Structure of a heat-stable enterotoxin produced by a human strain of *Escherichia coli*

Differences from the toxin of another human strain suggest the presence of compensated amino acid exchanges

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The amino acid sequence of a heat-stable enterotoxin produced by a human strain of enterotoxinogenic *Escherichia coli* was determined to be (disregarding the sulfide bridges):

Asn–Thr–Phe–Tyr–Cys–Cys–Glu–Leu–Cys–Cys–Asn–Pro–Ala–Cys–Ala–Gly–Cys–Tyr

Compensated amino acid exchanges have occurred in positions 11 and 18 in relation to the structure of a heat-stable enterotoxin from a different human strain of *Escherichia coli*

Heat-stable enterotoxin Escherichia coli, *pathogenic for man* *Amino acid sequence* *Compensated amino acid exchange*

1. INTRODUCTION

Enterotoxinogenic *Escherichia coli* is a common cause of diarrhoea in man and animals [1]. Two types of enterotoxin are produced, either alone or in combination [1]. One is a heat-labile, large enterotoxin composed of one 25500-M_r subunit and four or five 11000-M_r subunits [2]. Its mechanism of action appears to be similar to that of cholera toxin since it exerts its effects by stimulating the adenylate cyclase within the epithelial cells of the small intestine [3]. The other type of enterotoxin is a heat-stable, smaller polypeptide of about 2000–5000 M_r [4–6] and its mechanism of action may involve the guanylate cyclase system [7]. Host susceptibility, as well as molecular mass, amino acid composition and methanol solubility vary for heat-stable enterotoxins from different strains [4–6,8].

The primary structure of a purified heat-stable enterotoxin (ST) produced by *E. coli* pathogenic for man, has now been determined. The amino acid sequence of this human enterotoxin is in full agreement with that deduced from a nucleotide sequence for a porcine heat-stable enterotoxin [9]. However, in relation to another human enterotoxin [10], two amino acid exchanges have occurred. Structurally, these constitute compensated exchanges, which together with additional variations allow further conclusions on the relative importance of different positions of the toxin.

2. MATERIALS AND METHODS

The heat-stable enterotoxin produced by human strain C57/26C2 of *E. coli*, was purified by steps of hydrophobic interaction chromatography, exclusion chromatography, and ion-exchange chro-

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matography, as in [11], yielding the expected amino acid composition, potency, and biological activity.

Toxin (210 μg) was dissolved in 1.4 ml 0.4 M Tris–HCl (pH 8.15) containing 2 mM EDTA and 6 M guanidine–HCl. The solution was kept under nitrogen, and 25 μl 0.5 M dithiothreitol was added. After reduction for 2 h at 37°C, iodo[^{14}C]acetate (35 μCi) was added. The carboxymethylation was carried out for 2 h in the dark at room temperature under nitrogen, after which the reaction was stopped by 100 μl 2-mercaptoethanol. After another 10 min at 37°C, the product was dialyzed against 30% acetic acid in Spectrapor 6 dialysis tubing (M_r cut off: 1000).

The amino acid composition was determined with a Beckman 121 M analyzer after hydrolysis at 110°C for 24 h in evacuated tubes containing 6 M HCl with 0.5% phenol.

Sequence degradations of about 50 nmol carboxymethylated toxin were carried out in a Beckman 890 D liquid-phase sequencer, using a 0.1 M Quadrol peptide program in the presence of Polybrene, added together with 200 nmol glycine and pre-cycled [12]. Phenylthiohydantoin derivatives were identified by high-pressure liquid chromatography in a Hewlett-Packard 1084 B instrument on a Nucleosil C_{18} column using an acetate/acetonitrile gradient [13] supplemented where possible by thin-layer chromatography [14], and for carboxymethylcysteine by radioactivity measurements.

3. RESULTS

The heat-stable enterotoxin was [^{14}C]carboxymethylated and submitted to degradations in a liquid-phase sequencer. The structure obtained is given in fig.1. Residues were identified up to and including the C-terminus in position 18. The composition from this structure is identical to the analytical composition from acid hydrolysis of the toxin [11], showing that the structure is complete and consistent with all data.

