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Cistrons Encoding *Escherichia coli* Heat-Labile Toxin

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The structure and products of the two cistrons encoding the *Escherichia coli* heat-labile toxin (LT) were studied. The LT deoxyribonucleic acid (DNA) region had been isolated as part of a DNA fragment from the plasmid P307, and this fragment was joined to the cloning vector pBR313. Deletion mutations of various lengths were introduced into the LT DNA region and into the adjacent DNA sequences. Analysis of the deletions indicated that the maximum size of the LT DNA region was 1.2×10^6 daltons. Two proteins of 11,500 daltons and 25,500 daltons had been shown to be encoded by the LT DNA region. The functions of these LT gene products were investigated. The 11,500-dalton protein had an adsorption activity for Y-1 adrenal cells, and this protein was shown to form aggregates of four or five monomers. The 25,500-dalton protein was shown to have an adenylate cyclase-activating activity. The two cistrons encoding for each of the LT proteins have been located on a genetic map of the LT DNA region. Both cistrons are probably transcribed from the same promoter.

Escherichia coli that cause a cholera-like disease in humans and farm animals elaborate two distinct enterotoxins (15). One toxin shares partial immunological identity with the enterotoxin produced by *Vibrio cholerae* (2, 14). This *E. coli* toxin is known as the heat-labile toxin (LT), and it also shares several biochemical properties with cholera toxin, including its mechanism of action (10, 11). Although LT has been studied for the past 10 years, the molecular nature of this toxin has largely remained an enigma. We reasoned that it would simplify a complex problem to study LT in a defined genetic system. The LT DNA region was known to be a part of large, transmissible plasmids, and these plasmids could be transferred from wild-type *E. coli* into the extensively characterized *E. coli* K-12 strain (22). However, the plasmids that encode LT are large, at least 50×10^6 daltons in mass (23). These plasmids have a coding capacity for about 100 average-size proteins (30,000 daltons per protein). An ideal situation would be to isolate the LT DNA region on a small carrier plasmid and to introduce this genome into *E. coli* K-12.

So et al. (24) used molecular cloning to isolate the LT DNA region on a 5.8×10^6-dalton DNA fragment and inserted it onto the carrier plasmid pBR322. *E. coli* carrying the cloned toxin were found to be hypertoxin producers due to the amplification of the LT DNA region. Dallas and Falkow (4) identified two LT gene-encoded proteins by analyzing the cloned LT DNA region in minicells. In this communication, we described the localization of the LT cistrons on a genetic map of the LT DNA region. We have determined that the maximum size of the LT DNA region is 1.2×10^6 daltons. We also present data that corroborate our earlier report on the molecular nature of LT, and we describe several properties of the LT components.

MATERIALS AND METHODS

Bacterial strains and plasmids. All strains used were derivatives of *E. coli* K-12. C600 is *thr leu thi lac supE*. DS410 is *thr thi minA minB rpsL*. EWD030 is a recombinant plasmid that encodes LT synthesis and ampicillin resistance (24). pBR313 and pBR322 encode for ampicillin resistance and tetracycline resistance (1).

Isolation of plasmid DNA. Plasmid DNA was isolated as described by So et al. (24).

Restriction enzyme reactions. All restriction enzymes were obtained from Miles Laboratories, and complete digestion conditions were performed as specified by the supplier. Partial plasmid digest reaction conditions were determined empirically and varied with the enzyme batch. Partial digests were obtained by varying the time, temperature, enzyme concentration, and DNA concentration of the reaction mixture.

DNA gel electrophoresis. Vertical agarose gels were run according to the method described by Greene et al. (13). Vertical gels of polyacrylamide were formed with 30% acrylamide and 0.8% *N,N'*-methylene-bisacrylamide (Biorad) and were polymerized according to the method described by Laemmli (17). Gels were

electrophoresed at 120 V in Tris-borate buffer (89 mM Tris base, 2.5 mM EDTA, 89 mM boric acid) and stained in a 25-μg/ml solution of ethidium bromide. The molecular mass of specific DNA fragments was determined using *Eco*RI-cleaved F plasmid DNA (E. Ohtsubo, personal communication) and *Hin*dIII-cleaved simian virus 40 DNA (19) as standards.

Ligation conditions. Polynucleotide ligase from bacteriophage T4-infected cells was a gift from H. W. Boyer. Ligation reaction conditions were as described by So et al. (24). The restriction enzymes *Eco*RI, *Bam*HI, and *Hin*dIII make staggered DNA strand breaks, and the DNA fragments have unpaired, complementary bases at each end. Ligation reactions using these cohesive-ended DNA fragments were carried out at 13°C for 18 h. The restriction enzymes *Hpa*I and *Hin*cII make DNA strand breaks that produce fragments in which all nucleotides are base paired. Ligation reactions using these flush-ended fragments were carried out at 20°C for 18 h (20).

