Purification and Characterization of a Neu5Acα2→6Galβ1→4GlcNAc and HSO_3^-→6Galβ1→4GlcNAc Specific Lectin in Tuberous Roots of *Trichosanthes japonica*†

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ABSTRACT: Two lectins were purified from tuberous roots of *Trichosanthes japonica*. The major lectin, which was named TJA-II, interacted with Fucα1→2Galβ/GalNAcβ1→ groups, and the other one, which passed through a porcine stomach mucin–Sepharose 4B column, was purified by sequential chromatography on a human α_1-antitrypsin–Sepharose 4B column and named TJA-I. The molecular mass of TJA-I was determined to be 70 kDa by sodium dodecyl sulfate gel electrophoresis. TJA-I is a heterodimer of 38-kDa (36-kDa) and 32-kDa (30-kDa) subunits with disulfide linkage(s), and the difference between 38 and 36 kDa, and between 32 and 30 kDa, is due to secondary degradation of the carboxyl-terminal side. It was determined by equilibrium dialysis that TJA-I has four equal binding sites per molecule, and the association constant toward tritium-labeled Neu5Acα2→6Galβ1→4GlcNAcβ1→3Galβ1→4Glc_{OT} is $K_a = 8.0 \times 10^5$ M^{-1}. The precise carbohydrate binding specificity was studied using hemagglutinating inhibition assay and immobilized TJA-I. A series of oligosaccharides possessing a Neu5Acα2→6Galβ1→4GlcNAc or HSO_3^-→6Galβ1→4GlcNAc group showed tremendously stronger binding ability than oligosaccharides with a Galβ1→4GlcNAc group, indicating that TJA-I basically recognizes an *N*-acetyllactosamine residue and that the binding strength increases on substitution of the β-galactosyl residue at the C-6 position with a sialic acid or sulfate. A TJA-I column is useful for separating oligosaccharides and glycoproteins with a Neu5Acα2→6 (or HSO_3^-→6) Galβ1→4GlcNAc group from those with a Neu5Acα2→3 (or HSO_3^-→3) Galβ1→4GlcNAc group.

Investigation of lectin–carbohydrate interactions is essential for understanding cell biological processes such as differentiation, metastasis, development, proliferation, and cell–cell adhesion, because these "signals" are mediated by lectin-like molecules on the external surface of cells through binding to specific types of sugar structures on the membranes of other cells (Rademacher et al., 1988). Since Morell et al. (1968) first reported that the clearance of serum glycoproteins is mediated by galactose binding proteins localized on the surface of liver parenchymal cells, many mammalian lectins have been found and studied (Ashwell & Harford, 1982).

In recent years, it has become apparent that the carbohydrate binding specificity of lectins for sugar chains on the cell surface is far more complicated than the original sugar specificity proposed on the basis of the results of hapten inhibition studies (Townsend et al., 1986; Philips et al., 1990). Although mammalian lectins are unstable and difficult to purify, lectins in plants and invertebrates are stable and reliable models of mammalian lectins. In particular, it has been elucidated that several *N*-acetyllactosamine specific lectins show various unique carbohydrate binding characteristics, as observed in phytohemagglutinins E_4 and L_4 (Kobata & Yamashita, 1989; Bierhuizen et al., 1988), *Datura stramonium* agglutinin (Cummings & Kornfeld, 1984; Yamashita et al., 1987), and *Allomyrina dichotoma* lectin-II (Yamashita et al., 1988, 1989a).

We found that two lectins exist in the tuberous roots of *Trichosanthes japonica*, which are named TJA-I and TJA-II. TJA-I showed high affinity to sugar chains with the Neu5Acα2→6Galβ1→4GlcNAc or HSO_3^-→6Galβ1→4GlcNAc sequence in preliminary experiments. We will describe in this paper the purification of TJA-I, its physicochemical properties, and its precise carbohydrate binding specificity.

