Nucleotide Sequence of the Epidermolytic Toxin A Gene of 
*Staphylococcus aureus*

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The nucleotide sequence of the *eta* gene, which codes for the epidermolytic toxin serotype A of *Staphylococcus aureus* TC16, is reported. The coding sequence of 840 nucleotides specifies a protein which, when secreted, has a predicted molecular weight of 26,950. The sequence of *eta* and the deduced amino acid sequence of the toxin have been compared with those of epidermolytic toxin serotype B. The coding sequences have 52% identical residues, and the polypeptides have 40% identical residues. Amino acid residues have been conserved in the areas of the proteins which correspond to major hydrophobic domains, whereas the regions likely to specify antigenic determinants occur in hydrophilic sequences that have diverged. The level of expression of epidermolytic toxin A in *S. aureus* 8325-4 was shown to be dependent on the integrity of a regulatory gene called *agr*.

Strains of *Staphylococcus aureus* which cause the staphylococcal scalded skin syndrome secrete an extracellular toxic protein called epidermolytic toxin or exfoliative toxin (ET; reviewed by Elias et al. [10]). The purified forms of the toxin can induce skin exfoliation experimentally by splitting of the epidermis in the plane of the stratum granulosum (3). Two serologically distinct forms of the toxin (ETA and ETB) have been described (4, 14). They differ in molecular mass (30 and 29.5 kilodaltons, respectively), pI values, and heat stability (4, 27). Some amino acid sequence homology was observed in N-terminal residues of the two serotypes, but the toxins differed considerably in tryptic peptide maps and amino acid composition (4, 14).

The genes coding for ETA and ETB (*eta* and *etb*, respectively) have recently been cloned in *Escherichia coli* phage and plasmid vectors (12, 18, 25, 26). The *eta* gene is known to be located in the chromosome of *S. aureus* (25), whereas large plasmids have been implicated in the expression of ETB (24). However, the *etb* gene has only recently been shown unequivocally to be plasmid linked (12, 26).

The nucleotide sequence of the *eta* gene cloned from a scalded skin syndrome-associated strain of *S. aureus* is described in this paper. The accompanying paper (18) reports the cloning and sequencing of *eta* from another strain of *S. aureus* as well as the sequence of the *etb* gene.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and bacteriophages.** The bacterial strains and plasmids are listed in Table 1. The phage vectors M13tg130 and M13tg131 (16) were obtained from Amersham Corp. (Amersham, United Kingdom).

**Bacteriological media, chemicals, and enzymes.** *S. aureus* was routinely grown in Trypticase soy broth and agar (BBL Microbiology Systems, Cockeysville, Md.). Bernheimer-Schwartz broth (5) was used for ETA expression studies. *E. coli* strains were routinely cultured in L broth and agar (19). *E. coli* 71-18 was routinely subcultured on M9 minimal salts agar supplemented with glucose, proline, and thiamine (19). YT×2 broth and H agar (34) were used when propagating M13.

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Amino acid sequence of eta. Alignment of the chemically derived N-terminal amino acid sequence of ETA (14) with the translation product of the eta gene reported here suggests that the signal peptide comprises 38 residues. It has features in common with the signal peptides of other secreted proteins from S. aureus (7, 15).

One discrepancy between the deduced amino acid sequence and chemical analysis is that the previously reported C-terminal residue is lysine (4). Perhaps the C-terminal asparagine and glutamic acid residues are removed proteolytically in S. aureus culture supernatants. The predicted molecular weight of the mature form of the toxin (including the C-terminal N and E residues) is 26,950, which is close to the value of 26,500 obtained electrophoretically (13).

Expression of eta. We previously demonstrated (25) that expression of the eta gene cloned on a 4-kb fragment from S. aureus TC16 was regulated by a locus called the accessory gene regulator (agr) (9, 28). One of the BAL 31-generated deletions constructed here (delta-15) included the structural gene eta and 256 base pairs of DNA upstream from the ATG codon (Fig. 1). It was subcloned from the M13tg vector into pUC18 and pUC19 to yield pETA7 and pETA8, respectively. Both plasmids synthesized about 10 µg of toxin per 10^10 E. coli cells. The orientation-independent expression of ETA in E. coli suggests that the native promoter is present and functional in these plasmids.

