Developmentally Regulated Expression of a Peptide:N-Glycanase during Germination of Rice Seeds (Oryza sativa) and Its Purification and Characterization*

(Received for publication, July 15, 1999, and in revised form, September 17, 1999)

Tschining Chang, Meng-Chiang Kuo, Kay-Hooi Khoo, Sadako Inoue, and Yasuo Inoue‡

From the Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan

Peptide:N-glycanase (PNGase; EC 3.5.1.52) activity was detected in dormant rice seeds (Oryza sativa) and the imbibed rice grains. Time-course studies revealed that the enzyme activity remained almost constant until about 30 h after imbibition in both of endosperm- and embryo tissue-containing areas, and started to increase only in growing germ part, reached a peak at about 3-day stage, followed by a gradual decrease concomitant with a sharp increase in the coleoptile. The specific activity increased about 6-fold at about 3-day stage. PNGase was purified to electrophoretic homogeneity from the extracts of germinated rice seeds at 24 h, and the apparent molecular weight of the purified enzyme, estimated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), was about 80,000. The purified enzyme was designated PNGase Os to denote its origin. The N-terminal sequence of the 10 residues was determined to be SYN-VASVAGL. The purified PNGase Os in SDS-PAGE appeared as a rather broad band, consistent with the presence of multiple glycoforms as indicated by chromatographic behavior on a Sephadex G-75 column. PNGase expressed in coleoptile under anoxia condition was also purified, and both of the purified enzymes were found to exhibit very similar, if not identical, electrophoretic mobility in SDS-PAGE. PNGase Os exhibited a broad pH-activity profile with an optimum of 4–5 and, interestingly, was significantly inactivated by K⁺ and Na⁺ at near the physiological concentration, 100 mM. These results are discussed in relation to other work.

* This work was supported by National Science Council Grants 87-2311-B-001-121 and 88-2311-B-001-010. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Fax: 886-2-2788-9759; E-mail: syinoue@gate.sinica.edu.tw.

‡ The abbreviations used are: PNGase, peptide:N-glycanase; DTT, dithiothreitol; ConA, concanavalin A; PMSF, phenylmethylsulfonyl fluoride; FAB, fast atom bombardment; PAGE, polyacrylamide gel electrophoresis; MES, 4-morpholineethanesulfonic acid; MOPS, 4-morpholinepropanesulfonic acid.

© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
submerged under water, a condition being referred to as "anoxia," rice seedlings generate a gaseous hormone, ethylene, which is known to promote the growth of coleoptile in the dark (32). Germination is a carefully coordinated complex process involving both cell proliferation and differentiation. During germination of plant seeds, a number of temporally regulated morphological and biochemical events occur. The developmental regulation of proteins and nucleic acids during germination makes these systems good paradigms for studies on differential gene expression and regulation of the synthesis of macromolecules.

As a part of our long range goal to define the functional roles of PNGase in biological systems, we began to study the rice (Oryza sativa) germination with the special reference to the possible involvement of PNGase in releasing free oligosaccharides and thereby their acting as elicitor or chemical signals which trigger rice germination and development such as coleoptile cell growth under anoxia conditions. In this study, we found a rice storage-PNGase referred to as PNGase Os, and its developmentally regulated expression in growing germ and coleoptile of rice during germination. Here we report its identification, purification and characterization.

EXPERIMENTAL PROCEDURES

Rice Grains—Rice grains were kindly provided through the Institute of Botany, Academia Sinica, Taipei, Taiwan. Before each experiment, the grain was washed three times with the deionized water (Millipore Mini-Q SP system, Bedford, MA), sterilized with 0.5% hypochlorite for 10 min, and then thoroughly washed again with 1 liter of deionized water/100 g of rice grains, each time, for six times. These clean rice grains were kept in a sterilized box and submerged in 5-cm-deep water. The whole box was placed in an incubation chamber and kept at 30 °C in the dark. For purification of PNGase Os, rice grains imbibed for 24 h were washed with de-ionized water 4 °C and wet grains were wiped with paper towel. Coleoptiles were collected when the growing germ protruded and their length was 30–35 mm. The harvested coleoptiles were washed with de-ionized water at 4 °C, transferred into liquid nitrogen, and kept until further treatment.

