Determination of the Primary Structure of Cholera Toxin B Subunit*

CHUN-YEN LAI
From the Roche Institute of Molecular Biology, Nutley, New Jersey 07110

The primary structure of cholera toxin B subunit, responsible for the binding of the toxin to cell surface ganglioside Gm₁, has been determined as:

```
Thr-Pro-Gln-Ile-Thr-Asp-Leu-Cys-Ala-Glu-Tyr-His-Asn-Thr-Gln-Ile-Thr-Leu
Asn-Asn-Ile-Phe-Ser-Tyr-Thr-Glu-Ser-Leu-Ala-Gly-Lys-Arg-Glu-Met-Ala-Ile-Ile
Thr-Phe-Lys-Asn-Ala-Thr-Phe-Glu-Val-Glu-Val-Pro-Gly-Ser-Gln-His-Ile-Asp-Ser
Gln-Lys-Ala-Ile-Glu-Arg-Met-Lys-Asn-Thr-Leu-Arg-Ile-Ala-Tyr-Leu-Thr-Glu-Ala
Lys-Val-Glu-Lys-Leu-Cys-Val-Asn-Asn-Thr-Asp-His-Leu-Ile-Ile-Asp-Ile-Ile-Ser
103
Met-Ala-Asn
```

The number and the sequence of amino acid residues has been established by separation and analyses of the peptides obtained through enzymatic and chemical cleavage of the B subunit. The chymotryptic peptides from the citraconylated B subunit proved to be useful in the alignment of the tryptic peptide.

The binding of cholera toxin to cell surfaces is the first step in the intoxication reaction (for review, see Ref. 1), and the B subunit of the toxin has been implicated in the process (1-3). We have previously achieved a complete separation of the cholera toxin subunits (4) and demonstrated that the B subunit is a polypeptide of Mₑ = 10,000 containing one intrachain disulfide bond and also that 1 mol of the holotoxin contains 5 or 6 mol of the subunit and 1 mol of the other subunit, A. Preliminary reports on the primary structure of cholera toxin B subunit have recently been published from three laboratories, including our own, with small discrepancies (5-7). In this paper, we present a detailed account of the determination of the sequence of 103 amino acid residues in this protein. The data on the separation and analyses of the peptides, obtained from chemically modified or unmodified B subunit through enzymic or BrCN cleavages, should also be of interest in future studies on the active centers of the binding subunit.

MATERIALS AND METHODS
Cholera toxin was initially obtained from the Geographic Medicine Branch, National Institute of Allergy and Infectious Diseases, through the courtesy of Dr. Carl E. Miller, and later prepared in this laboratory from the lyophilized culture filtrate of Vibrio cholerae provided by the same source. B subunit was prepared by gel filtration of cholera toxin on Sephadex G-75 in 5% HCOOH as described previously (4). The isolated B subunit was reduced and S-carboxymethylated with 3-iodo-[14C]acetic acid in 6 M guanidine HCl essentially as described by Crestfield (9) and used as the starting material for the sequence analyses.

Enzymes used as reagents were obtained from Worthington Biochemical, Freehold, N. J.; tosylphenylalanyl chloromethyl ketone-treated-trypsin, α-chymotrypsin, and carboxypeptidases A and B (DFP-CoA and DFP-CoD); thermolysin was from CalBiochem, La Jolla, Calif.; A-protease was from Pierce Chemical, Rockford, Ill. Reagents used in the Edman degradation procedures were of "Sequan grade" from the Pierce Chemical Co. Pyridine, benzene, ethyl acetate, and trifluoroacetic acid were distilled and used within 6 months. Other reagents were of the highest quality available commercially and used without further purification.

Ion exchange chromatography on Dowex 50-X2 (Bio-Rad AG 50-X2) or Dowex 1-X2 (Bio-Rad AG 1-X2) was performed essentially as described by Schreuder (10). High voltage paper electrophoresis was performed with a Savant apparatus (Savant Instruments, Hicksville, N. Y.) on Whatman No. 3MM paper at the pH indicated. Detection of peptides using fluorescamine (11, 12) and Edman degradation (13, 14) of peptides were as described previously. The amino acid residues removed at each step were identified by regeneration of the residue (15) and amino acid analysis. Under the conditions of regeneration (150° for 4 h in 5.7 N HCl, 0.1% SnCl₂), anilinothiazolinones of threonine give rise to ε-amino-butyric acid (εABA), serine to alanine, and isoleucine, partially to alloisoleucine, respectively (15). A portion of the peptide was saved after each step and analyzed for the remaining amino acids when identification of the removed residue failed. Amino acid analyses were performed with a Jeol 6AH or 5AH automatic amino acid analyzer (Jeo USA, Cranford, N. J.) having a range of detection above 2 x 10⁻⁶ M. The presence of side chain amide groups in peptides containing acidic amino acids was determined by the method of Offord (16) from the electrophoretic mobility of the peptides at pH 6.5.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Primary Structure of Cholera Toxin B Subunit

