

α -C-Mannosyltryptophan is not recognized by conventional mannose-binding lectins

Toshio Nishikawa,^{a,d,*} Shigeo Kajii,^a Chihiro Sato,^b Zenta Yasukawa,^c Ken Kitajima^c and Minoru Isobe^a

^aLaboratory of Organic Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

^bLaboratory of Molecular Bioregulation, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601 Japan

^cBioscience and Biotechnology Center, Nagoya University, Chikusa, Nagoya 464-8601, Japan

^dPRESTO, Japan Science and Technology Agency, Honcho 4-1-8, Kawaguchi, Saitama, Japan

Received 14 January 2004; revised 4 February 2004; accepted 4 February 2004

Abstract— α -C-Mannosyltryptophan (C-Man-Trp) is a novel, naturally occurring C-linked carbohydrate–protein linkage first found in 1994 from human ribonuclease 2. Since then, a number of C-Man-Trp residue have been found from several important proteins such as interleukin 12 β , components of complement system, thrombospondin-1, and erythropoietin receptor, however, the biological functions have remained unknown even though its biosynthetic pathway has been revealed. In order to find a clue as to the biological functions, we examined the affinity of C-Man-Trp with conventional mannose lectin such as concanavarin A (Con A) and mannose-binding lectin (MBL). The affinity of C-Man-Trp with Con A, a typical mannose-binding lectin from plant was examined using a Con A-Sepharose column. Unlike *p*-nitrophenyl- α -O-Man, C-Man-Trp was not retained on the column. MBL-C, a major mannose-binding lectin purified from mouse serum, did not bind with N-biotinylated C-Man-Trp, judging from ELISA based assay. These results imply that C-Man-Trp may be recognized with the other specific proteins associated with its unknown biological functions.

© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

Carbohydrate parts of glycoproteins and glycolipids have been found to play important roles in a variety of biological phenomena such as fertilization, embryonic development, differentiation, neurogenesis, immune response, and inflammation.^{1,2} Most of the carbohydrate moiety are linked to protein through *N*-glycosidic bond with asparagine and *O*-glycosidic bond with serine or threonine.³ In 1994, however, Trp-7 residue of ribonuclease 2 isolated from human urine was found to be α -C-mannosylated at second position of the indole (1 in Fig. 1).^{4,5} Interestingly, the mannose moiety of C-Man-Trp adopt not an usual ⁴C₁, but a mixture of some conformations including unusual ¹C₄, judging from

extensive analysis of the NMR spectra. C-Man-Trp is the first example of naturally occurring C-glycosyl amino acid found in proteins, although many synthetic C-glycosyl amino acids have been reported.^{6,7} This novel post-translational modification is catalyzed by microsome-associated enzyme, ‘C-mannosyltransferase’, which has not been purified so far.⁸ This enzyme was found to recognize an amino acid sequence Trp-x-x-Trp to glycosylate the first Trp of this motif.⁹ These studies imply that this linkage is more common than expected in the early stage of this study, because the recognition sequence is contained in thrombospondin type 1 repeat whose sequence has been found in many proteins such as extracellular matrix proteins and proteins associated with complement systems, and in the WS motif whose sequence has been found in cytokine receptors.¹⁰ In fact, the C-Man-Trp residue has also been found in a variety of proteins such as recombinant interleukin 12,¹¹ human complement system,^{12,13} human platelet thrombospondin-1¹⁴ and recombinant erythropoietin receptor.¹⁵ Furthermore, the C-mannosyltransferase activity was

Keywords: C-mannosyltryptophan; Lectin; Mannose-binding lectin; Con A.