Preparation of Samples from Germinated Rice for Testing the PNGase Os Activity—Each aliquot of 100 grains at different stages of germination after hydration was washed with distilled water, cut horizontally along the germ, and hulls removed quickly. Hullled germinated rice was cut horizontally along the germ and with a blade (see Fig. 1B) and stored separately in liquid N2 until use. To analyze for PNGase activity, samples were ground into fine powder using a motor pre-cooled at 80 °C. The extracts of germinated rice seeds were prepared by treating the powdered samples twice with 10 ml of assay buffer (20 mM sodium acetate, 2 mM DTT, and 1 mM PMSF) and neutralized with 0.5N NaOH to pH 7.7. The total volume was 30 ml. The mixture was dialyzed against the assay buffer solution. The pooled supernatant was then chromatographed on a DEAE-Sephadex A-25 anion-exchange column (6.6 × 10 cm). The flow-through fractions were collected, and pH was adjusted to 5.5. The solution then was subjected to an affinity chromatography on a ConA-Sepharose column (1.5 × 5 cm) which was pre-washed with 10 mM sodium acetate buffer (pH 5.5) containing 100 mM KCl, 2 mM DTT, and 1 mM PMSF. Then, PNGase Os-containing fractions were eluted with 50 mM sodium acetate buffer solution (pH 5.5) containing 2 mM DTT and 200 mM glucose. The PNGase Os-containing fractions were combined and concentrated using a Centricon-30 (Amicon) to 1 ml, and then subjected to Sephadex G-75 column (1.6 × 11 cm) chromatography for further purification of the enzyme. The purity of the purified PNGase Os was checked by SDS-PAGE. The SDS-PAGE was performed routinely by using a NuPAGE™ electrophoresis system (Novex, San Diego, CA). All experimental procedures followed the protocol supplied by the manufacturer. In general, the gradient polyacrylamide gel (4–12%) was used, and MOPS SDS was used as the running buffer (final: 50 mM MOPS, 50 mM Tris-HCl, 3.5 mM SDS, and 1 mM EDTA, pH 7.7). Samples were mixed with an appropriate amount of sample buffer (4 ×) to a final concentrations of: 0.29 M sucrose, 0.25 M Tris-HCl, 69 mM SDS, 0.5 mM EDTA, 0.22 mM Serva Blue G250, and 0.17 mM Phenol Red, and then heated at 90 °C for 10 min. Electrophoresis was performed under a constant voltage of 200 V for 50 min.

The amino acid terminal sequence of 10 residues of PNGase Os was determined by a protein sequencer (model 492; Applied Biosystems, Inc., Foster City, CA).

FAB-Mass Spectrometric Analysis of the Free Oligosaccharide Derived from Stem Bromelain by Treatment with PNGase Os—Stem bromelain obtained from Sigma was used without further purification. The major N-glycan structure was previously characterized as Manα1,6Xylj1,2-Manj1,4GlcnAcj1,6Fucj1,3GlcNAc (33). To test the substrate specificity of PNGase Os, the stem bromelain glycoprotein was digested with trypsin and chymotrypsin to glycopeptides. Since aldon PNGase A (Roche Molecular Biochemicals) is known to act on the stem bromelain-derived glycopeptides to liberate the free N-glycan chain, this enzyme was used as a control for the product analysis by FAB-mass spectrometry. The glycopeptides were treated with the purified PNGase Os, and the free oligosaccharide products were separated from peptide fragments by passage through a Sep-Pak
De-N-glycosylating Enzyme in Germinating Rice Seeds

**RESULTS**

**Developmentally Regulated Enzyme Activity**—Morphological change of rice under the anoxia condition is quite different from that of dicot plant. The rice grains first develop the growing germ area and extend out from the grain on the third day (Fig. 1A). From that time on, the coleoptile began to develop quickly, and on day 6, reached its maximum length of 65–70 mm with the coleoptile length used as the morphological index to show the increasing expression of the enzyme activity. E and G denote endosperm and growing germ-containing regions. C represents coleoptile organ.

