DNA Sequencing of the *eta* Gene Coding for Staphylococcal Exfoliative Toxin Serotype A

By SUSUMU SAKURAI,¹ HITOSHI SUZUKI¹ AND ISAMU KONDO^{2*}

¹ Division of Molecular Genetics, The Jikei University School of Medicine, Japan

² Research Center, Taisho Pharmaceutical Co. Ltd, I-403, Yoshino-cho, Ohmiya-shi, 330 Japan

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We report the nucleotide sequence of a 1.45 kb segment containing the *eta* gene, coding for staphylococcal exfoliative toxin A (ETA), isolated from the recombinant plasmid pETA-J3. The coding region of 840 bp specified a polypeptide of 280 amino acid residues which included a putative 38 residue signal sequence. The amino acid composition deduced from the structural gene was in agreement with the results of peptide analysis of the ETA molecule reported by others. The sequence of the 35 N-terminal amino acid residues of ETA derived from *Staphylococcus aureus* strain ZM was also consistent with that deduced from the DNA sequencing.

INTRODUCTION

Infection of human neonates with certain *Staphylococcus aureus* strains can cause Ritter's disease, also known as the staphylococcal scalded skin syndrome (SSSS). Since Melish & Glasgow (1970) established a mouse model for bioassay of SSSS, rapid progress has been made in molecular studies of the disease, and it is now well established that the exfoliative toxin (ET) is the causative agent of SSSS (Melish & Glasgow, 1970; Kapral & Miller, 1971; Arbuthnott *et al.* 1971; Kondo *et al.*, 1973, 1974, 1975). The most conclusive evidence for this is that administration of highly purified ET to mice reproduces the typical Nikolsky sign (NK), exfoliation of the epidermis seen in patients with Ritter's disease. Some inbred mice are able to produce antibodies to ET while others are non-producers. However, all mouse strains so far tested are susceptible to this toxin (Kondo & Sakurai, 1980). Therefore, the mechanism of ET pathogenesis is independent of the immune response of the hosts. When rabbits were immunized with ET, a new toxin serotype (ETB), non-crossreactive to the existing ET (ETA), was discovered (Kondo *et al.*, 1973). Structural differences between serotypes ETA and ETB are further suggested by differences in heat stability (Kondo *et al.*, 1973, 1974). Also ETA is a metallo-toxin, containing one copper ion per molecule, whereas no metal is detectable in the ETB molecule (Kondo & Sakurai, 1980). ETA is heterogeneous in isoelectric point while ETB is homogeneous (Kondo *et al.*, 1974). In addition, ETA is coded by a chromosomal gene while ETB is plasmid-specified (Rogolsky *et al.*, 1974, 1976; Wiley & Rogolsky, 1977; Jackson & Iandolo, 1986; O'Toole & Foster, 1986*a, b*).

Although ETA and ETB are antigenically distinct, they presumably share some structures important for toxinogenesis. Therefore, we cloned the gene coding for ETA, *eta*, into pUC9 and transformed *Escherichia coli* C600 with the recombinant plasmid pETA, in order to analyse the properties of ETA. ETA produced by the *E. coli* transformant, cETA, was indistinguishable from the ETA produced by *Staphylococcus aureus*, sETA, in its physicochemical and serological properties and its ability to cause exfoliation. cETA did not remain in the periplasmic space of the *E. coli* transformants, and more than 97% of the toxin became extracellular (Sakurai *et al.*, 1987).

Abbreviations: ETA, ETB, exfoliative toxin A, B; ORF, open reading frame.

Fig. 1. Restriction map and sequencing strategy of the *AluI*-*TaqI* fragment of pETA-J3. Abbreviations of restriction endonucleases are as follows: A, *AluI*; B, *Bam*HI; E, *Eco*RI; Q, *TaqI*; S, *Sau*3AI; R, *RsaI*; D, *DraI*. The arrows indicate the DNA segments inserted into the replicative-form DNA of M13mp18 for sequencing.

In this communication, we report the sequence of the entire coding region as well as the putative promoter of *eta*. We have also determined the N-terminal amino acid sequence of sETA protein.

METHODS

Bacterial strains and media. *S. aureus* strain ZM, an ETA-producing strain, and *E. coli* transformant strain *Ceta*, carrying the cloned *eta* gene, were as described previously (Kondo *et al.*, 1973; Sakurai *et al.*, 1987). Phage M13mp18 and *E. coli* JM109 were included in a sequencing kit obtained from Takara Shuzo (Tokyo).

