The Primary Structure of Staphylococcal Enterotoxin B

III. THE CYANOGEN BROMIDE PEPTIDES OF REDUCED AND AMINOETHYLATED ENTEROTOXIN B, AND THE COMPLETE AMINO ACID SEQUENCE*

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I-YIH HUANG† AND MERLIN S. BERGDOLL

From the Food Research Institute and the Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706

SUMMARY

Cyanogen bromide treatment of reduced, aminoethylated enterotoxin B yielded seven major peptides plus homoserine and homoserine lactone, as was expected from the number of methionine residues present in the enterotoxin B molecule. The cyanogen bromide peptides were fractionated on Sephadex G-50 and further purified by rechromatography on Sephadex G-50 or paper electrophoresis at pH 1.9. They contained a total of 239 amino acid residues.

Tryptic peptides of each of the cyanogen bromide peptides were purified and their amino acid composition was determined. The order of these peptides in each cyanogen bromide peptide and the order of the cyanogen bromide peptides in enterotoxin B were established.

Based on the information from tryptic, chymotryptic, and cyanogen bromide peptides, the complete amino acid sequence of enterotoxin B was established. Staphylococcal enterotoxin B is a single polypeptide chain containing 239 amino acid residues.

The amino acid sequence (partial or complete) of the tryptic and chymotryptic peptides of enterotoxin B has been elucidated (1, 2). These peptides account for 239 amino acid residues. Because of the large number of single residue overlaps, the complete amino acid sequence of enterotoxin B could not be determined. In order to link all of the tryptic peptides in one polypeptide chain, additional overlapping peptide information was essential. Chemical cleavage of the methionyl bonds of the protein by cyanogen bromide (3) was chosen as a method to obtain the needed information. Enterotoxin B contains 8 residues of methionine, but since in two instances 2 methionine residues appear together in the polypeptide chain (1) seven major peptides plus free homoserine and homoserine lactone (2 moles per mole of enterotoxin B) should be obtained.

The present paper describes the isolation, purification, and characterization of enterotoxin B peptides obtained after treatment with cyanogen bromide on reduced, aminoethylated enterotoxin B, and the complete amino acid sequence of enterotoxin B elucidated by utilizing information obtained from tryptic, chymotryptic, and cyanogen bromide digestion of enterotoxin B.

MATERIALS AND METHODS

Materials—All materials used in this work have been described elsewhere (1, 2, 4).

Reduction and Aminoethylation of Enterotoxin B—The procedures used for reduction and aminoethylolation of enterotoxin B were essentially the same as those reported previously (4). The NH₂ and COOH termini and amino acid composition of reduced, aminoethylated enterotoxin B have also been described previously (4).

Cyanogen Bromide Cleavage of Enterotoxin B—The procedure used for the cleavage of methionyl bonds in reduced, aminoethylated enterotoxin B was essentially that of Steers et al. (5).

Separation of Cyanogen Bromide Peptides of Enterotoxin B—The cyanogen bromide peptides were dissolved in 20 ml of 1 N formic acid and applied to a column (1.9 x 150 cm) of Sephadex G-50 which had been equilibrated in 1 N formic acid. The column was developed with 1 N formic acid at a flow rate of 15 to 20 ml per hour at room temperature. The effluent fractions (5.2 ml) were collected automatically. The peptides in each fraction were detected by measurement of the absorption at 277 mµ and ninhydrin (6). Appropriate fractions were pooled and dried in a rotary evaporator under reduced pressure at 40°. Further purification of the fraction was carried out by rechromatography of one-eighth of the fraction on the same column of Sephadex under the same conditions or by paper electrophoresis at pH 1.9 as described below.

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† Present address, Immunochemistry Research Department, Evanston Hospital, Northwestern University, Evanston, Illinois 60201.
or DNP-glycine. For 6 hours. The latter condition was useful for DNP-proline described previously (1). Hydrolysis of DNP-peptides was each cyanogen bromide peptide was determined by a method by paper electrophoresis at pH 1.9 followed by paper chromatography (Solvent I) unless otherwise indicated.