The free N-glycans thus obtained were converted to permethyl derivatives by treatment with NaOH/dimethyl sulfoxide slurry (34), and the molecular mass profiles were examined by measuring their FAB-mass spectra on an Autospec OA-TOF mass spectrometer (Micromass, Manchester, United Kingdom) fitted with a cesium ion gun operated at 25 kV. Samples were dissolved in methanol for loading onto the probe tip coated with monothioglycerol as matrix.

### TABLE I

| Purification step | Protein mg | Activity units | Specific activity units/mg | Yield % |
|------------------|------------|----------------|---------------------------|--------|
| Original extract | 12,000     | 40             | 3.3 $\times 10^{-3}$      | 100    |
| DEAE-Sephadex A-25 | 9,000     | 29             | 3.2 $\times 10^{-3}$      | 72     |
| ConA-Sepharose    | 2,500      | 9              | 3.6 $\times 10^{-3}$      | 22     |
| Sephadex G-75     | 0.8        | 4.5            | 5.6                       | 11     |

The effect of mono- and divalent cations on the purified PNGase Os activity was examined and is summarized in Table II. Mg$^{2+}$ showed the greatest stimulation of this activity, Mn$^{2+}$ could substitute partially for Mg$^{2+}$, while Cu$^{2+}$, Fe$^{3+}$, and Zn$^{2+}$ showed no significant stimulation, and PNGase Os was partially inhibited by Cu$^{2+}$. We also carried out experiment to examine if silver ion exerts any effect on PNGase Os activity. In contrast to l-929 PNGase (11), the presence of EDTA did not affect the growth of PNGase Os activity caused by the presence of 2 mM Cu$^{2+}$ was perhaps not due to the binding of this particular divalent metal ion to an active thiol group. Interestingly, this enzyme was significantly inactivated by K$^+$ and Na$^+$ at near the physiological concentration, 100 mM. The activity was not inhibited by EDTA, showing that PNGase Os does not require divalent cations for its activity.

PNGase Os was shown to deglycosylate bromelain glycopeptide which contains the xylose residue linked β-1→2 to β-mannose and fucose residue linked α-1→3 to the innermost proximal N-acetylglucosamine residue. FAB-mass spectrometry analysis demonstrated that the N-glycan released from the bromelain peptide is a wide spectrum of different glycoforms (see below).
glycopeptide substrate by PNGase Os was similar to those obtained by treatment with PNGase A, with the molecular mass of the major product corresponded to (Xyl)$_1$(Man)$_2$(GlcNAc)$_2$(Fuc)$_1$ ($m/z$ 1301 for [M + Na]$^+$ of permethyl derivative). Thus, PNGase Os does not share the substrate specificity with the previously well characterized bacterial-origin PNGase F, but rather shares with plant-derived PNGase A in capability of releasing N-glycan chains having the α1→3 fucosylated core.

Unusual features of PNGase Os was revealed when chromatographed on a column of Sephadex G-75. Fig. 4 shows the elution profiles of PNGase Os under different conditions. The apparent molecular size for PNGase Os, determined by gel filtration using 100 or 140 mM KCl, was found to be in the range of 33,000, whereas it was estimated to be approximately 58,000 when eluted with 500 mM KCl or 100 mM KCl containing 0.2 M glucose (Fig. 4). The results can be interpreted if one considers that PNGase Os binds with relatively high affinity to the Sephadex gel, and as was found previously for L-929 PNGase, PNGase Os could serve not only as an enzyme but also as a carbohydrate recognition protein in vivo, although we need to identify the presence of a high affinity binding site for carbohydrate. Even in the presence of 0.2 M glucose, PNGase Os showed a smaller molecular weight as compared with the enzyme at low salt concentration; such observed behavior may be due to the interaction between the enzyme and Sephadex gel, the presence of which retards the elution of PNGase Os from Sephadex G-75. Shortage of materials did not allow us to carry out further work to obtain a more definitive answer to this problem.

