ELSEVIER

FEBS Letters 342 (1994) 334–338

FEBS 13892

FEBS LETTERS

Strong affinity of *Maackia amurensis* hemagglutinin (MAH) for sialic acid-containing Ser/Thr-linked carbohydrate chains of N-terminal octapeptides from human glycophorin A

Yukiko Konami, Kazuo Yamamoto, Toshiaki Osawa**, Taturo Irimura*

Division of Chemical Toxicology and Immunochemistry, Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

Received 1 February 1994; revised version received 9 March 1994

Abstract

The interaction of the *Maackia amurensis* hemagglutinin (MAH) with various glycopeptides and oligosaccharides was investigated by means of immobilized lectin affinity chromatography. An amino terminal octapeptide obtained from human glycophorin A having three Neu5Acα2→3Galβ1→3(Neu5Acα2→6)GalNAc tetrasaccharide chains, designated as CB-II, was found to have an extremely strong affinity for MAH. Therefore, it is strongly suggested that hemagglutination by MAH was caused by its interaction with Ser/Thr-linked carbohydrate chains of human glycophorin A on erythrocyte membranes.

Key words: *Maackia amurensis* hemagglutinin; Lectin affinity chromatography; Carbohydrate-binding specificity; Human glycophorin A; Sialic acid-containing Ser/Thr-linked carbohydrate chain

1. Introduction

Two isolectins were previously purified from *Maackia amurensis* seeds in this laboratory [1]. They were designated as *Maackia amurensis* hemagglutinin (MAH) and *Maackia amurensis* leukoagglutinin (MAL) because of their agglutination activity towards different blood cells [1]. Between 1974 and 1976 we described the carbohydrate binding specificity of these isolectins and the similarity of their carbohydrate binding specificity with other lectins as revealed by hemagglutination inhibition tests and competitive binding assays to human erythrocytes [2,3]. The results strongly suggested that MAH preferentially binds Ser/Thr-linked carbohydrate chains whereas MAL binds Asn-linked carbohydrate chains [2,3]. In 1988 and 1991, additional studies on the carbohydrate binding specificity of MAL were performed in other laboratories [4,5]. As a result of these studies, it became clear that MAL strongly binds carbohydrate chains containing sialic acid, particularly the Neu5Acα2→3Galβ1→4GlcNAc sequence. However, the detailed carbohydrate binding specificity of another isolectin, MAH, was not extensively studied. In our early work, porcine submaxillary gland mucin (PSM) was found to be a strong haptenic inhibitor of MAH-induced hemagglutination and the reactivity was attributed to its sialic acid-containing Ser/Thr-linked carbohydrate chains [1]. Therefore, contribution of sialyl residues in the ligand activity for MAH was also likely. In the present study, we have investigated the carbohydrate binding specificity of MAH by means of immobilized affinity chromatography with various glycopeptides and oligosaccharides having defined carbohydrate structures.

2. Materials and methods

2.1. Materials

The seeds of *Maackia amurensis* were obtained from F.W. Schumacher, Sandwich, MA, USA. Sepharose 4B and Sephadex G-25 were purchased from Pharmacia, Uppsala, Sweden. Fetuin from bovine serum and α2→3 specific sialidase from *Salmonella typhimurium* were obtained from Sigma, St. Louis, MO, USA. Pronase from *Streptomyces griseus* was purchased from Kaken Kagaku, Tokyo, Japan. [^{14}C]Acetic anhydride was obtained from New England Nuclear, Boston, MA, USA.

2.2. Preparation of specific affinity absorbent, fetuin-glycopeptide-Sepharose 4B

Kawaguchi et al. [1] partially purified MAH by affinity chromatography on a column of Sepharose 4B containing covalently bound glycopeptides derived from porcine thyroglobulin and observed that MAH preferentially binds to a structure in the sialic acid-containing

*Corresponding author. Fax: (81) (3) 3815 9344.

** *Present address:* Yakult Central Institute for Microbiological Research, Yaho, Kunitachi-shi, Tokyo 186, Japan.

Abbreviations: Fetuin-GP-Sepharose, fetuin-glycopeptides-Sepharose 4B; MAH, *Maackia amurensis* hemagglutinin; MAL, *Maackia amurensis* leukoagglutinin; PSM, porcine submaxillary mucin.