Hydrolysis of cyanogen bromide peptides plus 2 moles of homoserine lactone was digested with trypsin and Morton (8) was used to determine the content of tryptophan and threonine, respectively.

Determinations of 10 and 5% were made for losses of serine and homoserine lactone per mole of protein were obtained from the treatment with cyanogen bromide. This is what was expected since enterotoxin B contains two pairs of methionine residues (1). Gel filtration on Sephadex G-50 was found to be the most satisfactory method for fractionating the mixture of cyanogen bromide peptides, the results of which are shown in Fig. 1. An aliquot of every fifth effluent sample was examined by paper electrophoresis at pH 5.6 and 1.0 and by end group analysis. On the basis of these qualitative tests, fractions were pooled as shown in Fig. 1 and dried in a rotary evaporator under reduced pressure. These pooled fractions were reexamined by paper electrophoresis and by end group analysis.

End group analysis indicated the presence of two peptides in Fraction A, one with lysine and the other with tyrosine as the NH2 terminus. These two peptides could not be separated by rechromatography on Sephadex G-50. Tryptic digestion of this fraction showed Fraction A to be the aggregated mixture of the two peptides purified from Fractions B and D described below.

| Fragment Number | BrCN I | BrCN II | BrCN III | BrCN IV | BrCN V | BrCN VI | BrCN VII | BrCN VIII | BrCN IX | Total |
|-----------------|--------|---------|-----------|---------|--------|---------|----------|-----------|---------|-------|
| Lys             | 3.0(3) | 12.9(13)| 9.0(9)    | 2.0(2)  | 1.0(1) | 5.1(5)  | 33       |           |         |       |
| His             | 0.9(1) | 1.8(2)  | 1.9(2)    |         |        |         |          |           |         | 5     |
| Arg             |        | 1.8(2)  | 2.6(3)    |         |        |         |          |           |         | 5     |
| Asp             | 2.2(2) | 19.5(20)| 14.4(14)  | 2.2(2)  | 3.0(3) | 2.2(2)  | 44       |           |         |       |
| Thr             | 1.0(1) | 4.0(4)  | 5.6(6)    |         |        |         |          |           |         | 13    |
| Ser             | 3.0(3) | 4.9(5)  | 4.0(4)    | 1.0(1)  | 1.0(1) | 1.0(1)  | 20       |           |         |       |
| Glu a           | 3.0(3) | 1.0(1)  | 10.0(10)  | 1.0(1)  | 1.0(1) | 1.0(1)  | 20       |           |         |       |
| Pro             | 2.8(3) | 2.4(2)  | 5.0(5)    | 1.1(1)  |        |         |          |           |         | 9     |
| Gly             | 1.0(1) | 2.8(3)  | 1.3(1)    | 1.0(1)  |        |         |          |           |         | 5     |
| Cys b           |        | 1.9(2)  |          |         |        |         |          |           |         | 2     |
| Val             | 6.0(7) | 6.1(6)  |          |         |        |         |          |           |         |       |
| Met             | 0.0    | 0.0     | 0.0       | 0.0     | 0.0    | 0.0     | 0.0      | 3.2(3)    | 9.1    | 16    |
| Ile             | 4.9(5) | 3.2(3)  |          |         |        |         |          |           |         |       |
| Leu             | 5.2(3) | 7.2(7)  |          | 1.0(1)  | 1.0(1) | 1.0(1)  | 16       |           |         |       |
| Tyr             | 9.1(9) | 8.6(9)  | 1.1(1)    |         |        |         |          |           |         | 11    |
| Phe             | 0.9(1) | 5.0(5)  | 5.2(5)    | 1.0(1)  |        |         |          |           |         | 12    |
| Trp c           |        |        | 1.0(1)    |         |        |         |          |           |         | 1     |
| Homoserine + homoserine lactone d | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 1.0(1) | 0.9(1) | 0.0(0) | 8    |
| NH2 terminus    | Glu    | Glu     | Lys       | Tyr     | N.D./  | Pro     | Tyr      | Val       | 239    |       |

a Glutamic acid emerged with homoserine from the 60-cm column on the amino acid analyzer; therefore, residues per molecule for glutamic acid were estimated by considering the amount of homoserine.

b Determined as S-γ-aminomethylcysteine with 16-cm column of amino acid analyzer (4).

c Qualitatively examined on paper by Ehrlich’s reagent and, if positive, spectrophotometric determination in 0.1 NaOH was made.

d Ehrlich’s reagent-negative.

e Homoserine lactone emerged after NH3 as a single peak but homoserine emerged with glutamic acid. Residues per molecule for homoserine and homoserine lactone were estimated by twice the amount of homoserine lactone.

f Not determined.