The C-terminal amino acid of the enterotoxin is tyrosine. Asparagine is identified as the N-terminal residue, and also in position 11. The 6 cysteine derivatives are found at positions 5, 6, 9, 10, 14 and 17, and no basic amino acids are present in the toxin.

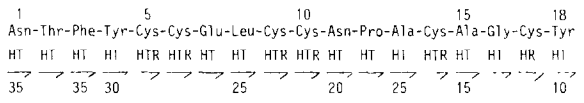

Fig.1. Results of sequence analysis of heat-stable enterotoxin produced by *Escherichia coli*, strain C57/26C2, pathogenic for man: (——→) residues identified by sequencer degradations; (H) identification by high performance liquid chromatography (with values in nmol stable residues recovered from application of 50 nmol toxin); (T) by thin-layer chromatography; (R) by ^{14}C measurements.

4. DISCUSSION

The amino acid sequence of carboxymethylated heat-stable enterotoxin from the human strain C57/26C2 has been determined. It consists of 18 residues and is in full agreement with the amino acid composition; 6 of the positions are occupied by the cysteine derivatives. Treatment of the native peptide with reducing agents such as dithiothreitol, destroys the biological activity, which suggests the presence of disulfide linkages in the intact molecule. Such a compact structure with 3 disulfide bridges in 18 residues, probably accounts for the pH and heat stability, as well as for the hydrophobic properties of the toxin [11].

Previous reports show the existence of heat-stable enterotoxins of M_r 2000–10000 [4–6,15], which suggests that the ST molecule is synthesized as a precursor, and that it, in common with many exported proteins, is subject to specific proteolytic processing associated with transmembrane transport. The report [9] of a porcine ST, for which the amino acid sequence was deduced from the nucleotide sequence, agrees with our report. The last 18 amino acid residues prior to the stop codon are identical to the present sequence. However, the sequence of a human ST isolated from another strain [10], differs from our structure in positions 11 and 18. We find asparagine in position 11 and tyrosine in position 18, while the other sequence has tyrosine in position 11 and asparagine in position 18. Consequently, the differences in positions 11 and 18 are not unique to human and porcine strains but a property of the toxin not related to the host origin. Two further sequences of heat-stable enterotoxins produced by different human strains have been established, one

