INFECTION AND IMMUNITY, May 1987, p. 1321–1323
0019-9567/87/051321-03$02.00/0

Vol. 55, No. 5

NOTES

Expression of the S-1 Catalytic Subunit of Pertussis Toxin in *Escherichia coli*

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Received 29 September 1986/Accepted 15 January 1987

The S-1 subunit of pertussis toxin was expressed as a fusion protein in a strain of *Escherichia coli* deficient in protein degradation. The fusion protein reacted with anti-pertussis toxin antibody, and, like authentic pertussis toxin, it ADP-ribosylated a 41,000-molecular-weight membrane protein from human erythrocytes.

Pertussis toxin (molecular weight, 105,060) is composed of noncovalently associated subunits designated S-1, S-2, S-3, S-4, and S-5 (M_rs of 26,220, 21,920, 21,860, 12,060, and 10,940, respectively) (7, 8). The toxin contains one copy of each subunit except S-4, which is present in two copies. The S-1 subunit catalyzes transfer of the ADP-ribose moiety of NAD to the α subunit of the Gi regulatory component of the adenylate cyclase complex (8). ADP-ribosylation of Gi decreases the response to inhibitory agents of adenylate cyclase and increases the response to stimulatory agents (2).

Recently, two laboratories have cloned and sequenced the pertussis toxin genes (3, 4, 7), but attempts to express the gene products in *Escherichia coli* were unsuccessful (3). Possible explanations for the lack of expression include unusual codon usage and improper spacing of RNA polymerase binding sites.

We have expressed the S-1 subunit as a fusion protein in *E. coli*. This protein contains the first six amino acids of beta-galactosidase and five amino acids from the pUC18 polylinker followed by amino acids 2 through 235 of the S-1 subunit. Expression of this fusion protein is controlled by the beta-galactosidase promoter, thus eliminating the requirement for the transactivation gene product that promotes expression of pertussis toxin in *Bordetella pertussis* (9).

A 4.7-kilobase *Eco*RI DNA fragment which contained the entire pertussis toxin genome has been subcloned into pEMBL8$^+$ (7) and was used as the source of DNA for the construction of the S-1 fusion protein. This plasmid has been designated pT101 (R. Rappuoli, personal communication). (Rappouoli has also expressed a fusion protein of S-1 protein by using an RNA polymerase fusion gene in *E. coli* [submitted for publication].) The S-1 fusion gene was constructed in two steps. First, a *Pst*I fragment which contained the entire S-1 gene and the 5′ portion of S-2 was isolated from pT101 and digested with *Sau*3A and *Sal*I. The digested fragments were then subcloned into pUC18 (10; purchased from Pharmacia, Inc., Piscataway, N.J.), which had been digested with *Bam*HI and *Sal*I. A transformant was isolated which contained the *Sau*3A-*Sal*I gene fragment encoding for amino acids 2 through 106 of the S-1 subunit. This transformant was termed pUCSau/Sal. Next, a *Sph*I gene fragment which contained the complementary 3′-terminal region of the S-1 gene was isolated from the original *Pst*I fragment and inserted into pUCSau/Sal, which had been digested with the same enzyme. Restriction endonuclease analysis identified a transformant which possessed the *Sph*I fragment in the proper orientation relative to the 5′ S-1 gene sequence. This transformant was designated pUCS-1 (Fig. 1).

When pUCS-1 was transformed into *E. coli* JM103 (5), the tranformants produced the S-1 subunit, but expression was low (Fig. 2). The presence of lower-molecular-weight immunoreactive peptides suggested that the fusion protein produced was being degraded within its surrogate host. In other studies, we have observed that certain deletion mutants of *Pseudomonas aeruginosa* exotoxin A were unstable in *E. coli* JM103 but stable in a host strain mutated in the *htp*R and *lon* genes, the products of which are involved in intracellular protein degradation (1; C. Guidi-Rontani and R. J. Collier, unpublished results). When pUCS-1 was transformed into *E. coli* LC137 [*htpR*(AmTs) *lonR9*(Ts) *lac*(Am) *trp*(Am) *pho*(Am) *rpsL supC*(Ts) *mal*(Am) *tsx*::Tn*10*], obtained from Alfred L. Goldberg, Harvard Medical School, yields of S-1 subunit obtained were at least 10-fold higher than those obtained with *E. coli* JM103 as determined by Western blot analysis. Also, we did not detect lower-molecular-weight peptides immunoreactive with anti-pertussis toxin. During this experiment, *E. coli* JM103 grew to an A_{595} of 2.3, while *E. coli* LC137 grew to an A_{595} of 0.9. Cells grew at the same rate with or without either pUCS-1 or isopropylthiogalactoside (IPTG). In *E. coli* LC137, the S-1 subunit was expressed in the presence or absence of IPTG, which is consistent with the fact that this strain did not possess the *lac* I_q mutation. This mutation causes overproduction of the *lac* repressor and consequent repression of the beta-galactosidase promoter on the high-copy-number pUC plasmid.