Roman numerals, starting from the NH2 terminus of enterotoxin B, BrCN I, BrCN II, etc.

Tryptophan—The spectrophotometric method of Goodwin and Morton (8) was used to determine the content of tryptophan with the use of 0.1 NaOH. The cyanogen bromide and tryptic peptides which contained tryptophan were detected on paper chromatograms with Ehrlich’s reagent (9).

Nomenclature—The peptides obtained from cyanogen bromide digestion of reduced, aminoethylated enterotoxin B were given

1 The abbreviation used is: DNP-, 2,4-dinitrophenyl-.
Fig. 1. Fractionation of cyanogen bromide fragments of reduced, aminomethylated enterotoxin B. A, reduced, aminomethylated enterotoxin B (900 mg) was treated with cyanogen bromide. The cyanogen bromide fragments were applied to a column (1.9 X 150 cm) of Sephadex G-50 equilibrated with 1 N acetic acid. The effluent fractions were determined at 277 nm (——) and by the ninhydrin method (——). The fракtions between arrows indicate which portions of the effluent fractions were pooled. Fractions B and D were rechromatographed on the same column of Sephadex G-50 under same conditions but different sample size, one-eighth of original, to give the patterns shown in B and C. B, Fraction B. C, Fraction D. The arrows in B and C indicate those fractions from each column which were pooled.

Fig. 2. Chromatographic fractionation of tryptic peptides from a tryptic digest of Peptide BrCN III. Digest was chromatographed on a column of Dowex 50W-X2 (0.9 X 60 cm) with the starting buffer, 0.17 m pyridine acetate, pH 4.8, and limit buffer, 8.5 m pyridine acetate, pH 5.0. The details of the procedures were described by Konigsberg and Hill (10). Peptides were numbered according to the elution order and are identical with those described in the text (I = BrCN III-T-1, etc.).

Purification of Fraction B, which contained both NH2-terminal lysine (+ + + +) and tyrosine (+ +), yielded BrCN III which contained only lysine as its NH2 terminus (Fig. 1B).

Purification of Fraction C, which contained both NH2-terminal lysine (+ + + +) and tyrosine (+ + + +), yielded BrCN III and BrCN IV (described below); however, the purity was not sufficient for further studies.

Purification of Fraction D, which contained both NH2-terminal lysine (+ +) and tyrosine (+ + + +), yielded BrCN IV which contained only tyrosine as its NH2 terminus (Fig. 1C).

Purification of Fraction E, which contained NH2-terminal glutamic acid (+ + + +) and valine (+ + + +), by paper electrophoresis at pH 1.9 yielded BrCN I and BrCN IX with glutamic acid and valine, respectively, as the NH2 terminus. Peptide BrCN IX did not contain homoserine or homoserine lactone and hydrazinolysis revealed lysine to be the COOH terminus; therefore, this peptide is the COOH-terminal part of enterotoxin B.

Purification of Fraction F, in which DNP-proline (+) was detected as the NH2 terminus (12 N HCl, 6-hour hydrolysis), by paper electrophoresis at pH 1.9 yielded BrCN VI.

Purification of Fraction G, which contained NH2-terminal glutamic acid (+ + + +), tyrosine (+ + + +), and unknown yellow spots (may be DNP-homoserine and its lactone), by paper electrophoresis at pH 1.9 yielded peptides BrCN II (glutamic acid as the NH2 terminus), BrCN VIII (tyrosine as the NH2 terminus), and BrCN V and BrCN VII (free homoserine and homoserine lactone).

A summary of the properties of the cyanogen bromide peptides and their designation is given in Table I. Peptides BrCN I and BrCN II contain glutamic acid as the NH2 terminus; however, it was determined from the tryptic and chymotryptic peptides (1, 2) that BrCN I is the NH2-terminal part of enterotoxin B.

