Binding Characteristics of $^3$H-Dihydroalprenolol to $\beta$-Adrenergic Receptors of Rat Brain: Influence of Exo- and Endo-Glycosidases and Glycopeptidase

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Abstract—The significance of carbohydrate moieties containing the $\beta$-adrenoceptor molecule in the rat brain was examined using radioligand binding assay methods. Thus, this experiment was designed to assess the effects of exoglycosidase ($\alpha$-D-mannosidase and neuraminidase), endoglycosidase (endoglycosidase D and endoglycosidase H), and glycopeptidase A on the affinity of $\beta$-adrenoceptor. The main reason why five kinds of enzymes were used in the present study is that they can hydrolyze different carbohydrate molecules from cell membranes. Rat brain was used and $\beta$-adrenoceptor binding assay was carried out using $^3$H-dihydroalprenolol ($^3$H-DHA) as a ligand. $^3$H-DHA binding to $\beta$-adrenoceptors was sensitive to very low concentration of endoglycosidase H and glycopeptidase A, thus indicating that the treatments with these enzymes of rat brain membrane appear to decrease the number of $\beta$-receptor binding sites. On the other hand, the treatment with neuraminidase, endoglycosidase H, and glycopeptidase A of the membrane induced lower values of the dissociation constant ($K_d$) than those of the control. $\alpha$-D-mannosidase and endoglycosidase D are without effect in spite of the removal of hexose contents and total carbohydrate contents with these treatments, respectively. These results imply that complex type N-linked acidic carbohydrate chains containing neuraminic acid and high mannose type N-linked carbohydrate chains, which are hydrolyzed with endoglycosidase H and glycopeptidase A, of the rat brain membrane containing $\beta$-adrenoceptor molecules play a crucial role in the drug-receptor interaction.

Our previous reports (1–3) have been shown that carbohydrate molecules of the membrane extensively participate the drug-receptor interaction in $\beta$-adrenoceptors of the rat brain and the myocardium. Those papers clarified that the addition of lectins (concanavalin A and Phaseolus vulgaris phytohemagglutinin) and polymeric effectors (polymyxin B, DNA and heparin) induced significant changes in the affinity and/or the number of $\beta$-adrenoceptors of the rat brain and the removal of sialic acid from the rat myocardium by pre-treatment with neuraminidase was also found to increase the experimental reproducibility of the radioligand binding assay method for $\beta$-adrenoceptors. Stiles and coworkers (4) also suggest that the $\beta$-adrenoceptor molecule is a glycoprotein containing complex and/or high mannose type N-linked carbohydrate chain. It is of interest to assess which carbohydrate molecules participate in the drug-receptor interaction of $\beta$-adrenoceptors. In addition, it is also very important to find the relationship between the removal of different carbohydrate molecules from the $\beta$-adrenoceptors and changes in their affinity ($K_d$ and $B_{max}$). Thus, the object of this experiment is to assess the effects of the removal of carbohydrate chains in rat brain membranes on the value of $K_d$ and $B_{max}$ of $\beta$-adrenoceptors.
Materials and Methods

Materials: $^{3}$H-dihydroalprenolol ($^{3}$H-DHA) (104.8 Ci/mmole) was purchased from New England Nuclear Corp. Neuraminidase from Clostridium perfringens was purchased from Sigma Chem. Co. Endoglycosidase D (Endo D) from Diplococcus pneumoniae, endoglycosidase H (Endo H) from Streptomyces griseus, $\alpha$-D-mannosidase from jack bean, and glycopeptidase A (Glyco A) from almonds were purchased from Seikagaku Kogyo Co., Ltd. As depicted in Fig. 1, these enzymes degrade the N-linked carbohydrate chains in a glycoprotein.

The preparation of membrane-enriched fraction: The membrane-enriched fraction from the rat brain was prepared using the modified method as described previously (3). In brief, after the removal of the rat brain, the cerebellum and cerebral cortex were minced and homogenized with a glass homogenizer. The homogenate was filtered through 4 layers of gauze, and the filtrate was centrifuged at 40,000 g for 30 min. The resultant pellets were rinsed at once and homogenized with a glass homogenizer using 20 ml of 0.1 M citric acid/sodium phosphate buffer (pH 6.0) for the treatment by the enzymes.