When cell extracts were subjected to differential centrifugation, 95% of the S-1 subunit was found in the particulate fraction (Fig. 3). Insoluble S-1 subunits could be solubilized (90%) by dissolving the particulate fraction in 7 M urea and then dialyzing it in 0.1 M $NaHCO_3$.

The solubilized S-1 subunit exhibited ADP-ribosyltransferase activity with the same specificity as that of authentic

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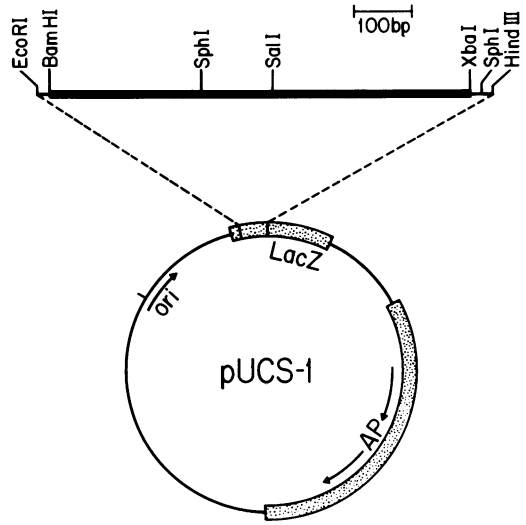

FIG. 1. Restriction map of pUCS-1. The highlighted portion of the expanded segment of DNA encodes for amino acids 2 through 235 of the S-1 subunit (*Bam*HI to *Xba*I). Restriction sites flanking the S-1 structural gene originated from the pUC18 vector. bp, Base pairs.

pertussis toxin; both the fusion protein and pertussis toxin ADP-ribosylated a 41,000-molecular-weight protein in human erythrocyte membranes (6) corresponding to the α subunit of the Gi protein of adenylate cyclase (Fig. 4). The apparent specific activity of the fusion protein was about 25% of that of authentic pertussis toxin. Specific activity was measured as the amount of Gi protein ADP-ribosylated per equivalent of S-1 protein under controlled conditions. After

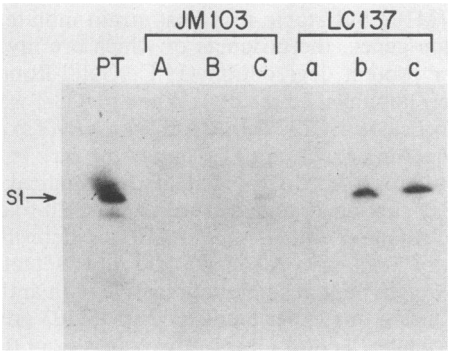

FIG. 2. Expression of S-1 subunit in *E. coli* JM103 and LC137 transformed with pUCS-1. Cultures of *E. coli* JM103 or LC137, with or without pUCS-1, were prepared by 100-fold dilution of overnight cultures and grown for 2 h at 32°C. IPTG (1 mM final concentration) was added to certain of the cultures, and 4 h later, the cells were harvested. Cell growth reached A_{595} values of 2.3 for *E. coli* JM103 and 0.9 for LC137. Growth rates were the same in the presence and absence of either pUCS-1 or IPTG. Cells were disrupted by sonic oscillation, and 38 μg of the cell extract protein (Coomassie Protein Assay, Pierce Chemical Co., Rockford, Ill.) was subjected to 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of beta-mercaptoethanol. Proteins were transferred to nitrocellulose sheets, which were probed with ^{125}I-labeled anti-pertussis toxin and autoradiographed. Lanes A and a, Cells without plasmid; lanes B and b, cells carrying pUCS-1, not IPTG induced; lanes C and c, cells carrying pUCS-1, IPTG induced; lane PT, 0.4 ng of pertussis toxin.