Transformation. Plasmid DNA was introduced into bacterial strains according to the method described by Lederberg and Cohen (18).

Toxin determination. The assay for LT activity was essentially as described by Donta et al. (5).

Minicell preparation. Minicells were isolated and proteins were radioisotopically labeled according to the method described by Dougan and Sherratt (6).

Polyacrylamide gel electrophoresis. Polyacrylamide protein gels were prepared according to the method of Laemmli (17). Typically, 10 to 15% linear-gradient sodium dodecyl sulfate (SDS)-polyacrylamide gels were prepared. Gels were developed at 20 mA for 2 h and then 30 mA for the remainder of the electrophoresis. Current was constant. After staining the gels were dried onto Whatman 3MM filter paper, and Kodac X-Omat R film was exposed for from 3 days to 3 weeks at room temperature. Molecular mass standards were phosphorylase *b* (rabbit muscle), 100,000 daltons; catalase (beef liver), 58,500 daltons; alcohol dehydrogenase (horse liver), 41,000 daltons; DNase I (bovine pancrease), 31,000; RNase A (bovine pancrease), 13,600 daltons (Boehringer). Cholera toxin (Schwarz/Mann) was also used as a standard.

Adenylate cyclase-activating activity. Extracts of *E. coli* harboring various plasmids were prepared as outlined by Evans et al. (7). Pigeon erythrocyte lysate assays were performed as described by Gill and King (11). Sizes of the enzymatically active proteins were determined by fractionating the polymyxin extracts on polyacrylamide gels. Proteins in the extract were precipitated by ammonium sulfate (75%) and redissolved in 0.5% SDS-5 mM dithiothreitol. A volume corresponding to about 10 ml of the original culture was mixed with dansylated myoglobin (12) and fractionated on a 7.5 to 15% SDS-polyacrylamide gel (17). The positions occupied by dansylated myoglobin (17,000) and myoglobin dimer (34,000) were marked under UV illumination. The interval between these markers was excised and sliced into 21 1-mm segments. Each slice was soaked overnight in 0.2 ml of saline containing 0.1% ovalbumin and 2 mM dithiothreitol. The extracts were assayed for their ability to activate adenylate cyclase by incubating 5 μl for 30 min at 25°C with 45 μl of a freshly prepared pigeon erythrocyte lysate supplemented with 5 mM nicotinamide adenine dinucleotide, 5 mM ATP, and 0.025% SDS (12). The positions marked for subunit A and fragment A_1 of cholera toxin were obtained by alignment with parallel gel tracks that were stained, a procedure that is uncertain to about one half a slice.

Guidelines employed for recombinant DNA experiments. The experiments reported here were performed using P2-EK1 conditions as specified in the *Guidelines for Recombinant DNA Technology* published by the U.S. National Institutes of Health.

RESULTS

Isolation of EWD020. The cloned LT DNA region was recloned from EWD030 into the larger cloning vehicle pBR313 (Fig. 1). EWD030 and pBR313 were cut with *Bam*HI, and the DNA fragments were mixed and joined by T4 ligase. The ligation mixture was used to transform C600, and ampicillin-resistant (Ap^r), tetracycline-sensitive transformants were identified. These transformants were assayed for LT production using the Y-1 adrenal cell system. Plasmid DNA was isolated from several LT^+ transformants, the DNA was cleaved with *Bam*HI, and the DNA fragments were separated on a 0.7% agarose gel. The identity of the cloning vehicle moiety of the LT^+ plasmids was easily determined since pBR313 was 5.8×10^6 daltons in mass and pBR322 (the cloning vector portion of EWD030) was 2.6×10^6 daltons in mass (1). A plasmid composed of pBR313 and the cloned LT DNA region (5.8×10^6 daltons) was isolated and designated EWD020 (Fig. 1). EWD020 had two *Eco*RI recognition sites: one in pBR313 and one in the cloned DNA. The insertion orientation of the cloned DNA in pBR313 could be determined since the two *Eco*RI sites were located asymmetrically (Fig. 1). Recombinant molecules containing both orientations of the cloned DNA were identified. It was found that the LT phenotype was expressed regardless of the orientation of the cloned DNA in pBR313. This result suggested that the LT cistrons were transcribed from their original promoter.