MATERIALS AND METHODS[1]

RESULTS

Purification of TJA-I. The results of affiniy chromatography of crude TJA on a porcine stomach mucin–Sepharose are shown in Figure 1A. One-tenth of the hemagglutinating activity passed through the column, which was termed TJA-I. The pass-through fraction on the porcine stomach mucin–Sepharose column was then applied to an human α_1-antitrypsin column, most of the hemagglutinating activity being adsorbed to the column and eluted with PBS containing 0.1 M lactose (Figure 1B). TJA-I bound to the α_1-antitrypsin column was applied to a second porcine stomach mucin–Sepharose column to remove contaminating TJA-II (Figure 1C). Finally, 4.4 mg of TJA-I was obtained from 20 g of *T. japonica* tuberous roots, as summarized in Table I.

Molecular Mass of TJA-I. The molecular mass of TJA-I was determined by Sephadex G-100 gel filtration (1.6 × 90 cm) in the presence of 20 mM lactose. The lectin was eluted as a single band at an elution volume corresponding to 60 kDa (data not shown).

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[1] Portions of this paper (including the Materials and Methods, Table III, and Figures 1, 4, and 5) are presented in the supplementary material.

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Table I: Purification of *T. japonica* Agglutinin I

purification step	total protein[a] (mg)	total agglutination act.[b] (titer × mL)	specific act. (titer/mg/mL)	purification (x-fold)
crude extract from 20-g root tubers	435.2	2560	5.9	1.0
35–55% ammonium sulfate precipitate	207.4	1536	7.4	1.3
pass-through fraction on porcine stomach mucin affinity column	80.0	1280	16.0	2.7
lactose eluate from α_1-antitrypsin affinity column	7.0	1280	182.9	31.0
pass-through fraction from the second porcine stomach mucin affinity column	4.4	1280	290.9	49.3

[a] Protein was determined by the method of Lowry et al. (1951). [b] The hemagglutination titer was determined with human O erythrocytes in the presence of porcine stomach mucin (0.5 mg/mL).

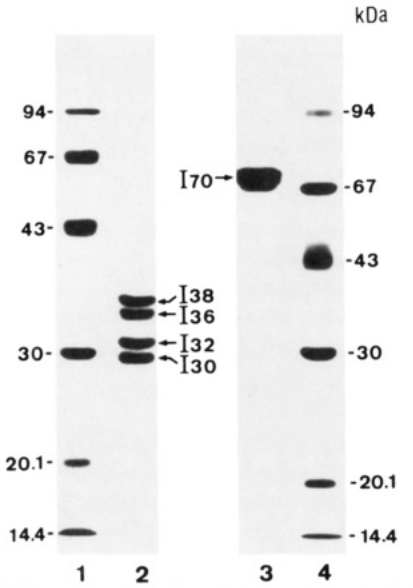

FIGURE 2: SDS–polyacrylamide gel electrophoresis of the purified TJA-I. Lanes 1 and 2, samples reduced with 2% 2-mercaptoethanol; lanes 3 and 4, unreduced samples; lanes 2 and 3, TJA-I (5 μg); lanes 1 and 4, molecular mass standards: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). The gels were stained with Coomassie Brilliant Blue R-250.

On SDS–PAGE in the absence of 2-mercaptoethanol, TJA-I gave a rather broad single band around 70 kDa (Figure 2, lane 3). In the presence of 2-mercaptoethanol, it gave four apparent bands, which were termed subunits I_{38} (38 kDa), I_{36} (36 kDa), I_{32} (32 kDa), and I_{30} (30 kDa), respectively (Figure 2, lane 2). These results indicate that the active lectin exists as a heterodimer with disulfide bond(s).

Amino Acid and Monosaccharide Compositions of TJA-I Subunits. The amino acid and monosaccharide compositions of the four subunits electroblotted onto a PVDF membrane were determined according to the methods described in Materials and Methods. The four subunits had similar amino acid compositions including high concentrations of hydroxyl amino acids and hydrophobic amino acids. The monosaccharide compositions and contents of the four subunits were similar to one another, consisting of mannose, xylose, fucose, and *N*-acetylglucosamine residues.