SSDI 0014-5793(94)00277-3

carbohydrate chains of the PSM molecule. Because bovine fetuin was known to contain Ser/Thr-linked carbohydrate chains having either the structure Neu5Acα2→3Galβ1→3GalNAc or Neu5Acα2→3Galβ1→3(Neu5Acα2→6)GalNAc and Asn-linked carbohydrate chains having both α2→6 and α2→3 specific sialic acid linkages [6,7], we used a mixture of fetuin glycopeptides as the affinity absorbent. Bovine fetuin was exhaustively digested with pronase according to the procedure described by Fukuda and Egami [8]. The obtained glycopeptides were purified by gel filtration on a column of Sephadex G-25. A mixture of glycopeptides prepared from bovine fetuin was coupled to cyanogen bromide-activated Sepharose 4B according to a previously described method (Fetuin-GP-Sepharose) [9].

2.3. Purification of Maackia amurensis hemagglutinin (MAH)

MAH was isolated and purified from *Maackia amurensis* seeds by the method of Kawaguchi et al. [1] with modifications. The seeds were finely ground in an electric seed mill and extracted overnight with 0.9% NaCl solution. Ammonium sulfate was added to the extract, and the 30%–80% ammonium sulfate precipitate was collected and dialyzed against water. The dialyzed material was applied to an affinity column of Fetuin-GP-Sepharose, washed with 50 mM Tris-HCl, pH 7.5, and eluted with 50 mM glycine-HCl, pH 3.0. The eluted protein was dialyzed against 50 mM phosphate buffer, pH 5.0, and applied to a SP-Toyopearl ion exchange column. After a large peak was eluted with the same buffer, the absorbed protein was eluted with the same buffer containing 1 N NaCl. The eluate containing MAH was collected, pooled, and lyophilized. The affinity-isolated MAH was further purified by reversed-phase high-performance liquid chromatography on a column of C_{18} using a linear gradient (0–100%) of 2-propanol/acetonitrile (7:3) in distilled water containing 0.1% TFA in 60 min at a flow rate of 1 ml/min. The purified MAH was free from contamination of MAH as judged by polyacrylamide gel electrophoresis in the absence or in the presence of b-mercaptoethanol and by the determination of the N-terminal amino acid sequence.

2.4. Preparation of MAH-Sepharose 4B column

The purified MAH was coupled to cyanogen bromide-activated Sepharose 4B [10] at a concentration of 3.6 mg/ml gels. Radiolabeled oligosaccharide or glycopeptide samples (2,000 cpm in 100 ml) were loaded onto a column of MAH-Sepharose 4B (0.6 × 4.0 cm) equilibrated with 10 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl. After the column was eluted with the same buffer, bound material was eluted with 50 mM glycine-HCl, pH 5.0, or 0.1 N NaOH. Fractions (0.2 ml) were collected at a flow rate of 0.6 ml/h at room temperature. The radioactivity of each fraction was measured on a liquid scintillation counter.