Localization of PNGase Os in the Coleoptile Cell—The coleoptile segments contain only few layers of cells. Therefore, the freeze-fracture method could easily disrupt the cells. The washing medium (5 ml each; three times) of the disrupted coleoptile that passed through the membrane of pore size 0.2 μm was expected to contain material solubilized from cell wall, cytosol, and small non-disrupted suborganelles. In this fraction, PNGase Os activity was present of at about 20% of the total in the coleoptile (Fig. 5, lane F1). The tissue that did not pass through the membrane mostly consisted of the fibrous cell wall. Low salt medium (washing medium) was not able to extract additional PNGase Os activity (Fig. 5, lane F2). Next, the disrupted coleoptile was centrifuged at 16,000 rpm (about 25,000 × g) for 10 min to precipitate large cell debris and/or high density suborganelles. The supernatant contained material solubilized from cell wall, cytosol, and small non-disrupted suborganelles. In this soluble fraction (C1) approximately 20% of the total PNGase Os activity was found when low salt medium was used (Fig. 5, lane C1), and additional 10% of the enzyme activity was extracted from the precipitate by use of the medium containing 100 mM KCl (Fig. 5, lane C2). Treatment of the extensively washed cell wall fiber fraction with pectinase and cellulase resulted in exudation of about 80% and 70% of the total enzyme activity by the filtration and centrifugation methods, respectively (Fig. 5, lanes F3 and C3), indicating that a major proportion of PNGase Os was associated with cell wall material of coleoptile.

De-N-glycosylating Enzyme in Germinating Rice Seeds

**TABLE II**

| Metal ion | Concentration (mM) | Relative activity (%) |
|-----------|-------------------|----------------------|
| EDTA      | 2                 | 100                  |
| Mg$^{2+}$ | 2                 | 138                  |
| Ca$^{2+}$ | 2                 | 114                  |
| Cu$^{2+}$ | 2                 | 63                   |
| Fe$^{3+}$ | 2                 | 109                  |
| Mn$^{2+}$ | 2                 | 122                  |
| Zn$^{2+}$ | 2                 | 110                  |
| Ag$^+$    | 2                 | 96                   |
| K$^+$     | 100               | 51                   |
| Na$^+$    | 100               | 28                   |

Gase, *i.e.* PNGase F, but rather shares with plant-derived PNGase A in capability of releasing N-glycan chains having the α1→3 fucosylated core.

Fig. 2. SDS-PAGE of samples from various purification steps of PNGase Os. *Lane 1*, protein makers, from top: myosin, β-galactosidase, phosphorylase b, bovine serum albumin, glutamic dehydrogenase, lactate dehydrogenase, carbonic anhydrase, trypsin inhibitor, lysozyme, and aprotinin. *Lane 2*, extract from imbibed rice grains. *Lane 3*, samples of rice extract were further purified by passing through a DEAE A-25 column. *Lane 4*, eluant from ConA column by 0.2 M glucose. *Lane 5*, purified PNGase Os (0.5 μg) from G-75 column.

Fig. 3. Effects of the environmental parameters on PNGase Os activity. (A) pH; (B) temperature.
De-N-glycosylating Enzyme in Germinating Rice Seeds

FIG. 4. Chromatographic behavior of PNGase Os on a column of Sephadex G-75. The purified enzyme (500 μg) were passed through the G-75 column, which was pre-equilibrated with buffer containing 0.14 M KCl or 0.1 M KCl together with 0.2 mM Glc.