ET-broth (Sakurai *et al.*, 1987) and L-broth (Miller, 1972) were used for growth of *S. aureus* and *E. coli* respectively. ET-agar and L-agar were also prepared by adding 15 g or, for soft agar, 5 g purified agar to 1 litre of ET-broth and L-broth, respectively.

DNA sequencing. The recombinant plasmid, pETA-J3, was described previously (Sakurai *et al.*, 1987). A 1.45 kb DNA fragment containing the *eta* gene was isolated from pETA-J3 by *AluI* and *TaqI* digestion. This segment was cleaved with restriction endonucleases *AluI*, *TaqI*, *Sau*3AI, *RsaI* and *DraI* to give fragments of suitable size for sequencing. These were ligated to the replicative-form DNA of M13mp18 and used to transfect *E. coli* JM109. The phages isolated from colourless plaques were used to infect JM109. Single-stranded DNA was purified and sequenced by the dideoxy chain termination method (Sanger *et al.*, 1977, 1980; Sanger, 1981; Messing & Vieira, 1982) using [α - 32 P]dCTP (ICN Radiochemicals). The DNA sequence was analysed using the DNASIS computer program (Hitachi Software Engineering Co.).

Determination of N-terminal amino acid sequence of ETA. The ETA produced by *S. aureus* strain ZM was purified by HPLC on a Hitachi 3013-0 column, and subjected to N-terminal sequence determination by the method of Tarr (1986) with a gas-phase protein sequencer (model 470A, ABI Co.) and on-line PTH amino acid analyser (model 120A PTH Analyzer, ABI Co.).

RESULTS AND DISCUSSION

Nucleotide sequence of the *eta* gene. The restriction map of the *eta* fragment and the sequencing strategy are shown in Fig. 1. The nucleotide sequence of the 1454 bp fragment containing the *eta* gene was obtained in both strands (Fig. 2). One open reading frame (ORF), extending from residue 374 to residue 1213, was identified by computer analysis. The TAA codon at position

Fig. 2. Nucleotide sequence of the *AluI*-*TaqI* fragment containing the *eta* gene. The amino acids of the polypeptide deduced from the nucleotide sequence are shown with single-letter symbols under the triplet codons. Nucleotide sequence numbers are shown on the right; amino acid sequence numbers are shown below the appropriate amino acid. Three sets of -35 and -10 regions corresponding to the consensus sequence of the *E. coli* promoter are indicated with solid lines above the respective sequences. SD, ribosome-binding region (Shine-Dalgarno sequence). Four inverted sequences are underlined with arrows indicating the direction of the inversion. The stop codon for the translation of mRNA (TAA) and the termination codon for mRNA transcription are indicated by asterisks and dotted underlining respectively.

1214 is considered to be the translation termination codon. This 840 bp sequence codes for a polypeptide of 280 amino acid residues (Fig. 2). Two promoter-like sequences were recognized in sequences 5' to this ORF stretch, at nucleotides 235–273 and 295–333. Several plausible –35 and –10 regions corresponding to the consensus sequence of the *E. coli* promoter (Hawley & McClure, 1983) were apparent, each separated by 16–18 bp (Fig. 2). A potential ribosome-binding site (Shine–Dalgarno sequence), AGGATG, was identified starting at position 361 with optimal spacing (7 bp) from the initiation codon ATG.

Table 1. *N*-terminal amino acid sequence of ETA from *S. aureus* strain ZM

The sequence shown was determined chemically. It is identical to that deduced from the *eta* nucleotide sequence, the N-terminal Glu residue corresponding to the 39th amino acid in the sequence shown in Fig. 2.

Residue no.	Amino acid	Residue no.	Amino acid	Residue no.	Amino acid
1	Glu	13	Lys	25	Leu
2	Val	14	Trp	26	Pro
3	Ser (Thr)*	15	Asn (Asp)*	27	Lys
4	Ala	16	Lys	28	Glu
5	Glu	17	Tyr	29	Leu
6	Glu	18	Tyr	30	Phe
7	Ile	19	Gly	31	Ser
8	Lys	20	Val	32	Lys
9	Lys	21	Asn (unknown)*	33	Val
10	His	22	Ala (Asx)*	34	Asp
11	Glu	23	Phe	35	Glu
12	Glu	24	Asn		

* Johnson *et al.* (1979) sequenced the N-terminus of ETA from *S. aureus* strain TA, as far as residue 23. That sequence was identical to the one shown except for the amino acids in parentheses.

