Vol. 198, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1994 Pages 251-256

Interaction of a Novel Tn (GalNAcα1→Ser/Thr) Glycoprotein with Gal, GalNAc and GlcNAc Specific Lectins

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Received November 26, 1993

SUMMARY: A naturally occurring Tn glycoprotein (Native ASG-Tn) with GalNAcα1→Ser/Thr as the only carbohydrate side chains, has been prepared from armadillo submandibular glands. In a quantitative preciptin assay, this glycoprotein completely precipitated *Maclura pomifera* (MPA), *Vicia villosa* B_4 (VVL-B_4) and *Artocarpus integrifolia* (Jacalin, AIL). It also reacted well with *Helix pomatia* (HPL) and *Wistaria floribunda* (WFL) and precipitated over 75% of the lectin nitrogen added, but poorly with *Ricinus communis* agglutinin (RCA_I), ricin, peanut (*Arachis hypogaea*, PNA), *Abrus precatorius* agglutinin (APA) and *Triticum vulgaris* (WGA). This finding suggests that this novel Tn-glycoprotein may serve as a useful reagent for differentiating Tn and T specific monoclonal antibodies and lectins.

The Tn determinant infers the structure of GalNAcα1→Ser(Thr) in the peptide core as the major *O*-glycosidic linkage in glycoproteins. At the red cell surface, Tn transformation indicates an acquired disorder characterized by the exposure of normally cryptic GalNAc residues α1→linked to the hydroxyl of Ser or Thr on membrane sialo-glycoproteins (1). It is the result of a selective deficiency of the 3-β-D-galactosyltransferase involved in the biosynthesis of the **T** structure: Galβ1→3GalNAcα→Ser(Thr) (2). The Tn antigen can be detected at the cell surface of erythrocytes, granulocytes, platelets,

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Abbreviations : Gal, D-galactopyranose; Glc, D-glucopyranose; LFuc or Fuc, L-fucopyranose; GalNAc, 2-acetamido-2-deoxy-D-galactopyranose; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; GalNAc-ol, 2-acetamido-2-deoxy-D-galactitol;
MWCO, Molecular weight cut off. Abbreviations of lectins and lectin determinants are illustrated in Table I. ASG-A, armadillo submandibular glycoprotein, fraction A (13); ASG-Tn, the desialized ASG-A; native ASG-Tn or native Tn, a naturally occurring Tn glycoprotein isolated from the extract of 0.01 M PBS pH 6.8 after removal of ASG-A, one of the sialic acid containing glycoproteins in armadillo submandibular glands.

0006-291X/94 $5.00

and B and T lymphocytes of patients presenting the Tn syndrome (3). This antigen has also been proposed as a marker of cancerous tissues (4,5).

During the past two decades, many water soluble glycoproteins and polysaccharides have been used to study the binding property of lectins (6-12), especially glycoproteins bearing **F** (GalNAcα1→3GalNAc), **A** (GalNAcα1→3Gal), $\mathbf{A_f}$ (GalNAcα1→3[Fucα1→2]Gal), **Tn** (GalNAcα1→Ser/Thr), **B** (Galα1→3Gal), **E** (Galα1→4Gal), **I/II** (Galβ1→3/4GlcNAc), **L** (Galβ1→4Glc) and **T** (Galβ1→3GalNAc) determinants (6-8). For this purpose, many related glycoproteins have been searched for in our laboratory. In our previous work, we isolated two sialic acid containing glycoproteins : ASG-A and ASG-B from the armadillo submandibular glands (13). Recently, we found a new glycoprotein (Native ASG-Tn), in which GalNAcα1→Ser/Thr was the only type of carbohydrate side chain in the 0.01 M PBS, pH 6.8 extract after removal of ASG-A. This glycoprotein had a core protein composed mainly of six amino acids (Thr, Ser, Ala, Glu, Gly and Val). When the binding property of this glycoprotein was analyzed by quantative preciptin assay (14, 15), it reacted well with all **Tn** and **F/A** specific lectins tested, but it did not precipitate **T** (Galβ→3GalNAc) specific lectins such as PNA and APA. This finding suggests that this glycoprotein may be useful as an reagent for differentiating **Tn** and **T** specific monoclonal antibodies and/or lectins.