* Corresponding author. Tel.: +81-52-789-4112; fax: +81-52-789-4111; e-mail: nishikawa@agr.nagoya-u.ac.jp

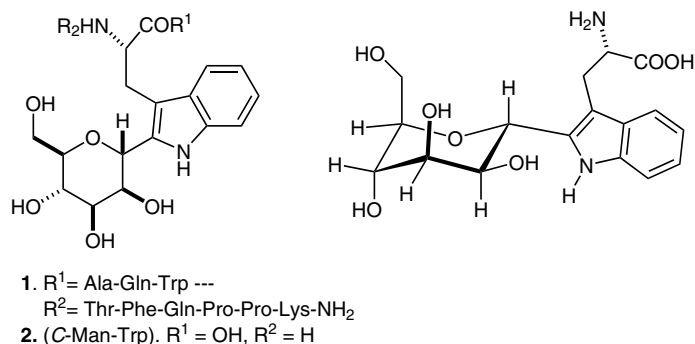


Figure 1. Chemical structure of α -*C*-mannosyltryptophan.

detected in many organisms such as mammals, nematodes, birds, amphibians, fish, but not in *Escherichia coli*, insect or yeast.^{10,16} On the other hand, monomeric *C*-Man-Trp (**2**, in Fig. 1) was isolated not only from human urine,^{17,18} but also from marine organisms.^{19,20} Monomeric *C*-Man-Trp in blood was reported to be a potential candidate as a marker molecule for diagnosing renal function.²¹ Despite these extensive studies, the biological function of this novel modification has not been clarified, although some possibilities were discussed.¹⁰ To clue the biological functions, authentic *C*-Man-Trp-containing compounds as well as probes for these structures are absolutely necessary. However, since sufficient amount of such compounds are not available from natural sources or enzymatic synthesis at present, we as well as others have studied on chemical synthesis of *C*-Man-Trp^{22–24} and its analogs.²⁵ The presence of D-mannose in *C*-Man-Trp residue prompted us to examine whether *C*-Man-Trp could be recognized by conventional α -mannose-binding lectins. The study might provide some clues not only for the biological functions of *C*-Man-Trp in proteins, but also for the significance of monomeric *C*-Man-Trp in blood and urine. In this paper, we describe the affinity of *C*-Man-Trp with typical α -mannose-binding lectins using synthetic *C*-Man-Trp and its derivative.

2. Results and discussion

2.1. *C*-Man-Trp and *C*-Man-Ind were not recognized by a plant lectin Con A

Concanavarin A (Con A) is a plant lectin that can bind to monomeric and polymeric forms of α -mannosides.²⁶ To examine if *C*-Man-Trp is recognized by a typical mannose binding lectin Con A, we employed Con A-Sepharose column, which was reported to retain monomeric α -*O*-mannoside.²⁶ In our experiments, 20% of the applied O-Man-pNP were recovered in fractions 18–22, which was eluted with 1.0 M α -MeMan and 78% O-Man-pNP passed through the column, because of retention capacity of the column (Fig. 2A). On the other hand, *C*-Man-Trp was not retained in the column as shown in Figure 2B. It is thus concluded that *C*-Man-Trp is not recognized by Con A.

