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# Correction of Partial Amino Acid Sequence of Erabutoxins

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The amino acid sequences of erabutoxins a and b were re-examined. The previously reported sequence of Ser-Glu at positions 21 and 22 of erabutoxins was corrected to Glu-Ser.

The amino acid sequences of erabutoxins a, b and c were reported by Sato & Tamiya (1971) and by Tamiya & Abe (1972). Since then, more than 50 snake neurotoxins have been isolated and sequenced (cf. Maeda & Tamiya, 1974, 1976). A comparison of these sequences, and consideration of the likely base changes involved, led us to re-examine the erabutoxin sequences at positions 21 and 22, where serine and glutamic acid occur. The present paper describes the revision of the sequence 21–22 to Glu-Ser instead of, as previously reported, Ser-Glu.

## Experimental and Results

Erabutoxins a and b were prepared as described by Tamiya & Arai (1966) and reduced and *S*-carboxymethylated as described by Sato & Tamiya (1971). The reduced and *S*-carboxymethylated erabutoxin a (30mg) was digested with trypsin (twice-crystallized; Sigma Chemical Co., St. Louis, MO, U.S.A.) in 2ml of 0.05M-Tris/HCl buffer, pH8.0, for 16h at 37°C. The enzyme/substrate ratio was 1:60 (w/w). The tryptic-peptide mixture was subjected to gel filtration on a column (1.2cm×250cm) of Sephadex G-25 (fine grade) in 0.1M-acetic acid, at a flow rate of 15ml/h. The peptide fraction that was eluted at 203–220ml was collected, evaporated to dryness and applied to a column (1.2cm×15cm) of DEAE-cellulose equilibrated with 0.01M-Tris/HCl buffer, pH8.2. The NaCl concentration in the elution buffer was increased to 0.5M exponentially. The flow rate was 40ml/h. Peptide $T_{3Ea}$ (residues 16–27; Sato & Tamiya, 1971) was eluted at the NaCl concentration of 0.12M.

Direct Edman degradation (Iwanaga *et al.*, 1969) of peptide $T_{3Ea}$ (1.0$\mu$mol) revealed the *N*-terminal sequence of the peptide to be Thr-CmCys-Pro-Ser-Gly-Glu-Ser-. The spectrophotometrically deter-

Table 1. *Amino acid compositions of peptides* $T_{3Ea}$ *and* $T_{3Eb}$ *and their fragments obtained by Staphylococcus aureus V8 proteinase digestion*

The results are expressed as molar proportions, the amino acid whose value is marked * being used as the standard for the particular peptide or fragment.

| | $T_{3Ea}$ | $T_{3Ea}$-a | $T_{3Ea}$-c | $T_{3Eb}$ | $T_{3Eb}$-a | $T_{3Eb}$-c |
|---|---|---|---|---|---|---|
| Lysine | 0.92 (1) | | 1.09 (1) | 0.96 (1) | | 1.14 (1) |
| Histidine | | | | 0.89 (1) | | 1.00* (1) |
| *S*-Carboxymethylcysteine† | 1.86 (2) | 0.47 (1) | 0.43 (1) | 1.65 (2) | 0.36 (1) | 0.47 (1) |
| Aspartic acid | 1.05 (1) | | 1.00* (1) | | | |
| Threonine | 0.99 (1) | 0.89 (1) | | 0.99 (1) | 0.89 (1) | |
| Serine | 2.72 (3) | 1.18 (1) | 1.62 (2) | 2.87 (3) | 1.10 (1) | 1.90 (2) |
| Glutamic acid | 1.00* (1) | 1.00* (1) | | 1.00* (1) | 1.00* (1) | |
| Proline | 1.09 (1) | 0.83 (1) | | 1.29 (1) | 1.06 (1) | |
| Glycine | 0.96 (1) | 1.11 (1) | | 1.09 (1) | 1.16 (1) | |
| Tyrosine | 0.32 (1) | | 0.61 (1) | 0.77 (1) | | 0.61 (1) |
| Total | 12 | 6 | 6 | 12 | 6 | 6 |
| Yield (%) | 60‡ | 53§ | 77§ | 33‡ | 42§ | 20§ |
| Mobility‖ | −30 | −51 | 7 | −10 | −47 | 50 |

† This amino acid tends to give low recovery values.
‡ Expressed as a percentage of reduced and *S*-carboxymethylated erabutoxin a or b.
§ Expressed as a percentage of peptide $T_{3Ea}$ or $T_{3Eb}$.
‖ The mobility of each peptide on paper electrophoresis at pH4.8 is given relative to that of arginine (=100).