Only BrCN IV gave a positive test for tryptophan and, as discussed below, yielded a peptide upon tryptic digestion which corresponded to the single Ehrlich-positive peptide purified by paper electrophoresis at pH 1.9. The mole ratio of tyrosine to tryptophan was found by spectrophotometric analysis to be 9 for BrCN IV and 1 for the Ehrlich-positive tryptic peptide.

Digestion of Cyanogen Bromide Peptides with Trypsin

The major cyanogen bromide peptides, BrCN I, BrCN III, BrCN IV, BrCN VI, and BrCN IX, were further analyzed by digestion with trypsin. The resultant tryptic peptides from BrCN I, BrCN IV, and BrCN VI were purified by paper electrophoresis at pH 1.9 followed by paper chromatography (Solvent I) (1). The peptides from BrCN III were chromatographed initially on a column of Dowex 50W-X2 (7) (Fig. 2) followed by paper chromatography (Solvent I) except for Peptide BrCN III-T-15; this peptide was insoluble and was removed by centrifugation and purified by paper electrophoresis at pH 1.9. The peptides from BrCN IX were purified by paper chromatography only (Solvent I).

Peptide BrCN I—This peptide contains 21 amino acid residues and when digested with trypsin for 24 hours gave three peptides, BrCN I-T-1 (Thr, 1.02; G1y, 0.97; Lue, 1.01; Phe, 0.87; homo...
**Peptide BrCN II**—As shown in Table I, this peptide contains only 3 residues with glutamic acid as its NH₂ terminal. The sequence of this peptide which is a part of OT-18 (1) is shown in Fig. 3.

**Peptide BrCN III**—The amino acid composition and NH₂ terminal are outlined in Table I. Digestion of this peptide with trypsin gave 15 tryptic peptides, BrCN III-T-1, (Lys, 1.20; Asp, 2.08; Ala, 1.00; Leu, 1.00), BrCN III-T-2 (Lys, 1.00; Asp, 1.00; Thr, 1.00), BrCN III-T-3 (Lys, 1.00; Asp, 1.00; neutral at pH 5.6), BrCN III-T-4 (Lys, 1.14; His, 0.82; Asp, 3.80; Thr, 1.64; Ser, 1.00; Glu, 1.00; Ile, 0.88), BrCN III-T-5 (Lys, 2.00; His, 0.95; Asp, 4.10; Ser, 0.98; Ala, 1.00; Val, 3.00; Ile, 1.00; Leu, 1.00; Tyr, 0.95; dinitrophenylation: bis-DNP-lysine), BrCN III-T-6 (Lys, 1.00; Asp, 1.24; basic at pH 5.6), BrCN III-T-7 (Thr, 1.00; Cys (as aminoethylcysteine), 1.00; homoserine and homoisoleucine lactone, 0.90), BrCN III-T-8 (Lys, 1.00), BrCN III-T-9 (Lys, 1.00; Glu, 1.00; Val, 0.90; Phe, 1.00), BrCN III-T-10 (Arg, 0.80; Asp, 3.00; Gly, 1.06; Val, 1.02; Leu, 0.98; Tyr, 0.99), BrCN III-T-11 (Arg, 1.00), BrCN III-T-12 (Lys, 1.00; Tyr, 1.10 (analyzed on parent effluent fraction)), BrCN III-T-13 (Cys (as aminoethylcysteine), 1.10; Asp, 2.00; Gly, 1.00; Phe, 1.00; Ala, 0.93; Val, 1.00; Tyr, 2.63; Phe, 1.00), BrCN III-T-14 (Lys, 0.96; Ser, 0.98; Tyr, 1.00; Phe, 1.04), BrCN III-T-15 (Lys, 1.00; Asp, 2.00; Ser, 1.60; Glu, 1.00; Ile, 2.70; Leu, 2.00; Tyr, 1.95; Phe, 2.00).

