Primary Structure of Diphtheria Toxin Fragment B: Structural Similarities with Lipid-binding Domains

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ABSTRACT  Two different lipid-associating domains have been identified in the B fragment of diphtheria toxin using automated Edman degradation of its cyanogen bromide peptides, secondary structure prediction analysis, and comparisons with known phospholipid-interacting proteins.

The first domain is located in the highly hydrophilic (polarity index [PI] = 61.0%) 9,000-dalton N-terminal region of fragment B. This region shows primary and predicted secondary structures dramatically similar to those found for the phospholipid headgroup-binding domains of human apolipoprotein A1 (surface lipid-associating domain).

The second domain is located in the highly hydrophobic (PI = 32.4%) middle region of fragment B. Its structure resembles that found for the membranous domain of intrinsic membrane proteins (transverse lipid-associating domain).

In contrast, the hydrophilic C-terminal 8,000-dalton region of fragment B (PI = 53.8%) does not show structural similarity with lipid-associating domains.

Diphtheria toxin (62,000 daltons) is a typical example of the group of toxic proteins that use receptor-mediated internalization to reach their cytoplasmic targets (20). The proteolytically activated molecule consists of two functionally distinct fragments linked together by a disulfide bridge. The C-terminal fragment B (40,700 daltons) binds to specific eukaryotic cell-membrane receptors and mediates the entry of the enzymically active N-terminal fragment A (21,150 daltons) into the cytoplasm where A catalytically ADP-ribosylates elongation factor 2 and, thereby, inhibits protein synthesis of the cell (9).

The primary structure of fragment A and most of its catalytic properties are known (11, 14, 17, 18). Fragment B has been shown to contain two functional regions, (a) the 17,000-dalton C-terminal region, which is involved in the binding to the specific cell receptors (25), and (b) the 23,000-dalton N-terminal fragment A, which participates in the interaction of fragment B with the cell membrane bilayer (1, 2). However, neither the molecular mechanism of this interaction nor the type of interaction is known.

Finding answers to these questions would be helped by a knowledge of the amino acid sequence of fragment B. In a previous work (12), we isolated and characterized the cyanogen bromide peptides of fragment B; their proposed molecular weights and alignment were: CB4 (4,700 daltons), CB2 (14,000 daltons), CB5 (2,500 daltons), CB1 (12,000 daltons), and CB3 (8,000 daltons) (Fig. 4).

This work reports, for the first time, a partial amino acid sequence of fragment B which includes the N-terminal amino acid sequence of peptides CB2, CB1, and CB3, and the complete primary structure of peptides CB4 and CB5.

Comparison of these sequences with those of known lipid-associating proteins and conformational predictions allowed us to identify two distinct lipid-associating domains: a hydrophilic surface lipid-associating domain, as found in serum apolipoproteins, located at the N-terminus of fragment B, and a hydrophobic transverse lipid-associating domain, as found in intrinsic membrane proteins, located in the middle region of fragment B.

MATERIALS AND METHODS

Materials

Partially purified diphtheria toxin was purchased from Connaught Laboratories, Toronto, Canada, and purified to homogeneity, according to Michel and Dirkx (17), in the presence of 20 mM phenylmethanesulfonyl fluoride. Polyamide thin-layer sheets (F 1.700) were obtained from Schleicher and Schuell, GmbH, D-3354 Dassel, W. Germany. Reagents and solvents for automated amino acid sequence analysis, all sequanal grade, were obtained from E. Merck D-1600 Darmstadt, W. Germany. All other chemicals were analytical grade.

Methods

The cyanogen bromide peptides of diphtheria toxin fragment B were prepared according to Falmagne et al. (12). Automated Edman degradation was performed...
dicted secondary structures are presented using the following sym-
notations: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenyl-
alanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophane; Y, tyrosine. Predicted secondary structures are presented using the following symbols: α-helix (α), β-sheet (β), β-turns or loop (γ), nondefined (—) conformations. In case of ambiguity, both conformations are presented.

RESULTS

Amino Acid Sequence Analysis

All amino acid sequences were determined by automated Edman degradation of 50-200 nmol of peptides. Fig. 1 shows the N-terminal sequences of the large CNBr peptides CB1 (12,000 daltons), CB2 (14,100 daltons), and CB3 (8,000 daltons) and the complete sequences of the smaller CNBr peptides CB4 (41 residues) and CB5 (25 residues).

The sequence of CB5 was completed by C-terminal degradation with carboxypeptidases A and B (10). To obtain its complete sequence, CB4 was further cleaved at its arginyl residue with trypsin after citraconylation of the lysines and at the tryptophanyl residue with cyanogen bromide in the presence of heptafluorobutyric acid (19).