FIG. 1. Separation of tryptic peptides from cholera toxin B subunit. A, gel filtration of the tryptic digest of S-carboxymethyl B subunit (12.5 mg) on Sephadex G-25F (2.2 x 90 cm) in 0.1 M NH,HCO,. The flow rate was 40 ml/h. Aliquots (50 μl) from every other tube (2.4 ml) were assayed with fluorescamine after hydrolysis (11). Fractions T1 and T3 contained cysteine residues as evidenced by the radioactivity in the peaks (from the 15C-carboxymethyl group). Each fraction was lyophilized and purified further (B, C, and D). B, DEAE-cellulose chromatography of Fraction T1 (A). The column (1 x 26 cm) was eluted with a linear gradient of 0.05 to 0.15 M NH,HCO,. pH 8.5, in a total of 400 ml. A 0.1-ml aliquot from every other tube (1.8 ml) was analyzed for peptides. Peptides T1a, T1b, and T1c were essentially pure as judged by the NH,-terminal analysis. Peptide T1c contained one of the 2 cysteine residues (labeled with 15CChlodoacetate) in the B subunit (BT[Cys]-1, (18)). C, Dowex 50-X2 chromatography of Fraction T2 (A). The column (0.9 x 50 cm) was eluted with a linear gradient of pyridine acetate buffer, 0.2 M (pH 3) to 2 M (pH 5), in a total of 1000 ml. A 0.15-ml aliquot was analyzed. Peptide T3c contained the other cysteine residue (BT[Cys]-2 (18)). Peptides T3c' and T3c'' had amino acid compositions identical to those of T3c. The results indicate that the 2 Asn residues in T3c have lost one and two amide groups, forming T3c' and T3c'', respectively.

Maleylation and citraconylation of the B polypeptide were carried out essentially as described previously (17). In a typical experiment, 11 mg of S-carboxymethylated B chain dissolved in 0.6 ml 8 M guanidine HCl was adjusted to pH 8.5 and mixed with 0.6 ml 0.2 M NaHCO,. While stirring with a magnetic stirrer, 12 μl of citraconic anhydride (Pierce Chemical) was added in 3-μl aliquots, maintaining the pH at 8.5 by addition of approximately 0.1 ml of 0.5 M NaOH each time. For maleylation, 50% (w/v) solution of maleic anhydride in acetone was added in 5-μl aliquots, eight times, maintaining the pH at 9.0 with 2 M NaOH (5 μl for each addition). After the reaction, the mixture was desalted on a column (1 x 26 cm) of Sephadex G-25F in 0.1 M NH,HCO, and used directly for the proteolytic digestion.

RESULTS

Properties of Cholera Toxin B Subunit

B subunit, as isolated by gel filtration of purified cholera toxin in 5% HCOOH (4), is insoluble at neutral pH values unless it is first dissolved in 8 M urea followed by dialysis to remove urea. The optical absorbance at 280 nm (A280) of a 0.1% solution in 70% HCOOH was determined to be 0.95; the dry weight of the sample was determined by amino acid analysis of an aliquot and summation of the residue weights.1 The results of analyses on NH2 and COOH termini of the B subunit (4, 5) established that the B subunit was a single-chain polypeptide.

1 D. Chang and C. Y. Lai, manuscript in preparation.

Separation and Sequence Analyses2 of Tryptic Peptides from B Subunit

Complete separation of the peptides resulting from the digestion of the S-carboxymethylated B subunit with trypsin (1% w/w of substrate, pH 8.5, 23° for 3 h) was achieved by gel filtration followed by ion exchange chromatography on DEAE-cellulose or Dowex 50-X2 as shown in Fig. 1. The main component of T4 (Fig. 1A) was arginine, which was separated and identified by paper electrophoresis. The separation patterns shown in Fig. 1 were reproducible from experiment to experiment except for the yields of peptides, which varied somewhat with the conditions of tryptic digestion. Purity of peptides was tested by paper electrophoresis. The peak, T2g (Fig. 1C), was due to two peptides, T2g1 and T2g2, which were further separated by Dowex 50 chromatography (see Fig. 5 in the miniprint supplement). The overall yields of peptides obtained in one series of separation steps are shown in Table I.

