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A CNBR PEPTIDE LOCATED IN THE MIDDLE REGION OF DIPHTHERIA TOXIN FRAGMENT B INDUCES CONDUCTANCE CHANGE IN LIPID BILAYERS.

POSSIBLE ROLE OF AN AMPHIPATHIC HELICAL SEGMENT

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SUMMARY

Conductance measurements on planar lipid bilayers demonstrate that CB1, a CNBr peptide of diphtheria toxin fragment B located in its middle region, possesses the unique property to destabilize the lipid bilayer organization. It is suggested that a segment of 25 amino acids in the N-terminal sequence of CB1 could be responsible for this effect. Its very low polarity, its predicted amphipathic helical structure and a helix length corresponding to the thickness of the hydrocarbon region of the lipid bilayer should specifically favor its insertion in the membrane. The existence of such a transverse lipid-associating domain could confer upon the molecule the properties leading to the anchoring of diphtheria toxin in the cytoplasmic membrane.

INTRODUCTION

Like membrane proteins, diphtheria toxin fragment B has been shown to interact with non-ionic detergents (1) and synthetic model membranes (2) through a hydrophobic domain localized in its 23 000 daltons N-terminal region. However, the segment of dipththeria toxin fragment B responsible for this interaction with the lipid bilayer has never been identified experimentally.

Amino acid sequence and theoretical structural analyses of the five cyanogen bromide peptides of fragment B (CB1 to 5) has allowed to predict two possible lipid-associating domains (3).

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In the present paper, we report about the unique property of CB1, the CNBr peptide located in the middle of the B chain, to induce voltage-dependent increase in ion conductance across a lipid bilayer reflecting insertion of the peptide into the lipid core. Peptide CB1 was completely sequenced in order to detect regions which conform to the requirements allowing their insertion in the lipid bilayer. An amphipathic helical structure resembling that found for the membranous domain of intrinsic membrane proteins has been theoretically predicted only for the N-terminal region (3); no other regions of CB1 exhibit similar structure-function relationships. The results are discussed in terms of anchoring of diphtheria toxin in the cytoplasmic membrane.

MATERIALS AND METHODS

Diphtheria toxin (Connaught Laboratories, Toronto), its A and B fragments, and the CNBr peptides of fragment B (CB1 to 5) (4) were solubilized in 0.1 M NH_4HCO_3, extensively dialyzed against this solution and filtered through a 0,42 µ Millipore filter.

Amino acid sequence data were obtained by automated Edman degradation (5) of CNBr peptide CB1 and of the peptides produced by cleavage of CB1 at its two tryptophanyl residues according to Ozols and Gerard (6) and at its arginyl and lysyl residues with trypsin.

Glycerol monooleate (GMO) was a Sigma product. N-decane was redistilled before use. Planar lipid bilayers were formed from a solution of GMO dissolved in decane on a 1.3 mm diameter aperture in a Teflon cell separating two aqueous phases (3.5 cm^3) (7-9). Membrane conductances (G_m) were determined by measuring the specific current (I_m/cm^2) as a function of an imposed potential difference (V_m). All the CNBr peptides of fragment B were added in each chamber to a final concentration of 10^{-8} M. The aqueous phase was 0.1 M NH_4HCO_3 pH 7.8 containing 0.15 M NaCl.

RESULTS AND DISCUSSION

Addition of fragment B (final concentration: 10^{-8}M) to the solution bathing the GMO bilayer (pH 7.8) induced a three-fold increase in the bilayer conductance, whereas no conductance change was observed with either diphtheria toxin or fragment A (Table I). To identify the hydrophobic region responsible for this effect, we prepared the CNBr peptides of fragment B and added each of them separately to the aqueous solution. Only one of the peptides, CB1, induced a 16-fold increase of membrane conductance; no effect was detected with the four other peptides. An ohmic relationship was observed in all cases up to 30 mV (Fig. 1) and identical results were obtained with reverse polarity. The increase in conductance

TABLE I. Effect of diphtheria toxin, its A and B fragments, and the CNBr peptides of fragment B on the conductance of GMO planar bilayer membrane. The protein and peptide concentrations were 10^{-8}M. Membranes were formed in 0.1 M NH_4HCO_3 pH 7.8 containing 0.15 M NaCl. In parentheses are the numbers of experiments.

Protein or peptides	Conductance (x 10^7 mho/cm^2)
None	0.5 ± 0.10 (8)
Diphtheria toxin	0.5 ± 0.10 (4)
Fragment A	0.5 ± 0.10 (5)
Fragment B	1.6 ± 0.32 (11)
CB1	7.9 ± 1.20 (12)
CB2	0.5 ± 0.10 (8)
CB3	0.5 ± 0.10 (6)
CB4	0.5 ± 0.10 (6)
CB5	0.5 ± 0.10 (8)