N-terminal amino acid sequence and putative signal peptide. The 35 amino acid residues constituting the N-terminal sequence of mature ETA purified from *S. aureus* ZM culture supernate were determined (Table 1). The first amino acid, glutamic acid (E), corresponds to the 39th residue in the amino acid sequence deduced from the nucleotide sequence of the cloned *eta* gene; the following 34 residues were identical to those deduced from the DNA sequence. Since the 39th amino acid was identified as the N-terminal residue of the mature toxin, the signal sequence is presumed to consist of the preceding 38 amino acid residues (Fig. 2).

The hydropathy profile of the *eta* gene product (Fig. 3) shows that the N-terminal sequence contains a markedly hydrophobic stretch in the middle. Although this putative signal peptide is long, it is still within the range for a signal peptide of a protein excreted from Gram-positive bacteria. For example, protein A contains a signal sequence of 36 amino acid residues (Uhlen *et al.*, 1983) whereas ETB has 31 (Jackson & Iandolo, 1986).

Molecular mass and amino acid composition of ETA predicted from the DNA sequence. The amino acid composition of ETA predicted from the *eta* gene structure is in close agreement with that of ETA derived from *S. aureus* strain TA reported by Johnson *et al.* (1979), with only minor differences: the predicted *eta* gene product contains four more Val residues, two more Leu residues and one more Gly residue, but two fewer Glu + Gln residues. The total number of amino acid residues of the predicted *eta* gene product is 242, five more than reported by Johnson *et al.* (1979). The molecular mass of ETA calculated from these 242 amino acids (26.9 kDa) is somewhat larger than that (24 kDa) estimated by gel filtration in our previous experiments (Kondo *et al.*, 1973).

Potential secondary structure in the 5' and 3' flanking regions of the eta gene. The 5' flanking regions contain two inverted repeats (Figs 2 and 4). Both partially overlap the -10 region of the putative promoters and thus may play regulatory roles. Inverted repeats were also identified at two positions in the 3' flanking region: the one farthest from the ORF was GC rich and was followed by seven T residues; it may correspond to a ρ -dependent transcription terminator (Rosenberg & Court, 1979).

In conclusion, several characteristic structures of the *eta* gene and its product were identified: double promotor regions, two inverted sequences in both the 5' and the 3' flanking regions, a long signal sequence consisting of 38 amino acid residues, and an N-terminal segment

containing a markedly hydrophobic region in the middle. However, it is not yet known what domains in the nucleotide sequence of this gene play essential roles in coding for the biological properties of ETA.

We have recently cloned the gene coding for ETB (*etb*) and determined the DNA sequence (unpublished results). It was different from the *etb* sequence reported by Jackson & Iandolo (1986), but Lee *et al.* (1987) have presented a revised DNA sequence of *etb* in addition to the sequence of the *eta* gene, and our data on *eta* are in agreement with their *eta* sequence except for minor differences in 5' and 3' flanking regions. The DNA sequence of *etb* which we recently obtained is also in agreement with their revised *etb* sequence. The comparative analyses between the *eta* and *etb* genes cloned in our department are now being carried out.