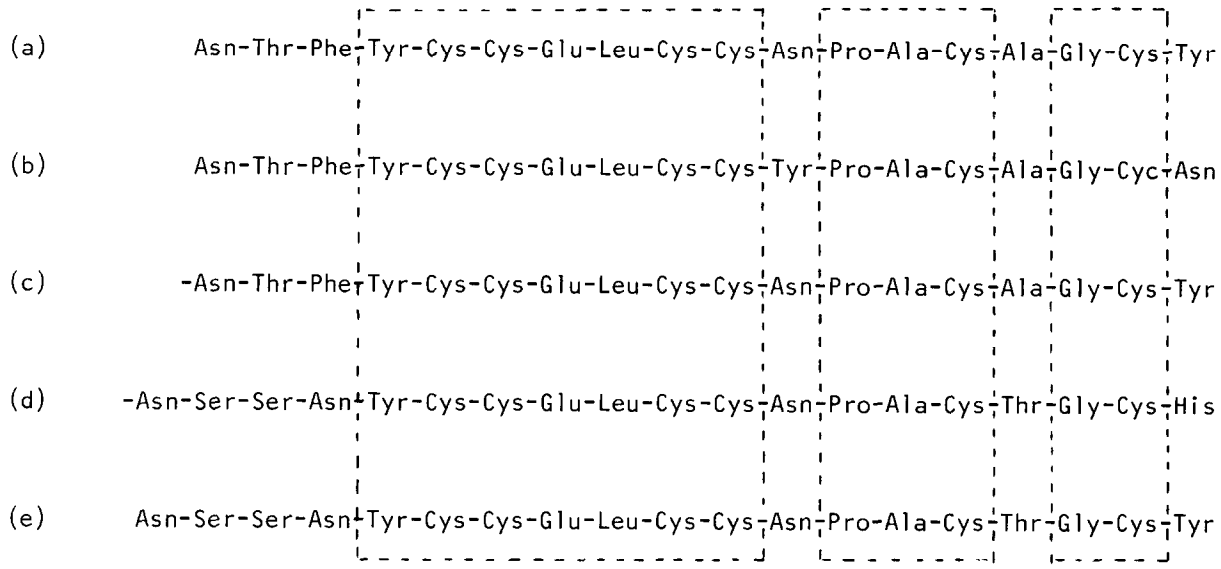

Fig.2. Comparison of structures of heat-stable enterotoxins produced by *Escherichia coli*: (a) present structure; (b) human ST from [10]; (c) porcine ST deduced from a nucleotide sequence [9]; (d) human ST deduced from a nucleotide sequence [16]; (e) human ST from [17]; hitherto invariable residues are boxed.

directly determined and the other deduced from the nucleotide sequence [16,17]. Both these toxins contain 19 amino acid residues, by having an extra N-terminal residue in relation to the present sequence. The structures are compared in fig.2.

Interestingly, the 18-residue human ST that differs in positions 11 and 18 from our sequence, exhibits differences suggesting the presence of compensated amino acid exchanges. Apparently, asparagine and tyrosine can complement each other at these positions in the two toxins. Taking all toxins into account, they also exhibit single differences in these positions, again of a compensated nature.

Positions 11 and 18 may therefore be ascribed a certain function. They can apparently accept some exchanges, in contrast to most cysteine-adjacent positions (which are hitherto invariable) and a few other positions also containing single exchanges. Independent of the functional correlation, the present results show a new structure for human ST, and indicate two interesting positions, 11 and 18, which may have compensated functions in the active enterotoxin.

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REFERENCES

[1] Sack, R.B. (1980) J. Infect. Dis. 142, 279–286.
[2] Gill, D.M., Clements, J.D., Robertson, D.C. and Finkelstein, R.A. (1981) Infect. Immun. 33, 677–682.
[3] Holmgren, J. and Lönnroth, L. (1980) in: Cholera and related diarrhoeas (Ouchterlony, Ö. and Holmgren, J. eds) pp.88–103, Karger, Basel.
[4] Alderete, J.F. and Robertson, D.C. (1978) Infect. Immun. 19, 1021–1030.
[5] Staples, S.J., Asher, S.E. and Giannella, R.A. (1980) J. Biol. Chem. 255, 4716–4721.
[6] Takeda, Y., Takeda, T., Yano, T., Yamamoto, K. and Miwatani, T. (1979) Infect. Immun. 25, 978–985.
[7] Field, M., Graf, L.H. jr, Laird, W.J. and Smith, P.L. (1978) Proc. Natl. Acad. Sci. USA 75, 2800–2804.
[8] Burgess, M.N., Bywater, R.J., Cowley, C.M., Mullan, N.A. and Newsome, P.M. (1978) Infect. Immun. 21, 526–531.
[9] So, M. and McCarthy, B.J. (1980) Proc. Natl. Acad. Sci. USA 77, 4011–4015.

[10] Chan, S.-K. and Giannella, R.A. (1981) J. Biol. Chem. 256, 7744–7746.
[11] Rönnberg, B. and Wadström, T. (1983) submitted.
[12] Jörnvall, H. and Philipson, L. (1980) Eur. J. Biochem. 104, 237–247.
[13] Zimmerman, C.L., Appella, E. and Pisano, J.J. (1977) Anal. Biochem. 77, 569–573.
[14] Inagami, T. and Murakami, K. (1972) Anal. Biochem. 47, 501–504.
[15] Lathe, R., Hirth, P., De Wilde, M., Harford, N. and Lecocq, J.-P. (1980) Nature 284, 473–474.
[16] Moseley, S.L., Echeverria, P. and Falkow, S. (1981) The 17th Joint Conf. US-Japan Cooperative Medical Science Program, Cholera Panel, Baltimore MD.
[17] Aimoto, S., Takao, T., Shimonishi, Y., Hara, S., Takeda, T., Takeda, Y. and Miwatani, T. (1982) Eur. J. Biochem. 129, 257–263.