Results of sequence analyses on the tryptic peptides are summarized in tables in the miniprint supplement.2 The analytical data including Figs. 5 to 7 and Tables 3 through 19 are presented in miniprint immediately following this paper. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document 77M-233, cite author(s), and include a check or money order for $3.60 per set of photocopies.

1 0.2M [PfB*]
2 D. I. Iag"7 I I ' I T-3
600 i -i-
0 10 20 30 40 TUBE NUMBERS
100 120
0 20 40 TUBE NUMBERS
100 120
0 20 40 TUBE NUMBERS
100 120
0 20 40 TUBE NUMBERS
100 120

Downloaded from http://www.jbc.org/ by guest on October 15, 2017
Amino acid sequences of some tryptic peptides were deduced from the structural information on the chymotryptic peptides containing part of their sequence or the fragments derived from them, or both. Indices to these data are also provided in Table I.

**Chymotryptic Peptides of B Subunit**

In order to obtain information on the arrangement of the tryptic peptides in the B subunit (B chain), the latter was hydrolyzed with chymotrypsin and the resulting peptides were isolated (Fig. 2). Only the chromatograms which have

<p>| Table I: Tryptic and chymotryptic peptides from cholera toxin B subunit: index to figures and tables for their isolation and analyses |
|---------------------------------|--------------------|-----------------|------------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Peptides*</th>
<th>Residue number</th>
<th>Separation</th>
<th>Overall yields</th>
<th>Analyses*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1c</td>
<td>1-23</td>
<td>Fig. 1D</td>
<td>44 (%)</td>
<td>Ref. 10</td>
</tr>
<tr>
<td>T2h</td>
<td>24-34</td>
<td>Fig. 1C</td>
<td>52 (%)</td>
<td>Table 3</td>
</tr>
<tr>
<td>T2f</td>
<td>24-35</td>
<td>Fig. 1C</td>
<td>25 (%)</td>
<td>Table 3</td>
</tr>
<tr>
<td>T2f</td>
<td>36-43</td>
<td>Fig. 1C</td>
<td>33 (%)</td>
<td>Table 4</td>
</tr>
<tr>
<td>T1b</td>
<td>44-62</td>
<td>Fig. 1B</td>
<td>41 (%)</td>
<td>Table 5</td>
</tr>
<tr>
<td>T1a</td>
<td>44-63</td>
<td>Fig. 1H</td>
<td>35 (%)</td>
<td>Table 5</td>
</tr>
<tr>
<td>T2i</td>
<td>64-67</td>
<td>Fig. 1C</td>
<td>90 (%)</td>
<td>Table 6</td>
</tr>
<tr>
<td>T2j, k</td>
<td>68-73</td>
<td>Fig. 1C</td>
<td>46 (%)</td>
<td>Table 7</td>
</tr>
<tr>
<td>T2g</td>
<td>74-61</td>
<td>Fig. 1C, Fig. 5</td>
<td>52 (%)</td>
<td>Table 8</td>
</tr>
<tr>
<td>T2g</td>
<td>82-84</td>
<td>Fig. 1C, Fig. 5</td>
<td>56 (%)</td>
<td>Table 9</td>
</tr>
<tr>
<td>T3c</td>
<td>85-91</td>
<td>Fig. 1D</td>
<td>37 (%)</td>
<td>Ref. 18</td>
</tr>
<tr>
<td>T2e</td>
<td>92-103</td>
<td>Fig. 1C</td>
<td>42 (%)</td>
<td>Table 10</td>
</tr>
<tr>
<td>C2i</td>
<td>19-25</td>
<td>Fig. 2B</td>
<td>45 (%)</td>
<td>Table 11</td>
</tr>
<tr>
<td>C2k</td>
<td>32-37</td>
<td>Fig. 2R</td>
<td>27 (%)</td>
<td>Table 12</td>
</tr>
<tr>
<td>C2f</td>
<td>43-48</td>
<td>Fig. 3C</td>
<td>18 (%)</td>
<td>Table 13</td>
</tr>
<tr>
<td>C2b</td>
<td>49-72</td>
<td>Fig. 3B</td>
<td>43 (%)</td>
<td>Table 14</td>
</tr>
<tr>
<td>C3a</td>
<td>73-76</td>
<td>Fig. 3A</td>
<td>53 (%)</td>
<td>Table 16</td>
</tr>
<tr>
<td>C3b</td>
<td>77-88</td>
<td>Fig. 3B</td>
<td>60 (%)</td>
<td>Table 16</td>
</tr>
<tr>
<td>C3c</td>
<td>89-94</td>
<td>Fig. 3C</td>
<td>23 (%)</td>
<td>Table 17</td>
</tr>
<tr>
<td>C2a</td>
<td>95-103</td>
<td>Fig. 2B</td>
<td>23 (%)</td>
<td>Table 18</td>
</tr>
</tbody>
</table>