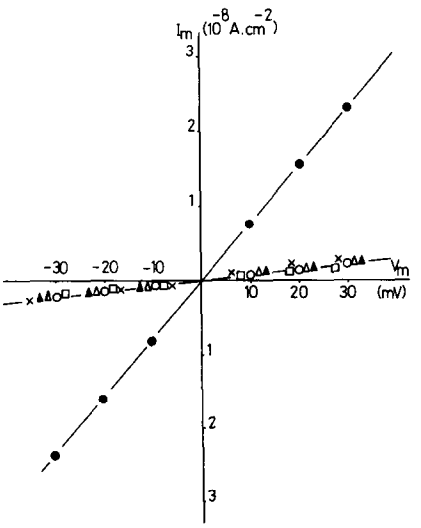

FIGURE 1. Current-voltage characteristics of GMO planar bilayer membranes in the presence of CB1 (●), CB2 (Δ), CB3 (▲), CB4 (□), CB5 (×), and in the absence of CNBr peptide (○). The peptide concentration was 10^{-8}M. Membranes were formed in 0.1 M NH_4HCO_3 pH 7.8 containing 0.15M NaCl.

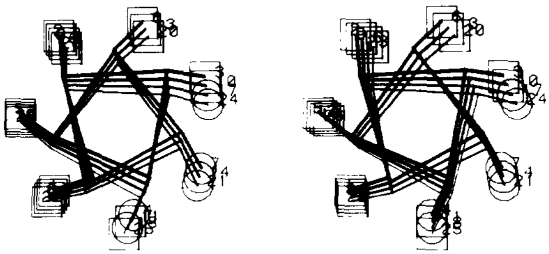

FIGURE 2. Computer-constructed stereo view of the hydrophobic α-helical amphipathic N-terminal segment of CB1 (residues 7-32). The helix is presented with its axis perpendicular to the plane of the image. Squares and circles represent respectively hydrophobic and hydrophilic amino acid residues.

supposes a change in the permeation of ions across the bilayer as a result of the insertion of a peptide into the lipid bilayer (8).

In a recent paper (3), we have theoretically predicted the presence in the N-terminal 40 residues amino acid sequence of CB1 of a segment (residues 7-32) that has an amphipathic α-helical structure (Fig. 2) characterized by a very low polarity (polarity index (10)= 0.27), specific of hydrophobic segments of intrinsic membrane proteins, and a length of 35 Å corresponding to the thickness of the bilayer apolar phase. Examination of the first complete sequence of peptide CB1 (Fig. 3) confirms the uniqueness of this amphipathic helical hydrophobic domain. The remaining 98 residues amino acid sequence is more hydrophilic (po-

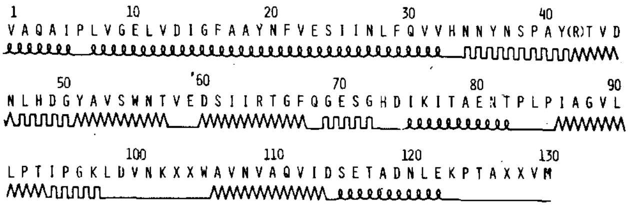

FIGURE 3. Amino acid sequence of CB1. One letter amino acid abbreviations used are: A(Alanine), C(Cysteine), D(Aspartic acid), E(Glutamic acid), F(Phenylalanine), G(Glycine), H(Histidine), I (Isoleucine), K(Lysine), L(Leucine), M(Methionine), N(Asparagine), P(Proline), Q(Glutamine), R(Arginine), S(Serine), T(Threonine), V(Valine), W(Tryptophane) and Y(Tyrosine). The predicted secondary structure is presented using the following symbols (16): α-helix (ℓ), β-pleated sheet (Λ), β-turns or loops (⨅) and non defined (—) conformations.

larity index (10)=0.46) and contains no stretch of apolar residues of similar length and endowed with similar structural properties.

To explain the conductance pattern, one can suppose that the insertion of the amphipathic N-terminal segment of CB1 into the bilayer could change the lipid organization leading to its destabilization. It is also possible that several amphipathic segments could aggregate and form a channel, since CB1 has the property to form dimers in aqueous solution (4). It would, however, be premature to prefer one of these possibilities even if amphipathic helices aggregated through their polar faces within the membrane can form multimeric structure which span the membrane perpendicularly to its plane as reported for glycophorin (11). Interestingly, transmembrane, voltage-dependent channel formation has recently been reported for diphtheria toxin (12, 13), provided the pH of the medium is lower than 6.0. At pH 7.8 and equal final molar concentration (10^{-8}M) CB1 induced a more drastic conductance change than the whole fragment B (Table I) while no change was observed with diphtheria toxin, in agreement with the results of Kagan and Finkelstein (12) and Donovan et al. (13). These effects could be due to the cryptic orientation of CB1 in fragment B and diphtheria toxin which would not allow an easy insertion in the lipid bilayer. It has been suggested that low pH could modify the toxin conformation so as to expose a hydrophobic domain responsible for insertion in lipid bilayers (14, 15). Our conductance measurements demonstrate that CB1 possesses the unique property to destabilize the lipid bilayer. This capacity could be associated to a hydrophobic α-helical amphipathic segment of a length of 35 Å corresponding to the thickness of the bilayer apolar phase. We propose that this segment, located in the middle of the fragment B molecule, could constitute a part of the hydrophobic domain leading to the anchoring of diphtheria toxin in the cytoplasmic membrane.

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