N-Terminal Amino Acid Sequences of TJA-I Subunits. The N-terminal amino acid sequences of the four subunits derived from TJA-I can be classified into two groups with completely different N–terminal sequences, as follows: I_{38} (I_{36}), Asn-Pro-Pro-Tyr-Leu-Glu-Ala-Arg-Thr-; I_{32} (I_{30}), Arg-Gly-Phe-Pro-Phe-Ala-Ala-Tyr-Val-Val-Tyr-Ile-Gln-Arg-. The sequences of I_{38} and I_{36}, and of I_{32} and I_{30}, were homologous, respectively. These results indicate that the difference between I_{38} and I_{36} and that between I_{32} and I_{30}, respectively, may be due to secondary degradation of the respective carboxyl-terminal sides and, therefore, that TJA-I must be a heterodimer of I_{38} and I_{32} with disulfide bond(s). Such posttranslational modification of carboxyl termini has also been reported for natural human interferon-γ (Rinderknecht et al., 1984).

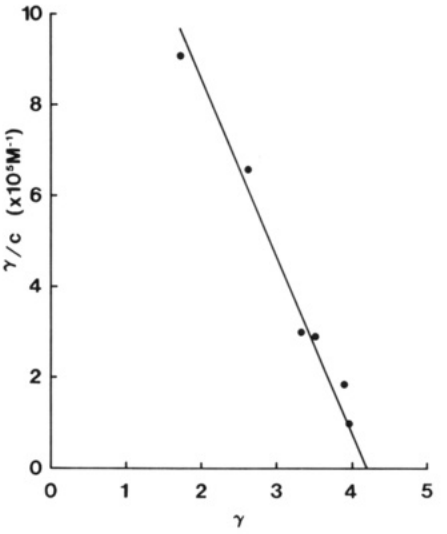

FIGURE 3: Binding of Neu5Acα2→6Galβ1→4GlcNAcβ1→3Galβ1→4Glc$_{OT}$ (LST-c$_{OT}$) to TJA-I. The results obtained on equilibrium dialysis were plotted according to Scatchard. γ denotes mol of bound sugar/mol of the lectin, and *c* denotes mol of unbound sugar.

Equilibrium Dialysis. Figure 3 shows Scatchard plots of tritiated Neu5Acα2→6Galβ1→4GlcNAcβ1→3Galβ1→4Glc$_{OT}$ (LST-c$_{OT}$)[2] binding with TJA-I. The plots gave a straight line, indicating homogeneity with respect to the affinity for this sugar. The association constant was calculated to be 8.0×10^5 M^{-1}, and TJA-I was estimated to have four binding sites/molecule.

Hemagglutination Inhibition Assay. In order to elucidate the carbohydrate binding specificity of TJA-I, a hemagglutination inhibition assay was performed (Table II). D-Galactose and *N*-acetylgalactosamine, and their derivatives, were inhibitory in the range of 25–100 mM. Among the epimers of D-galactose, D-glucose (C-4 epimer) and D-gulose (C-3 epimer) were noninhibitory. On the other hand, D-talose (C-2 epimer) showed less inhibitory potency than D-galactose, and 6-deoxy-D-galactose (D-fucose) displayed the same inhibitory potency as D-galactose. Other monosaccharides, including *N*-acetylneuraminic acid, had no effect on the activity up to a concentration of 200 mM, as shown in Table II. These results indicated that the equatorial hydroxyl group at the C-3 position and the axial hydroxyl group at the C-4 position of the pyranose ring are the most important loci for the binding of D-galactose to TJA-I.

Furthermore, hemagglutination inhibitory activity of *N*-acetyllactosamine and lactose showed 15 and 8 times higher affinity than D-galactose to TJA-, respectively, indicating that TJA-I fundamentally recognizes *N*-acetyllactosamine/lactose residues in oligosaccharides. Interestingly, 6′-sialyllactose and

[2] Subscript OT is used in this paper to denote NaB^{3}H$_4$-reduced oligosaccharides.

