PRIMARY STRUCTURE OF THE B SUBUNIT OF CHOLERA ENTEROTOXIN

Y. NAKASHIMA, P. NAPIORKOWSKI, D. E. SCHAFER* and W. H. KONIGSBERG

*Department of Molecular Biophysics and Biochemistry and *Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510,* and **Veterans Administration Hospital, West Haven, Connecticut 06516, USA*

Received 27 July 1976

1. Introduction

Cholera enterotoxin, a protein secreted by the bacterium *Vibrio cholerae,* activates adenylate cyclase in a wide variety of vertebrate cell types [1]. The toxin consists of two different types of subunit, known as the A ('active') and B ('binding') subunits, associated in non-covalent fashion [2]. Each toxin molecule probably contains one A and five B subunits [3]. Under suitable conditions the A subunit is sufficient to activate adenylate cyclase in plasma membrane fragments [4,5]. The B subunit has a strong affinity for G_{M_1} ganglioside in plasma membranes, and this interaction seems to be necessary for the function of the toxin in intact cells [6]. Nearly all the antigenicity of the intact toxin resides in the B subunit [2]. We have determined the primary structure of the B subunit as a step toward understanding structure–function relationships in cholera enterotoxin.

2. Material and methods

Purified cholera enterotoxin [7] was at first obtained through the US Cholera Panel, National Institutes of Health, and later purchased from Schwarz/Mann Chemical Co.

The B subunit was separated from the A subunit by treating the toxin in 6 M urea containing 10% formic acid followed by gel filtration on Sephadex G-75 with elution in 10% formic acid. The purity of the B subunit, as determined by SDS-polyacrylamide gel electrophoresis, was greater than 95%.

Oxidation, reduction, radioalkylation, and cleavage by CNBr and BNPS skatole (2-nitrophenylsulfenyl)-3-methyl-3′-bromoindolenine) were performed by standard methods [8–12]. Tryptic, chymotryptic, and thermolytic digestions were carried out by standard procedures [13]. The resulting peptides were separated by gel filtration, ion-exchange chromatography, and paper electrophoresis.

Automated Edman degradation was performed on a J.E.O.L. JAS-47K Sequence Analyzer and a Beckman 890B Sequenator, using a standard protein and peptide program with a single cleavage step [14]. The resulting PTH-amino acid derivatives were identified by gas chromatography, thin-layer chromatography, and amino acid analysis after HI hydrolysis [15–17]. Smaller peptides were sequenced by the dansyl Edman method [18]. Carboxypeptidase A and B digestion was used routinely for carboxy-terminal sequence analysis [19].

3. Results and discussion

The B subunit of cholera enterotoxin contains 103 amino acid residues, and has a calculated molecular weight of 11 600 daltons. The primary structure that we propose is shown in fig.1. There are three methionine residues which are sites for CNBr cleavage, one tryptophan residue that can be cleaved by BNPS skatole, and two half-cystine residues which form an internal disulfide bridge.

Evidence in support of the proposed sequence was obtained by means of several different experimental approaches. An automatic sequenator run was made on the intact B subunit, giving the sequence of the first 50 residues (fig.1, seq. 1). The B subunit was also