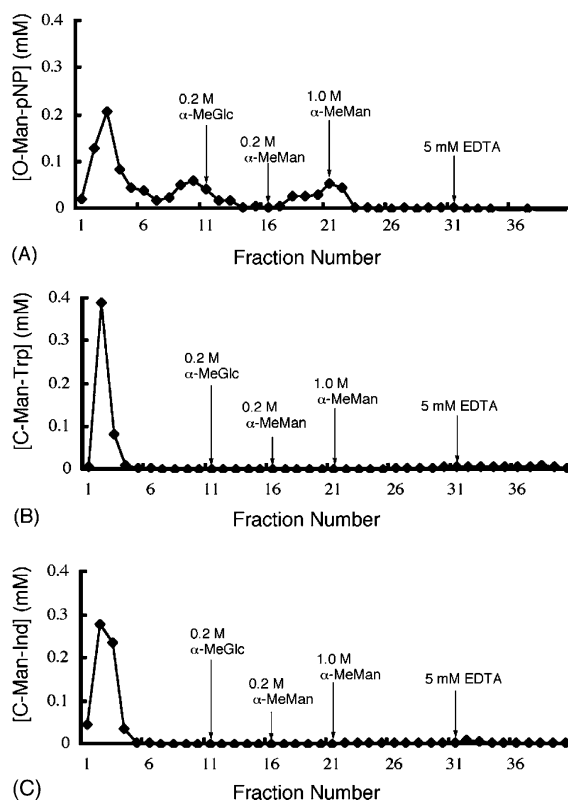
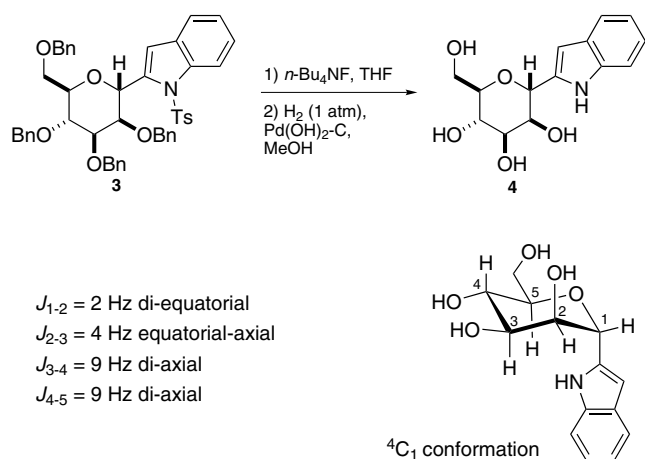


Figure 2. Affinity chromatograms on Con A-Sepharose column with O-Man-pNP (A), *C*-Man-Trp (B), and *C*-Man-Indole (C). (A) The concentration of O-Man-pNP was determined by measuring absorbance at 405 nm after hydrolysis of the mannoside. O-Man-pNP was retained in the column and eluted in fractions 18–22. O-Man-pNP was also eluted in the pass-through fractions because of overloading of the mannoside. (B) The concentration of *C*-Man-Trp was determined spectrophotometrically at 280 nm. In the pass-through fractions, 78% of the loaded *C*-Man-Trp were detected. *C*-Man-Trp was not found in any fractions eluted with α -MeGlc, α -MeMan or EDTA. (C) The concentration of *C*-Man-Indole was determined spectrophotometrically at 280 nm. In the pass-through fractions, 86% of the loaded *C*-Man-Indole were detected. *C*-Man-Indole was not found in any other fractions.

To obtain a further insight into recognition of the α -*C*-mannoside by Con A, the same lectin chromatography was performed for *C*-Man-Indole **4**, which was readily prepared from a synthetic intermediate for *C*-Man-Trp



Scheme 1. Synthesis and conformation of α -C-mannosylindole.

(Scheme 1). As shown in Figure 2C, C-Man-Indole was not recognized by Con A either.

As ^1H NMR analysis of C-Man-Indole revealed that the conformation of the mannose moiety is 4C_1 (diagnostic coupling constants are shown in Scheme 1), which is the same as that of usual α -O-mannoside, the indole moiety prevented the interaction with Con A, although some other α -C-mannosides were reported to bind to Con A.^{27,28} Thus, the reason that C-Man-Trp does not bind to Con A is the unusual mannose conformation as well as the structure of aglycon (Trp).

2.2. C-Man-Trp was not recognized by MBL-C

Since C-Man-Trp residue has been found only from animal, we then examined the affinity of C-Man-Trp with typical animal lectin. In mouse serum, two C-type lectins, named as mannose-binding lectins MBL-C and -A, are known to occur, and can recognize both monomeric and polymeric forms of α -mannosides.²⁹ Among them, MBL-C is a major serum MBL and 4–7 times more abundant in weight than MBL-A.³⁰ It is noteworthy that MBL activates complement system,³¹