MATERIALS AND METHODS

Chemical & reagents. Neutral sugars, *N*-acetylneuraminic acid, *N*-glycolylneuraminic acid, hexosamine and cetyltrimethylammonium bromide (Cetavlon) were products of Sigma Chemical Co., St. Louis, MO, U.S.A.; hydroxyapatite (Bio-Gal HTP) was purchased from Bio-Rad, Richmond, CA, U.S.A.

Lectins. *Ricinus communis* agglutinin (RCA_1) and ricin were purchased from Boehringer Mannheim Biochemical, Germany; peanut lectin (PNA), wheat germ agglutinin (WGA) and *Vicia Villosa* B_4 ($VVA-B_4$), from Sigma Chemical Co., St. Louis. MO, U.S.A. The *Maclura pomifera* (MPA), *Helix pomatia* (HPA), *Dolichos biflorus* (DBA) and *Wistaria floribunda* (WFA) lectins were purified by adsorption to insoluble polyleucyl hog gastric (A+H) mucin (16-18), and eluted by melibiose (12), GalNAc (19) and lactose (20), respectively. The mistletoe lectin-I (ML-I), provided by Dr. Uwe Pfüller, Universität Witten/Herdecke, Institute of Phytochemistry, Berlin (Germany), was isolated from ground plant material mistletoe grown on the locust tree (*Robinia pseudoacacia*) by an acid-treated agarose affinity chromatography with 0.15 M NaCl as eluant (21). *Abrus precatorius* agglutinin (APA) as well as abrin-a, given by Drs. L.P. Chow and J.Y. Lin, Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan, were purified from the seeds of *Abrus precatorius* (Jequirity bean) by Sepharose 4B and DEAE-cellulose column chromatographies (22).

Native Tn glycoprotein. Submandibular glands of armadillos, captured on the Gulf coast of Mexico, were provided by Dr. E. Storrs of Gulf South Research Institute (New Iberia, LA, U.S.A.). Four glands of 4-6 g each were used in this work. The Tn glycoprotein was prepared by a modification of the method of Tettamenti & Pigman (23), as described previously (13). After ASG-A was eluted, it was extracted with 0.01 M PBS at pH 6.8 from the hydroxyapatite-glycoprotein complex until the hexosamine content had dropped to baseline level. This material was filtered through MWCO 3.0×10^4 membrane, dialyzed against H_2O (MWCO 1.2×10^4), and lyophilized. The

contaminating ASG-A in this fraction was removed by addition of Cetavlon to a final conc. of 3% and the clot (ASG-A-cetavlon complex) was spun down by low speed centrifugation. For desialylation, a sample of the glycoprotein, dissolved in 0.01 M HCl, was hydrolysed at 80°C for 40 min and dialyzed at 4°C against 20 vol. of water for 48 h, with frequent changes of water. The nondialyzable material was collected, and freeze-dried (24).

Analytical procedures. Protein content was measured by the procedure of Lowry et al. (25), with crystalline bovine serum albumin as the standard, and by summation of the amino acids (24). Sialic acid was determined by resorcinol (26). Total hexosamine was determined by the Elson-Morgan method, as described by Boas (27), after hydrolysis of the samples in 2 N HCl for 2 h at 104°C. The ratio galactosamine/glucosamine was determined by means of an amino acid analyzer (28). For the determination of fucose, the cysteine/H_2SO_4 reaction of Dische & Shettles (29) was used, with a heating period of 10 mins. Galactose was analyzed according to Dische & Danilchenko (30). The absence of nucleic acids and tryptophan was demonstrated by U.V. absorption at 260 and 280 nm, with yeast RNA and tryptophan as the reference materials, respectively. Amino acid composition was analyzed with a Beckman 6300 Amino Acid Analyzer at the Service Center, National Science Council at the National Taiwan University, Taipei, Taiwan. Alkaline β-elimination-borohydride reduction was performed by the condition of Carlson (31, 32), and the liberated oligosaccharides were purified by the procedures described by Wu et al. (32).