Isolation of deletion derivatives of EWD020. A more precise location of the LT cistrons on EWD020 was determined by analyzing deletion derivatives of the plasmid. Specific DNA fragments were deleted from EWD020, and the effects of the deletions on the expression of the LT cistrons were determined. The restriction enzyme *Hpa*I cut EWD020 into two fragments (Fig. 1). The smaller fragment (3.0×10^6 daltons) could be deleted from the plasmid without affecting the expression of ampicillin resistance or plasmid replication (1). EWD020 was cleaved with *Hpa*I, the fragment ends were

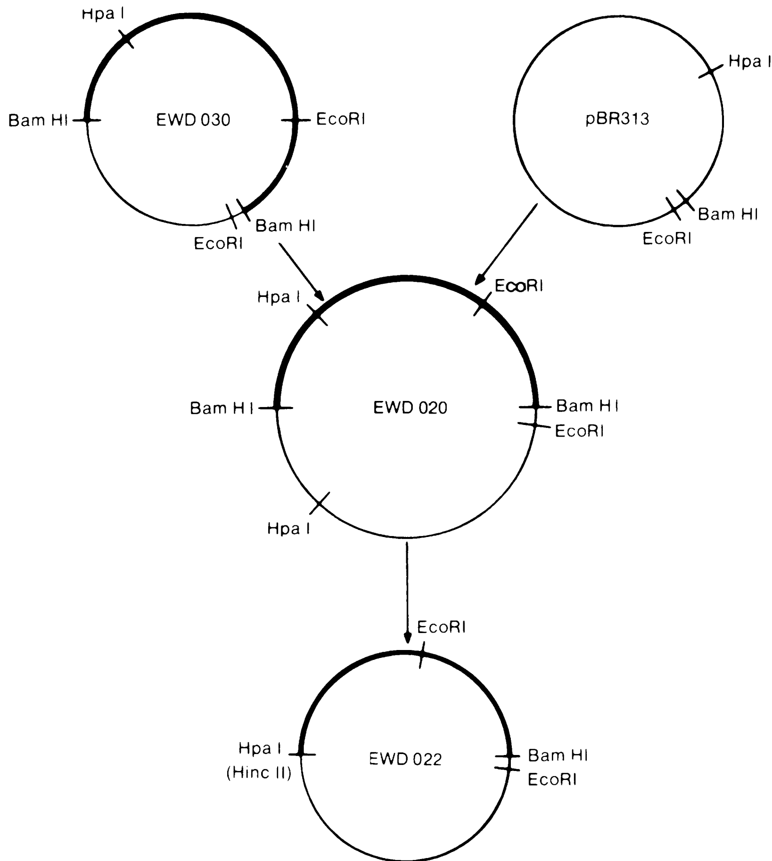

FIG. 1. *Diagram showing the derivation of EWD022. The heavy line denotes cloned LT DNA from P307, and the light line denotes cloning vehicle DNA. The 5.8 × 10⁶-dalton DNA fragment from EWD030 was recloned into pBR313. This hybrid plasmid was designated EWD020. The small HpaI DNA fragment of EWD020 was deleted using HpaI to form the plasmid EWD022, which was found to encode LT. The DNA recognition site for HincII is contained in the DNA recognition site for HpaI; therefore, all HpaI recognition sites are cleaved by HincII.*

joined by T4 ligase, and the ligation mix was used to transform C600. Plasmid DNA was isolated from 10 Ap^r transformants, the DNA was cut with *Hpa*I, and the DNA fragments were separated on a 0.7% agarose gel. Nine of the transformants had a deletion of the 3.0×10^6-dalton *Hpa*I fragment. The deletion plasmid was designated EWD022 (Fig. 1), and strains carrying the plasmid were found to synthesize LT by the Y-1 adrenal cell assay system.

The restriction enzyme *Hinc*II cleaved EWD022 into eight fragments (Fig. 2). The molecular masses of the fragments were 2.30×10^6, 1.90×10^6, 1.65×10^6, 1.55×10^6, 0.61×10^6, 0.47×10^6, 0.19×10^6, and 0.16×10^6 daltons, respectively. Two *Hinc*II sites were known to be located in the cloning vehicle (1). Specific *Hinc*II DNA fragments were deleted from EWD022 by making partial plasmid digests. EWD022 DNA fragments composed of two or more contiguous *Hinc*II fragments were generated by altering the *Hinc*II enzyme reaction conditions. The partial plasmid digests were ligated and transformed into C600. Deletion plasmids were identified by isolating plasmid DNA from Ap^r transformants, cutting the DNA with *Hinc*II, and separating the DNA fragments on a 7% polyacrylamide gel. The four deletion plasmids shown in Fig. 2 were identified among 80 Ap^r transformants that were screened. *E. coli* carrying EWD235 were found to be LT^+, and the plasmid had a 1.9×10^6-dalton DNA fragment deleted. Strains carrying the plasmids EWD200, EWD210, and EWD247 were all phenotypically LT^-. EWD200 had 0.47×10^6- and 0.19×10^6-dalton fragments deleted, EWD210 had a 0.16×10^6-dalton fragment deleted, and EWD247 had 1.9×10^6- and 0.61×10^6-dalton fragments deleted.