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      1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17
Ea. Arg-Ile-Cmc-Phe-Asn-Gln-His-Ser-Ser-Gln-Pro-Gln-Thr-Thr-Lys-Thr-Cmc-
Eb. Arg-Ile-Cmc-Phe-Asn-Gln-His-Ser-Ser-Gln-Pro-Gln-Thr-Thr-Lys-Thr-Cmc-
Ec. Arg-Ile-Cmc-Phe-Asn-Gln-His-Ser-Ser-Gln-Pro-Gln-Thr-Thr-Lys-Thr-Cmc-

     18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
   Pro-Ser-Gly-Glu-Ser-Ser-Cmc-Tyr-Asn-Lys-Gln-Trp-Ser-Asp-Phe-Arg-Gly-
   Pro-Ser-Gly-Glu-Ser-Ser-Cmc-Tyr-His-Lys-Gln-Trp-Ser-Asp-Phe-Arg-Gly-
   Pro-Ser-Gly-Glu-Ser-Ser-Cmc-Tyr-His-Lys-Gln-Trp-Ser-Asp-Phe-Arg-Gly-

     35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51
   Thr-Ile-Ile-Glu-Arg-Gly-Cmc-Gly-Cmc-Pro-Thr-Val-Lys-Pro-Gly-Ile-Lys-
   Thr-Ile-Ile-Glu-Arg-Gly-Cmc-Gly-Cmc-Pro-Thr-Val-Lys-Pro-Gly-Ile-Lys-
   Thr-Ile-Ile-Glu-Arg-Gly-Cmc-Gly-Cmc-Pro-Thr-Val-Lys-Pro-Gly-Ile-Asn-

     52 53 54 55 56 57 58 59 60 61 62
   Leu-Ser-Cmc-Cmc-Glu-Ser-Glu-Val-Cmc-Asn-Asn
   Leu-Ser-Cmc-Cmc-Glu-Ser-Glu-Val-Cmc-Asn-Asn
   Leu-Ser-Cmc-Cmc-Glu-Ser-Glu-Val-Cmc-Asn-Asn
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Fig. 1. *Amino acid sequences of erabutoxins a (Ea), b (Eb) and c (Ec)*
The residues shown in *italics* indicate the part of the sequence corrected in the present paper. Abbreviation: Cmc, *S*-carboxymethylcysteine.

mined yield of the phenylthiohydantoin derivative of glutamic acid at step 6 was 0.5 μmol. The sequence was also confirmed subtractively at steps 5, 6 and 7 of degradation.

Peptide $T_{3Ea}$ (approx. 1 mg) was dissolved in 0.1 ml of 0.1 M-ammonium bicarbonate buffer, pH 7.8, and digested with 20 μg of *Staphylococcus aureus* V8 proteinase (Seikagaku Kogyo Co., Tokyo, Japan) for 16 h at 37°C. Under these conditions the enzyme cleaves the peptide bonds at the carboxyl side of glutamic acid residues (Houmard & Drapeau, 1972). The digest was freeze-dried; freeze-drying was repeated twice after the addition of water (each 0.5 ml). The resulting peptides, $T_{3Ea}$-a, $T_{3Ea}$-b and $T_{3Ea}$-c, were separated preparatively by paper electrophoresis at pH 4.8, for 90 min (Maeda & Tamiya, 1976). The amino acid compositions and the electrophoretic mobilities of the peptides are shown in Table 1. Peptide $T_{3Ea}$-b was found to be the original peptide.

Tryptic peptide $T_{3Eb}$ was prepared from reduced and *S*-carboxymethylated erabutoxin b (10 mg), and digested with *S. aureus* V8 proteinase as described above. The amino acid composition of the resulting hexapeptides, $T_{3Eb}$-a and $T_{3Eb}$-c, are also shown in Table 1.

## Discussion

The results show that the residues 21 and 22 of erabutoxins a and b are glutamic acid and serine respectively (Fig. 1).

Sato & Tamiya (1971) split the peptide $T_{3Eb}$ with chymotrypsin and a proteinase (Proctase B) successively, and obtained peptide $T_3$-$C_1$-Pr [20–23; Gly-(Glu,Ser)-Ser]. They carried out a subtractive Edman degradation and deduced the sequence Gly-Ser-Glu-Ser. Contamination by glutamic acid arising from incomplete Edman degradation and decomposition of serine on hydrolysis might have led to this erroneous conclusion.

*S. aureus* V8 proteinase was, because of its specificity, especially useful in establishing the position of the glutamic acid residue.

The amino acid sequence of the corresponding part of the erabutoxin c (Tamiya & Abe, 1972) was deduced by homology. Its sequence must therefore also be corrected (Fig. 1).

Recently, Tsernoglou *et al.* (1977) suggested the same sequence as that now proposed after X-ray crystallography of erabutoxin b.

## References

Houmard, J. & Drapeau, G. R. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3506–3509
Iwanaga, S., Wallén, P., Gröndahl, N. J., Henshen, A. & Blombäck, B. (1969) *Eur. J. Biochem.* **8**, 189–199
Maeda, N. & Tamiya, N. (1974) *Biochem. J.* **141**, 389–400
Maeda, N. & Tamiya, N. (1976) *Biochem. J.* **153**, 79–87
Sato, S. & Tamiya, N. (1971) *Biochem. J.* **122**, 453–461
Tamiya, N. & Abe, H. (1972) *Biochem. J.* **130**, 547–555
Tamiya, N. & Arai, H. (1966) *Biochem. J.* **99**, 624–630
Tsernoglou, D., Petsko, A. & Tu, A. T. (1977) *Biochim. Biophys. Acta* **491**, 605–608