Acrylamide gel electropheresis. Discontinuous 5-10% gradient sodium dodecylsulfate polyacrylamide gel (PAGE) (33) and 0.5-4.5 μg Tn antigen was used for electropheresis. The resulting bands were revealed by silver staining (0.5 μg) or Coomassie Brilliant Blue R-250 (4.5 μg).

Immunochemical assays. Quantitative precipitin assay was performed by a microprecipitin technique (14) using 4.9 to 6.3 μg of lectin nitrogen (N) for each determination: total N in the washed precipitates was estimated by the ninhydrin method (15).

RESULT AND DISCUSSION

An armadillo salivary glycoprotein containing only **Tn** (GalNAcα1→ Ser/Thr) as carbohydrate side chains was isolated from the 0.01 M PBS pH 6.8 extract after removal of ASG-A, which is one of the sialic acid containing glycoproteins in armadillo submanbular glands. GalNAc-ol was the only sugar liberated by alkaline β-elimination and borohydride reduction. We defined this novel fraction as Native ASG-Tn or native Tn, and the desialylated ASG-A as ASG-Tn. The protein content, as determined by the method of Lowry (25), was nearly 1.70 times higher than when determined by the sum of the amino acids (24). The native Tn was composed of 30.6% GalNAc and 59.6% amino acids, with six amino acids constituting (Thr+Ser, 55%; Ala, 6%; Glu, 10%; Gly 14.5%; and Val 13.2%) 98.7 mole% of the protein core. Thus, the component difference between ASG-Tn and ASG-A (13.3% sialic acid, 26.6% GalNAc and total amino acids, 53.5%) was in the sialic acid content of the latter. The molecular weight has not been established, but it was the nondialyzable fraction of MWCO 1.2×10^4 and the filtrable fraction of MWCO 3.0×10^4. ASG-Tn run on PAGE gels (5-10%), stained neither with Coomassie Brilliant Blue nor with silver nitrate. The binding property of native ASG-Tn was analyzed by interaction with thirteen Gal-, GalNAc- and GlcNAc- specific lectins (Fig I and Table I)

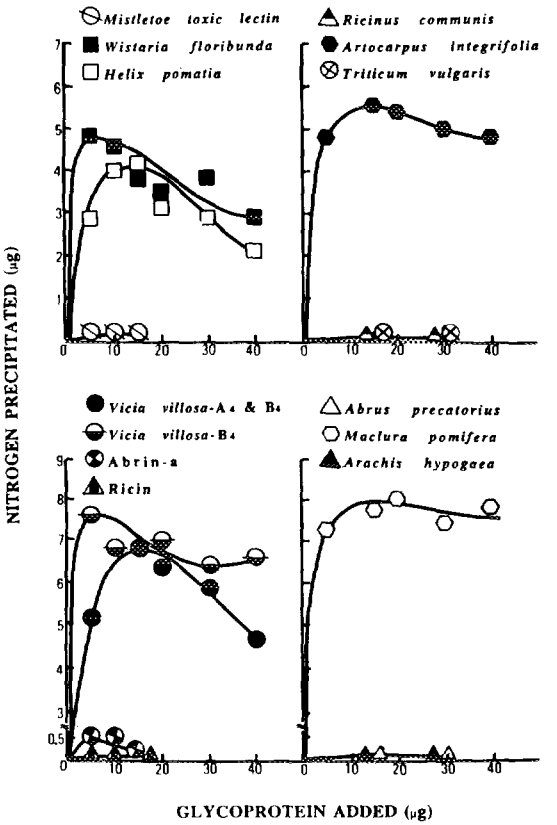

Fig. I. Quantitative Precipitation Curves of Native Armadillo Submandibular Tn Glycoprotein (Native ASG-Tn) with Gal, GalNAc and GlcNAc Specific Lectins. The amount of lectin nitrogen added is listed in Table I. Total volume: 300 μl.