Treatment of the brain membrane with the various enzymes: The prepared membrane-enriched fraction (about 8 mg of protein) was treated for 1 hr at 37°C with or without neuraminidase (0.1 U), $\alpha$-D-mannosidase (0.1 U), Endo D (0.01 U), Endo H (0.01 U) or Glyco A (0.1 mU) in total volume of 4 ml of 0.1 M citric acid/sodium phosphate buffer (pH 6.0). The membrane-enriched fraction was then centrifuged at 40,000 g for 30 min, and the resultant pellets were rinsed once with 75 mM Tris-HCl, 25 mM MgCl$_2$, pH 7.2, and homogenized with a glass homogenizer.

Fig. 1. Structures of N-linked carbohydrate chains in glycoproteins are shown, and each of the enzymes used in the present study cleaves carbohydrate chains as indicated by the arrows. The common structure of N-linked carbohydrate chains is framed in A. B-D show high mannose type (B), complex type (C), and hybrid type carbohydrate chain (D). Endo D hydrolyzes the complex and hybrid type carbohydrate chain. Endo H hydrolyzes the high mannose and hybrid type carbohydrate chain. Glyco A hydrolyzes the complex, high mannose, and hybrid type carbohydrate chain.
glass homogenizer using 8 ml of the same buffer. The enzyme-treated and untreated (control) membrane fractions were stored at 4°C and used within 1 hr for the β-adrenoceptor binding assay. Subsequently, an aliquot of the prepared membrane-enriched fraction was stored at -80°C for the determination of the carbohydrate composition.

**Binding assay:** Binding assay was carried out by the method described previously (3). In brief, the β-adrenoceptor binding assay was carried out in duplicate with 3H-DHA in the presence (non-specific) and absence (total) of 100 nM dl-propranolol. For 3H-DHA binding, 0.25 ml of membrane suspension (about 0.25 mg) was incubated with shaking for 30 min at 23°C with various concentrations of 3H-DHA in a total volume of 0.5 ml containing 60 mM Tris-HCl and 20 mM MgCl₂ (pH 7.2). At the end of the incubation period, the incubation medium was immediately filtered through a GF/C glass fiber filter using the improved method (3), which was continuously filtered, washed, and air-dried. The filter was added to 5 ml of Tt 76 scintillation fluid. The difference in mean values between total and non-specific binding was taken as the specific binding. All binding assays were performed within 5 hr after the removal of the rat brain.

**Analytical methods:** Protein was determined by the method of Lowry et al. (5) using bovine serum albumin as a standard. Hexose was determined by the method of Dimler et al. (Anthrone method) (6) as mannose. Hexuronic acid was determined by the method of Bitter and Muir (7) as glucuronic acid. Methyl pentose was determined by the method of Dische and Shettles (8) as fucose. Sialic acid was determined by the method of Warren (9) as N-acetyl neuraminic acid. Hexosamine was determined by the method of Wagner (10) as glucosamine. Significant differences were analyzed using the paired Student’s t-test.

**Results**

The data presented in Table 1 show the effects of exoglycosidase, endoglycosidase, and glycopeptidase on the number of β-adrenoceptor binding sites and the affinity of 3H-DHA and β-adrenoceptor binding obtained by Scatchard analysis. The values of K_d and Bmax of the control without enzyme treatment were not different from each other. The Glyco A and Endo H significantly caused a decrease (39.9 and 63.3%, respectively) in the density of β-adrenoceptors (Bmax) and a decrease (48.2 and 62.2%, respectively) in the value of dissociation constant (K_d). The neuraminidase caused a decrease (49.2%) in the value of K_d, but only small change was observed in the density of β-adrenoceptors. α-D-mannosidase and Endo D had no effect on the values of K_d and Bmax of β-adrenoceptors. The values of the Hill coefficient with or without the treatment of various enzymes was equal to unity.

Figure 2 shows the Lineweaver-Burk plots of the drug-receptor interaction using the rat brain membrane with or without the treatment of enzymes. The treatment of neuraminidase showed competitive type activation, and the treatment of Glyco A and Endo H showed uncompetitive type inhibition.