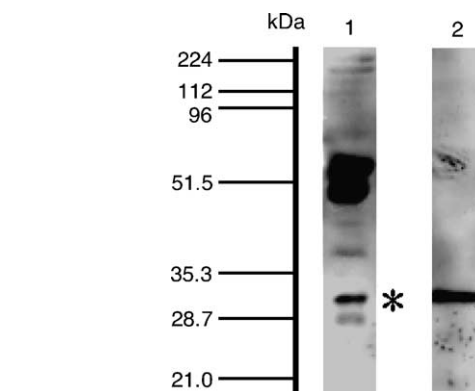
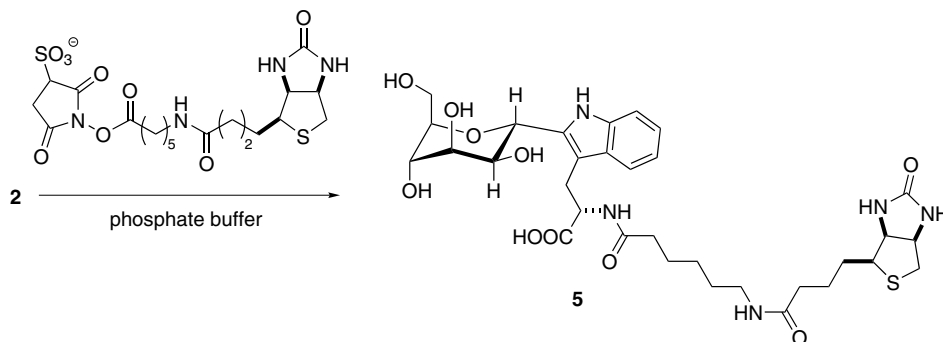


Figure 3. Western blotting of the purified MBL-C using anti-MBL-C. Mouse serum and purified MBL-C were subjected to SDS-PAGE/immunoblotting using anti-MBL-C as a primary antibody. Lane 1, whole serum; lane 2, purified MBL-C; In lane 1, MBL-C is marked by the asterisk, and the strong bands around 50–60 kDa appear to be intrinsic immunoglobulins in mouse serum.

whose several component proteins were heavily C-mannosylated.^{12,13} To examine binding ability of MBL-C to C-Man-Trp directly, MBL-C was purified from mouse sera by serial purification steps including the mannan-Sepharose column chromatography²⁹ (Fig. 3), and tested for binding activity to C-Man-Trp residues by the enzyme-linked immunosorbent assay (ELISA)-based assay,³² by utilizing biotinylated C-Man-Trp prepared from C-Man-Trp (Scheme 2). No binding was observed (data not shown), indicating that MBL-C has no ability to bind the C-Man-Trp residues.

In summary, this study clarifies that the novel C-Man-Trp cannot be recognized by conventional mannose-binding lectins such as Con A and MBL-C. These results led us to suppose that the C-Man-Trp should be recognized by the other specific proteins, which are involved in unknown biological functions of C-Man-Trp. Our preliminary experiments found that several proteins in mouse serum bound to C-Man-Trp immobilized on Sepharose.³³ Efforts to identify the C-Man-Trp-binding proteins from mammal are currently underway in our laboratories.



Scheme 2. Preparation of biotinyl C-Man-Trp.

3. Experimental section

3.1. General

Proton nuclear magnetic resonance (^1H NMR) spectra were recorded on a Bruker ARX-400 (400 MHz) spectrometer. Chemical shifts of all compounds were assigned using *t*-BuOH ($\delta = 1.22$ ppm) as an internal standard. Data are reported as follows; chemical shift, integration, multiplicity (s=singlet, d=doublet, t=triplet, m=multiplet), coupling constant(s), and assignment. High resolution mass spectra (HRMS) were recorded on a JEOL JMS-LCmate LC/MS system, and reported in *m/z*. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel coated glass plates 60F₂₅₄ (Merck, Art 1.05715) using UV light as visualizing agent and 7% ethanolic phosphomolybdic acid, or *p*-anisaldehyde solution and heated as developing agents. Merck silica gel 60 (particle size 0.063–0.2 mm ASTM) were used for open-column chromatography. Preparative TLC were carried out on 0.5 mm Merck silica gel plates (60F₂₅₄, Art 1.05744). All commercially available reagents were used as received.