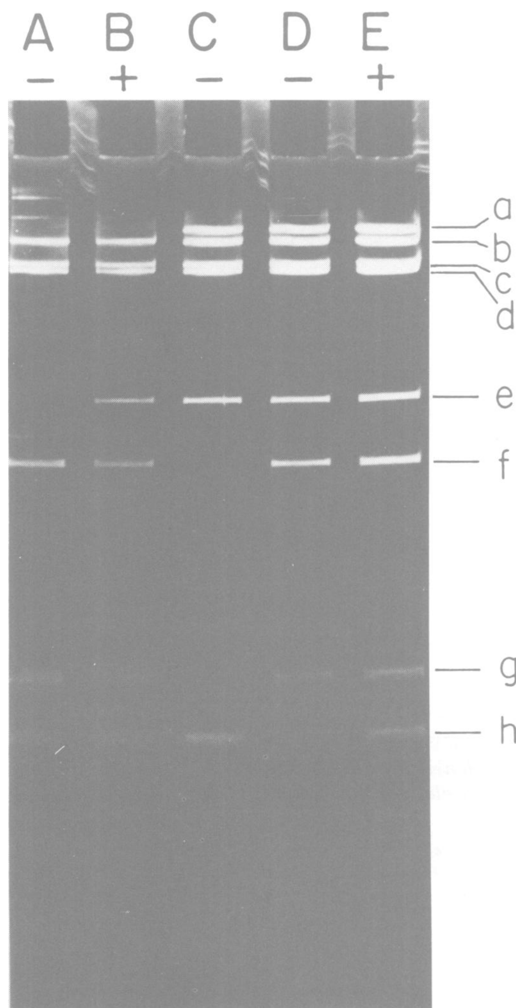

Fig. 2. *HincII cleavage of EWD022 and HincII deletion plasmids. Plasmid DNA was purified, cut with HincII, and electrophoresed through a vertical 7% polyacrylamide gel: (A) EWD247, (B) EWD235, (C) EWD200, (D) EWD210, (E) EWD022. The + or − indicates whether the plasmid encoded functional LT as assayed in the Y-1 adrenal cell test. All plasmids were constructed from EWD022 by deleting one or more HincII DNA fragments.*

Similarly, *Hin*dIII-generated DNA fragments were also deleted from EWD022. Two unique deletion plasmids were isolated. C600 carrying the plasmid EWD300 were phenotypically LT^+, and C600 carrying EWD306 were phenotypically LT^-. EWD300 had a 1.7×10^6-dalton fragment deleted and EWD306 had a 2.5×10^6-dalton fragment deleted. The *Hinc*II and *Hin*dIII recognition sites were located on EWD022 and are schematically shown in Fig. 3.

Only two deletion plasmids derived from EWD022 converted C600 to the LT^+ phenotype. EWD235 had a 1.9×10^6-dalton *Hinc*II fragment deleted, and EWD300 had a 1.7×10^6-dalton *Hin*dIII fragment deleted. These two DNA fragments did not overlap each other. A plasmid in which both of these fragments were deleted would result in an LT^+ plasmid which contained only 1.2×10^6 daltons of cloned DNA. EWD235, which contained three *Hin*dIII sites, required only a deletion of the 1.7×10^6-dalton *Hin*dIII fragment to form such an LT^+ plasmid. Consequently, EWD235 was cut with *Hin*dIII using enzyme reaction conditions that made partial plasmid digests, and the DNA fragments were ligated with T4 ligase. EWD235 also contained a single *Bam*HI site that was located in the 1.7 $\times 10^6$-dalton *Hin*dIII DNA fragment. Plasmids with this DNA fragment deleted would not be linearized after digestion with *Bam*HI. Since linear molecules transform at 1/100 to 1/1,000 the frequency of circular DNA molecules (3), cleavage of the ligated molecules with *Bam*HI would be expected to increase the frequency with which deletion plasmids were isolated from a transformant population. The ligation mixture from the *Hin*dIII partial digestion experiment was cut with *Bam*HI and then used to transform *E. coli* C600. A plasmid was isolated that contained only the cloning vehicle and the 1.2×10^6-dalton LT DNA region. This plasmid, EWD299, converted C600 to the LT^+ phenotype.

EWD299 was shown to have two *Eco*RI recognition sites and two *Hin*dIII recognition sites. A further deletion of EWD299 was made by deleting the DNA bounded by the two *Eco*RI sites using methods that already have been described. This new plasmid was phenotypically LT^- and was designated EWD500. Likewise, the DNA bounded by the two *Hin*dIII sites in EWD299 was deleted; the plasmid generated was also phenotypically LT^-, and it was designated EWD501. Figure 4 is a schematic diagram that identifies the relative position of each deletion and indicates whether strains carrying a specific plasmid synthesize LT.