Table II: Inhibition by Various Sugars of the Hemagglutinating Activity of *T. japonica* Agglutinin I with Human O Erythrocytes

sugar[a]	concentration[b] (mM)
D-galactose	50 (1)[c]
D-talose (C-2 epimer)	100 (0.5)
D-gulose (C-3 epimer)	no inhibn at 200 mM
D-glucose (C-4 epimer)	no inhibn at 200 mM
D-fucose (6-deoxy-D-galactose)	50 (1)
methyl α-D-galactoside	25 (2)
methyl β-D-galactoside	25 (2)
N-acetyl-D-galactosamine	100 (0.5)
methyl α-D-*N*-acetylgalactosaminide	100 (0.5)
methyl β-D-*N*-acetylgalactosaminide	50 (1)
D-galactosamine hydrochloride	200 (0.25)
N-acetylneuraminic acid	no inhibn at 200 mM
lactose (Galβ1→4Glc)	6.5 (8)
N-acetyllactosamine (Galβ1→4GlcNAc)	3.25 (15)
6′-sialyllactose (Neu5Acα2→6Galβ1→4Glc)	0.40 (125)
6′-sialyllacto-*N*-neotetraose (LST-c) (Neu5Acα2→6Galβ1→4GlcNAcβ1→3Galβ1→4Glc)	0.025 (2000)

[a] Saccharose, L-sorbose, D-arabinose, methyl α-D-mannoside, methyl α-D-glucoside, D-fructose, D-mannose, L-rhamnose, D-xylose, trehalose, gentiobiose, palatinose, D-ribose, salicin, L-fucose, D-cellobiose, D-α-galacturonic acid, α-glucuronic acid, and D-glucosamine hydrochloride showed no inhibition even at 200 mM, respectively. [b] Minimum concentration required for complete inhibition of hemagglutination with titer 4 of *T. japonica* agglutinin I. [c] The relative inhibitory ability was calculated in relation to galactose.

6′-sialyllacto-*N*-neotetraose (LST-c) showed 125 and 2000 times higher hemagglutination inhibitory activity than that of D-galactose, although *N*-acetylneuraminic acid did not show any hemagglutination inhibitory effect even up to a concentration of 200 mM. These results indicate that TJA-I principally recognizes not *N*-acetylneuraminic acid but *N*-acetyllactosamine/lactose and the hydroxyl group at C-6 position of the β-galactose residue should be substituted with *N*-acetylneuraminic acid to increase the binding strength to TJA-I.

Carbohydrate Binding Specificity of an Immobilized TJA-I Column. The precise carbohydrate binding specificity of TJA-I was determined from the behavior of various oligosaccharides on TJA-I–Sepharose column chromatography at 4 and 20 °C. Representative elution profiles are shown in Figure 4, and the structures examined are given in Table III. Oligosaccharides **1**, **2**, and **5**–**9** in Table III passed through the TJA-I–Sepharose column at 20 °C, as shown by dotted lines in Figure 4A–E. When the column temperature was lowered to 4 °C, the following interesting data were obtained. Oligosaccharides **1**, **2**, and **5** possessing 1 mol of *N*-acetyllactosamine residue per molecule were slightly retarded on the column (Figure 4A, solid line, and Table III). In contrast, oligosaccharide **3** with a terminal Galβ1→3GlcNAc group and oligosaccharide **4** with a terminal Galβ1→6Gal group passed through the column without interaction (Figure 4B, solid line, Table III), indicating that the terminal *N*-acetyllactosaminyl structure is important. The behavior of complex-type oligosaccharides **5**–**8** (Figure 4A, C–E, solid line, Table III) indicated that the elution volumes of complex-type oligosaccharides increase in proportion to the number of Galβ1→4GlcNAc residues per molecule. The elution volume of bisected biantennary oligosaccharide **9** was decreased compared to that of nonbisected biantennary oligosaccharide **6**.