Peptide T-7 contains homoserine and homoserine lactone which establishes it as the COOH terminus of BrCN III. Peptide T-7 is identical with the 3 NH₂-terminal residues of OT-1 (1). Trypsin did not cleave the bond between S-β-aminoethylcysteine and homoserine or homoserine lactone which is consistent with the failure of trypsin to cleave the peptide chain at the carboxyl side of lysine or arginine when these residues were adjacent to the COOH terminus of the peptide (2). Peptides BrCN III-T-1, BrCN III-T-2, BrCN III-T-3, BrCN III-T-4, BrCN III-T-6, BrCN III-T-8, BrCN III-T-9, BrCN III-T-10, BrCN III-T-11, BrCN III-T-12, and BrCN III-T-15 have the same amino acid compositions as Peptides OT-2, OT-3, OT-5, OT-8, OT-10, OT-12, OT-16, OT-18, OT-22, OT-17, OT-24, and OT-29, respectively, and T-13 and T-14 combined, the same composition as OT-30 except for the replacement of cysteic acid in OT-30 with S-β-aminoethylcysteine which is susceptible to tryptic digestion. Peptide

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**Fig. 4.** Order of tryptic peptides and amino acid sequence of BrCN II and III. Proof of sequence is derived from the overlapping tryptic (OT) and chymotryptic (RC and OC) peptides (1, 2). Continuous lines indicate the sequences were actually determined; dashed lines indicate that only the compositions were determined.
Fig. 5. Order of tryptic peptides and amino acid sequence of BrCN IV and V. Proof of sequences is derived from the overlapping tryptic (OT) and chymotryptic (RC and OC) peptides (1, 2). Continuous lines indicate that the sequences were actually determined; dashed lines indicate that only the compositions were determined.

Fig. 6. Order of tryptic peptides and amino acid sequence of BrCN V and BrCN VI. Proof of sequence is derived from the overlapping tryptic (OT) and chymotryptic (RC and OC) peptides (1, 2). Continuous lines indicate that the sequences were actually determined; dashed lines indicate that only the compositions were determined.

Fig. 7. Order of tryptic peptides and amino acid sequence of BrCN VII, BrCN VIII, and BrCN IX. Proof of sequence is derived from the overlapping tryptic (OT) and chymotryptic (RC and OC) peptides (1, 2). Continuous lines indicate that the sequences were actually determined; dashed lines indicate that only the compositions were determined.
FIG. 8. The complete amino acid sequence of enterotoxin B. Evidence of sequence is derived from Figs. 3 to 7. Only the order of the tryptic peptides (OT) are included. Roman numerals are placed on each methionine residue to express the COOH terminus of each cyanogen peptide. Peptide BrCN IX, the COOH-terminal peptide of enterotoxin B (from residue 223 to 239), is not expressed.
BrCN III-T-5 has the same composition as OT-19 except for an extra lysine residue in T-5. This lysine residue is the NH₂ terminus of T-5 and thus the NH₂ terminus of BrCN III (Table I). The evidence for the sequence of BrCN III is given in Fig. 4, and the evidence that Peptides BrCN I, BrCN II, and BrCN III will appear in that order in the enterotoxin B chain is outlined in Figs. 3 and 4. Although the overlap of OT-3 and OT-22 by RC-39 is a single leucine residue, T-10 (OT-22) is the only tryptic peptide in BrCN III with leucine as the NH₂-terminal residue. All of the residues in Peptides BrCN I, BrCN II, and BrCN III can be assigned to known tryptic peptides with only one possible arrangement for these peptides (Figs. 3 and 4).