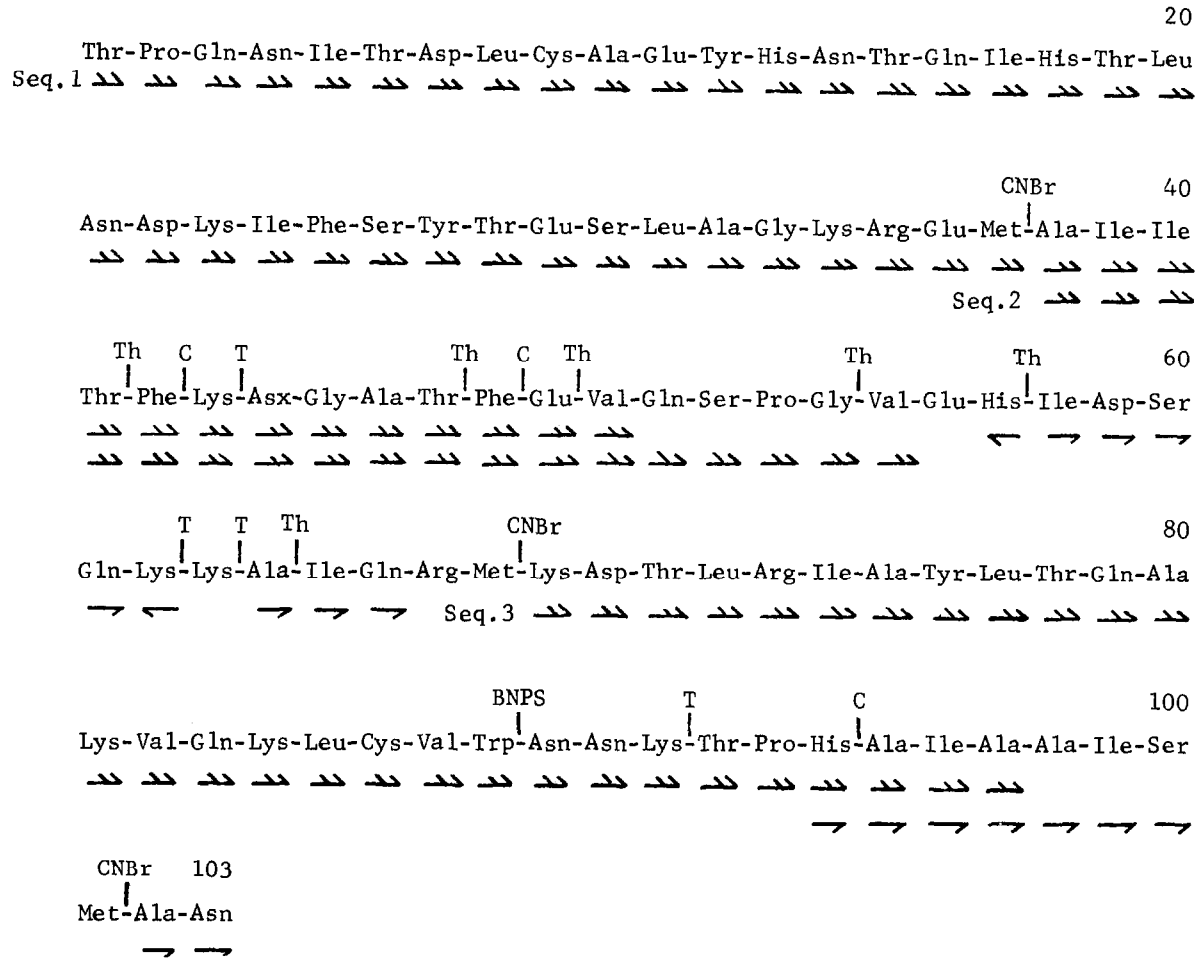

Fig.1. Primary structure of the B subunit of cholera enterotoxin. The sequence shown was obtained by automatic sequencing (Seq. 1, 2, and 3 —), manual Edman degradation (—>) and carboxypeptidase digestion (<—). C, T, Th, CNBr, and BNPS indicate the sites of cleavage by chymotrypsin, trypsin, thermolysin, CNBr, and BNPS skatole.

cleaved with CNBr, and the products separated into three fractions by gel filtration on Sephadex G-75. The sum of the amino acid compositions of the three fragments agreed with the composition of the intact B subunit. Furthermore, the three methionine residues were accounted for as homoserine or homoserine lactone in the isolated CNBr fragments.

The low molecular weight fraction from CNBr cleavage consisted of the dipeptide Ala–Asn, which was assigned to the COOH-terminus of the B subunit since it was the only CNBr fragment lacking homoserine or homoserine lactone. The middle fraction was a peptide having 31 residues. Seventeen residues from the NH_2-terminus of this peptide were determined by automatic sequencing procedures; the first thirteen of the seventeen residues matched those determined as residues 38–50 from the original sequenator run on the intact B subunit, thus establishing this peptide as consisting of residues 38–68. Since automatic sequencing from the NH_2-terminus of this fragment terminated at residue 54, the sequence of the remaining portion (55–68) was established after isolation of the tryptic, chymotryptic, and thermolytic peptides derived from this fragment.

The fraction of the highest molecular weight consisted of two peptides, (1–37) and (69–101), linked

by a disulfide bridge. The primary structure of residues 69–97 was established by automatic sequencing of this high molecular weight fraction, (1–37)–S–S–(69–101). When the NH_2 group of residue 1 was succinylated in the intact B subunit prior to CNBr cleavage, a unique sequence from 69–97 was obtained (fig.1, seq. 3). The remaining residues (98–101) and the structure of the peptide (69–101) were confirmed by the isolation and sequencing of tryptic, chymotryptic, and thermolytic peptides derived from this region and the peptide obtained by BNPS skatole cleavage (fig.1).

Finally, amide groups of Asx and Glx residues were assigned by thin-layer chromatography of PTH-amino acid derivatives obtained from the sequenator and by the mobility of the smaller peptides containing Asx or Glx after paper electrophoresis at pH 6.5 [16,20]. Difficulty was encountered in the Edman degradation at the Asx-Gly sequence (residues 44–45). The acidic conditions used to separate the A and B subunits may have resulted in cyclization here, giving a succinimideglycyl moiety, which cleaves in such a way as to favor the formation of a β peptide bond not susceptible to Edman degradation [13,21–22]. This is known to occur mainly with asparaginylglycine residues. On the basis of these observations, therefore, we suggest that residue 44 is asparagine.

All of the sequence data that we have obtained, both from long sequenator runs and from enzymatic digestion, are internally consistent and are in agreement with the sequence of residues 1–42 as determined by Kurosky et al. [23] and with the sequences around the half-cystine residues which were determined by Lai et al. [24].

On the basis of a slight but significant degree of sequence homology, it has recently been suggested that the β subunit of thyrotropic hormone (TSH) and analogous anterior pituitary and placental hormones may function in a manner similar to the B subunit of cholera enterotoxin, despite differences in binding specificity to various gangliosides [25–26]. This hypothesis has been strengthened by observation of competitive binding between TSH and cholera enterotoxin [27]. It will be of great interest to study the details of the relationship between the B subunit and G_{M_1} ganglioside in cell membranes, as well as the B subunit–A subunit interaction. It is hoped that the primary structure of the B subunit will provide significant information that will help in relating the structure and function of cholera enterotoxin as well as providing a basis for further comparative studies with pituitary hormones and other enterotoxins.

Acknowledgements

We thank Dr Paul L. Fletcher, Jr., and Mr. Gary Davis for assistance with some of the automatic sequence analyses. This investigation was supported by the Veterans Administration and by grants NSF-GB-32348 and USPHS GM-12607.

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