Interestingly, oligosaccharides **10**–**12** bound very strongly to the column at 4 and 20 °C and were eluted with PBS–NaN_3 containing 0.1 M lactose (Figure 4F and Table III). These results indicated that oligosaccharides possessing a Neu5Acα2→6Galβ1→4GlcNAc(Glc) group interact more strongly with a TJA-I column than ones possessing a terminal Galβ1→4GlcNAc(Glc) group. Furthermore, these sialylated oligosaccharides were not eluted with the buffer containing 0.2 M *N*-acetylneuraminic acid, and oligosaccharide **13** containing a HSO_3^-→6Galβ1→4GlcNAc group also strongly interacted with the column at 4 and 20 °C (Figure 4F and Table III). These results indicated that the sialic acid and sulfate residues of the Neu5Acα2(HSO_3^-)→6Galβ1→4GlcNAc group may only play an auxiliary role, either by causing the Galβ1→4GlcNAc group to assume a conformation that better fits the binding site of TJA-I or by interacting with some charged amino acids in the lectin to facilitate the binding of the oligosaccharide group.

On the other hand, oligosaccharide **14** with the terminal galactose residues substituted at the C-2 position with a fucosyl residue, oligosaccharide **15** with the subterminal *N*-acetylglucosamine residues substituted at the C-3 position with a fucosyl residue, oligosaccharides **16** and **17** with the terminal β-galactose residues substituted at the C-3 position with an *N*-acetylneuraminic acid or α-galactose residue, and oligosaccharide **18** possessing terminal β-*N*-acetylgalactosamine residues passed through the column at 4 and 20 °C, indicating that the terminal Galβ1→4GlcNAc group at least should not be substituted at the C-2 or C-3 position to interact with the TJA-I column.

Behavior of Human Serum Glycoproteins on TJA-I–Sepharose and Allo A-II–Sepharose Columns. Although the carbohydrate binding specificity of Allo A-II–Sepharose was similar to that of TJA-I–Sepharose as previously reported (Yamashita et al., 1988), the elution profiles of human serum glycoproteins on columns of these two lectins were somewhat different.

Most human serum glycoproteins contain bi-, tri-, and tetraantennary complex-type sugar chains with Neu5Acα2→6Galβ1→4GlcNAc groups. When these sialylated glycoproteins and the corresponding asialoglycoproteins were applied to TJA-I and Allo A-II columns, respectively, the sialylated glycoproteins were adsorbed to both columns and eluted with PBS–NaN_3 containing lactose (Figure 5, ●). On the other hand, the behavior of asialoglycoproteins on Allo A-II and TJA-I columns was different (Figure 5, ○). Asialoglycoproteins were not bound to the TJA-I column (Figure 5A,B, ○), but were greatly retarded or adsorbed to the Allo A-II column and eluted with PBS–NaN_3 containing 10 mM lactose (Figure 5C,D, ○). These results indicate that association constants of oligosaccharides possessing Galβ1→4GlcNAc residues as to Allo A-II are larger than those of TJA-I. Accordingly, an Allo A-II–Sepharose column is useful for separating sialylated and desialylated human serum glycoproteins from serum albumin, while a TJA-I–Sepharose one can be used to separate desialylated glycoproteins and glycoproteins possessing Neu5Acα2→6Galβ1→4GlcNAc groups.