Peptide BrCN IV—The amino acid composition and NH₂ terminus are outlined in Table I. Tryptic digestion of this peptide gave 12 peptides, BrCN IV-T-1 (Asp, 3.16; Ser, 0.98; Glu, 2.00; Ile, 0.99; Tyr, 1.00; Phe, 2.00; Trp, 1.00; homoserine and homoserine lactone, 1.00), BrCN IV-T-2 (Lys, 1.00; Asp, 3.00; Thr, 0.65; Ser, 0.75; Glu, 1.12; Val, 1.00; Leu, 2.00; Phe, 1.00), BrCN IV-T-3 (Lys, 1.00; Asp, 2.20; Thr, 0.75; Ser, 0.98; Glu, 1.95; Pro, 0.80; Gly, 1.12; Ile, 1.02; Leu, 1.00; Tyr, 2.80; Phe, 1.00), BrCN IV-T-4 (Arg, 0.90; Asp, 1.10; Thr, 1.76; Glu, 2.24; Ala, 1.00; Val, 1.00; Leu, 2.00; Tyr, 0.95), BrCN IV-T-5 (Lys, 1.00; His, 0.94; Asp, 3.09; Thr, 1.00; Glu, 2.09; Gly, 3.22; Val, 1.00; Leu, 1.00; Tyr, 0.73), BrCN IV-T-6 (Lys, 1.00; Asp, 0.77; Glu, 1.10; Gly, 1.10; Val, 1.05; Phe, 0.99), BrCN IV-T-7 (Arg, 1.00; Thr, 0.58; Ser, 0.55; Val, 1.12; Ile, 1.00), BrCN IV-T-8 (Asp, 0.94; Lys, 1.06; basic at pH 5.6), BrCN IV-T-9 (Lys, 1.00), BrCN IV-T-10 (Arg, 1.00; Tyr, 2.00, (hydrolyzed by leucine aminopeptidase and carboxypeptidase A and B)), BrCN IV-T-11 (Lys, 1.00; His, 0.55; Val, 1.00; Leu, 1.00; Tyr, 0.59), and BrCN IV-T-12 (Lys, 1.00; lysyl-lysine (minor component)). The order in which these peptides appear in BrCN IV is given in Fig. 5. Peptide BrCN IV-T-1 was the only peptide that gave a positive reaction to Ehrlich’s reagent. Spectrophotometric analysis established the more ratio of tyrosine to tryptophan as 1:1. The location of tryptophan in this peptide is given in Fig. 5. This peptide is the COOH terminus of BrCN IV since it is the only tryptic peptide which contains homoserine and homoserine lactone. The composition of Peptides BrCN IV-T-2, BrCN IV-T-3, BrCN IV-T-4, BrCN IV-T-5, BrCN IV-T-6, BrCN IV-T-7, BrCN IV-T-8, BrCN IV-T-9, BrCN IV-T-10, and BrCN IV-T-11 is identical with Peptides OT-3, OT-22, OT-12, OT-11, OT-21, OT-16, OT-6, OT-26, and OT-27, respectively (1). Peptide BrCN IV-T-12, lysyl-lysine, is evidence that at least 3 consecutive lysine residues are present in BrCN IV which is confirmed by OCD (Fig. 5). Peptide BrCN IV-T-5 has the same amino acid composition as OT-1 except for the 3 NH₂-terminal residues of OT-1. This establishes T-5 as the NH₂-terminal part of BrCN IV. Peptide OT-20 is placed on the carboxyl side of OT-1 since OT-26 (T-10) is the only tryptic peptide in BrCN IV with tyrosine as the NH₂ terminus.

Peptides BrCN V and BrCN VII—Digestion with cyanogen bromide gave 2 moles of homoserine and homoserine lactone as was expected from the presence of two pairs of methionine residues in the enterotoxin B molecule (OT-28 (Fig. 5) and OT-23 (Fig. 6)) (1, 2).

Peptide BrCN VI—The amino acid composition and NH₂ terminus are outlined in Table I. Tryptic digestion of this peptide gave three peptides, BrCN VI-T-1 (Leu, 1.00; Tyr, 0.92; homoserine and homoserine lactone, 0.95), BrCN VI-T-2 (Lys, 1.00; Asp, 1.10; Pro, 1.80; Gly, 1.10; Ala, 1.03), and BrCN VI-T-3 (Lys, 1.00; Asp, 1.10; Ser, 1.07; Glu, 1.10; Phe, 1.00). The order of appearance of these peptides in BrCN VI is outlined in Fig. 6. Peptide BrCN VI-T-1 is the COOH terminus of BrCN VI since it contains homoserine and homoserine lactone. Peptide BrCN VI-T-3 has the same amino acid composition as OT-10, and T-2 the same composition as the COOH-terminal part of OT-28 (Fig. 6).