FIG. 5. The localization test of the PNGase Os activity in the coleoptile. Filtration method (F1, F2, and F3) and centrifugation method (C1, C2, and C3) were used to locate the expressed enzyme activity (see “Experimental Procedures”). F1 and C1, activity presented in the membrane-filterable fraction and supernatant of the coleoptile extract each. F2 and C2, the activity in further extracts by null-ion buffer of the non-filterable fraction and pellet from experiment of F1 and C1, respectively. F3 and C3, activity presented in the portion treated with pectinase and cellulase of non-filterable fraction and pellet each. Lane PC, substrate only. Lane SF, reaction product of the commercial PNGase F.

DISCUSSION

PNGase was purified from rice seeds at 24 h after the onset of germination. It was purified to electrophoretic homogeneity, and the $M_r$ of the purified enzyme, estimated by SDS-PAGE, was approximately 80,000. The purified enzyme was designated as PNGase Os to indicate its origin. Its $N$-terminal amino acid sequence (10 residues) was determined as SYNVASVAGL to show its purity. The elution pattern from the ConA-Sepharose column showed a broad and polydisperse peak eluted in the range of 5–200 mM glucose (data not shown), and the main peak appeared at 20–50 mM glucose. Although several other explanations could be considered, the data suggested that a single enzyme protein may be variably glycosylated and PNGase Os is thus represented by a wide spectrum of glycoforms. This finding was consistent with the result showing that the purified sample of PNGase Os migrated on SDS-PAGE as a broad band (see Fig. 2).

We have investigated the developmental change of expression of PNGase activity in rice seeds from day 0 of imbibition to day 6 of embryogenesis. Dormant seeds were shaved to contain a low level of the PNGase activity, and the same level was maintained from the time of imbibition for nearly 30 h (Fig. 1B). At an early stage of germination (0–12 h), the observed enlargement of rice grain was merely due to the cell growth but not due to cell division. The rather constant level of PNGase activity in the region containing endosperm of germinating rice seeds strongly indicated that the enzyme was pre-stored and assumed to be involved in de-N-glycosylating storage glycoproteins, thereby facilitating the proteolytic breakdown of the glycoproteins immediately after the onset of germination to provide amino acid nutrients. We found that PNGase activity in growing germ of the developing rice seeds began to increase markedly about 2 days after the initiation of germination, reached the maximum level at the 3-day stage, and then declined (Fig. 1, A and B). At this period of time in rice seedling development, coleoptile cells ceased division and the cell elongation was driven mainly by the uptake of water. The high specific activity of PNGase was found in coleoptile, which was more than 5-fold relative to the level in the first 30-h stage. The activity was localized in the coleoptile cell wall and the enzyme purified therefrom migrated with the same apparent molecular mass (80 kDa) of PNGase Os on SDS gels. The finding of PNGase in the coleoptile cell wall should be of physiological significance and of interest from the following points of view.

(a) Since the apoplast of coleoptile where PNGase activity is localized is not a typical digestive suborganelle in plant cells, the function of PNGase may not be to facilitate proteolytic breakdown of storage glycoproteins. More likely, its function may be to produce free glycan chains that have been reported to be involved in the regulation of plant growth and development.

(b) When auxin, one of the plant hormones, activated a plant cell, the apoplast area was always acidified first (36, 37), and this should be accompanied by K+ ion flux inward into the cytosol. The properties clarified for the purified PNGase Os in this study can be related to the physiology of the germinating rice seed. The pH optima for PNGase Os (pH 4–5.5) correlated with the pH of the apoplast at the times in rice seedling development when the PNGase Os was produced. These results indicate that acidification of the cell wall should enhance the activity of the PNGase nearby. If we combine this with the change in K+ concentration to become lower upon acidification, the PNGase Os activity can be further augmented (Table II). One can also consider other regulatory effect(s) resulting from dual properties of PNGase Os that could serve not only as enzyme but also as a lectin-like protein as revealed in this study (Fig. 4). It may be noted that we also found such dual properties in our previous studies on animal-derived l-929 PNGase (16). Thus, PNGase Os in multiple glycoforms may have distinct differences in enzymatic characteristics, which may also be related to its functional role during germination and early development.