Effects of plasmid deletions on LT expression. We recently reported LT to be a multimeric toxin composed of two proteins of 25,500 daltons (25.5K) and 11,500 daltons (11.5K) (4). Plasmid deletions that did not affect LT expression would not be expected to affect either of these two proteins, but deletions that did affect LT expression might be expected to affect one or both of these proteins. The effect of a given deletion on the LT proteins could be established by analyzing plasmid-specified proteins in minicells (8) (Fig. 5). It was found that

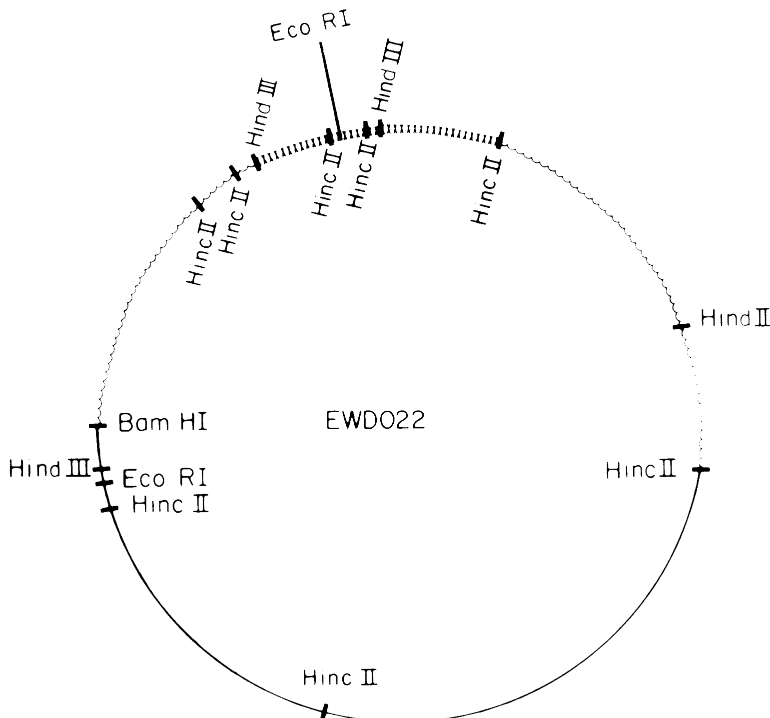

FIG. 3. *Diagram of restriction enzyme recognition sites in EWD022. The solid line represents DNA from the cloning vehicle, pBR313. The wavy line denotes DNA cloned from P307. The crossed line denotes that region of the cloned DNA that contained the LT DNA region. Deletions that extended into this region resulted in the loss of the LT⁺ phenotype.*

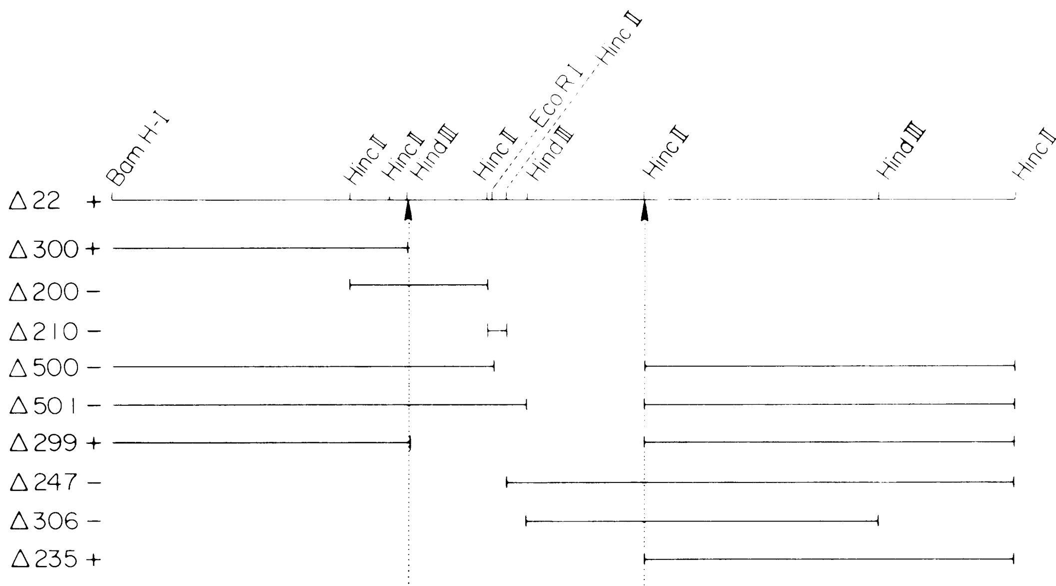

FIG. 4. *Diagram showing the relative positions of deletions in EWD022. Generation of the deletion plasmid derivatives is explained in the text. The designation for each plasmid is shown on the far left and the + or − indicates whether the plasmid encoded functional LT. Only the DNA fragment originally cloned from P307 is shown. Deleted regions are shown by solid lines, and restriction enzyme recognition sites are also shown. The LT DNA region was found to be located between the dotted lines. This region is 1.2×10^6 daltons in mass.*