Peptide BrCN VIII—This peptide contains 6 amino acid residues including homoserine and homoserine lactone with tyrosine as the NH₂ terminus (Table I). The sequence of this peptide is outlined in Fig. 7.

Peptide BrCN IX—The amino acid composition and NH₂ terminus are outlined in Table I. This peptide is the COOH terminal part of enterotoxin B since it did not contain homoserine or homoserine lactone. Tryptic digestion of this peptide gave four peptides and free lysine, BrCN IX-T-1 (Lys, 2.00), BrCN IX-T-2 (Lys, 1.00; minor component), BrCN IX-T-3 (Lys, 1.05; Asp, 1.00; Ser, 0.98; Val, 1.00), BrCN IX-T-4 (Lys, 0.98; Asp, 1.02; Val, 1.00), and BrCN IX-T-5 (Lys, 1.00; Thr, 1.96; Glu, 1.00; Val, 0.99; Ile, 0.90; Leu, 1.10; Tyr, 0.90). Peptide BrCN IX-T-4 has the same amino acid composition as OT-4. Peptide BrCN IX-T-5 is the only peptide obtained from tryptic digestion of the cyanogen bromide peptides that was not obtained from tryptic digestion of intact enterotoxin B. Apparently, in the latter digestion the chain was broken at the tyrosine residue. To complete the information on the tryptic peptides, T-5 has been labeled OT-15 as described previously (1).

Complete Amino Acid Sequence

The complete amino acid sequence of enterotoxin B is outlined in Fig. 8. It is derived from Figs. 3 to 7 in which the order of placement of the cyanogen bromide peptides is outlined. All of the residues in the cyanogen bromide peptides were assignable to known tryptic peptides and there was only one possible way to arrange each tryptic peptide into the cyanogen bromide peptides.

DISCUSSION

The results of the amino acid sequence studies show that enterotoxin B contains 239 amino acid residues with a molecular weight of 28,494. This is very near the molecular weight of 29,000 (242 residues) calculated from the amino acid composition by the minimum molecular weight method based on the presence of 2 residues of half-cystine. Three different molecular weights have been published for enterotoxin B, 24,000 ± 3,000 (11), 35,300 (299 residues) (12), and 30,000 ± 1,000 (252 residues) (13). The first value was calculated from the sedimentation data with an assumed value for the diffusion constant which happened to be about 20% high. This value was more or less confirmed by minimum molecular weight calculations based on 1 residue of tryptophan. Later values obtained for tryptophan were interpreted as indication of the presence of 2 tryptophan residues (12, 13). Actually, the tryptophan values proved to be high as only one tryptophan residue was revealed by the sequence studies. It should be expected that the tryptophan values would be inaccurate since the ratio of tryptine to tryptophan in enterotoxin B is 21:1. The value of 35,300, reported by Spero et al. (12), was obtained at least in part from the half-cystine value which was about 20% lower than that reported by Bergdoll et al. (13). The 30,000 value reported by the latter workers was a compromise between the value calcu-
listed with the sedimentation data and that with the half-cystine value.

The difference in the number of residues of the different amino acids previously reported (12, 13) and the numbers actually found was essentially proportionate to the molecular weights used in calculating the number of residues. The number of amide groups in the molecule is greater than the number reported (12, 13), giving an excess of nine basic groups instead of five to eight as had been calculated.

An earlier attempt to determine the terminal amino acid sequences (13) gave different results than this detailed study revealed. This may have been due to inadequacies of the earlier procedures.

Information about the conformation of enterotoxin B indicates it to be a very compact molecule (12, 14). It has been revealed that the 2 half-cystine residues appear in the enterotoxin molecule as 1 cystine residue since no free ─SH groups are present (12, 13). This linkage is not essential, however, for maintaining the conformation or biological activity of the enterotoxin since the ─S─S─ linkage can be reduced and methylated without appreciably affecting either the conformation or the activity (15).

Since the half-cystine residues are readily available for reduction, it would appear that the middle part of the enterotoxin chain is exposed since these 2 residues are Nos. 92 and 112 in the chain. There is an excess of basic residues in this portion of the chain including a pair of lysine residues and a ─Lys─Arg─Lys─ series.

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