2.5. Preparation of labeled glycopeptides and oligosaccharides

Asn-linked glycopeptides were prepared by repeated pronase digestion of the corresponding glycoproteins [11]. In some cases, oligosaccharides were released from glycopeptides by hydrazinolysis as previously described [11]. Triantennary disialo complex-type Asn-linked oligosaccharides (PTG UB tri, **a**) were prepared from porcine thyroglobulin by the method described in a previous paper [12]. Biantennary disialo complex-type Asn-linked glycopeptides were prepared from human serum transferrin (hTf bi, **b**) as previously described [11]. Desialylated biantennary complex-type Asn-linked glycopeptides (hTf bi (−SA), **c**) were prepared after incubation of the sialylated glycopeptides (hTf bi, **b**) with α2→3 specific *Salmonella typhimurium* sialidase (0.2–0.5 units) in 0.1 M sodium acetate buffer, pH 5.5, for 20 h. Biantennary disialo complex-type Asn-linked oligosaccharides having a bisecting *N*-acetylglucosamine residue and a fucose residue linked to the innermost *N*-acetylglucosamine residue (hGph bi, **d**) were isolated from a tryptic fragment, T1, of human glycophorin A [13]. Poly-*N*-acetyllactosamine type carbohydrate chains were obtained from human erythrocyte membrane Band 3 glycoprotein (N-1, **e**) [14]. The N-terminal octapeptides from human erythrocyte glycophorin A (CB-II) were prepared from the tryptic fragment, T1, according to the method of Prohaska et al. by cyanogen bromide cleavage [15]. The structures of all oligosaccharides and glycopeptides used in this study were confirmed by compositional analyses, methylation analyses, and sequential glycosidase digestions [11–14]. Fig. 1 shows the structures of these Asn-linked glycopeptides and oligosaccharides. The glycopeptides (5~ 50 μg) were acetylated with [^{14}C]acetic anhydride (2 mCi/mmol) in 0.1 ml of 4.5 M sodium acetate at room temperature for 5 min. Labeled glycopeptides were isolated by gel filtration on Sephadex G-25. The oligosaccharides were labeled by reduction with NaB^3H_4 according to the method previously described [16]. Specific activity of ^{14}C-labeled glycopeptides and ^{3}H-labeled oligosaccharides were approximately 7.5×10^{10} and 8.9×10^{10} dpm/mmol, respectively.

2.6. Modification of glycopeptides CB-II

The sialic acid residues of intact [^{14}C]CB-II was removed by mild acid hydrolysis in 50 mM HCl at 80°C for 1 h. Asialo, agalacto [^{14}C]CB-II was obtained after Smith periodate degradation of asialo [^{14}C]CB-II [17]. The α2→3 linked sialic acid residues of [^{14}C]CB-II were removed by α2→3 specific sialidase from *Salmonella typhimurium* (Sigma). The removal of the sialic acid residues was confirmed by the eluting position of the anion-exchange chromatography on a Mono Q column (Pharmacia, Sweden). Fig. 2 summarizes the structures of the derivatives of CB-II used in this study.

3. Results

3.1. Affinity chromatography of glycopeptides with Ser/Thr-linked carbohydrate chains on immobilized MAH

In order to elucidate the carbohydrate-binding specificity of MAH, affinity chromatography of various glycopeptides and oligosaccharides on the immobilized MAH column was performed. Fig. 3 shows the elution profiles of glycopeptide CB-II and the digestion products from CB-II. Intact [^{14}C]CB-II, obtained by cyanogen bromide cleavage of human glycophorin A, constitutes the N-terminal portion of this glycoprotein and contains three Ser/Thr-linked tetrasaccharides. Intact [^{14}C]CB-II showed a very strong affinity for the MAH column and could not be eluted with lactose (which was known to inhibit hemagglutination by MAH at high concentrations) or Gly-HCl buffer, pH 3.0. Bound glycopeptides were eluted using 0.1 N NaOH as shown in this figure. The removal of a single sialic acid residue attached to galactosyl residues in each tetrasaccharide of intact [^{14}C]CB-II by digestion with α2→3 specific sialidase markedly decreased the affinity of the obtained glycopeptides, ([^{14}C]CB-II (−2,3 SA)), for the MAH-Sepharose column. The affinity of [^{14}C]CB-II (−2,3 SA) is much weaker than that of intact [^{14}C]CB-II judging from the slight retardation from the MAH-Sepharose column instead of the strong binding of intact [^{14}C]CB-II. Desialylated [^{14}C]CB-II (CB-II (−SA)) and asialo, agalacto-[^{14}C]CB-II (CB-II (−SA, −Gal)) did not show retardation from the column.