all LT^+ plasmids encoded the 11.5K protein but none of the LT^- plasmids encoded this protein (Table 1). It was not possible to make such a direct determination for the 25.5K protein. The presence of this protein was usually obscured on the autoradiographs by the presence of β-lactamase, another plasmid-specified protein, which has a molecular mass of 26K (6). Careful analysis of the proteins specified by the LT^+ plasmids revealed that all LT^+ plasmids also encoded a 21.5K protein (Fig. 5). The effect of a deletion on the 25.5K protein was determined by assaying for adenylate cyclase-activating activity. Gill et al. (10) had demonstrated that crude LT preparations had an adenylate cyclase-activating activity. Extracts were prepared from strains carrying LT^+ or LT^- deletion plasmids, and the samples were tested in pigeon erythrocyte lysates for adenylate cyclase-activating activity (Table 2). All LT^+ plasmids were found to encode an adenylate cyclase-activating activity. In addition, several of the phenotypically LT^-

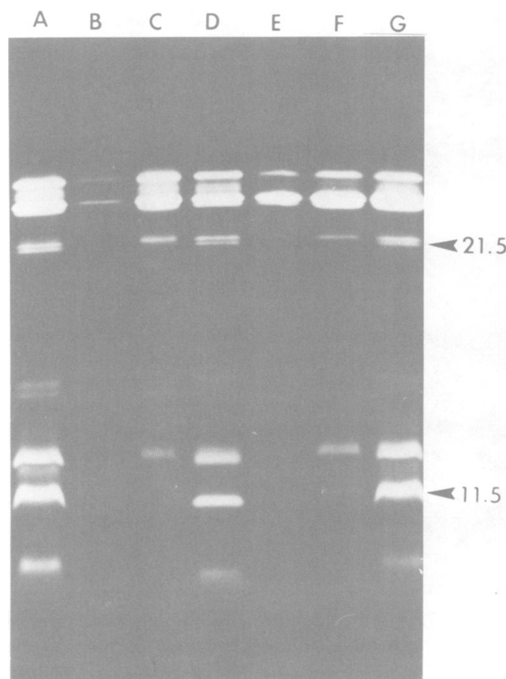

FIG. 5. *Autoradiograph of a polyacrylamide gel containing minicell plasmid proteins encoded by the deletion plasmids. Minicell samples were prepared as described in the text and all samples were fully denatured by boiling in 0.3% SDS in the presence of 3% β-mercaptoethanol: (A) EWD300, (B) EWD200, (C) EWD210, (D) EWD299, (E) EWD247, (F) EWD306, and (G) EWD235. Two proteins of masses 21.5K and 11.5K were always encoded by LT^+ plasmids.*

TABLE 1. *Properties encoded by deletion plasmids*

Plasmid	11.5K protein	Toxin test	Adenylate cyclase test
EWD235	+	+	+
EWD306[a]	−	−	−
EWD247[a]	−	−	−
EWD300	+	+	+
EWD200[b]	−	−	+
EWD500[b]	−	−	+
EWD210[b]	−	−	+
EWD501[b]	−	−	−
EWD299	+	+	+

[a] LT^- plasmids with deletions extending from the right into the LT DNA region (Fig. 4).

[b] LT^- plasmids with deletions extending from the left into the LT DNA region (Fig. 4).

TABLE 2. *Plasmid-encoded adenylate cyclase-activating activity*

Plasmid	Activity equivalent (ng of cholera toxin)[a]
EWD022	30
EWD299	45
EWD235	10
EWD306	0
EWD247	0
EWD300	60
EWD200	15
EWD500	15
EWD210	540
EWD501	0
pBR313	0

[a] Amount of cholera toxin (nanograms) that would have given the same enzymic activity as the LT released from 50 ml of culture.

plasmids were also shown to encode for an adenylate cyclase-activating activity. An estimate of the molecular mass of the protein with an adenylate cyclase-activating activity was determined by resolving the proteins in the LT extracts on SDS-polyacrylamide gels, fractionating the gels, and assaying each fraction for adenylate cyclase-activating activity (Fig. 6). Two peaks of activity were found. One peak corresponded to a protein of 25.5K, and the other (minor) peak corresponded to a protein 21.5K in mass. These results suggested that the 25.5K protein had an adenylate cyclase-activating activity.