* Peptides are listed in order of their location from the NH₂ terminus of the B chain. Information on the overlaps is provided by analyses of peptide C2i-cC2a.

Figs. 5 through 7 and Tables 3 through 19 are found in the miniprint supplement.

To circumvent this problem, the S-carboxymethylated B chain was first citraconylated as described under "Materials and Methods" and then treated with chymotrypsin in 0.1 M NH₄HCO₃ for 4 h at 35°C. It was anticipated that the modification of the lysine side chains would prevent the hydrolysis of bonds adjacent to this residue. After the preliminary fractionation by gel filtration on Sephadex G-25F in 0.1 M NH₄HCO₃ (Fig. 3A), the pooled fractions were lyophilized, and the residue was dissolved in 1% HCOOH to effect removal of the citraconyl group (30 min at 23°C) and then separated by ion exchange chromatography (Fig. 3, B and C). The chymotryptic peptides obtained from the citraconylated B chain were designated with the prefix "cC," in a similar fashion as the "T" (tryptic) and "C" (chymotryptic) peptides (Figs. 1 and 2, respectively). The fraction cC3 (Fig. 3A) contained one major peptide which was purified by high voltage paper electrophoresis at pH 3.5, with 53% yield (residues 73 to 76).

The chymotryptic peptides found to contain amino acid residues which overlap two or more tryptic peptides were analyzed for their sequences, and the results are summarized in the miniprint supplement.² Indices to these data are provided in Table I.

**Alignment of Tryptic Peptides**

Peptides Derived from NH₂ and COOH Termini: The amino acid sequence at the NH₂ terminus of cholera toxin B subunit was determined as Thr-Pro-Glx- by Edman degradation, in which threonine (42%, as α-aminobutyric acid) and proline (75%) were successively released. The third residue in sequence was identified as 5-dimethylaminonaphthalene-1-sulfonic acid (DNS-Glu). The only tryptic peptide with cysteine, has previously been established (BT(cys)-1, see Ref. 18).

### Table of Contents

- **Primary Structure of Cholera Toxin B Subunit**
- **Chymotryptic Peptides of B Subunit**
- **Alignment of Tryptic Peptides**

---

A. Gel filtration of the chymotryptic digest of S-carboxymethyl B subunit (7 mg) on Sephadex G-25F. The column (1 x 150 cm) was eluted with 0.1 M NH₄HCO₃ at the rate of 8 ml/h and fractions of 2 ml were collected. Aliquots of 80 µl from every other tube were analyzed with fluorescamine as previously described (11). Fractions C1-C4 were lyophilized and subjected to further separations. B, Dowex 50-X2 chromatography of Fraction C2 from the gel filtration (A). The column (0.0 x 60 cm) was eluted with a linear gradient of pyridine acetate buffer, 0.1 N (pH 3, starting) to 1 N (pH 5) and 8 N (pH 6.5), as indicated in the figure. The flow rate was 10.6 ml/h and 1.8-ml fractions were collected. Aliquots of 0.1 ml from every other tube were analyzed for peptides. Peptides contained in the fractions indicated with bars were found to be essentially pure.
The COOH-terminal sequence of subunit B was established by three lines of evidence. (a) Carboxypeptidase digestion: maleylated subunit B (0.8 mg in 1 ml of 0.2 M N-ethylmorpholine/HC1 buffer; pH 8.5) was treated with 8 μl of carboxypeptidase solution (2 μg/ml) at room temperature. At the indicated time, 150-μl aliquots were removed and added to 0.85 ml of 0.2 M sodium citrate buffer, pH 2.2, and analyzed with a Jeol 5AH amino acid analyzer using a program for physiological fluids. No amino acid was detected at 0 min. (b) Hydrazinolysis of subunit B: no amino acid was detected; the COOH terminus is either asparagine or glutamine. Approximately 0.5 mg of subunit B (50 nmol) was used. After extraction of hydrazides (14), the aqueous layer was analyzed with a Jeol 6AH amino acid analyzer, the limit of detection with which was 1 nmol. (c) BrCN cleavage of subunit B and gel filtration (supplement Fig. 2A): yielded a dipeptide, Ala-Asn (CN-3, over 95% recovery in three experiments, see supplement Table II for the analysis).