A shuttle plasmid (pETA9) carrying the delta-15 sequence was constructed and transformed into S. aureus 8325-4, ISP546, and RN4220. The agr⁺ host 8325-4 synthesized 2.8 mg of ETA per ml. In contrast the agr mutant ISP546 synthesized 80 µg/ml. This confirms that the expression of eta is regulated by the agr system in S. aureus (25, 28) and shows that both the regulatory site and promoter are present in delta-15. Similar experiments with the cloned etb gene showed that expression of ETB is also agr regulated (data not shown).

Comparison of the nucleotide sequence of eta and etb. The eta gene cloned from S. aureus UT002 (18) has complete residue identity with that reported here for S. aureus TC16. This identity extends 112 bases 5' and 139 bases 3' from the coding sequences. The etb gene has also recently been sequenced (18), and Fig. 2 shows the eta sequence aligned with that of etb. The coding sequences have 440 identical residues out of 840 (52%). This explains our previous observation that eta and etb did not hybridize in Southern blots performed at 80% stringency (26). A sequence of 29 base pairs containing dyad symmetry which is located upstream from the eta coding sequence has 28 identical residues (97%) with a sequence in the promoter-containing region of the etb clone.

Comparison of ETA and ETB. The aligned amino acid sequences of ETA and ETB (32) are shown in Fig. 3. The number of identical residues in the primary translation products is 113 out of 280 (40%). Interestingly, the hydropathy plots (17) of the two proteins are very similar in profile, with hydrophobic and hydrophilic domains occurring in the same regions (data not shown). Amino acid sequences have been conserved in certain areas of the proteins which correspond, in two of the cases, to major hydrophobic domains.

DISCUSSION

In this paper we report the DNA sequence of the gene coding for ETA cloned from S. aureus TC16, a strain which was isolated in Glasgow, Scotland, in 1969. In the accompanying paper (18) is presented the sequence of etb cloned from strain UT0002 which was isolated in New York in 1969. The coding sequences and over 100 nucleotides 5' and 3' of the genes were identical.

Regions where the amino acid sequences of ETA and ETB are strongly conserved include predominantly hydrophobic domains. These are probably important in the function of the toxins and could be involved in binding to receptors on the surface of granular cells of susceptible animals, in internalization of the toxin, or in binding to filaggrin in keratohyalin granules (31). The determinants responsible for the antigenic differences between ETA and ETB probably occur in the hydrophilic regions where amino acid sequences have diverged.

The codon usage pattern in eta is strongly biased toward A or T residues in the third position of degenerate codons. The
G+C content of this residue is 15%. This reflects the low 
G+C content of the chromosomal DNA of the host organ-
ism.

We have demonstrated that ETA is expressed at reason-
able levels in *E. coli* when cloned in both orientations in pUC 
vectors, presumably because the *eta* promoter is active. 
However, expression of ETB in *E. coli* is dependent on 
vector promoters (12, 26). This difference may be explained 
by the most likely candidate for the *eta* promoter being 
reasonably similar to the *E. coli* consensus promoter, where-
as no closely related sequence has been identified upstream 
from the *etb* gene (18).

The expression of several extracellular proteins by *S. 
aureus* is controlled positively by a regulatory locus called 
*agr* (6, 9, 28). In *agr* mutants the level of protein secreted 
in culture supernatants is much lower than in the wild type. 
There is a concomitant reduction in specific mRNA, at least 
in the cases of α-toxin and toxic shock syndrome toxin 1 
(28). It is possible that control is exerted at the level of 
transcriptional initiation, although other mechanisms are
FIG. 2. Alignment of \textit{eta} and \textit{etb} nucleotide sequences. (A) Alignment of part of the promoter-proximal region showing a 29-base-pair conserved sequence containing dyad symmetry. (B) Alignment of the \textit{eta} and \textit{etb} coding sequences. Alignments were performed by the method of Wilbur and Lipman (32) with K-tuple size 4, window size 20, gap penalty 7. Colons show identical residues.
possible. We have shown that the expression of the cloned eta and etb genes is subject to agr control in S. aureus 8325-4 (25). A 29-base pair sequence containing dyad symmetry is highly conserved in the promoter-proximal regions of eta and etb. It is present just upstream from the putative eta promoter and could conceivably be a recognition site for a regulatory protein. However, this remains highly speculative because the eta and etb promoters have not been identified by transcript mapping studies.

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LITERATURE CITED


