.7% agarose slab gels (0.04 M Tris, 0.2 M sodium acetate, 0.002 M EDTA, pH 7.8).

the Ent plasmid. To facilitate the selection of transconjugants, the plasmids of a human isolate of *E. coli* H10407 were phenotypically tagged by transposition from an F'tsiac::Tn5 plasmid as described by Sansonetti et al. (17). Tagged plasmids were subsequently transferred by conjugation to K-12 strain 711, and an LT-producing transconjugant was selected (16). The fact that the LT-producing transconjugant, designated 711(10407), did indeed receive plasmid(s) of human strain H10407 was demonstrated by agarose gel electrophoresis (Fig. 1). The transconjugant contained the two large plasmids (ca. 60 × 10⁶ daltons) from H10407. A plasmid of this size has previously been shown to contain the genes coding for enterotoxin production in H10407 (7).

LT was purified by the agarose affinity chromatography technique described by Clements and Finkelstein (3). Both porcine (F1LT) and human (10407-LT) enterotoxins behaved identically during chromatography on and elution from the agarose matrix. Cholera toxin was prepared by the method of Mekalanos et al. (15). The A and B subunits of cholera enterotoxin and of the *E. coli* enterotoxins (F1LT and 10407-LT) were isolated by gel filtration under dissociating conditions as described previously (4, 9). Antisera to cholera enterotoxin subunits and to F1LT were prepared by immunizing goats with 1 mg of purified antigen suspended in 5 ml of Freund complete adjuvant (2, 4). Antiserum to 10407-LT was prepared by immunizing a rabbit with 100 µg of purified 10407-LT suspended in 2 ml of Freund complete adjuvant.

F1LT and 10407-LT, examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under conditions of heating, thiol reduction, and thiol reduction after trypsin activation, behaved identically in terms of subunit number, arrangement, and size. The relative mobilities of the individual subunits were calculated and were found to be identical (Rf = 0.629 [A] and 0.939 [B monomer]). Comigration of the subunits of F1LT and 10407-LT was confirmed by coelectrophoresis of samples (Fig. 2) after heating in SDS (gel 1), thiol reduction (gel 2), and trypsin treatment plus thiol reduction (gel 3). In each case, a single band was present corresponding to the *E. coli* A and *E. coli* B subunits. In addition, each of the enterotoxins was maximally activated by pretreatment with trypsin, and each showed the same relative increase in biological activity when tested on Y-1 adrenal cells. We did not find the differences in migration of the B monomers on SDS-PAGE recently reported by other laboratories. Thus, although Geary et al. (5) and Takeda et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, B64, p. 28) observed differences in migration of B subunits after isolation from organisms of different genetic backgrounds cultured in the presence of lincomycin, in this study the B subunits were found to migrate as a single molecular species in SDS-PAGE after isolating...
FIG. 3. Reactions of monospecific antiserum to F1LT (A, center well) and to 10407-LT (B, center well) with F1LT (wells 1), 10407-LT (wells 4), and isolated subunits from each of the enterotoxins. Wells 2, 3, 5, and 6 contained 10 μg each of F1LT-A, 10407-LT-A, 10407-LT-B, and F1LT-B, respectively. (A) The reaction between antisera to F1LT (center well) and 10407-LT-B (well 5) was spurred over by the precipitin line formed between antisera to F1LT and F1LT-B (well 6), whereas the reaction between antisera to F1LT and 10407-A (well 3) appears to be continuous with the precipitin band formed between antisera to F1LT and F1LT-A (well 2). (B) The reaction between antisera to 10407-LT (center well) and F1LT-B (well 6) was spurred over by the precipitin line formed between antisera to 10407-LT and 10407-LT-B (well 5), whereas the reaction between antisera to 10407-LT and F1LT-A (well 2) appears to be continuous with the precipitin band formed between antisera to 10407-LT and 10407-LT-A (well 3).

The relationship of the F1LT and 10407-LT subunits was further investigated relative to the subunits of cholera toxin. In immunodiffusion reactions which tested F1LT-B and 10407-LT-B against antiserum to cholera B, each of the E. coli B subunits was immunologically identical (Fig. 4). This same reaction was observed when the E. coli A subunits were examined by immunodiffusion against antiserum to cholera A (data not shown). Therefore, each of the E. coli subunits possessed the same shared determinants relative to cholera, regardless of whether they were of porcine or human origin.

This study thus establishes that the immunological dissimilarity of human and porcine LT's described by previous investigators is attributable to differences in the B subunits only; the A subunits were immunologically indistinguishable, whereas the B subunits of the two enterotoxins cross-reacted with a reaction of partial identity. The differences appeared to be confined to the E. coli-specific regions of the molecules since A and B subunits of both E. coli enterotoxins were immunologically identical with respect to antiserum against the corresponding subunits of cholera toxin. Our findings are in accord with those recently described by Holmes et al. (R. K. Holmes, E. M. Twiddy, and M. G. Bramucci, Abstr. 17th Joint Conf. Cholera 1981, p. 43), who examined purified preparations of LT of human and porcine origin by radioimmunoassay and found that they con-

them from organisms grown in the absence of lincomycin and allowing expression of plasmid genes in an isogenic background. Whether the differences observed by others are a result of host-specific modification or a consequence of growth in the presence of lincomycin is not known.

After isolation by chromatography under dissociating conditions, the subunits of F1LT and 10407-LT were compared immunologically by immunodiffusion against homologous and heterologous antisera to each of the enterotoxins (Fig. 3). In Fig. 3A, the reaction between antisera to F1LT (center well) and 10407-LT-B (well 5) was spurred over by the precipitin line formed between antisera to F1LT and F1LT-B (well 6), whereas the reaction between antisera to F1LT and 10407-A (well 3) appeared to be continuous with the precipitin band formed between antisera to F1LT and F1LT-A (well 2). In Fig. 3B, the reaction between antisera to 10407-LT (center well) and F1LT-B (well 6) was spurred over by the precipitin line formed between antisera to 10407-LT and 10407-LT-B (well 5), whereas the reaction between antisera to 10407-LT and F1LT-A (well 2) appeared to be continuous with the precipitin band formed between antisera to 10407-LT and 10407-LT-A (well 3). Thus, with these antisera, the A subunits of F1LT and 10407-LT appeared to be immunologically identical, but each of the B subunits possessed unique and shared antigenic determinants.
tain nearly identical A polypeptide determinants and B determinants that were only partially cross-reacting.

It remains to be determined whether the immunological differences between porcine and human LTs affect their value as immunogens to provide protection against strains isolated from different species. We have shown that immunization with porcine LT given either alone or as a component of a cross-linked ST-LT vaccine provides equal, strong protection against multiple, heterologous serotypes of human LT-producing strains (10, 11). Whether human LT will prove to be more effective in providing protection against human strains is currently under investigation.

This study was supported by a grant from Johnson and Johnson Baby Products Co., Raritan, N.J.

LITERATURE CITED


