INFECTION AND IMMUNITY, Jan. 1993, p. 335–337
0019-9567/93/010335-03$02.00/0

Vol. 61, No. 1

Binding of Pertussis Toxin to Lipid Vesicles Containing Glycolipids

SALLY Z. HAUSMAN AND DRUSILLA L. BURNS*

Division of Bacterial Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892

Received 9 July 1992/Accepted 15 October 1992

The binding of pertussis toxin and its B oligomer to lipid vesicles containing glycosphingolipids was studied. Both pertussis toxin and the B oligomer bound to lipid vesicles containing ganglioside G_{D1a}. Binding of pertussis toxin to these vesicles decreased upon treatment of the vesicles with neuraminidase, suggesting that sialic acid residues are important for efficient binding of the toxin to G_{D1a}.

Pertussis toxin (PT), an exotoxin produced by *Bordetella pertussis*, has the A-B structure typical of many bacterial toxins. PT is composed of an enzymatically active A subunit (PTA) and a B oligomer (PTB) that is responsible for binding of the toxin to receptors on the surface of mammalian cells (10). PTB is made up of five subunits, S2, S3, S4, and S5, found in a 1:1:2:1 ratio (10), and appears to contain at least two receptor binding sites (9, 12).

PT has been reported to bind to both glycoproteins and glycolipids (2, 3, 9, 11). The major toxin receptors of Chinese hamster ovary cells are sialyllactosamine (NeuAc → Galβ4GlcNAc) residues of N-linked oligosaccharide chains on glycoproteins (3, 12). The terminal sialic acid residue appears to be crucial for binding of the toxin and subsequent intoxication of the cell (12). The toxin has also been shown to bind to mixtures of glycolipids isolated from cells of several types (9, 11). Thus, it is possible that PT binds to more than one type of receptor, each of which may carry any of several carbohydrate determinants. In the study reported here, we examined the ability of PT and PTB to bind to a number of purified glycosphingolipids incorporated into phospholipid vesicles to better define the type(s) of glycolipid that can serve as a receptor.

To study binding, we used a model membrane-receptor system that consisted of phospholipid vesicles into which glycolipids were incorporated. This model system was used in the hope that it might mimic a biological membrane. The vesicles containing glycolipids (Sigma Chemical Co., St. Louis, Mo., and Boehringer-Mannheim, Indianapolis, Ind.) were prepared as previously described (7). Briefly, dimyristoylphosphatidylcholine, cholesterol, and dicetyl phosphate (Sigma) in a molar ratio of 2:1.5:0.22 were dried under nitrogen in the presence of 150 μg of glycolipid. The dried preparation was resuspended in a solution of 75 mM KCl and 75 mM NaCl with the aid of glass beads. The vesicles were washed five times with 10 mM sodium citrate buffer (pH 6) containing 75 mM KCl and 75 mM NaCl to remove unincorporated starting materials. The presence of glycolipid in the preparations was verified by thin-layer chromatography on silica gel 60 plates (EM Science, Gibbstown, N.J.).

PT (purchased from the Michigan Department of Public Health) was separated into PTA and PTB as previously described (1). Lipid vesicles, with or without incorporated mixed brain gangliosides, were incubated with 5 μg of PT, 2.5 μg of PTA, or 5 μg of PTB in a total volume of 0.2 ml for 2 h at 37°C. The preparations were centrifuged, and supernatant fractions (containing free toxin subunits) and pellet fractions (containing lipid vesicles and bound toxin subunits) were obtained. The detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate was added to each fraction to give a final concentration of 2%. Proteins were precipitated by the addition of trichloroacetic acid (final concentration, 10% [wt/vol]). The precipitated material was suspended in 0.1 M Tris-HCl (pH 9) containing 100 mM dithiothreitol, 2% sodium dodecyl sulfate (SDS), and 20% glycerol and was then subjected to SDS-polyacrylamide gel electrophoresis (PAGE) essentially as described previously (8). Quantitative estimates of the amounts of toxin and toxin subunits bound to the vesicles were made by densitometric analysis of the gels. In the absence of ganglioside, some PT, a small amount of PTA, but little PTB bound to the lipid vesicles (Fig. 1). The mode of binding of PTA to lipid vesicles is unknown; however, this finding may have implications concerning the mechanism by which this subunit could interact with mammalian cell membranes and gain access to its target substrate within the cell (5). When mixed-brain gangliosides were incorporated into the vesicles, a significant proportion of both PT and PTB bound to the vesicles. Very little PTA bound to these vesicles.

To determine the identities of glycolipids that act as toxin receptors, we incorporated purified glycosphingolipids into the vesicles and examined the binding of PTB. The structures of oligosaccharide moieties of the glycosphingolipids investigated in this study are shown in Table 1. PTB bound to the vesicle preparations containing ganglioside G_{D1a} but did not bind to a significant extent to vesicles containing the other glycolipids examined (Table 1).

