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Lectinlike Properties of Pertussis Toxin

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We have examined the lectinlike properties of pertussis toxin by binding-inhibition assays and affinity chromatography of goose erythrocyte membranes. Although pertussis toxin and wheat germ agglutinin apparently recognize similar sugar sequences on glycoproteins, the binding activities of the two lectins are not identical.

The structure of pertussis toxin (PT) is well defined, but the role of its structure in pathophysiology is incompletely understood. While most of the biological effects of PT may be related to the ADP-ribosyltransferase activity of the S_1 (enzymatic) subunit (13), the mitogenic activity of PT is apparently the result of the lectinlike interaction of the basal components with lymphocyte receptors (12, 14). The lectinlike activity of the PT B oligomer may also contribute to the attachment of *Bordetella pertussis* organisms to respiratory epithelial cells (19). Accordingly, it is important to identify and characterize receptors for PT in order to fully understand its activity in the disease process.

In a previous report (3), we identified important sugar sequences involved in the lectinlike interaction of PT with the glycoprotein fetuin. Our studies suggested that PT binds to similar sugar sequences as the plant lectin, wheat germ agglutinin (WGA). However, we also showed that sugar sequence alone does not predict the ability of PT to bind to glycoproteins. For example, α1 acid glycoprotein contains fetuinlike sugar sequences (11) but bound less well than fetuin to PT. Bhavanandan and Katlic (5) reported a similar disparity between sugar sequence and binding affinity in the WGA system. Therefore, it appeared that PT and WGA receptor activity may depend on glycoprotein elements in addition to sugar sequences in the glycan units. In the present report, we explore the extent of functional homology between these two lectins by comparing the abilities of various glycoproteins and potential receptors to bind to PT and WGA.

A radioreceptor-binding-inhibition assay with fetuin-coated polystyrene tubes was used to measure the interaction of PT and WGA with several glycoproteins containing asparagine-linked glycans terminating in neuraminlactose. Biologically active, ^{125}I-labeled PT was prepared as described previously (4). WGA was iodinated by the Iodo Gen procedure described by Armstrong et al. (1), with the exception that the reaction was carried out in the presence of 0.4 M *N*-acetylglucosamine (GlcNAc) to prevent iodination damage to the WGA-binding sites.

Data obtained from the binding-inhibition experiments shown in Fig. 1 confirm and extend previous observations (3, 5). The graphs show that WGA and PT bind with variable avidity to five sialoglycoproteins containing similar sugar sequences. For example, fetuin, with a 50% binding-inhibition concentration (IC_{50}) of $9.5 \pm 1.6 \times 10^{-8}$ M, was approximately 500 times better than the worst inhibitor, transferrin (IC_{50}, $4.3 \pm 0.3 \times 10^{-5}$ M) in the PT system ($n = 2$). Glycophorin A, with an IC_{50} of $4.5 \pm 0.5 \times 10^{-9}$ M, was at least 20,000 times better than the worst inhibitor, transferrin (IC_{50}, $> 1.1 \pm 0 \times 10^{-4}$ M) in the WGA system ($n = 2$). This selectivity among ligands could permit PT to sample a subfraction of glycoprotein receptors from within a larger population bearing similar sugar sequences.

We next used affinity-isolation procedures to identify and compare PT and WGA receptors in goose erythrocyte membranes. Goose erythrocytes were chosen because the PT-mediated goose hemagglutination reaction is an assay for PT-binding activity (16). We also examined the interaction of goose erythrocyte membrane proteins with fetuin-agarose to evaluate nonspecific binding to an agarose-immobilized, nonlectin protein. Cholera toxin (CT)-agarose was also used because CT was previously shown to have a higher affinity for sialoglycolipids than for sialoglycoproteins (6). Details of the affinity-isolation techniques, solubilization of goose erythrocytes in Triton X-100, and the surface iodination procedure were reported previously (2, 7). PT- and CT-agarose were prepared with activated CH-Sepharose. WGA-agarose was obtained from Pharmacia, Inc., and fetuin-agarose was obtained from Sigma Chemical Co.