<table>
<thead>
<tr>
<th>Carboxypeptidase digestion</th>
<th>Residues released (mol/mol of subunit B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Asn (0.080), Ala (0.089), Met (0.032)</td>
</tr>
<tr>
<td>20</td>
<td>Asn (0.260), Ala (0.258), Met (0.111), Ile (trace)</td>
</tr>
<tr>
<td>40</td>
<td>Asn (0.388), Ala (0.469), Met (0.182), Ile (0.041), Ser (0.029)</td>
</tr>
<tr>
<td>90</td>
<td>Asn (0.414), Ala (0.524), Met (0.203), Ile (0.072), Ser (0.038)</td>
</tr>
</tbody>
</table>

Delineation of Sequence from Residue 1 to Residue 73 and Residues 74 through 103 - The carboxypeptidase peptide cC2f (residues 43 to 48) contained a single lysine residue overlap of the NH₂-terminal portion of peptide T1b (residues 44 to 62 (Table 19)). This lysine residue could either be the COOH terminus of T2f (residues 63 to 69), T3f (residues 68 to 74), or Lys-63 (T3al) since cleavage at the α-amino group of these lysines has been known to occur with chymotrypsin. However, isolation and partial sequence analyses of the large peptide C2g (residues 49 to 81), T2g (residues 64 to 73), and T2f (residues 64 to 73), showed that the single-residue overlap of peptide cC2f thus could only be at Lys-43.

Similarly, isolation and analyses of peptide cClf (residues 77 to 88; Fig. 3B; Table 16) was crucial in the alignment of T2g² (residues 74 to 81), T2g (residues 82 to 84) and T3e (residues 85 to 91). The overlap between the peptides T3c and T2e (residues 92 to 103, the COOH-terminal tryptic peptide) was found with peptide cC2i (Table 17), establishing the sequence of residue 1 through residue 43 (Fig. 4; 119).

The alignment of the tryptic peptides in the entire B chain must therefore be, from the NH₂ terminus: Tic-T2h-T4a-T2f-T1b-T3al-T2i-T2k-T2g2-T2gl-T3c-T2e (Fig. 4). The linkage at the COOH terminus of the B chain (Table II). From the major fraction from the Sephadex column, a peptide with 37 amino acid residues was isolated by DEAE-Sephadex chromatography in 3 m urea (CN-2d/2e, Fig. 6D). This peptide was shown to have been derived from the NH₂ terminus of the B subunit on Edman degradation. Furthermore, tryptic digestion of this peptide (CN-2d) yielded peptides Tic, T2h, arginine, and a dipeptide, Glu-HSe, separable by gel filtration and paper electrophoresis. From this result and the partial structures of chymotryptic peptides, C2i, C2e, and C2k, the array of Tic (residues 1 to 23), T2h (residues 24 to 34), T4a (Arg-351, and T2f (residues 36 to 43) in that order from the NH₂ terminus was deduced, establishing the sequence of residue 1 through residue 43 (Fig. 4; 119).
FIG. 4. Derivation of the total primary structure of cholera toxin B subunit. Information used in the formulation of the primary structure is summarized here. The solid lines denote peptide segments with determined sequence. The broken lines denote segments identified by amino acid composition. The data on the separation and analyses of peptides may be found through the index in Table I.

The molecular weight of the cholera toxin B subunit calculated from the total primary structure is 11,604, nearly 20% higher than the values obtained by physicochemical methods (1, 2). Since the molecular weights of cholera toxin and the A subunit have been determined by equilibrium centrifugation as 84,000 and 28,000 to 30,000 (1, 4), respectively, the result...
from the structural study now indicates the presence of five B subunits/mol of choleragen toxin, rather than six. Based on the analyses of the molar ratio of cysteinyl residues in subunits B and A, and of the COOH-terminal dipeptides produced from the toxin on BrCN cleavage, we had previously proposed the existence of two molecular species of choleragen toxin, AB, and AB₂. Gill has recently reported a composition of AB₂ for choleragen toxin based on cross-linking studies (8).