3.2. Materials

α -C-mannosyltryptophan (C-Man-Trp) was chemically synthesized according to our method.^{21,22} Methyl- α -D-mannopyranoside (α -MeMan), methyl- α -D-glucopyranoside (α -MeGlc), and *p*-nitrophenyl- α -D-mannopyranoside (O-Man-pNP) were purchased from Sigma. Con A Sepharose gel and BrCN-activated Sepharose gel were purchased from Amersham Pharmacia Biotech. Yeast mannan was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Mannan-Sepharose was prepared by conjugating yeast mannan with BrCN-activated Sepharose according to the manufacturer's instruction. A monoclonal anti-mouse mannose-binding lectin C (MBL-C) antibody (14D12)²⁷ was provided by Dr. Steffen Thiel (University of Aarhus, Denmark).

3.3. Synthesis of mannosyl indole

A solution of *N*-Ts-mannosylindole **3** (50 mg, 0.06 mmol) and TBAF (1 M in THF, 0.33 mL, 0.33 mmol) in THF (1.6 mL) was heated under reflux for 2.5 h. The reaction was quenched with satd NH₄Cl solution, the mixture was extracted with AcOEt three times. The combined extract was washed with water and brine, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (silica gel 5 g, AcOEt–hexane = 1:5) to give tetra-*O*-benzylmannosylindole (18.8 mg, 47%). The product (13 mg, 0.021 mmol) was dissolved in MeOH (1 mL) and 2 N HCl (5.3 μ L) and 20% Pd(OH)₂–C (13 mg) were added. The reaction vessel was filled with N₂ gas and the N₂ was replaced with H₂ gas. After vigorously stirring at room temperature for 20 min, the mixture was filtered through the pad of Hyflo Super-Cel, the filtrate was concentrated under reduced pressure. The crude product was purified on preparative TLC (CH₂Cl₂–MeOH = 4:1)

to give C-mannosylindole **4** (4.3 mg, 74%). ^1H NMR (400 MHz, D₂O) δ 3.30 (1H, ddd, *J* = 9, 6, 2 Hz, H-5'), 3.73 (1H, t, *J* = 9 Hz, H-4'), 3.76 (1H, dd, *J* = 12, 6 Hz, H_A-6'), 3.86 (1H, dd, *J* = 12, 2 Hz, H_B-6'), 3.93 (1H, dd, *J* = 9, 4 Hz, H-3'), 4.63 (1H, dd, *J* = 4, 2 Hz, H-2'), 5.26 (1H, d, *J* = 2 Hz, H-1'), 6.54 (1H, s, H-3), 7.13 (1H, t, *J* = 8 Hz, indole), 7.23 (1H, t, *J* = 8 Hz, indole), 7.48 (1H, d, *J* = 8 Hz, indole), 7.64 (1H, d, *J* = 8 Hz, indole). ^{13}C NMR (100 MHz, D₂O) δ 62.8, 68.9, 71.5, 73.2, 76.2, 77.3, 102.3, 113.2, 121.6, 122.2, 124.1, 129.2, 136.2, 137.9. HRMS (FAB) calcd for C₁₄H₁₈N₁O₅S (M+H), 280.1185, found: 280.1177.