3.2. Affinity chromatography of glycopeptides with Asn-linked carbohydrate chains on immobilized MAH

Elution profiles of Asn-linked carbohydrate chains on the immobilized MAH column were also compared. Triantennary disialo complex-type Asn-linked oligosaccharides from porcine thyroglobulin (PTG UB tri, **a**) [11], biantennary disialo complex-type Asn-linked glycopeptides (hTf bi, **b**) and desialylated biantennary complex-type Asn-linked glycopeptides (hTf bi (−SA), **c**) from human serum transferrin [12], biantennary disialo

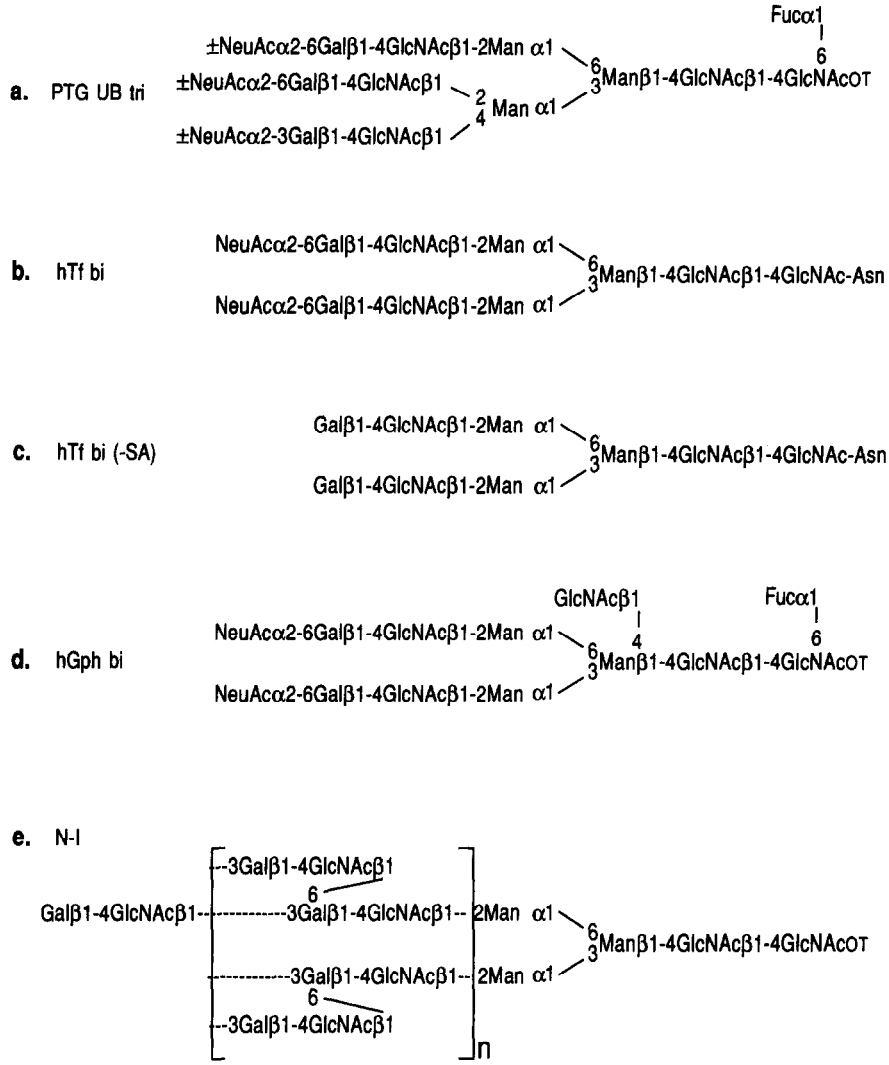

Fig. 1. Structures of various glycopeptides and oligosaccharides containing Asn-linked carbohydrate chains used in this study. Details are described in section 2.

complex-type Asn-linked oligosaccharides having a bisecting *N*-acetylglucosamine residue and a fucose residue linked to the innermost *N*-acetylglucosamine residue (hGph bi, **d**) [13], and poly-*N*-acetyllactosamine type carbohydrate chains obtained from human erythrocyte membrane Band 3 glycoprotein (N–I, **e**) [14] were used. Retardation was not observed with any of these ^{14}C-labeled glycopeptides or ^{3}H-labeled oligosaccharides. It should be noted that the oligosaccharides (hGph, bi, **d**) did not bind MAH despite the fact that it was derived from human glycophorin A. The carbohydrate chains from another major erythrocyte membrane glycoprotein, Band 3 glycoprotein (N–I **e**), did not have affinity for this column.