(c) Although our results demonstrated that rice seed PNGase stored in its quiescence and purified from the imbibed rice at an early stage (24 h) of germination was electrophoretically identical to that expressed in and isolated from coleoptile, their biochemical identity is still an open question. Furthermore, whether or not the major glycoform of the PNGase Os prestored in rice seed is identical with that of the enzyme expressed in coleoptile remains to be elucidated.

(d) In addition to possible function of generating free glycan, PNGase-catalyzed de-N-glycosylation of plant glycoproteins was proposed as a possible mechanism for regulating protein activity by removing N-glycans and converting glycosylated Asn residues to the Asp residues (9, 28, 38, 39). A specific PNGase seems to have a specific function during early embryogenesis (e.g. germination) and the subsequent ontogenesis (e.g. post-germinative development) as we have shown for the embryonic development of fish (40).
All these arguments appear to concur that PNGase has yet undefined physiological role in plant and animal cells and to confirm our previous proposal that the N-glycosylation/de-N-glycosylation system should occur more commonly than presently recognized in living organisms (28, 38, 39). Possible involvement of PNGase in the processes that initiate and control the metabolic activities of plant and animal development by free oligosaccharides liberated from glycoproteins has been substantiated by their accumulation, although the underlying molecular mechanisms are in most cases unknown. The results of this study suggest that free oligosaccharide(s) liberated from N-linked glycoproteins present in the growing cell wall could be involved in the second phase of the auxin-induced cell elongation, and/or that these unconjugated glycans could regulate expression of genes of key enzymes or functional proteins by mechanisms different from the known hormone-controlled ones. Thus, an interplay of free oligosaccharides, hormones, and key protein genes is considered to be operating in the maturation, quiescence, and germination of plant seeds.

The function of the PNGase-catalyzed deglycosylation during rice germination and post-germinative developmental processes is unknown. Elucidation of a possible functional interplay between PNGase-catalyzed oligosaccharide formation, production of chemical signals such as the fruit ripening hormone, ethylene, and auxin action is our target goal and currently under way. Results of our preliminary studies indicate that free oligosaccharides are present in rice seeds and that their chemical composition based on sugar analysis is also in agreement with the substrate specificity of PNGase Os demonstrated in this study.