Table 2 shows the data of carbohydrate composition and protein concentration of the rat brain membrane with or without the
### Table 1. Effects of glycosidases and glycopeptidase on the β-adrenoceptor of rat brain

|                | B<sub>max</sub> (fmoles/mg protein) | K<sub>d</sub> (nM) | Hill coefficient |
|----------------|-------------------------------------|-------------------|-----------------|
| Control        | 729.8 ± 88.5                        | 12.03 ± 2.14      | 1.05 ± 0.05     |
| Neuraminidase  | 517.0 ± 54.3                        | 6.11 ± 0.50**     | 1.00 ± 0.03     |
| α-Mannosidase  | 864.5 ± 85.8                        | 13.34 ± 1.06      | 0.98 ± 0.03     |
| Control        | 681.6 ± 110.6                       | 14.43 ± 1.97      | 0.98 ± 0.01     |
| Glyco A        | 409.6 ± 58.7**                      | 7.47 ± 1.56*      | 1.00 ± 0.01     |
| Endo D         | 664.2 ± 68.0                        | 15.09 ± 1.46      | 1.00 ± 0.01     |
| Endo H         | 249.9 ± 64.9***                     | 5.46 ± 1.42***    | 1.01 ± 0.02     |

The data obtained by Scatchard analysis. The value (n) represents the number of experiments. Numbers are given as the mean ± S.E. Paired Student's t-test. *P<0.05 vs. control, **P<0.01 vs. control, ***P<0.001 vs. control.

### Table 2. Carbohydrate composition of the membrane fractions treated with glycosidase and glycopeptidase

|                | Protein (mg/g tissue) | Hexose | Hexuronic acid | Methyl pentose (μg/mg protein) | Sialic acid | Hexosamine | Total         |
|----------------|-----------------------|--------|----------------|---------------------------------|-------------|-------------|---------------|
| Control        | 78.98 ± 3.68          | 71.72 ± 10.70 | 15.26 ± 2.33 | 16.81 ± 1.98                    | 16.97 ± 1.54 | 57.93 ± 1.71 | 178.69 ± 16.05 |
| Neuraminidase  | 70.26 ± 3.58***       | 72.81 ± 9.47  | 15.27 ± 2.53 | 17.12 ± 1.35                    | 12.51 ± 1.26*** | 56.60 ± 3.19 | 174.31 ± 14.60 |
| α-Mannosidase  | 73.02 ± 2.29          | 60.03 ± 9.50* | 12.99 ± 1.34 | 16.02 ± 0.73                    | 17.18 ± 0.44 | 56.81 ± 4.47 | 163.03 ± 5.20  |
| Control        | 77.63 ± 2.54          | 71.82 ± 11.23 | 17.37 ± 0.89 | 15.23 ± 1.54                    | 16.27 ± 0.86 | 59.94 ± 2.29 | 180.63 ± 11.28 |
| Glyco A        | 74.75 ± 1.39          | 59.13 ± 2.44  | 15.75 ± 0.47 | 14.76 ± 0.63                    | 15.69 ± 0.84 | 51.72 ± 3.14* | 157.05 ± 4.84* |
| Endo D         | 80.46 ± 2.46          | 52.68 ± 3.50  | 13.08 ± 1.46 | 13.31 ± 0.77                    | 15.89 ± 0.55 | 50.53 ± 5.09* | 145.49 ± 8.19** |
| Endo H         | 76.82 ± 4.29          | 62.34 ± 9.28  | 18.14 ± 1.16 | 13.74 ± 0.39                    | 16.26 ± 0.48 | 52.90 ± 4.49 | 163.38 ± 9.20* |

The value (n) represents the number of experiments. Numbers are given as the mean ± S.E. Each carbohydrate concentration is shown as μg/mg protein. Paired Students t-test. *P<0.05 vs. control, **P<0.01 vs. control, ***P<0.001 vs. control.
treatment of enzymes. The values of membrane protein yield and content of each carbohydrate of the control without enzyme treatment were not different from each other. Neuraminidase treatment caused significant decrease in the amount of protein and sialic acid. Other enzyme treatments, \( \alpha\)-D-mannosidase, Endo D, Endo H and glyco A, did not cause any changes in the amount of protein. \( \alpha\)-D-mannosidase treatment decreased the amount of hexose, whereas Endo D, Endo H and Glyco A significantly decreased the amount of total carbohydrate.