4. Discussion

There are several lectins of plant or animal origins that recognize carbohydrate chains containing sialic acid [12,17–26]. Among a variety of legume lectins, however, lectins from *Maackia amurensis* seeds seem to be the only exceptions that bind sialylated carbohydrate chains. The carbohydrate structure required for an isolectin from these seeds, MAH, is not known. In the present report, we propose that MAH binds to sialylated carbohydrate chains with different structural features from the carbohydrate chains recognized by MAL.

It is known that MAH binds Ser/Thr-linked oligosaccharides from the porcine submaxillary gland glycoprotein [1]. In competitive binding assays to human erythrocytes, this lectin was found to compete with *Bauhinia purpurea* agglutinin, that binds Ser/Thr-linked carbohydrate chains on the surface of human erythrocytes [2,13]. Thus, it was suggested that glycophorin A (PAS-1 glycoprotein) [27] consisted of the major binding site for MAH on erythrocyte surfaces. MAH did not effectively compete with MAL or with *Ricinus communis* agglutinin-

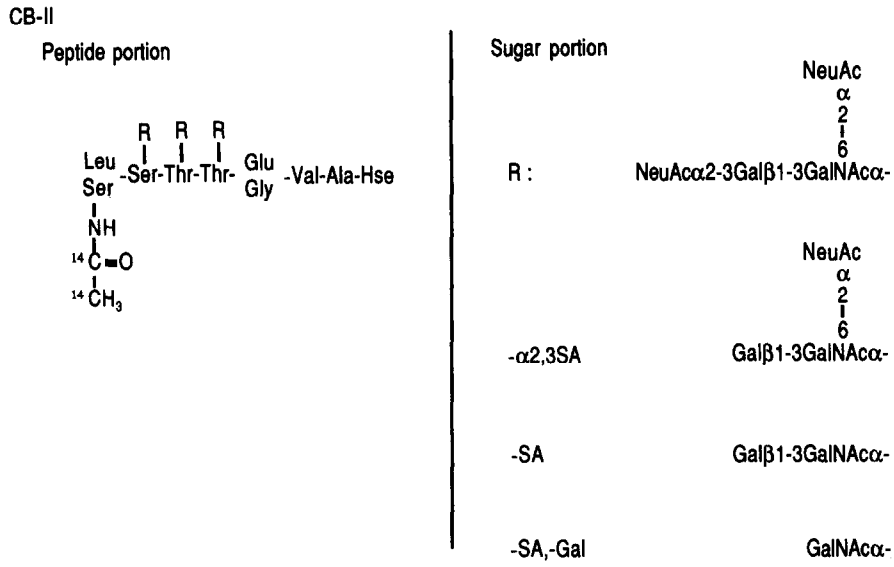

Fig. 2. Structures of CB-II and its derivatives obtained after sequential degradation of CB-II. Details are described in section 2.

I [2]. Our present results extended these previous findings by proving that sialylated Ser/Thr-linked carbohydrate chains at the amino terminal domain of human glycophorin A possessed a high affinity for MAH. Sialic acid linked to position 3 of the subterminal galactose in the tetrasaccharide unit Neu5Acα2→3Galβ1→3(Neu5Acα2→6)GalNAc was essential to the binding. [^{14}C]CB-II (−2,3 SA) obtained after the removal of a single sialic acid residue attached to C-3 of galactose residues of the intact [^{14}C]CB-II did not show a strong affinity for the MAH-Sepharose column, suggesting that a sialic acid residue linked to position 6 of the *N*-acetylgalactosamine in the tetrasaccharide unit did not contribute to the strong interaction between MAH and CB-II. Alternatively, two sialic acid residues in a single Ser/Thr-linked carbohydrate chain in this glycopeptides were

