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SPECIFICITY IN PROTEIN-MEMBRANE ASSOCIATIONS: THE INTERACTION OF GANGLIOSIDES WITH *ESCHERICHIA COLI* HEAT-LABILE ENTEROTOXIN AND CHOLERAGEN

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Gangliosides, which are complex glycospingolipids, have been implicated in the receptor-mediated effects of numerous agents, including enterotoxins and glycoprotein hormones (6). The nonpolar portion of these compounds, consisting of an *N*-acyl fatty acid derivative of sphingosine, is believed to serve as an anchor in the plasma membrane, whereas the carbohydrate moiety, which is exposed to the extracellular space, is believed to be responsible for receptor specificity. One of the more fully characterized ganglioside-protein interactions is that between ganglioside G_{M1} (galactosy-*N*-acetylgalactosaminyl-[*N*-acetyl-neuraminyl]-galactosylglucosylceramide) and the enterotoxin of *Vibrio cholerae* that is responsible for the clinical characteristics of cholera (3). Choleratoxin or choleragen (CT) is composed of two types of subunits held together by noncovalent interactions. The *A* component, which is believed to be composed of two polypeptide chains joined by a single disulfide bond, activates adenylate cyclase through a NAD-dependent ADP-ribosylation reaction (9). The *B* component, of which there are 4–6 molecules in the intact toxin, is responsible for the specific interaction between the toxin and G_{M1} (3, 10). Thus the initial event in the action of CT on cells is the binding of the *B* component to the oligosaccharide moiety of G_{M1}. The remaining events are not known with certainty; the A_1 subunit is currently believed to be inserted into the membrane bilayer (presumably after reduction of the disulfide bond that links it to A_2) and ultimately ADP-ribosylates a regulatory protein in the cyclase system.

The effects of a heat-labile enterotoxin of *Escherichia coli* (LT) on cells resemble in many ways those of CT. LT also appears to be structurally similar to CT in that it contains two types of subunits with molecular weights similar to those found for CT (11). The catalytic *A* subunit of LT is however believed to be a single polypeptide chain (5, 7) rather than a disulfide linked dimer as found for CT (1, 2, 3). In view of the similar structure and mode of action of these two enterotoxins we have investigated the interaction between LT and G_{M1}.

RESULTS AND DISCUSSION

Incorporation of purified G_{M1} into G_{M1}-deficient C6 glioma cells increased the subsequent binding of ^{125}I-LT or ^{125}I-CT. This increase was blocked for both toxins by prior treatment of G_{M1}-treated cells with unlabeled CT. Treatment of the cells with G_{D1a} (*N*-acetylneuraminylgalactosyl-*N*-acetylgalatosaminyl-[*N*-acetylneuraminyl]-galactosylglucosylceramide, G_{M2} (*N*-acetylgalactosaminyl-[*N*-acetylneuraminyl]-galactosylglucosylceramide) or G_{D1b} (galactosyl-*N*-acetylgalactosaminyl-[*N*-acetyl-neuraminyl-*N*-acetylneuraminyl]-galactosylglucosyl-

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ceramide) had no effect on ^{125}I-LT or ^{125}I-CT interaction with the cells.

Interpretation of data concerning interactions between LT and isolated gangliosides is complicated by ganglioside self-association and possible nonspecific hydrophobic interactions between the toxin and the ceramide moiety of gangliosides. These complications can be avoided by use of the isolated oligosaccharide moiety in binding studies. G_{M1}-oligosaccharide caused a concentration-dependent "blue shift" in the tryptophanyl fluorescence spectrum of LT and the corresponding isolated *B* subunit (LT_B). The wavelength of maximum fluorescence shifted to 328 nm at saturating oligosaccharide concentrations (~ 5:1 oligosaccharide:toxin) which is similar to that observed previously for CT and CT_B (4). Increases in fluorescence intensity at 328 nm on interaction of LT or LT_B with G_{M1}-oligosaccharide paralleled closely that observed upon interaction of CT (or CT_B) with G_{M1}-oligosaccharide. The oligosaccharides of gangliosides G_{M3} and G_{D1a} did not cause changes in the fluorescence spectrum of LT or CT.

LT possesses ADP-ribosyltransferase activity; guanidine compounds and proteins have been shown to serve as ADP-ribose acceptors (3). Treatment of purified LT with trypsin resulted in a 200% increase in ADP-ribosyltransferase activity in cell-free systems; the final specific activity was similar to that of untreated CT (8). Presumably trypsin acts on the intact *A* subunit of LT, generating a fragment with ADP-ribosyltransferase activity similar to the A_1 subunit of CT. These results are consistent with the hypothesis that LT has a single, unnicked polypeptide chain which is relatively inactive without protease treatment; it should be noted, however, that a reducing agent is still required for full LT activity.

In summary, these combined results are consistent with a mode of action similar for CT and LT. Specificity for action on target cells resides in the *B* subunits of LT and CT and the oligosaccharide moiety of G_{M1}, which appears to serve as the cell surface receptor for both toxins. The major difference between the two enterotoxins is that because of the presence of an unnicked *A* subunit in LT, trypsin treatment is required for full expression of enzymatic activity. This may account for differences in the effects of LT and CT on target cells.

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