DISCUSSION

Our results clearly demonstrate that TJA-I requires the presence of a terminal Neu5Acα2→6Galβ1→4GlcNAc or HSO_3^-→6Galβ1→4GlcNAc sequence for high-affinity binding, as summarized in Figure 6. Accordingly, a TJA-I–Sepharose column is a very useful tool for the separation and analysis of various oligosaccharides and glycoproteins possessing the Neu5Acα2→6Galβ1→4GlcNAc or HSO_3^-→6Galβ1→4GlcNAc sequence, especially in its ability to distinguish α2,6-linked isomers from α2,3-linked ones in

Weak binding (TJA-I^r fraction at 4 °C)
Galβ1→4GlcNAcβ1→R

Strong binding (TJA-I$^+$ fraction at 4 °C and 20 °C)
Neu5Acα2→6Galβ1→4GlcNAcβ1→R
HSO_3^-→6Galβ1→4GlcNAcβ1→R

Pass-through (TJA-I$^-$ fraction at 4 °C and 20 °C)
Galβ1→3GlcNAcβ1→R
Galβ1→6Galβ1→4Glc$_{OT}$
Neu5Acα2→3Galβ1→4GlcNAcβ1→R
Fucα1→2Galβ1→4GlcNAc β1→R
Galα1→3Galβ1→4GlcNAcβ1→R
Galβ1→4(Fucα1→3)GlcNAc β1→R
GalNAcβ1→4GlcNAcβ1→R

R : H or sugars

FIGURE 6: Summary of the carbohydrate binding specificity of immobilized TJA-I.

combination with a *Macckia amurensis* lectin (MAL)–Sepharose column, because the MAL–Sepharose column interacts with sugar chains possessing the Siaα2→3Galβ1→4GlcNAc structure (Knibbs et al., 1991). These sugar chains are retarded or bound to the column and eluted with buffer containing 0.4 M lactose (data not shown), indicating that a β-galactose residue is basically necessary for binding to a MAL column, similar to the cases of TJA-I and Allo A-II. However, substutition of the hydroxyl group at the C-3 position enhances the affinity of oligosaccharides to the MAL column.

Although a *Sambucus nigra* agglutinin (SNA)–Sepharose column does not interact with sugar chains possessing a terminal β-galactose residue, the addition of a sialic acid residue at the C-6 position of terminal sugar chains increases their binding constants 3 orders and the adsorbed oligosaccharides can be eluted with 0.1 M lactose in PBS–NaN_3- (Shibuya et al., 1987). The binding specificity of the SNA–Sepharose column is similar to those of the TJA-I–Sepharose and Allo A-II–Sepharose columns. However, sialyl-Tn antigen (Siaα2→6GalNAc) binds to the SNA colunn, but not to the TJA-I or Allo A-II column (data not shown). Because the Allo A-II and SNA columns also interact with oligosaccharide **13** possessing a HSO_3^-→6Galβ1→4GlcNAc group, these lectins do not have binding sites that directly accommodate a sialic acid or sulfate, but rather the sialic acid or sulfate causes the Galβ1→4GlcNAc to assume a conformation that binds better to their binding pockets. It is possible that the charge on the sialic acid or sulfate interacts with some charged amino acids in the binding pocket.

Accordingly, by using the TJA-I, SNA, and MAL columns with different sugar binding specificities together with the results of sialidase digestion, the sialyl linkages of the oligosaccharides and glycoproteins can be assigned as follows:

SNA^+ TJA-I^-: Siaα2→6GalNAc

TJA-I^+ MAL^-: Siaα2→6Galβ1→4GlcNAc

TJA-I^- $MAL^{r\ or\ +}$: Siaα2→3Galβ1→4GlcNAc

The sugar chains which still bind to the TJA-I column after sialidase digestion but pass through the column after methanolysis most possibly contain a sulfuric acid residue linked to the C-6 position of their terminal β-galactose residue.

SUPPLEMENTARY MATERIAL AVAILABLE

Materials and Methods, Figures 1, 4, and 5, and Table III are presented (9 pages). Ordering information is given on any current masthead page.

REFERENCES

Ashwell, G., & Harford, J. (1982) *Annu. Rev. Biochem. 51*, 531–554.

Bierhuizen, M., Edzes, H., Shiphorst, W., van den Eijnden, D. H., & van Dijk, W. (1988) *Glycoconjugate J. 5*, 85–97.