**Discussion**

Our laboratory has shown that the carbohydrate molecules in cell membranes are largely implicated in the drug-receptor interaction of \( \beta\)-adrenoceptors (1-3). To clarify the importance of carbohydrate chains in the drug-receptor interaction, one previous study was carried out to assess the changes in values of \( K_d \) and \( B_{max} \) of \( \beta\)-adrenoceptors in rat brain when different kinds of lectins were added in the \( \beta\)-adrenoceptor binding assay medium (3). We also reported that the changes in the negative or positive charges of the cell surface by the addition of polymeric effectors like polymyxin B, heparin or DNA induced alterations in the affinity of \( \text{H-DHA} \) to \( \beta\)-adrenoceptors in rat brain (4-6), suggesting that the charges in the carbohydrate chains, phospholipids and proteins participate in the \( \beta\)-adrenoceptor binding sites (2). Thus, in contrast to our previous studies, this study was performed to evaluate the effects of removal of carbohydrate moieties from the rat brain membrane containing \( \beta\)-adrenoceptor molecules on the drug-receptor interaction.

It is well known that cell surface carbohydrate is involved in receptor function, cell-to-cell interaction, recognition of surface molecules and cellular growth regulation (11, 12). The cell surface in the rat brain contains glycoproteins (13, 14), glycolipids (14-17), and glycosaminoglycans (17). Margolis et al. (13) reported that the carbohydrate chain of the glycoprotein prepared from rat brain contained sialic acid, galactose, N-acetyl glucosamine, mannose and fucose. As shown in Table 2, the results presented here also showed that almost the same carbohydrate molecules exist in the membrane-enriched fraction of the rat brain. The hydrolytic enzymes used in the present study could clearly remove carbohydrate molecules from the rat brain. Thus, it is clear that the removal of carbohydrate chains from the rat brain influenced the \( \text{H-DHA} \) binding to \( \beta\)-adrenoceptor in the rat brain, and it is of interest to note that the carbohydrate composition analyzed in the present study plays an important role in drug-receptor interaction.

The data presented here demonstrated that the treatments with Endo H and Glyco A or neuraminidase, Endo H and Glyco A of rat brain membrane decreased the number of \( \beta\)-adrenoceptor binding sites (\( B_{max} \)) or the value of \( K_d \) respectively (Table 1). As shown in Fig. 1, the common N-linked carbohydrate chains in the glycoprotein can be grouped into three types: complex, high mannose and hybrid type. Each enzyme treatment removed the carbohydrate residues from the membrane which could be specifically hydrolyzed with enzyme. As expected, the neuraminidase removed the sialic acid moieties and the \( \alpha\)-D-mannosidase removed the hexose moieties from the rat brain membrane. The treatment with endo-type enzymes, Endo D, Endo H and Glyco A, decreased the total carbohydrate contents (Table 2). Thus, the data obtained here may suggest that the carbohydrate moieties of the rat brain containing \( \beta\)-adrenoceptors are at least the complex type N-linked acidic carbohydrate chain and the high mannose type N-linked carbohydrate chain. These possibilities are supported by the following additional facts: (1) The complex type carbohydrate chain could be hydrolyzed with neuraminidase (18, 19), Endo D (20, 21), and Glyco A (22-24). (2) The high mannose type carbohydrate chain could be hydrolyzed with \( \alpha\)-D-mannosidase (25, 26), Endo H (19, 27-29), and Glyco A (22-24). (3) The hybrid type carbohydrate chain composed of high mannose and the complex type N-linked carbohydrate chain was hydrolyzed with all enzymes used in the present study. (4) Neuraminidase (18, 19) and \( \alpha\)-D-mannosidase (25, 26) are exo-type enzymes and Endo D (20, 21), Endo H (19, 27-29), and Glyco A (22-24) are endo-type enzymes.
enzymes. (5) The neuraminidase treatment removed sialic acid, but treatment with Endo D did not cause any changes in the sialic acid contents. (6) Endo H and/or Glyco A reduced the total carbohydrate contents, but α-D-mannosidase reduced only the hexose content. (7) Stiles et al. (4) showed that the mammalian β-adrenoceptor molecules from the hamster lung and the rat erythrocyte were deglycosylated by the treatment with endoglycosidase F which was capable of removing both complex and high mannose type N-linked carbohydrate chains, and the agarose-conjugated concanavalin A or wheat germ agglutinin was bound to these β-adrenoceptors. (8) Our previous report (3) showed that lectins (concanavalin A and Phaseolus vulgaris agglutinin) bound to β-adrenoceptors induced an alteration in the value of Bₘₐₓ and Kₘₐₓ.