Although the protein bands indicated by the solid dots beside lane 3 of Fig. 2B from the WGA-agarose column were very intense on the autoradiogram, they represented only a fraction of the proteins present in the iodinated goose erythrocyte extract (Fig. 2B, far right lane). In contrast, the unmarked, lower-molecular-weight (less than 93,000 [93K protein] erythrocyte proteins detected in the WGA-agarose GlcNAc fractions were clearly the dominant species in the total erythrocyte extract and were also present in small amounts in the final wash fractions obtained from all four of the affinity columns (Fig. 2A). Therefore, the WGA-agarose column was able to selectively bind a group of goose erythrocyte receptors that appeared to contain WGA-specific glycan units, because they were readily released from the column by GlcNAc. The 127K protein (Fig. 2B, arrowhead) may have represented a high-affinity WGA receptor which was not released by GlcNAc or a protein which bound to WGA-agarose by an alternate mechanism.

Only the lower-molecular-weight protein species were released from PT-agarose by GlcNAc. In addition, most of the lower-molecular-weight proteins observed in Fig. 2B, lane 1, were observed in the GlcNAc and diethanolamine fractions obtained from the CT-agarose columns and, to a

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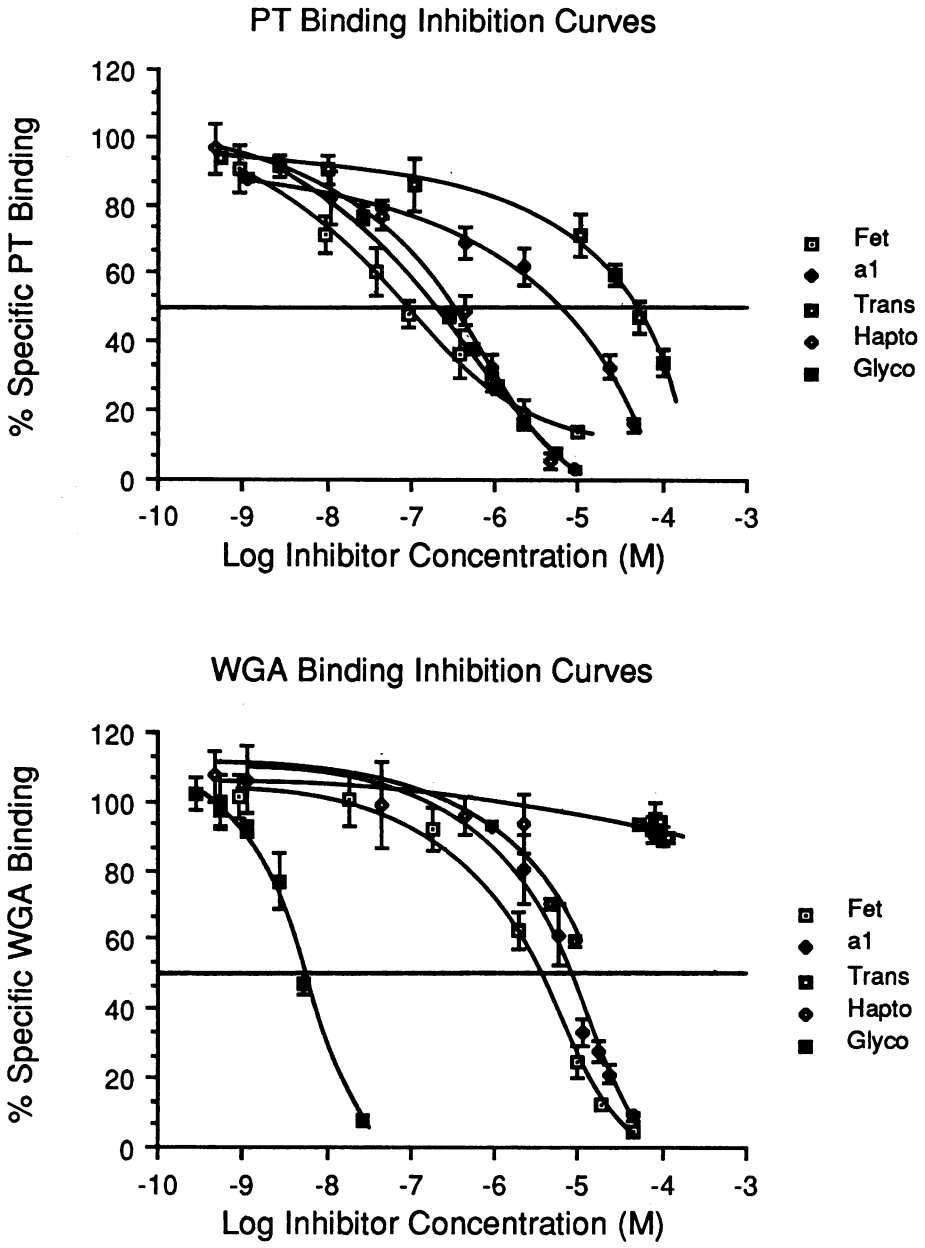