Cummings, R. D., & Kornfeld, S. (1984) *J. Biol. Chem. 259*, 6253–6260.

Hitoi, A., Yamashita, K., Niwata, Y., Irie, M., Kochibe, N., & Kobata, A. (1987) *J. Biochem. (Tokyo) 101*, 29–41.

Knibbs, R. N., Goldstein, I. J., Ratchiffe, R. M., & Shibuya, N. (1991) *J. Biol. Chem. 266*, 83–88.

Kobata, A. (1972) *Methods Enzymol. 28*, 262–271.

Kobata, A., & Yamashita, K. (1989) *Methods Enzymol. 179*, 46–54.

Laemmli, U. K. (1970) *Nature 227*, 680–685.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Morell, A. G., Irvine, R. A., Sternlieb, I., Scheinberg, I. H., & Ashwell, G. (1968) *J. Biol. Chem. 243*, 155–159.

Ohkura, T., Isobe, T., Yamashita, K., & Kobata, A. (1985) *Biochemistry 24*, 503–508.

Philips, M. L., Nudelman, E., Sighal, A. K., Gaeta, F. C. A., Perez, M., Hakomori, S. I., & Paulson, J. C. (1990) *Science 250*, 1130–1132.

Rademacher, T. W., Parekh, R. B., & Dwek, R. A. (1988) *Annu. Rev. Biochem. 57*, 785–838.

Rinderknecht, E., O'Connor, B. H., & Rodriguez, H. (1984) *J. Biol. Chem. 259*, 6790–6797.

Shibuya, N., Goldstein, I. J., Broekaert, W. F., Lubaki, M. N., Paeters, B., & Peumans, W. J. (1987) *Arch. Biochem. Biophys. 254*, 1–8.

Takeuchi, M., Takasaki, S., & Kobata, A. (1987) *J. Chromatogr. 400*, 207–213.

Tanaka, N., Nakada, H., Itoh, N., Mizuno, Y., Takanishi, M., Kawasaki, T., Tate, S., Inagaki, F., & Yamashina, I. (1992) *J. Biochem. 112*, 68–74.

Townsend, R. R., Hardy, M. R., Wong, T. C., & Lee, Y. C. (1986) *Biochemistry 25*, 5716–5725.

Umetsu, K., Kosaka, S., & Suzuki, T. (1979) *Tohoku J. Exp. Med. 129*, 161–167.

Umetsu, K., Kosaka, S., & Suzuki, T. (1985) *Biochem. Int. 10*, 549–552.

Yamashita, K., & Kobata, A. (1974) *Arch. Biochem. Biophys. 55*, 35–67.

Yamashita, K., Tachibana, Y., Takeda, S., Matsuda, I., Arashima, S., & Kobata, A. (1979) *J. Biol. Chem. 254*, 4820–4827.

Yamashita, K., Liang, C.-J., Funakoshi, S., & Kobata, A. (1981) *J. Biol. Chem. 256*, 1283–1289.

Yamashita, K., Hitoi, A., & Kobata, A. (1983) *J. Biol. Chem. 258*, 14753–14755.

Yamashita, K., Totani, K., Ohkura, T., Takasaki, S., Goldstein, I. J., & Kobata, A. (1987) *J. Biol. Chem. 262*, 1602–1607.

Yamashita, K., Umetsu, K., Suzuki, T., Iwaki, Y., Endo, T., & Kobata, A. (1988) *J. Biol. Chem. 263*, 17483–17489.

Yamashita, K., Kobata, A., Suzuki, T., & Umetsu, K. (1989a) *Methods Enzymol. 179*, 331–341.

Yamashita, K., Totani, K., Iwaki, Y., Kuroki, M., Matsuoka, Y., Endo, T., & Kobata, A. (1989b) *J. Biol. Chem. 264*, 17873–17881.

Yoshima, H., Matsumoto, A., Mizuochi, T., Kawasaki, T., & Kobata, A. (1981) *J. Biol. Chem. 256*, 8476–8484.