and quantitated by the precipitin assay. It was found that this glycoprotein completely precipitated three **Tn** specific lectins — *Maclura pomifera* agglutinin (MPA), *Vicia villosa* B_4 (VVA-B_4) agglutinin and *Artocarpus integrifolia* (Jacalin, AIL) requiring less than 1 μg for 50% precipitation. This glycoprotein is the first reagent that has the ability to completely precipitate MPA and Jacalin. These two lectins are also reacting with the **T** sequence (6-8), indicating that these lectins have the ability to recognize crypto-Tn determinants. Thus, the carbohydrate specificity of MPA and Jacalin can be classified as having dual specificities : **Tn** and **T** determinants. As expected, native ASG-Tn was the best reagent to precipitate VVA-B_4, but it reacted poorly with PNA, RCA_1, ricin, APA and WGA. Native-Tn also reacted well with two A specific lectins — *Helix pomatia* (edible snail) and *Wistaria floribunda* (WFA), and precipated 76% and 95% of the lectin nitrogen added,

Table I

Comparative Precipitation Activities of Native Armadillo Salivary Tn Glycoprotein with Gal, GalNAc and GlcNAc Specific Lectins

Lectin	Carbohydrate specificity[b] (6-7)	Amount of lectin used for precipitation reaction (μg N)	Maximum lectin N precipitated[a]	Amount of glycoprotein required for 50% precipitation (μg)
Helix pomatia (edible snail, HPA)	**F > A (≥ A_f) ≥ Tn.T.**	5.3	4.1 (76.4%)	4.5
Wistaria floribunda (WFA)	**A (> A_f), F > Tn, I(II)**	5.0	4.8 (94.9%)	< 0.5
Vicia villosa-A_4 & B_4 (VVA-A_4 & B_4)	**A + Tn**	5.5	6.8 (123.6%)	1.5
Arachis hypogaea (peanut, PNA)	**T >> I(II)**	6.0	1.0 (< 1.0%)	
Abrus precatorius agglutinin (APA)	**T > I/II > E > B > Tn**	5.5	0.1 (< 1.0%)	
Maclura pomifera agglutinin (MPA)	**T > Tn**	6.2	8.0 (128.0%)	< 0.5
Artocarpus integrifolia (Jacalin)	**T > Tn >>> I(II)**	5.3	5.5 (104.0%)	1.0
Vicia villosa-B_4 (VVA-B_4)	**Tn only**	6.3	7.6 (120.0%)	< 1.0
Abrin a	**Galα1→**	5.7	0.43 (< 1.0%)	
Ricin	**T, I/II, Lac > E & B**	4.9	0	
Mistletoe toxic lectin-I (ML-I)	**E, L, T, I/II**	5.1	0	
Ricinus communis agglutinin (RCA$_I$)	**II > I > B > T >> Tn**	5.9	0	
Triticum vulgaris (wheat germ, WGA)	**C**	5.0	0.02 (< 1.0%)	

a The value in parentheses indicates the % of μg N precipitated at maximum when the amount of lectin N added is expressed as 100%.

b Carbohydrate specificity of lectins as expressed by lectin determinants — **F**, GalNAcα1→3GalNAc; **A**, GalNAcα1→3Gal; **A_f**, GalNAcα1→3(Fucα1→2)Gal; **Tn**, GalNAcα1→Ser/Thr; **B**, Galα1→3Gal; **E**, Galα1→4Gal; **I/II**, Galβ1→3/4GlcNAc; **L**, Galβ1→4Glc; **T**, Galβ1→3GalNAc; **C**, GlcNAcβ1→4GlcNAc (chitin disaccharide) (6-8).

respectively. From these results, it is clear that native ASG-Tn is one of the best reagents to study the differential binding properties of **T**, **Tn** and **A_f** specific lectins as well as related monoclonal antibodies.

ACKNOWLEDGMENTS

This work was supported by Grants from the Chang-Gung Medical Research Plan (CMRP No. 293), Kwei-san, Tao-yuan, Taiwan, National Science Council (NSC 82-0412-B-182-088 and 82-0418-B-182-009), and the National Health Institutes (DOH 83-HR-316 and DOH 83-HR-209), Department of Health, Taipei, Taiwan. The authors thank Jung-Chin Lin for her secretarial assistance in preparing this manuscript.

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