FIG. 1. Inhibition curves for ^{125}I-labeled PT (top) and ^{125}I-labeled WGA (bottom) binding to fetuin-coated polystyrene tubes. The final concentration of ^{125}I-labeled PT or WGA in the assays was approximately 10^{-9} M. Background binding was determined in polystyrene tubes coated with bovine serum albumin alone, and the maximum (100%) binding in each experiment was measured in tubes containing no inhibitor. Binding at each concentration of inhibitor was performed in triplicate, and the error bars represent the standard error of the mean for each point. The glycoprotein inhibitors used in the experiments were fetuin (Fet), α1 acid glycoprotein (a1), transferrin (Trans), haptoglobin (Hapto), and glycophorin A (Glyco).

lesser extent, from the fetuin-agarose columns (Fig. 2B, lanes 5, 6, 7, and 8). However, all of the higher-molecular-weight goose erythrocyte proteins released from WGA-agarose in the presence of GlcNAc appeared in the PT-agarose-diethanolamine fractions. The failure of GlcNAc to release PT-bound erythrocyte receptors clearly reflects a major difference in the behavior of PT- and WGA-agarose. This finding was also consistent with our observation (3) that GlcNAc was unable to inhibit goose erythrocyte agglutination.

To investigate the reason for the binding of the lower-molecular-weight group of proteins to PT-agarose, iodinated goose erythrocyte extracts were passed through an affinity column consisting of histone-agarose (Fig. 3). Cationic histones were chosen because we suspected that binding of the low-molecular-weight species may be the result of electrostatic interactions between negative charges on sialoglycoproteins and positive charges on WGA, PT, and CT (10, 15, 18). In addition, we increased the NaCl concentration in the buffer solutions used in the affinity-isolation procedure to inhibit ionic interactions.

The lower-molecular-weight proteins observed in Fig. 2B were also clearly visible in the final wash and in diethanolamine fractions from the histone-agarose column (Fig. 3,

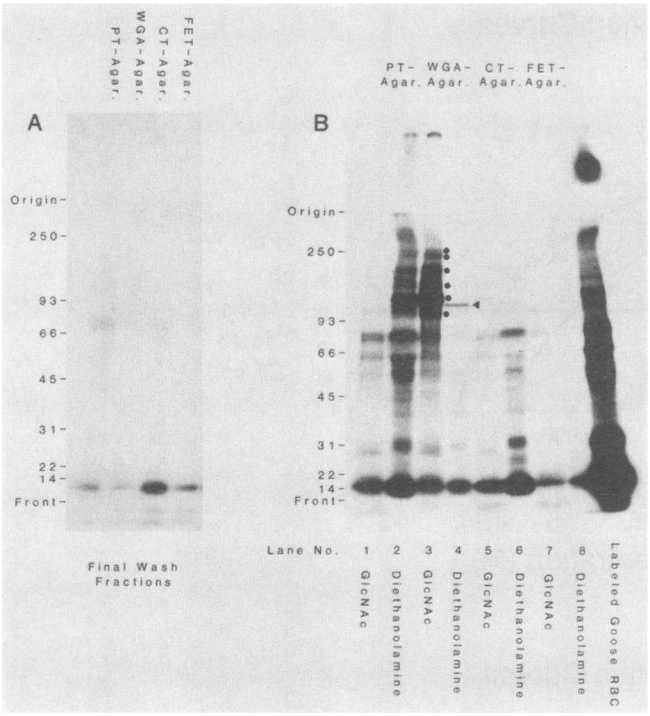

FIG. 2. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of the final wash fractions obtained from PT-agarose (PT-Agar.), WGA-agarose (WGA-Agar.), CT-agarose (CT-Agar.), or fetuin-agarose (FET-Agar.) affinity columns. (B) Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of bound, ^{125}I-labeled goose erythrocyte proteins released from the indicated columns by solutions containing 0.4 M GlcNAc or 50 mM diethanolamine (pH 11.5) containing 0.15 M NaCl. The PT-agarose and CT-agarose columns contained approximately 100 μg of protein, the WGA-agarose column contained approximately 165 μg of WGA, and the fetuin-agarose column contained approximately 230 μg of fetuin (based on a sialic acid content of 13.6 sialic acid residues per mol of fetuin [17]). The affinity-chromatography samples were analyzed on 7.5 to 15% linear acrylamide separating gels in the presence of 2-mercaptoethanol as described earlier (1, 8). The starting material that was loaded onto the columns was analyzed in the far right lane. The positions of the protein standards are indicated by their molecular weights (10^3) on the left side of the panels. The α-spectrin band in human erythrocyte membranes was used for the 250K-molecular-weight marker protein (9).