3.4. Synthesis of Biotinyl C-Man-Trp

To a solution of Man-Trp **2** (3.3 mg, 9.0 μ mol) in phosphate buffer (2.0 mL, 0.1 M, pH 7.2) was added EZ-LinkTM sulfo-NHS-LC-Biotin (15.0 mg, 27.0 μ mol, PIERCE). After stirring at the room temperature for 2 h, the reaction was quenched with 28% ammonia (1.1 mL). The resulting mixture was stirred at the same temperature for 1.5 h, concentrated under reduced pressure. The residue was chromatographed on preparative TLC (CHCl₃–MeOH/H₂O = 65:65:15). The product was further purified on a column of Cosmosil 75C₁₈ (gel 200 μ L, elution with H₂O–MeOH (3:1)) to give biotinyl Man-Trp **5** (2.6 mg 41%). ^1H NMR (400 MHz, D₂O) δ 0.71–0.85 (2H, m), 1.14–1.26 (2H, m), 1.14–1.26 (2H, m), 1.30–1.41 (2H, m), 1.50–1.75 (2H, m), 1.50–1.75 (2H, m), 2.05 (2H, t, *J* = 7 Hz), 2.22 (2H, t, *J* = 7 Hz), 2.72 (1H, d, *J* = 13 Hz), 2.92 (1H, dd, *J* = 13, 5 Hz), 2.90–3.00 (2H, m), 3.19 (1H, dd, *J* = 14, 5, 10.5 Hz, H_A- β), 3.22–3.30 (1H, m), 3.42 (1H, dd, *J* = 14.5, 5 Hz, H_B- β), 3.77 (1H, dd, *J* = 12, 3 Hz, H_A-6'), 3.80–3.86 (1H, m, H-5'), 3.96 (1H, dd, *J* = 6, 2 Hz, H-4'), 4.08 (1H, dd, *J* = 6, 3 Hz, H-3'), 4.17 (1H, dd, *J* = 12, 8 Hz, H_B-6'), 4.36 (1H, dd, *J* = 7, 5 Hz), 4.41 (1H, dd, *J* = 7, 3 Hz, H-2'), 4.55 (1H, dd, *J* = 7, 5 Hz), 4.57 (1H, dd, *J* = 10.5, 5 Hz, H- α), 5.22 (1H, d, *J* = 7 Hz, H-1'), 7.17 (1H, t, *J* = 7 Hz, H-6), 7.26 (1H, t, *J* = 7 Hz, H-5), 7.47 (1H, d, *J* = 7 Hz, H-4), 7.73 (1H, d, *J* = 7 Hz, H-7). HRMS (FAB) calcd for C₃₃H₄₇N₅O₁₀S (M+H), 706.3122, found: 706.3134.

3.5. Binding assay of Con A to C-Man-Trp

C-Man-Trp and *C-Man-Indole*: A Con A-Sepharose column (1 mL, 5 mm $\times$ 40 mm) was equilibrated with 10 mM Tris–HCl buffer (pH 7.4) containing 1 mM CaCl₂ and 1 mM MnCl₂ (Buffer A). An aqueous solution of *C-Man-Trp* or *C-Man-Indole* (2 mM, 0.3 mL) was loaded on the column. After standing for 10 min, the column was successively eluted with Buffer A (10 mL), 0.2 M α -MeGlc in Buffer A (5 mL), 0.2 M α -MeMan in Buffer A (5 mL), 1.0 M α -MeMan in Buffer A (10 mL), and 5 mM EDTA in 10 mM Tris–HCl buffer (pH 7.4) (10 mL). The eluate was collected in 1 mL portions. The concentrations of *C-Man-Trp* and *C-Man-Indole* were determined by absorbance at 280 nm: $E^{1\text{mM}, 1\text{cm}}$, 3.444 for *C-Man-Trp*; $E^{1\text{mM}, 1\text{cm}}$, 6.978 for *C-Man-Indole*. Unless otherwise stated, all procedures were conducted at 4 $^{\circ}\text{C}$.

O-Man-pNP: To the same column equilibrated with Buffer A was charged O-Man-pNP (3 mM, 0.3 mL). The column was successively eluted as described above. The eluate was collected in 1 mL portions. To determine the concentration of O-Man-pNP of each fraction, a 0.1 μ L aliquot was hydrolyzed in 0.1 mL of 1 N HCl at 100 °C for 30 min. Then 0.1 mL of 1 N NaOH and 1 mL of 1 M Na₂CO₃ buffer (pH 9.5) were added, and the absorbance at 405 nm was measured: $E^{1\text{mM}, 1\text{cm}}$, 2.458 at pH 9.5 for *p*-nitrophenol.