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REFERENCES

- ARBUTHNOTT, J. P., KENT, J., LYELL, A. & GEMMELL, C. G. (1971). Toxic epidermal necrolysis produced by an extracellular product of *Staphylococcus aureus*. *British Journal of Dermatology* **85**, 145-149.
- HAWLEY, D. K. & McCLURE, W. R. (1983). Compilation and analysis of *Escherichia coli* promoter DNA sequences. *Nucleic Acids Research* **11**, 2237-2255.
- JACKSON, M. P. & IANDOLO, J. J. (1986). Sequence of the exfoliative toxin B gene of *Staphylococcus aureus*. *Journal of Bacteriology* **166**, 574-580.
- JOHNSON, A. D., SPERO, L., CADES, J. S. & DE CICCO, B. T. (1979). Purification and characterization of different types of exfoliative toxin from *Staphylococcus aureus*. *Infection and Immunity* **24**, 679-684.
- KAPRAL, F. A. & MILLER, M. M. (1971). Product of *Staphylococcus aureus* responsible for the scalded skin syndrome. *Infection and Immunity* **4**, 541-545.
- KONDO, I. & SAKURAI, S. (1980). Studies on toxicity and immunology of staphylococcal exfoliatin A and B. In *Natural Toxins*, pp. 379-387. Edited by D. Eaker & T. Wadström. Oxford & New York: Pergamon Press.
- KONDO, I., SAKURAI, S. & SARAI, Y. (1973). Purification of exfoliatin produced by *Staphylococcus aureus* of bacteriophage group 2 and its physicochemical properties. *Infection and Immunity* **8**, 156-164.
- KONDO, I., SAKURAI, S. & SARAI, Y. (1974). New type of exfoliatin obtained from staphylococcal strains, belonging to phage groups other than group 2, isolated from patients with impetigo and Ritter's disease. *Infection and Immunity* **10**, 851-861.
- KONDO, I., SAKURAI, S., SARAI, Y. & FUTAKI, S. (1975). Two types of exfoliatin and their distribution in staphylococcal strains isolated from patients with scalded skin syndrome. *Journal of Clinical Microbiology* **1**, 397-400.
- KYTE, J. & DOOLITTLE, F. R. (1971). Product of *Staphylococcus aureus* responsible for the scalded skin syndrome. *Infection and Immunity* **4**, 541-545.
- LEE, C. Y., SCHMIDT, J. J., JOHNSON-WINGER, A. D., SPERO, L. & IANDOLO, J. J. (1987). Sequence determination and comparison of the exfoliative toxin A and toxin B cloned from *Staphylococcus aureus*. *Journal of Bacteriology* **169**, 3904-3909.
- MELISH, M. F. & GLASGOW, L. A. (1970). The staphylococcal scalded skin syndrome: development of an experimental model. *New England Journal of Medicine* **282**, 1114-1119.
- MESSING, J. & VIEIRA, J. (1982). A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. *Gene* **19**, 269-276.
- MILLER, J. H. (editor) (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- O'TOOLE, L. L. & FOSTER, T. J. (1986a). Epidermolytic toxin serotype B of *Staphylococcus aureus* is plasmid-encoded. *FEMS Microbiology Letters* **36**, 311-314.
- O'TOOLE, L. L. & FOSTER, T. J. (1986b). Molecular cloning and expression of epidermolytic toxin A gene of *Staphylococcus aureus*. *Microbial Pathogenesis* **1**, 583-594.
- ROGOLSKY, M., WARREN, R., WILEY, B. B., NAKAMURA, H. T. & GLASGOW, L. A. (1974). Nature of the genetic determinant controlling exfoliative toxin production in *Staphylococcus aureus*. *Journal of Bacteriology* **117**, 157-165.
- ROGOLSKY, M., WILEY, B. B. & GLASGOW, L. A. (1976). Phage group 2 staphylococcal strains with chromosomal and extrachromosomal genes for exfoliative toxin production. *Infection and Immunity* **13**, 44-52.
- ROSENBERG, M. & COURT, D. (1979). Regulatory sequences involved in the promotion and termination of RNA transcription. *Annual Review of Genetics* **13**, 319-353.
- SAKURAI, S., SUZUKI, H. & KONDO, I. (1987). Cloning of the gene coding for staphylococcal exfoliative toxin A and its expression in *Escherichia coli*. *FEMS Microbiology Letters* **42**, 63-67.
- SANGER, F. (1981). Determination of nucleotide sequences in DNA. *Science* **214**, 1205-1210.
- SANGER, F., NICKLEN, S. & COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **74**, 5463-5467.
- SANGER, F., COULSON, A. R., BARVELL, B. G., SMITH, A. J. H. & ROE, B. A. (1980). Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. Determination of nucleotide sequences in DNA. *Science* **214**, 1205-1210.

- TARR, G. E. (1986). A practical manual. In *Micro-characterization of Polypeptides*, pp. 155–194. Edited by J. E. Shively. Clifton, NJ: Humana Press.
- UHLEN, M., GUSS, B., NILSSON, B., GATENBECK, S., PHILIPSON, L. & LINDBERG, M. (1983). Complete sequence of the staphylococcal gene encoding protein A – a gene evolved through multiple duplications. *Journal of Biological Chemistry* **259**, 1695–1702.
- WILEY, B. B. & ROGOLSKY, M. (1977). Molecular and serological differentiation of staphylococcal exfoliative toxin synthesized under chromosomal and plasmid control. *Infection and Immunity* **18**, 487–494.