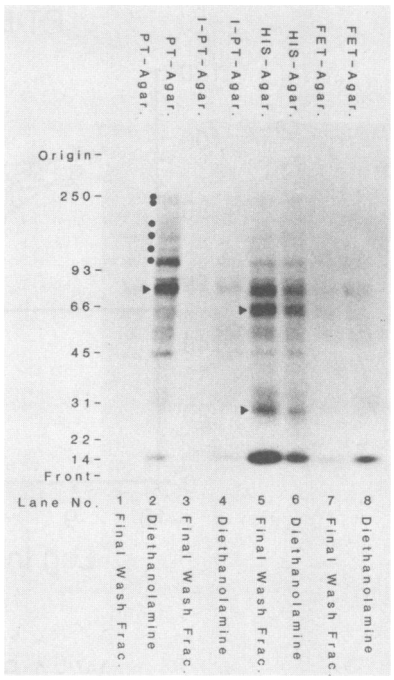

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of ^{125}I-labeled goose erythrocyte proteins in the final wash fractions (Final Wash Frac.) or released from PT-agarose (PT-Agar.), iodinated PT-agarose (I-PT-Agar.), histone-agarose (HIS-Agar.), or fetuin-agarose (FET-Agar.) affinity columns by 50 mM diethanolamine. The conditions for the affinity-chromatography procedure were the same as those described previously (2), with the exception that the concentration of NaCl was increased to 1 M in all of the buffer solutions. The histone-agarose columns contained approximately 100 μg of protein. Iodinated PT-agarose was prepared by exposing a portion of the PT-agarose preparation to NaI (40 μM) in the presence of chloramine T (7 mM) for 30 min. The iodination procedure was repeated three times. Receptors were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described in the legend to Fig. 2.

lanes 5 and 6) but not in the fractions obtained from negatively charged (17) fetuin-agarose (Fig. 3, lanes 7, 8). Therefore, it appeared that we may have been correct in our assumption that ionic interactions were responsible for the binding of the lower-molecular-weight proteins to PT-, WGA-, and CT-agarose. Although, in the presence of 1 M NaCl, some lower-molecular-weight species were detected in the diethanolamine fractions from PT-agarose (Fig. 3, lane 2), the 27K and 74K proteins observed in the histone-agarose diethanolamine fractions (Fig. 3, lane 5, arrowheads) and the PT-agarose-diethanolamine fractions from the experiment described earlier (Fig. 2B, lane 2) were not among them.

Except for a small amount of a 120K band in the histone-agarose fractions (Fig. 3, lanes 5 and 6), the high-molecular-weight species marked by the solid dots in Fig. 3 and corresponding to the high-molecular-weight species in Fig. 2B, lanes 2 and 3, were only observed in the diethanolamine fractions from the PT-agarose column. Therefore, it seemed unlikely that the high-molecular-weight protein species were binding to PT-agarose by simple electrostatic interactions.

Since we reported previously (4) that iodination reduced the receptor-binding activity of PT in goose erythrocytes and Chinese hamster ovary cells and it seemed unlikely that iodination would alter the capacity of PT for electrostatic interactions with negatively charged protein species, the Triton-solubilized goose erythrocytes were passed through iodinated PT-agarose. Polyclonal anti-PT immunoglobulin G was used to determine the amount of immunoreactive PT remaining in the iodinated PT-agarose preparations. Although the anti-PT immunoglobulin G-binding experiment demonstrated that iodinated PT-agarose affinity columns retained at least 65% of the PT present in the untreated PT-agarose preparations, iodinated PT-agarose (Fig. 3, lanes 3 and 4) failed to bind any of the goose erythrocyte membrane proteins seen in Fig. 3, lane 2. This confirmed that the PT receptors were specific for iodination-sensitive PT-binding sites and were not binding by nonspecific electrostatic interactions.

The 92K protein indicated by the arrowhead beside lane 2 in Fig. 3 was an enigma because, although it failed to bind to iodinated PT-agarose, it bound strongly to histone-agarose (Fig. 3, lane 6). It is possible that the 92K protein contained

sufficient negatively charged sialated glycan units to bind to histone-agarose by electrostatic interactions, even in the presence of 1 M NaCl, and to PT-agarose by stereospecific interaction with the lectinlike-binding sites.

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