3.6. Binding assay of MBL-C to C-Man-Trp

Purification of MBL-C: MBL-C was purified from mouse serum based on the method of Hansen et al.²⁹ Briefly, mouse serum was precipitated by 6.5% (w/v) polyethylene glycol in BBS/Ca (10 mM barbitol-NaOH (pH 7.4), 0.3 M NaCl, 0.01% Tween 20, 10 mM CaCl₂). The precipitate was dissolved in BBS/Ca and applied to 1 M ethanolamine-treated BrCN-activated Sepharose column and the pass-through fraction was applied to the mannan-Sepharose column. After washing the column with BBS/Ca, the bound MBL-C was eluted with 15 mM EDTA. The MBL-C thus purified was homogenous on SDS-PAGE, and amino acid sequence analysis showed that the MBL-C sample had the N-terminal sequence, Glu-Thr-Leu-Thr-Glu-Gly-Val, which is completely identical to the reported sequence of MBL-C.³⁴

ELISA-based method: MBL-C was dialyzed against PBS. The 96-well plate was coated with 50 μ L of MBL-C at 0–11.5 μ g/mL at 37 °C for 2 h. After washing three times with PBS, the wells were blocked with 1% BSA (pretreated with sodium periodate to eliminate glycan-containing impurities) in PBS at 4 °C overnight. After washing the wells twice with TBS (50 mM Tris-HCl buffer (pH 7.5), 0.15 M NaCl) and then with TBS containing 0.9 mM CaCl₂ (TBS/Ca), biotinylated C-Man-Trp (1 μ g/mL) or biotinylated Trp (1 μ g/mL) in TBS/Ca was added. After incubation at 37 °C for 2 h and washing four times with TBS/Ca containing 0.05% Tween 20, color development was performed using avidin-biotin-peroxidase complex (Vector Laboratories, CA) under the manufacturer's instruction as described previously.³²

Acknowledgements

We thank Dr. Steffen Thiel for his kind provision of the antibodies. This research was supported in part by a Grants-in-Aids for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (MEXT), and PRESTO, Japan Science and Technology Agency.