REFERENCES

1. Takahashi, N. (1977) Biochem. Biophys. Res. Commun. 76, 1194–1201
2. Taga, E. M., Wahed, A., and Van Etten, R. L. (1984) Biochemistry 23, 815–822
3. Sugiyama, K., Ishihara, H., Tejima, S., and Takahashi, N. (1983) Biochem. Biophys. Res. Commun. 112, 155–160
4. Yet, M.-G., and Wold, F. (1988) J. Biol. Chem. 263, 118–122
5. Flummer, T. H., Jr., Phelan, A. W., and Tarentino, A. L. (1987) Eur. J. Biochem. 163, 167–173
6. Flummer, T. H., Jr., Elder, J. H., Alexander, S., Phelan, A. W., and Tarentino, A. L. (1984) J. Biol. Chem. 259, 10790–10704
7. Seko, A., Kitajima, K., Inoue, S., and Inoue, Y. (1991) Biochem. Biophys. Res. Commun. 180, 1165–1171
8. Inoue, S. (1990) Trends Glycosci. Glycotechnol. 2, 225–234
9. Suzuki, T., Kitajima, K., Inoue, S., and Inoue, Y. (1994) Glycoconj. J. 12, 438–448
10. Suzuki, T., Seko, A., Kitajima, K., Inoue, S., and Inoue, Y. (1993) Biochem. Biophys. Res. Commun. 194, 1124–1130
11. Suzuki, T., Seko, A., Kitajima, K., Inoue, Y., and Inoue, S. (1994) J. Biol. Chem. 269, 17611–17618
12. Suzuki, T., Kitajima, K., Inoue, Y., and Inoue, S. (1995) J. Biol. Chem. 270, 15181–15186
13. Kitajima, K., Suzuki, T., Kouchi, Z., Inoue, S., and Inoue, Y. (1995) Arch. Biochem. Biophys. 319, 393–401
14. Suzuki, T., Kitajima, K., Emori, Y., Inoue, Y., and Inoue, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6244–6249
15. Seko, A., Kitajima, K., Iwamatsu, T., Inoue, Y., and Inoue, S. (1999) Glycobiology 9, 887–895
16. Suzuki, T., Kitajima, K., Inoue, S., and Inoue, Y. (1994) Glycoconj. J. 11, 469–476
17. Kopito, R. R. (1997) Cell 88, 427–430
18. Wiertz, E. J. H., Jones, T. R., Sun, L., Bogyo, M., Geuze, H. J., and Ploegh, H. L. (1996) Cell 85, 769–779
19. Hughes, E. A., Hammond, C., and Cresswell, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1896–1901
20. Halaban, R., Cheng, E., Zhang, Y., Moellmann, G., Hanlon, D., Michalak, M., Setaluri, V., and Hebert, D. N. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6210–6215
21. Huppa, J. B., and Ploegh, H. L. (1997) Immunity 7, 113–122
22. Yu, H., Kaung, G., Kobayashi, S., and Kopito, R. R. (1997) J. Biol. Chem. 272, 20890–20894
23. Suzuki, T., Park, H., Kitajima, K., and Lennarz, W. J. (1998) J. Biol. Chem. 273, 21526–21530
24. Suzuki, T., Yan, Q., and Lennarz, W. J. (1998) J. Biol. Chem. 273, 10683–10686
25. Priem, B., Sokolowan, J., Wieruszewski, J.-M., Streecker, G., Nazih, H., and Morvan, H. (1990) Glycoconj. J. 7, 121–132
26. Lhernould, S., Karamanos, Y., Bourgenie, S., Streecker, G., Julien, R., and Morvan, H. (1992) Glycoconj. J. 9, 191–197
27. Lhernould, S., Karamanos, Y., Lerouve, P., and Morvan, H. (1995) Glycoconj. J. 12, 84–98
28. Berger, S., Menudier, A., Julien, R., and Karamanos, Y. (1995) Biochimie 77, 751–760
29. Priem, B., Morvan, H., and Gross, K. C. (1994) Biochem. Soc. Trans. 22, 398–402
30. Priem, B., Gitti, R., Bush, C. A., and Gross, K. C. (1993) Plant Physiol. 102, 445–458
31. Priem, B., and Gross, K. C. (1993) Plant Physiol. 96, 398–401
32. Ishizawa, K., and Esashi, Y. (1984) Plant Cell Environ. 7, 239–245
33. Ishihara, H., Takahashi, N., Oguri, S., and Tejima, S. (1979) J. Biol. Chem. 254, 10715–10719
34. Delling, A., Khoo, R.-H., Panico, M., McDowell, R. A., and Morris, H. B. (1993) in Glycosciences: Status and Perspectives (Gabius, H., and Gabius, S., eds) pp. 187–222, Oxford University Press, New York
35. Ku, H. S., Suge, H., Rappaport, L., and Pratt, H. K. (1970) Proc. Natl. Acad. Sci. U. S. A. 67, 22110–22114
36. Ishihara, H., Takahashi, N., Oguri, S., and Tejima, S. (1979) J. Biol. Chem. 254, 10715–10719
37. Dell, A., Khoo, R.-H., Panico, M., McDowell, R. A., and Morris, H. B. (1993) in Glycbiology: A Practical Approach (Fukuda, M., and Kohata, A., eds) pp. 187–222, Oxford University Press, New York
38. Suzuki, T., Kitajima, K., Inoue, S., and Inoue, Y. (1994) Glycoconj. J. 4, 77–89
39. Suzuki, T., Kitajima, K., Inoue, S., and Inoue, Y. (1997) in Glycosciences: Status and Perspectives (Gabius, H., and Gabius, S., eds) pp. 122–131, Chapman & Hall, Weinheim, Germany
40. Seko, A., Kitajima, K., Inoue, Y., and Inoue, S. (1991) J. Biol. Chem. 266, 22110–22114