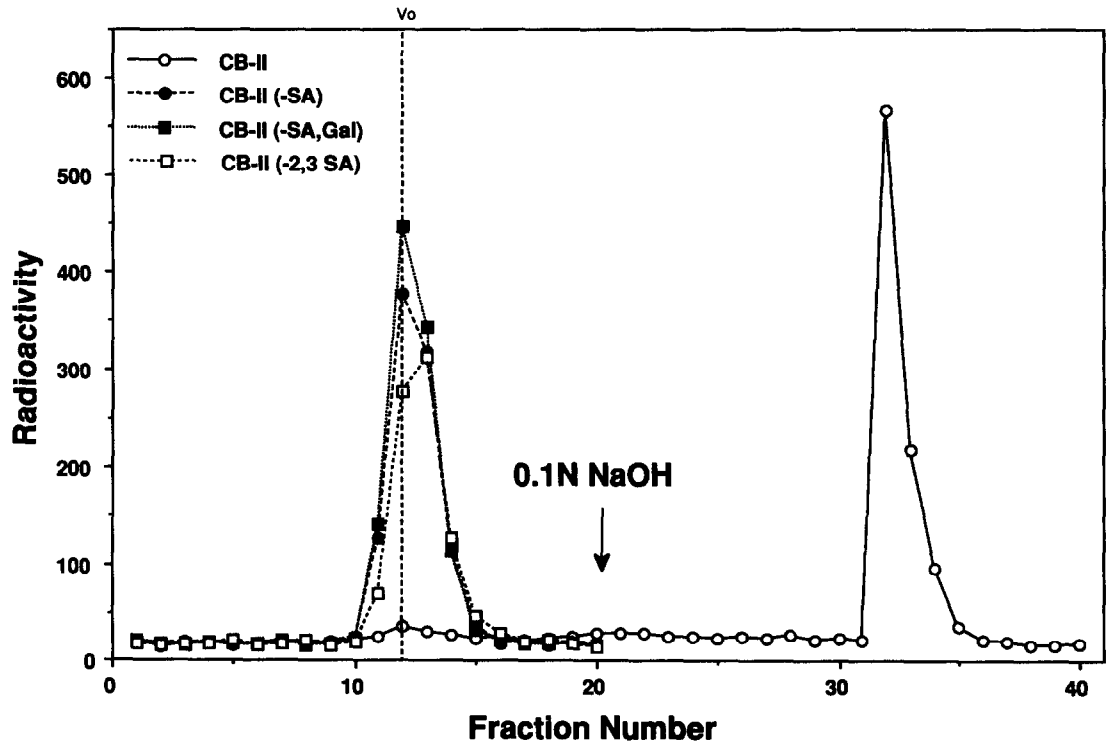

Fig. 3. Elution profiles of glycopeptide CB-II and its derivatives on a column of MAH-Sepharose. Open circle, CB-II with intact carbohydrate chains. Open square, CB-II after removal of 2→3-linked sialyl residues. Closed circle, CB-II after removal of all sialyl residues. Closed square, CB-II after removal of all sialyl and galactosyl residues. Arrow indicates the eluting position where the elution buffer was switched to 0.1 N NaOH. Vo indicates the position of the void volume fraction.

essential to the affinity. It remains to be elucidated whether a single carbohydrate chain alone had a strong affinity for MAH. It was not clear from the results whether a Ser/Thr residue was necessary. All Asn-linked carbohydrate chains used in this study did not show an affinity for MAH. A variety of Asn-linked carbohydrate chains other than those used in this study may be necessary before concluding that MAH does not interact with any Asn-linked carbohydrate chains. Another isolectin from *Maackia amurensis* seeds, MAL, is known to have a strong affinity for Neu5Acα2→3Galβ1→4GlcNAc sequences [4,5], and is not supposed to bind to Neu5Acα2→3Galβ1→3(Neu5Acα2→6)GalNAc tetrasaccharides. Intact [^{14}C]CB-II applied to a MAL-Sepharose 4B column under the same conditions described in this paper showed a slight retardation from the MAL-Sepharose column (data not shown).

As far as the interaction with human erythrocyte membrane surfaces is concerned, MAH is likely to interact only with Ser/Thr-linked carbohydrate chains of glycophorin A, because Asn-linked carbohydrate chains of glycophorin A or those of Band 3 glycoproteins did not have affinity for MAH. It would be very useful if MAH recognizes the heavily sialylated mucin-like domains of cell surface glycoproteins regardless of the core polypeptides structures.