References and notes

1. Lowe, J. B.; Marth, J. D. *Ann. Rev. Biochem.* **2003**, *72*, 643–691.
2. Gabius, H.-J.; Andre, S.; Kaltner, H.; Siebert, H.-C. *Biochim. Biophys. Acta* **2002**, *1572*, 165–177.
3. Taylor, C. M. *Tetrahedron* **1988**, *54*, 11317–11362.
4. Hofsteenge, J.; Müller, D. R.; de Beer, T.; Löffler, A.; Richter, W. J.; Vliegthart, J. F. G. *Biochemistry* **1994**, *33*, 13524–13530.
5. de Beer, T.; Vliegthart, J. F. G.; Löffler, A.; Hofsteenge, J. *Biochemistry* **1995**, *34*, 11785–11789.
6. Bertozzi, C.; Bendnarski, M. In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H., O'Neill, R. A., Eds.; Harwood Academic: 1996; pp 316–351.
7. Dondoni, A.; Marra, A. *Chem. Rev.* **2000**, *100*, 4395–4422.
8. Doucey, M.-A.; Hess, D.; Cacan, R.; Hofsteenge, J. *Mol. Biol. Cell* **1998**, *9*, 291–300.
9. Krieg, J.; Hartmann, S.; Vicentini, A.; Gläsner, W.; Hess, D.; Hofsteenge, J. *Mol. Biol. Cell* **1998**, *9*, 301–309.
10. Furmanek, A.; Hofsteenge, J. *Acta Biochim. Pol.* **2000**, *47*, 781–789.
11. Doucey, M.-A.; Hess, D.; Blommers, M. J. J.; Hofsteenge, J. *Glycobiology* **1999**, *9*, 435–441.
12. Hofsteenge, J.; Blommers, M.; Hess, D.; Furmanek, A.; Miroshnichenko, O. *J. Biol. Chem.* **1999**, *274*, 32786–32794.
13. Hartmann, S.; Hofsteenge, J. *J. Biol. Chem.* **2000**, *275*, 28569–28574.
14. Hofsteenge, J.; Huwiler, K. G.; Macek, B.; Hess, D.; Lawler, J.; Mosher, D. F.; Peter-Katalinic, J. *J. Biol. Chem.* **2001**, *276*, 6485–6498.
15. Furmanek, A.; Hess, D.; Rogniaux, H.; Hofsteenge, J. *Biochemistry* **2003**, *42*, 8452–8458.
16. Krieg, J.; Glasner, W.; Vicentini, A.; Doucy, M.-A.; Loeffler, A.; Hess, D.; Hofsteenge, J. *J. Biol. Chem.* **1997**, *272*, 26687–26692.
17. Horiuchi, K.; Yonekawa, O.; Iwahara, K.; Kanno, T.; Kurihara, T.; Fujise, Y. *Biochem. J.* **1994**, *115*, 362–366.
18. Gutsche, B.; Grun, C.; Scheutzw, D.; Herderich, M. *Biochem. J.* **1999**, *343*, 11–19.
19. Van Wagoner, R. M.; Jompa, J.; Tahir, A.; Ireland, C. M. *J. Nat. Prod.* **1999**, *62*, 794–797.
20. Garcia, A.; Lenis, L. A.; Jimenez, C.; Debitus, C.; Quinoa, E. R.; Riguera *Org. Lett.* **2000**, *2*, 2765–2767.
21. Takahira, R.; Yonemura, K.; Yonekawa, O.; Iwahara, K.; Kanno, T.; Fujise, Y.; Hishida, A. *Am. J. Med.* **2001**, *110*, 192–197.
22. Nishikawa, T.; Ishikawa, M.; Isobe, M. *Synlett* **1999**, 123–125.
23. Nishikawa, T.; Ishikawa, M.; Wada, K.; Isobe, M. *Synlett* **2001**, 945–947.
24. Manabe, S.; Ito, Y. *J. Am. Chem. Soc.* **1999**, *121*, 9754–9755.
25. Nishikawa, T.; Wada, K.; Isobe, M. *Biosci. Biotech. Biochem.* **2002**, *66*, 2273–2278.
26. Ohyama, Y.; Kasai, K.; Nomotom, H.; Inoue, Y. *J. Biol. Chem.* **1985**, *260*, 6882–6887.
27. Weatherman, R. V.; Mortell, K. H.; Chervenak, M.; Kiessling, L. L.; Toone, E. J. *Biochemistry* **1996**, *35*, 3619–3624.
28. Weatherman, R. V.; Kiessling, L. L. *J. Org. Chem.* **1996**, *61*, 534–538.
29. Hansen, S.; Thiel, S.; Willis, A.; Holmskov, U.; Jenseius, J. C. *J. Immunol.* **2000**, *164*, 2610–2618.
30. Liu, H.; Jensen, L.; Hansen, S.; Petersen, S. V.; Takahashi, K.; Ezekowitz, A. B.; Hansen, F. D.; Jenseius, J. C. J. C.; Thiel, S. *Scand. J. Immunol.* **2001**, *53*, 489–497.
31. Ikeda, K.; Sannoh, T.; Kawasaki, N.; Kawasaki, T.; Yamashina, I. *J. Biol. Chem.* **1987**, *262*, 7451–7454.
32. Sato, C.; Kitajima, K.; Inoue, S.; Seki, T.; Troy, F. A., II; Inoue, Y. *J. Biol. Chem.* **1995**, *270*, 18923–18928.
33. Nishikawa, T.; Kajii, S.; Wada, K.; Ishikawa, M.; Isobe, M.; Sato, C.; Yasukawa, Z.; Kitajima, K. The 24th

Annual Meeting of the Japanese Society of Carbohydrate Research, July 29–31, 2003, Yokohama, Japan, abstract, A3-05.

34. Sastry, K.; Zahedi, K.; Lelias, J.-M.; Whitehead, A. S.; Ezekowitz, R. A. B. *J. Immunol.* **1991**, *147*, 692–697.