In contrast to PTB, PTA bound to some types of vesicles, although variation was observed. Since PTA does not contain a receptor binding site, interaction of PTA with vesicles may be due primarily to hydrophobic interactions with the lipid vesicles. The variation in PTA binding that was observed might be due to changes in the physical nature of the vesicles occurring upon incorporation of the different glycolipids.

The binding of PT to glycolipids was not identical to the binding of PTB (Table 1). PT bound better to certain vesicle preparations, including vesicles that did not contain glycolipid, than did PTB. This finding may be due to interaction of PTA in the holotoxin molecule with the lipid vesicles or possibly to changes in the conformation of PTB induced by

* Corresponding author.

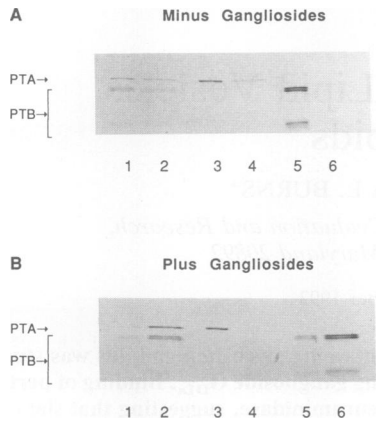

FIG. 1. Binding of PT and its subunits to lipid vesicles in the presence or absence of mixed brain gangliosides. PT (lanes 1 and 2), PTA (lanes 3 and 4), and PTB (lanes 5 and 6) were incubated with lipid vesicles (A) or lipid vesicles into which mixed brain gangliosides were incorporated (B). Unbound toxin or toxin subunit (lanes 1, 3, and 5) was separated from toxin or toxin subunit bound to the lipid vesicles (lanes 2, 4, and 6) as described in the text. Fractions were then subjected to SDS-PAGE.

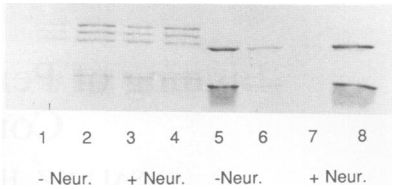

FIG. 2. Binding of PT and cholera toxin to vesicles containing G_{D1a} treated with neuraminidase. PT (lanes 1 through 4) or cholera toxin (lanes 5 through 8) was incubated with vesicles containing G_{D1a} that had not been treated (lanes 1, 2, 5, and 6) or that had been treated with neuraminidase (lanes 3, 4, 7, and 8) as described in the text. Toxin that had not bound to the vesicles (lanes 1, 3, 5, and 7) was separated from bound toxin (lanes 2, 4, 6, and 8) by centrifugation. Fractions were then subjected to SDS-PAGE.

PTA. Previously, the binding of PT to a variety of gangliosides that had been immobilized on a thin-layer chromatography plate was examined (3, 11). In one study (3) in which the binding of PT to well-defined glycosphingolipids was examined, no binding was detected. The discrepancy between those data and the data reported here may be due to the potential for our assay system to detect interactions of relatively low affinity between PT and gangliosides.

Although the presence of each of the glycolipids in the vesicle preparations was verified using thin layer chromatography, we were not able to quantify these compounds. It is possible that certain types of glycolipids were not incorporated into the vesicles to the same extent that other types of glycolipids were. Therefore, we cannot directly compare the relative abilities of different glycolipids to bind to PT and PTB. However, both PT and PTB clearly bind to vesicle preparations containing G_{D1a}.

Interestingly, both G_{D1a} and glycoprotein receptors for PT contain terminal sialic acid residues on their oligosaccharide chains (3). Although sialic acid residues have been shown to be crucial constituents of the glycoprotein receptor found on CHO cells (3, 12), the importance of this residue on glycolipid receptors is unknown. Therefore, we examined whether the sialic acid residues of G_{D1a} are also important for binding of PT. Vesicle preparations containing G_{D1a} were treated with neuraminidase (1 U) in 0.05 M sodium acetate (pH 5.5) containing 0.01 M $CaCl_2$ (0.35 ml) for 24 h at 37°C. The action of neuraminidase on G_{D1a} should convert at least some of this ganglioside to G_{M1} as the enzyme removes sialic acid residues from the glycolipid. The vesicles were treated with neuraminidase, washed, and suspended in 0.2 ml of 0.01 M Tris-HCl (pH 7.5), and then PT (5 μg) was added. PT bound significantly less well to vesicles that had been treated with neuraminidase than to control vesicles (Fig. 2). In contrast, the binding of cholera toxin, which does not bind to G_{D1a} but does bind to G_{M1} (4, 6), increases after neuraminidase treatment, demonstrating that G_{D1a} had been at least partially converted to G_{M1}. We also examined the ability of PTB to bind to vesicles containing G_{D1a} that had been treated with neuraminidase. In one such experiment, 65% of the total PTB bound to untreated vesicles containing G_{D1a}. After treatment of these vesicles with neuraminidase, no binding of B oligomer to the vesicles was detectable (data not shown). These findings are consistent with the idea that sialic acid residues may play a role in efficient pertussis toxin binding to glycolipids. Although the data reported here do