Aggregation of the 11.5K protein. Minicell protein preparations were fully reduced and denatured before electrophoresis. When samples from minicells carrying EWD299 were not fully denatured before electrophoresis, a new protein band was observed on autoradiographs (Fig. 7). The new band migrated at an apparent molecular mass of 49,000 daltons. Concomitant with the appearance of this band, the intensity of the band at 11.5K decreased. This result suggested

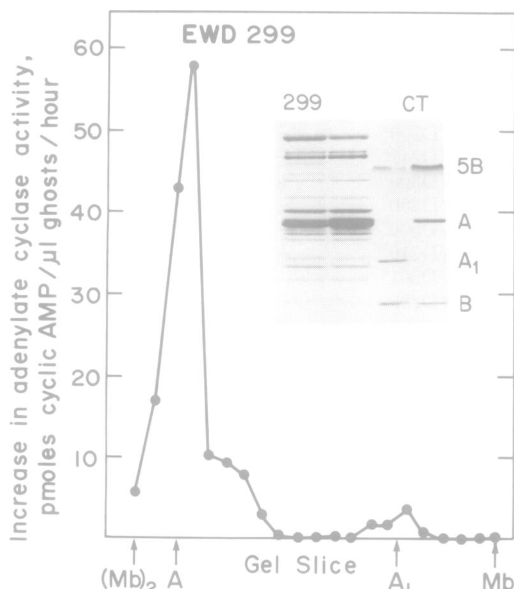

FIG. 6. *Adenylate cyclase-activating activity profile of an extract from a strain carrying EWD299. Adenylate cyclase activity was fractionated and assayed as described in the text. Myoglobin (Mb) and its dimer form [$(Mb)_2$] were used as molecular mass markers. The inset shows the stained gel before slicing. The wells marked CT contain cholera toxin either totally denatured or partially denatured. The letter designations are as follows: (5B) five aggregated B subunits; (A) unreduced subunit A; (A_1) fully reduced subunit A enzymatically active species; (B) fully disaggregated subunit B.*

that the 11.5K protein formed aggregates of four or five monomers that were stable in 0.3% SDS at room temperature.

DISCUSSION

The LT DNA region had been isolated by molecular cloning as part of a 5.8×10^6-dalton DNA fragment (24). To determine the size of the LT gene(s), we first reduced the size of the cloned LT DNA. By using in vitro deletion techniques, the cloned LT DNA was reduced in size from 5.8×10^6 daltons to 1.2×10^6 daltons. This small DNA fragment had a coding capacity for proteins of total mass of 60,000 daltons. Plasmids were also isolated which no longer encoded functional LT as measured with Y-1 adrenal cells. It was not known whether these deletion plasmids had an intact structural gene(s) and were mutated in controlling elements or whether they had a deletion in the LT structural gene(s).

The proteins encoded by the LT cistrons had been identified using an antiserum prepared against a crude LT preparation (4). An LT^+ plasmid, EWD299, was introduced into minicells, and the plasmid-encoded proteins were radioisotopically labeled. Two proteins of molecular mass 25.5K and 11.5K were found to react specifically with anti-LT serum. The two LT proteins were also found to react with anti-cholera toxin serum and anti-cholera toxin subunit B serum (4). These results indicated that LT was a multimeric protein composed of a 25.5K subunit and an 11.5K subunit. The data also indicated that LT formed a stable complex of the two subunits inside the cell (a cytoplasmic and periplasmic minicell fraction was used in the experiment). The 11.5K protein was found to be present in greater amounts than the 25.5K protein, as judged from the band intensities on the autoradiographs. This could be explained if the

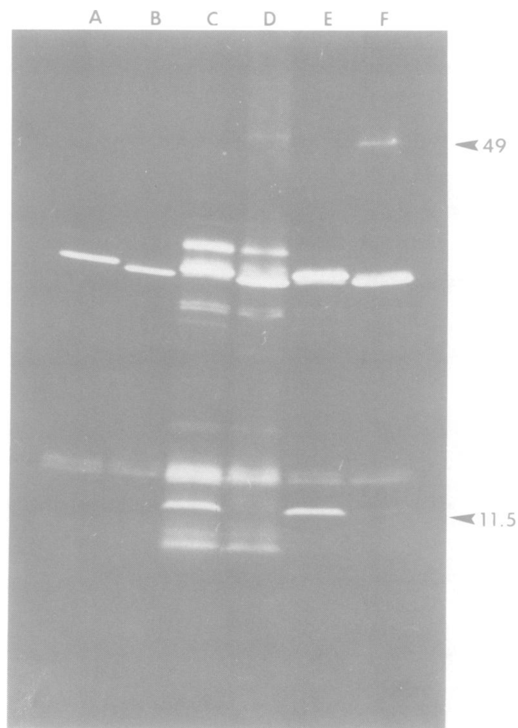

FIG. 7. *Autoradiograph of a polyacrylamide gel containing minicell plasmid protein either fully denatured or partially denatured. Minicell samples were prepared as described in the text. Half of each sample was boiled for 3 min in final sample buffer (fully denatured), and the other half was treated at room temperature with a final sample buffer that did not contain β-mercaptoethanol (partially denatured): (A) pBR313, fully denatured; (B) pBR313, partially denatured; (C) EWD299, fully denatured; (D) EWD299, partially denatured. Tracks E and F contain a cytoplasmic-periplasmic minicell fraction: (E) EWD299, fully denatured; (F) EWD299, partially denatured.*