Acknowledgements: This study was supported by the Hayashi Memorial Foundation for Female Natural Scientists and an Esso Research Grant for Women and Japan Health Science Foundation.

References

[1] Kawaguchi, T., Matsumoto, I. and Osawa, T. (1974) J. Biol. Chem. 249, 2786–2792.
[2] Kawaguchi, T., Matsumoto, I. and Osawa, T. (1974) Biochemistry 13, 3169–3173.
[3] Kawaguchi, T. and Osawa, T. (1976) Biochemistry 15, 4581–4586.
[4] Wang, W.-C. and Cummings, R.D. (1988) J. Biol. Chem. 263, 4576–4585.
[5] Knibbs, R.N., Goldstein, I.J., Ratcliffe, R.M. and Shibuya, N. (1991) J. Biol. Chem. 266, 83–88.
[6] Baenziger, J.U. and Fiete, D. (1979) J. Biol. Chem. 254, 789–795.
[7] Nilsson, B., Nordén, N.E. and Svensson, S. (1979) J. Biol. Chem. 254, 4545–4553.
[8] Fukuda, M. and Egami, F. (1971) Biochem. J. 123, 407–414.
[9] Matsumoto, I. and Osawa, T. (1972) Biochem. Biophys. Res. Commun. 46, 1810–1815.
[10] Matsumoto, I., Seno, N., Golovtchenko-Matsumoto, A.M. and Osawa, T. (1980) J. Biochem. (Tokyo) 87, 535–540.
[11] Yamamoto, K., Tsuji, T., Irimura, T. and Osawa, T. (1981) Biochem. J. 195, 701–713.
[12] Sueyoshi, S., Yamamoto, K. and Osawa, T. (1988) J. Biochem. 103, 894–899.
[13] Irimura, T., Tsuji, T., Tagami, S., Yamamoto, K. and Osawa, T. (1981) Biochemistry 20, 560–566.
[14] Tsuji, T., Irimura, T. and Osawa, T. (1980) Biochem. J. 187, 677–686.
[15] Prohaska, R., Koerner, T.A.W., Armitage, I.M. and Furthmayr, H. (1981) J. Biol. Chem. 256, 5781–5791.
[16] Sueyoshi, S., Tsuji, T. and Osawa, T. (1988) Carbohydr. Res. 178, 213–224.
[17] Roche, A.-C., Schauer, R. and Monsigny, M. (1975) FEBS Lett. 57, 245–249.
[18] Peters, B.P., Ebisu, S., Goldstein, I.J. and Flashner, M. (1979) Biochemistry 18, 5505–5511.
[19] Bhavanandan, V.P. and Katlic, A.W. (1979) J. Biol. Chem. 254, 4000–4008.
[20] Miller, R.L., Collawn, J.F. Jr. and Fish, W.W. (1982) J. Biol. Chem. 257, 7574–7580.
[21] Ravindranath, Higa, H.H., Cooper, E.L. and Paulson, J.C. (1985) J. Biol. Chem. 260, 8850–8856.
[22] Rogers, G.N., Herrler, G., Paulson, J.C. and Klenk, H-D. (1986) J. Biol. Chem. 261, 5947–5951.
[23] Shibuya, N., Goldstein, I.J., Broekaert, W.F., Nsimba-Lubaki, M., Peeters, B. and Peumans, W.J. (1987) J. Biol. Chem. 262, 1596– 1601.
[24] Yamashita, K., Umetsu, K., Suzuki, T., Iwaki, Y., Endo, T. and Kobata, A. (1988) J. Biol. Chem. 263, 17482–17489.
[25] N, Shibuya, Tazaki, K., Song, Z., Tarr, G.E., Goldstein, I.J. and Peumans, W.J. (1989) J. Biochem. 106, 1098–1103.
[26] Van den Berg, T.K., Brevé, J.P., Damoiseaux, J.G.M.C., Döpp, E.A., Kelm, S., Crocker, P.R., Dijkstra, C.D. and Kraal, G. (1992) J. Exp. Med. 176, 647–655.
[27] Marchesi, V.T., Furthmayr, H. and Tomita, M. (1976) Annu. Rev. Biochem. 45, 667–698.