TABLE 1. Binding of PT and its subunits to lipid vesicles containing glycosphingolipids

Glycolipid	Structure	Amount bound (% of total)[a]		
		PTB	PTA	PT
None		<1	16	36
G_{M1}	Galβ1-3GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1 → ceramide	<1	12	26
G_{M2}	GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1 → ceramide	3	5	12
G_{M3}	NeuAcα2-3Galβ1-4Glcβ1 → ceramide	<1	<1	5
Asialo-G_{M1}	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1 → ceramide	1	4	32
G_{D1a}	NeuAcα2-3Galβ1-3GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1 → ceramide	80	7	80
G_{D1b}	Galβ1-3GalNAcβ1-4[NeuAcα2-8,NeuAcα2-3]Galβ1-4Glcβ1 → ceramide	<1	<1	29
G_{D3}	NeuAcα2-8,NeuAcα2-3Galβ1-4Glcβ1 → ceramide	<1	<1	33
Globoside	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1 → ceramide	1	29	24
Sulfatides	Gal($3SO_4$)β1 → ceramide	<1	<1	17
Galactocerebrosides	Galβ1 → ceramide	<1	<1	17
Glucocerebrosides	Glcβ1 → ceramide	<1	<1	29
Lactocerebrosides	Galβ1-4Glcβ1 → ceramide	<1	23	48

[a] The amount of protein bound was determined by densitometric scanning of SDS-PAGE gels of protein fractions (bound and unbound to lipid fractions) as described in the text.

not address whether G_{D1a} acts as a functional receptor for PT on the surface of eucaryotic cells, these data provide information concerning the oligosaccharide structures to which PT is capable of binding.

REFERENCES

1. **Arciniega, J. L., D. L. Burns, E. Garcia-Ortigoza, and C. R. Manclark.** 1987. Immune response to the B oligomer of pertussis toxin. Infect. Immun. **55:**1132–1136.
2. **Armstrong, G. D., L. A. Howard, and M. S. Peppler.** 1988. Use of glycosyltransferases to restore pertussis toxin receptor activity to asialogalatofetuin. J. Biol. Chem. **263:**8677–8684.
3. **Brennan, M. J., J. L. David, J. G. Kenimer, and C. R. Manclark.** 1988. Lectin-like binding of pertussis toxin to a 165-kilodalton Chinese hamster ovary cell glycoprotein. J. Biol. Chem. **263:**4895–4899.
4. **Cuatrecasas, P.** 1973. Gangliosides and membrane receptors for cholera toxin. Biochemistry **12:**3558–3566.
5. **Hausman, S. Z., and D. L. Burns.** 1992. Interaction of pertussis toxin with cells and model membranes. J. Biol. Chem. **267:** 13735–13739.
6. **Holmgren, J., I. Lonnroth, and L. Svennerholm.** 1973. Tissue receptor for cholera exotoxin: postulated structure studies with G_{M1} ganglioside and related glycolipids. Infect. Immun. **8:**208–214.
7. **Kinsky, S. C., J. A. Haxby, D. A. Zopf, C. A. Alving, and C. B. Kinsky.** 1969. Complement-dependent damage to liposomes prepared from pure lipids and Forssman hapten. Biochemistry **8:**4149–4158.
8. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
9. **Saukkonen, K., W. N. Burnette, V. L. Mar, H. R. Masure, and E. I. Tuomanen.** 1992. Pertussis toxin has eucaryotic-like carbohydrate recognition domains. Proc. Natl. Acad. Sci. USA **89:**118–122.
10. **Tamura, M., K. Nogimori, S. Murai, M. Yajima, K. Ito, T. Katada, M. Ui, and S. Ishii.** 1982. Subunit structure of islet-activating protein, pertussis toxin, in conformity with A-B model. Biochemistry **21:**5516–5522.
11. **Tuomanen, E., H. Towbin, G. Rosenfelder, D. Braun, G. Hanson, G. Larson, and R. Hill.** 1988. Receptor analogs and monoclonal antibodies that inhibit adherence of *Bordetella pertussis* to human ciliated respiratory epithelial cells. J. Exp. Med. **168:**267–277.
12. **Witvliet, M. H., D. L. Burns, M. J. Brennan, J. T. Poolman, and C. R. Manclark.** 1989. Binding of pertussis toxin to eucaryotic cells and glycoproteins. Infect. Immun. **57:**3324–3330.