11.5K protein incorporated more radioisotope or if more of the 11.5K protein was synthesized as compared to the 25.5K protein. LT could be composed of several 11.5K proteins for every 25.5K protein, a situation identical to cholera toxin (9). Mild denaturing conditions (SDS at room temperature) dissociate subunit B from cholera toxin as a multimeric aggregate. When cytoplasmic LT^+ minicell fractions were treated at room temperature with SDS, a new band appeared on the autoradiographs at an apparent mass of 49,000 daltons. Concomitant with the appearance of this band, the intensity of the 11.5K band decreased. This result suggested that the 11.5K protein formed a stable aggregate of four or five monomers and that the native toxin might be composed of five 11.5K proteins and one 25.5K protein—a structure similar to cholera toxin (9). An assay that requires the function of both cholera toxin subunits is the Y-1 adrenal cell system. In this assay, subunit B binds the holotoxin to the target cell membrane and subunit A activates adenylate cyclase (11). The net result is rounding of the tissue culture cells. If the 11.5K protein were functionally analogous to cholera toxin subunit B, plasmids that did not encode the 11.5K protein would not be expected to encode a Y-1 cell-rounding activity. This was indeed found to be the case (Table 1). These data and data presented elsewhere (4) indicated that the 11.5K protein was structurally, functionally, and immunologically similar to cholera toxin subunit B. We consistently noted, however, that the mass of the LT protein was 500 daltons larger than cholera toxin subunit B. Similarly, a 25.5K protein was identified that was functionally similar to cholera toxin subunit A. However, unlike cholera toxin subunit A, the 25.5K protein was not split into two peptides in the presence of β-mercaptoethanol (at least not quantitatively). A 21.5K protein that also had an adenylate cyclase-activating activity was identified. This protein is probably a breakdown product of the 25.5K protein (either by proteolysis or reduction). It is possible that a small peptide released from the 25.5K protein was not discerned on our polyacrylamide gels. In summary, our results indicated that cholera toxin and LT share structural similarities. An LT composed of five 11.5K proteins and one 25.5K protein would have a molecular mass of 83,000 daltons. A comparison of cholera toxin and LT properties is summarized in Table 3.

A model showing the arrangement of the LT proteins on a schematic diagram of the LT DNA region is presented in Fig. 8. Deletions extending into the LT DNA region from the right abolish both toxin activity and adenylate cyclase-activating activity (Table 1). These results are consistent with the location of a common LT promoter on the right side of the LT DNA region. A deletion of the promoter would prevent transcription of both proteins. When the left end of the LT DNA region is deleted, the 11.5K protein is no longer synthesized (Table 1; plasmids are arranged in order of increased deletion of the left end of the LT DNA region). Deletions extending further into the LT DNA region abolish adenylate cyclase-activating activity. These results are consistent with the location of the 25.5K protein-encoding cistron proximal to the promoter.

The similarities between cholera toxin and LT

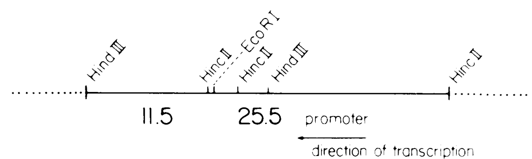

FIG. 8. *Diagram of the LT DNA region. The approximate position of the cistrons for the LT proteins is shown, and the relative position of the promoter for the LT cistrons is indicated.*

TABLE 3. *Properties of cholera toxin and LT*

Protein	Property	Cholera toxin	LT
Entire	Molecular mass	84,000	Predicted to be 83,000
Subunits			
A	Mass	28,000	25,500
	Secondary structure	24,000 and 5,000 polypeptides linked by a disulfide bond	None
	Activity	Adenylate cyclase activating	Adenylate cyclase activating
B	Mass	11,000[a]	11,500
	Immunogenicity	+	+
	Activity	Adsorption	Adsorption
	Aggregate formation	5 molecules	4–5 molecules

[a] The primary amino acid sequence has been determined and the compositional molecular mass is 11,590 daltons; however, this protein migrates at an apparent molecular mass of 11,000 daltons in our gel system (16).

pose an intriguing question. Did *E. coli* and *V. cholerae* at one time exchange genetic information? We now have the genetic knowledge and technology to answer this question. If the bacteria did exchange toxin determinants, there should be DNA sequence homology between the two toxin genes. Using DNA fragments from only the LT DNA region as hybridization probes, the relatedness of the toxin genes can be determined. The amino acid sequence of cholera toxin subunit B has been determined (16). Now that the 11.5K LT cistron has been located, the primary structure of the 11.5K protein can be determined by nucleotide sequencing, and a direct comparison of the primary structure of the two proteins should be possible.

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