On the other hand, the removal of sialic acid moieties from the rat cardiac membrane resulted in good reproducibility for the β-adrenoceptor binding assay and allowed an increase in the binding sites for the β-adrenoceptor (1). We also reported that when positively charged materials such as histone were added to the β-adrenoceptor binding assay medium, the amount of the non-specific binding increased regardless the specific binding (2). In the present study, the treatment of the rat brain membrane with neuraminidase induced a decrease of protein contents together with sialic acid contents and showed the competitive type activation of the β-adrenoceptors. Furthermore, Stiles and coworkers suggest that the treatment of the β-adrenoceptor molecule by neuraminidase caused the changes of molecular size of this molecule observed by electrophoresis in the presence of sodium dodecyl sulfate (4). Thus, these results obtained here imply that positively charged materials such as histone together with sialic acid moieties could be removed from the cell surface containing β-adrenoceptor molecules by the treatment with neuraminidase.

The effect of the enzyme treatments of the membrane containing the β-adrenoceptor molecule were irreversible. However, Lineweaver-Burk plots were used to examine the function of the carbohydrate chains as shown in Fig. 2. The neuraminidase treatment showed competitive activation. On the other hand, the treatments by Endo H and Glyco A showed uncompetitive inhibition. These facts imply that the complex type carbohydrate chain is localized at the active sites in the β-adrenoceptor molecule, and the innermost region of the high mannose type carbohydrate chain is localized around the active sites in the β-adrenoceptor molecule.

In conclusion, the present experiment using several kinds of enzymes to remove the N-linked carbohydrate molecules is a method with great potential, and evidence obtained in the present paper clearly imply the participation of the carbohydrate chain in ³H-DHA binding to β-adrenoceptors of the rat brain.