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Comparison of the 77-residues N-terminal hydrophilic sequence of fragment B with the sequence of human apolipoprotein A1 (Apo A1) revealed a significant degree of similarity (the probability that the similarity could be caused by chance equals 2 × 10^-7). Indeed, when residue 155 of Apo A1 is aligned on residue 1 of fragment B (Fig. 2), identical or homologous residues appear in 34% of the positions. Most homologies are clustered between residues 14-31 and 48-70 of fragment B, corresponding in Apo A1 to residues 168-185 (50% homology) and 202-223 (49% homology), respectively. Comparison of the conformational analyses of the homologous segments in the two proteins showed that α-helix is predicted for all of them.

To visualize the distribution of polar and apolar residues on the predicted helical segments, their helical nets were drawn. The projection of helix 16-30 of fragment B (Fig. 3A) clearly shows that this helix is amphipathic; it has a narrow hydrophobic face (residues Ile 16, Met 20, Ile 24, and Leu 27) surrounded by a large hydrophilic face (Fig. 3A, 2). On this face (Fig. 3A, 1), positively charged residues are at the periphery (residues Arg 17, Lys 19, 21, 23, and 28, and His 30) and negatively charged residues at the center (residues Asp 18, Glu 25 and 29), forming ion pairs or triplets. The helical segment 47-67 of
fragment B shows a similar distribution of its residues and is also amphipathic (not shown).

Such α-helical amphipathic domains with a narrow hydrophobic face and a large hydrophilic face containing ion pairs or triplets have been shown to be characteristic of serum lipoproteins and are responsible for their binding to phospholipid headgroups of phosphatidylcholine molecules (24), leading to reversible associations between proteins and surfaces of bilayers and to the formation of soluble lipid-protein complexes.

No statistically significant similarities were found between Apo A1 and the apolar middle region of fragment B nor its C-terminal region.

Structural Properties of the Hydrophobic Middle Region of Fragment B

The clustering of apolar residues in the N-terminal amino acid sequence of peptide CB1 is responsible for the high hydrophobicity of the middle region of fragment B. Conformational analysis of this sequence predicts helicity at residues 1–5 and 7–32, although β-pleated sheet structure is also probable at residues 19–30 (Fig. 1).

Like the helical segments of the N-terminal region of fragment B, helix 7–32 of CB1 is amphipathic, as shown by the distribution of its residues on the drawn helical net (Fig. 3B). However, the projection clearly shows that, for CB1, the hydrophobic face of the helix is the largest, with numerous apolar and bulky residues (Fig. 3B, 2); it surrounds a narrow polar face, containing few polar and neutral residues, which runs like a groove parallel to the axis of the helix (Fig. 3B, 1). This helix resembles the transverse lipid-associating domains of intrinsic membrane proteins, responsible for their anchoring in the membrane bilayer. As with them, its polarity is very low (polarity index = 26.9%) and the hydrophobic index of its large hydrophobic face is very high (hydrophobic index = 3.9), close to the value found for the apolar face of the membranous segment of glycophorin (23).

Structural Properties of the C-Terminal Polar Region of Fragment B

The N-terminal sequence of peptide CB3, located at the C-terminal end of fragment B, shows no structural similarities with both types of lipid-binding domains. It contains the disulfide bridge of fragment B (cys 2 and 12).

DISCUSSION

Our findings suggest that two different kinds of lipid-associating domains are present in the N-terminal sequence of diphtheria toxin fragment B endowed with lipid-binding properties.

The first one, located in the 9,000-dalton N-terminal region of B (Fig. 4), shows an apolipoprotein-like lipid-associating structure. This highly hydrophilic region of B could, thus, function like apolipoproteins when interacting with a biological membrane; the large polar face bearing ion pairs or triplets interacts specifically with the charged headgroups of phospholipids, and the molecule is stabilized parallel to the membrane by hydrophobic interactions of its narrow apolar face with the membrane lipid core. This is the general model proposed for the surface lipid-associating domain of plasma lipoproteins (23).

The second lipid-associating domain of fragment B, located in its middle region, is structurally related to the membranous segments of integral membrane proteins and designed to interact by its large apolar face with the hydrocarbon core of the phospholipids. This highly hydrophobic region of B could, thus, be a transverse domain involved in a process of membrane penetration. The 7–33 α-helix of CB1 would be ~35 Å long, which is approximately the thickness of the aqueous discontinuity of hydrated phospholipid bilayers.

The few negatively charged residues on the narrow polar face of this helix suggest stabilization of B in the membrane lipid core by interaction either with itself after formation of a multimeric complex or with preexisting integral membrane proteins, perhaps involved as carriers in the diphtheria toxin-receptor complex (13).

The C-terminal 8,000-dalton region of fragment B belongs to a receptor binding site of the diphtheria toxin molecule (25). It shows no structural similarities with the above lipid-interacting structures.

Here we suggest that the combination in diphtheria toxin fragment B molecule of a surface lipid-associating domain, as found in serum apolipoprotein, and a transverse lipid-associating domain, as found in intrinsic membrane proteins, can...
confer upon this molecule the dynamic properties leading to its association and anchoring in a cytoplasmic membrane.

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