The cleavage with BrCN resulted in three peptides of approximately equal sizes (residues 1 to 37, 38 to 68, and 69 to 101). Only the NH₂-terminal fragment could be isolated using DEAE-Sephadex chromatography in 3 M urea. Cleavage at the arginyl bonds, by tryptic digestion of the maleylated B subunit, also resulted in peptides of similar sizes (residues 1 to 35, 36 to 67, and 74 to 103) which could not be separated either on DEAE-cellulose or CM-Sephadex chromatography. The unseparated mixtures of peptides (residues 36 to 67 and 69 to 101 in the BrCN cleavage and the three fragments from the cleavage of arginyl bonds), however, have served to confirm the sequences around methionine or arginine residues. Edman degradation carried out on the mixture of peptides produced unequivocal identification of sequences next to these residues (see Fig. 4). Attempts have also been made to obtain peptide fragments through cleavage at the α-amide bond of the two cysteine residues of B subunit (18). Cyanylation of the —SH groups and subsequent cleavage according to Jacobson et al. (20) resulted in isolation of a peptide containing residues 86 to 103. Amino acid analyses of the peptide provided strong support for the linkage of T3c (B(Cys)-2, (18)) to T2c, the COOH-terminal tryptic peptide. This was important because both cysteine-containing tryptic peptides (T1c, 1–23; and T3c, 85–91) had the same COOH-terminal sequence, Asn-Asn-Lys.

The use of citraconic anhydride to modify lysine side chains before chymotryptic digestion offered a useful approach in obtaining peptide fragments for the alignment of tryptic peptides. The citraconylated proteins are soluble at pH 8 or above, where the enzymatic hydrolysis proceeds smoothly. With a soluble protein, the experimental manipulation is facilitated and can be carried out on a smaller scale. The blocking of lysine side chains appeared to reduce the rate of cleavage in the vicinity of these residues by chymotrypsin. Separation and analysis of e1Cl (residues 49 to 72) and eCl (residues 77 to 88) was crucial in delineating the sequence of residues 44 through 91 (Fig. 4). Our method of regenerating amino acids from their anilinothiazoliones, obtained in Edman degradations (15), has been successfully used in this study. While recoveries of amino acids in each step ranged from 40 to 99%, the residue removed in each degradation step could usually be identified unequivocally since the amount of the contaminating amino acids were generally 1 order of magnitude less. The low recoveries of amino acids appeared to correlate with the age of SnCl₂ solution added for hydrolysis. It is recommended that a fresh stock solution of SnCl₂ in 5.7 N HCl be prepared every 2 weeks.

The sequence of 103 amino acids residues reported here is in complete agreement with that determined independently by Kurosky et al. (21). The only discrepancy in the primary structure is the state of amidation of Glu-49; glutamine is assigned to this position in Kurosky's results. This, however, may be explained as a difference in the preparations of choleragen toxin used in the studies. Our preparation may have lost its amid group from Gln-49 during manipulation. Evidence for deamidation during sequence analyses has been obtained for Asn-44 and Asn-70.

The sequence reported by Nakashima et al. (7), on the other hand, differs from ours by 2 residues; serine and valine were reported for residues 52 and 55, respectively, while Kurosky et al. (21) and we found these residues in the reverse positions.

It is noteworthy that some repeating sequences are found in the primary structure of choleragen toxin B subunit. Such examples are: Asn-Asn-Lys (residues 21 to 23 and 89 to 91), Ala-Ile (residues 38 to 39, 64 to 65, 95 to 96, and 98 to 99) and though not in sequence; lysine, arginine, glutamic acid, methionine, alanine, and isoleucine in the regions of residues 34 to 39 and 64 to 69.

Acknowledgments—The author acknowledges the contribution of Dr. F. Mendez, Centro de Investigaciones Biologicas, Instituto de Biologia del Desarrollo, Madrid, Spain, in the sequence analyses of many of the tryptic peptides during his tenure as a Research Fellow at the Roche Institute. Thanks are also due to D. Chang, M. Wang, and A. Wodnar-Filipowicz for their assistance in various phases of this work.

REFERENCES

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primary Structure of Children's Table B Shirts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Description</td>
</tr>
<tr>
<td>1</td>
<td>Collar</td>
</tr>
<tr>
<td>2</td>
<td>Sleeve</td>
</tr>
<tr>
<td>3</td>
<td>Hem</td>
</tr>
</tbody>
</table>

*Note: This is a simplified representation of the primary structure of children's table B shirts.*
Determination of the primary structure of cholera toxin B subunit.
C Y Lai


Access the most updated version of this article at http://www.jbc.org/content/252/20/7249

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/20/7249.full.html#ref-list-1