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References
1 Nagatomo, T. and Sasaki, M.: Effects of neuraminidase and deoxyribonuclease on the β-adrenergic receptors in the rat heart. Japan. J. Pharmacol. 33, 481–484 (1983)
2 Tsuchihashi, H. and Nagatomo, T.: Influence of polymeric effectors on binding of ³H-dihydroalprenolol to β-adrenergic receptor of rat brain. Japan. J. Pharmacol. 38, 17–23 (1985)
3 Tsuchihashi, H. and Nagatomo, T.: Binding characteristics of ³H-dihydroalprenolol to β-adrenergic receptors of rat brain: Influence of lectins. Japan. J. Pharmacol. 38, 121–125 (1985)
4 Stiles, G.L., Benovic, J.L., Caron, M.G. and Lefkowitz, R.J.: Mammalian β-adrenergic receptors: Distinct glycoprotein populations containing high mannose or complex type carbohydrate chains. J. Biol. Chem. 259, 8655–8663 (1984)
5 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)
6 Dimler, R.J., Schaefer, W.C., Wise, C.S. and Rist, C.E.: Quantitative paper chromatography of D-glucose and its oligosaccharides. Anal. Chem. 24, 1411–1414 (1952)
7 Bitter, T. and Muir, H.: A modified uronic acid carbazole reaction. Anal. Biochem. 4, 330–334 (1962)
8 Dische, Z. and Shettles, L.B.: A specific color
reaction of methyl pentoses and a spectrophotometric micromethod for their determination. J. Biol. Chem. 175, 595–603 (1948)
9 Warren, L.: The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234, 1971–1975 (1959)
10 Wagner, W.D.: A more sensitive assay discriminating galactosamine and glucosamine in mixtures. Anal. Biochem. 94, 394–396 (1979)
11 Gahmberg, C.G.: Cell surface proteins: Changes during cell growth and malignant transformation. In Cell Surface Reviews, Vol. 3, Dynamic Aspects of Cell Surface Organization, Edited by Poste, G. and Nicolson, G.L., p. 371–421, Elsevier/North-Holland Biomedical Press, Amsterdam (1977)
12 Gahmberg, C.G.: Membrane glycoproteins and glycolipids: structure, localization and function of the carbohydrate. In Membrane Structure, Edited by Finean, J.B., and Michell, R.H., p. 127–160, Elsevier/North-Holland Biomedical Press, Amsterdam (1981)
13 Margolis, R.K. and Margolis, R.U.: Alkali-labile oligosaccharides of brain glycoproteins. Biochim. Biophys. Acta 304, 421–429 (1973)
14 Puro, K., Maury, P. and Huttunen, J.K.: Qualitative and quantitative patterns of gangliosides in extraneural tissues. Biochim. Biophys. Acta 187, 230–235 (1969)
15 Suzuki, K., Poduslo, S.E. and Norton, W.: Gangliosides in the myelin fraction of developing rats. Biochim. Biophys. Acta 144, 375–381 (1967)
16 Breckenridge, W.C., Gombos, G. and Morgan, I.G.: The lipid composition of adult rat brain synaptosomal plasma membranes. Biochim. Biophys. Acta 266, 695–707 (1977)
17 Constantopoulos, G., Rees, S., Cragg, B.G., Barranger, J.A. and Brady, R.O.: Experimental animal model for mucopoly saccharosis. Sulfatamin-induced glycosaminoglycan and sphingolipid accumulation in the rat. Proc. Natl. Acad. Sci. U.S.A. 77, 3700–3704 (1980)
18 Cassidy, J.T., Jourdian, G.W. and Roseman, S.: Sialidase from Clostridium perfringens. Methods Enzymol. 8, 680–685 (1966)
19 Varki, A. and Kornfeld, S.: The spectrum of anionic oligosaccharides released by endo-β-N-acetyl glucosaminidase from glycoproteins. Structural studies and interactions with the phosphomannosyl receptor. J. Biol. Chem. 258, 2808–2818 (1983)
20 Koide, N. and Muramatsu, T.: Endo-β-N-acetyl glucosaminidase acting on carbohydrate moieties of glycoproteins. Purification and properties of the enzyme from Diplococcus pneumoniae. J. Biol. Chem. 249, 4897–4904 (1974)
21 Tai, T., Yamashita, K., Ogata-Arakawa, M., Koide, N., Muramatsu, T., Iwashita, S. and Kobata, A.: Structural studies of two ovalbumin glycopeptides in relation to the endo-β-N-acetyl glucosaminidase specificity. J. Biol. Chem. 250, 8569–8575 (1975)
22 Takahashi, N.: Demonstration of a new amidase acting on glycopeptides. Biochim. Biophys. Res. Commun. 76, 1194–1201 (1977)
23 Takahashi, N., Toda, H., Nishibe, H. and Yamamoto, K.: Isolation and characterization of taka-aminase A apoprotein deglycosylated by digestion with almond glycopeptidase immobilized on Sepharose. Biochim. Biophys. Acta 707, 236–242 (1982)
24 Takahashi, N. and Nishibe, H.: Almond glycopeptidase acting on aspartyglycosylamine linkages. Biochim. Biophys. Acta 857, 457–467 (1981)
25 Li, Y.-T.: Presence of α-D-mannosidic linkage in glycoproteins. Liberation of α-mannose from various glycoproteins by α-mannosidase isolated from jack bean meal. J. Biol. Chem. 241, 1010–1012 (1966)
26 Li, Y.-T.: Studies on the glycosidases in jack bean meal. I. Isolation and properties of α-mannosidase. J. Biol. Chem. 242, 5474–5480 (1967)
27 Tarentino, A.L., Plummer, T.H., Jr. and Maley, F.: A β-mannosidic linkage in the unit A oligosaccharide of bovine thyroglobulin. J. Biol. Chem. 248, 5547–5548 (1973)
28 Tarentino, A.L. and Maley, F.: Purification and properties of an endo-β-N-acetyl glucosaminidase from Streptomyces griseus. J. Biol. Chem. 249, 811–817 (1974)
29 Tarentino, A.L., Pulmmar, Y.H., Jr. and Maley, F.: The release of intact oligosaccharides from specific glycoproteins by endo-β-N-acetyl glucosaminidase H. J. Biol. Chem. 249, 818–824 (1974)