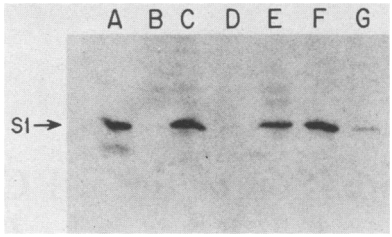

FIG. 3. Location and state of the S-1 subunit within *E. coli* LC137 carrying pUCS-1. Overnight cultures of *E. coli* LC137 carrying pUC18 or pUCS-1 were diluted 1/100 in L broth containing (per milliliter) 100 μg of ampicillin and 12.5 μg of tetracycline and shaken for 6 h at 32°C. Cells were collected and disrupted by sonic oscillation. Extracts were processed in various ways and then subjected to Western blot analysis as described in the legend to Fig. 2. Lane A, 0.4 ng of pertussis toxin; lanes B and C, total cell extracts of *E. coli*(pUC18) and *E. coli*(pUCS-1), respectively; lanes D and E, supernatant and particulate fractions, respectively, from centrifugation of pUCS-1 extract for 15 min at 15,000 × *g*. The particulate fraction from pUCS-1 extract was suspended in 7 M urea, dialyzed, and centrifuged as described above. Lane F, Urea-soluble fraction; lane G, urea-insoluble fraction.

incubation with either pertussis toxin or cell extracts containing the S-1 fusion protein, target erythrocyte membranes were separated by electrophoresis and subjected to autoradiography, as described in the legend to Fig. 4. The level of ADP-ribosylated Gi protein was then determined by densitometry of autoradiograms. S-1 protein in cell extracts was determined by densitometry of autoradiograms of Western blots, with authentic preparations of pertussis toxin as standards. Part or all of the difference in specific activity observed may have been due to incomplete renaturation of the insoluble fusion protein. Also, the presence of the 11 amino acid residues from the expression vector at the amino terminus of this fusion protein may have affected specific activity. *E. coli* LC137(pUCS-1) produced about 1 mg of the S-1 subunit per liter of culture when grown as described in the legend to Fig. 3. The S-1 subunit in these solubilized cell

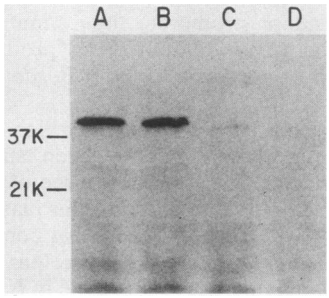

FIG. 4. ADP-ribosylation of human erythrocyte membranes. Reaction mixture (100 μl) contained 0.1 M Tris (pH 8.0), 20.0 mM dithiothreitol, 2.0 mM ATP, 10 μl of human erythrocyte membranes (40 μg of protein), 1 μCi of [^{32}P]NAD (specific activity, 667 Ci/mmol; New England Nuclear Corp., Boston, Mass.) and pertussis toxin or cell extracts as indicated. After incubation at 37°C for 30 min, membranes were precipitated by centrifugation and subjected to 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of beta-mercaptoethanol. The gel was fixed, stained, dried, and autoradiographed. Lane A, 0.4 ng of pertussis toxin; lane B, urea-soluble fraction from *E. coli* LC137(pUCS-1) (Fig. 3, lane F); lane C, urea-soluble fraction from *E. coli* LC137(pUC18); and lane D, no addition. Molecular weight markers are for diphtheria toxin fragments B (37K) and A (21K).

extracts showed no loss of enzymatic activity for a least 30 days during storage at 4°C.

The gene fusion described above provides a system for study of the enzymatic properties of pertussis toxin that is amenable to genetic manipulation. pUCS-1 contains several unique restriction sites within the S-1 gene (including *Bam*HI, *Sal*I, *Hinc*II, and *Xba*I) and outside the gene (*Eco*RI and *Hin*dIII). The enhanced yield of fusion protein obtained with a strain of *E. coli* deficient in protein degradation illustrates the potential advantage of such strains for expressing heterologous or genetically altered proteins.

This work was supported by Public Health Service grants AI-22021 from the National Institute of Allergy and Infectious Diseases and CA-39217 from the National Cancer Institute. Partial support was received from the Shipley Institute of Medicine.

We thank James L. Cowell and Charles R. Manclark (Division of Bacterial Products, Office of Biologics, Food and Drug Administration) for supplying anti-pertussis toxin antibody and a culture of *B. pertussis* 165.

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