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ALMOND GLYCOPEPTIDASE ACTING ON ASPARTYLGLYCOSYLAMINE LINKAGES

MULTIPLICITY AND SUBSTRATE SPECIFICITY

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Summary

The glycopeptidase preparation that has been isolated from almond emulsin and acts on β-aspartylglycosylamine linkages in glycopeptides was separated into three active fractions by DEAE-cellulose column chromatography. The three discrete species of glycopeptidase (Groups A, B and C) have been purified 30-, 136- and 99-fold, respectively. The optimum pH value of Group A was 6.0 and those of Groups B and C, 5.0. Isoelectric points of Groups A, B and C were pH 7.7, 8.6 and 8.7, respectively. All three glycopeptidases hydrolyzed quantitatively glycopeptides with 3-11 amino acid residues prepared from stem bromelain, ovalbumin and ovotransferrin. Group C preferred glycopeptides with shorter peptide chains, whereas Groups A and B preferred those with longer chains. Glycopeptidase Group A also hydrolyzed intact glycoproteins such as stem bromelain, ovalbumin, Taka-amylase A and desialylated human transferrin.

Introduction

The glycopeptidase from almond emulsin hydrolyzes β-aspartylglycosylamine linkages in glycopeptides with carbohydrate and oligopeptide moieties [1,2]. It acts neither on Asn-oligosaccharide nor on Asn-GlcNAc, which are substrates of an amidase (4-*N*-(2-β-D-glucosaminyl)-L-asparaginase,

Abbreviations: GlcNAc, *N*-acetylglucosamine, Asn^{OS}, Asn (oligosaccharide). 1 enzyme unit of glycopeptidase was defined as the amount of enzyme required to hydrolyze 1 μmol substrate per min under the standard conditions.

EC 3.5.1.26) [3]. The glycopeptidase is extremely useful for structural analysis of asparagine-linked oligosaccharides, as illustrated by the case of stem bromelain glycopeptides [4]. In the present paper, using the longer glycopeptide from stem bromelain Ala-Arg-Val-Pro-Arg-Asn-Asn^{os}-Glu-Ser-Ser-Met as a substrate, we demonstrate that two additional glycopeptidases are present in the same crude extract of almond emulsin, and that one of the new enzyme forms can act not only on glycopeptides but also on glycoproteins.

Materials and Methods

Substrates

Stem bromelain was prepared from commercial crude preparation (lot N-VI-1) from Jintan-Dorf Co., Osaka, by the method of Takahashi et al. [5]. Ovalbumin was prepared from fresh eggs by the method of Shepherd and Montgomery [6]. Taka-amylase A was a generous gift from Dr. T. Ikenaka, Osaka University. Human transferrin was obtained from Miles Laboratories, and was converted to desialylated transferrin by mild acid hydrolysis with 0.05 M H_2SO_4 at 80°C for 1 h, followed by Sephadex G-25 gel filtration.

The following glycopeptides of stem bromelain were prepared in our laboratory, The longest one, an undecapeptide, Ala-Arg-Val-Pro-Arg-Asn-Asn^{os}-Glu-Ser-Ser-Met, was obtained from the pepsin digest of stem bromelain [7]. Methionine was cleaved forming the decapeptide by pepsin digestion of the CNBr fragment [8]. Further tryptic digestion of the undecapeptide and the decapeptide yielded Asn-Asn^{os}-Glu-Ser-Ser-Met and Asn-Asn^{os}-Glu-Ser-Ser, respectively. A mixture of Asn^{os}-Glu-Ser and Asn-Asn^{os}-Glu-Ser was obtained by Pronase-P digestion, followed by carboxypeptidase digestion of stem bromelain. The mixture contained approx. 2 mol tetrapeptide and 5 mol tripeptide [9]. The oligosaccharides in these stem bromelain glycopeptides were composed of the following sugars (numbers in parentheses are expressed by molar ratio taking glutamic acid as 1.0): mannose (2.48), fucose (0.95), xylose (1.07) and *N*-acetylglucosamine (2.16) [10]. Ovalbumin glycopeptide, Glu-Glu-Lys-Tyr-Asn^{os}-Leu-Thr-Ser-Val was isolated from pepsin-digested ovalbumin by Sephadex G-25 gel filtration and by paper electrophoresis. The glycopeptide contained, on the average, 5.1 mol mannose and 3 mol *N*-acetylglucosamine/mol glycopeptide. Ovotransferrin glycopeptide, Gly-Leu-Ile-His-Asn^{os}-Arg was a generous gift from Dr. K. Hotta, Kitasato University. The glycopeptide contained, on the average, 3 mol mannose and 5—6 mol hexosamine/mol glycopeptide.

Other chemicals

p-Nitrophenyl derivatives of α-mannose, β-galactose, β-glucose, β-xylose and β-*N*-acetylglucosamine were purchased from Nakarai Chemicals. Actinomycete protease inhibitors, pepstatin, chymostatin and leupeptins were kindly given by Dr. T. Aoyagi, Institute for Microbial Chemistry. Sephadex G-200 and G-150 were purchased from Pharmacia Fine Chemicals AB, DEAE-cellulose (DE-52) from Whatman Ltd. and Ampholite from LKB.

Assay of the glycopeptidase using glycopeptides as substrates

For the enzyme purification the amount of the released ammonia was

determined. A glycopeptide (0.1 μmol) was incubated with the glycopeptidase (approx. 0.5 munit) in 30 μl 0.1 M citrate-phosphate buffer (pH 5.2), at 37°C for 3 h. After terminating the reaction by adding 5 μl 1 M HCl, the reaction mixture was incubated for an additional 3 h at 37°C. 3 ml of freshly prepared phosphate-buffered *o*-phthalaldehyde-mercaptoethanol was added to the reaction mixture. After standing for 45 min at room temperature, the fluorescence was measured, with excitation at 410 nm and emission at 470 nm, with a Hitachi spectrophotofluorimeter Model 204, as described by Taylor et al. [11]. The concentration of ammonia was regarded as equal to the amount of substrate hydrolyzed. When the enzyme was assayed by using tri- and tetrapeptides or undecapeptide from stem bromelain as substrate, the relase of ammonia was proportional to reaction time up to 3 h, when the release of ammonia was less than 0.04 μmol.

For the other analysis, oligosaccharides released were determined by orcinol-sulfuric acid reaction as described previously [2]. All assays comparing substrate specificity (Table II) and rates of reaction (Table I, Figs. 4 and 5) were carried out at substrate saturating conditions (10 mM).

Assay of the glycopeptidase with glycoproteins as substrates

Glycoprotein (5 nmol) was hydrolyzed with 0.2 munit glycopeptidase in 20 μl 0.2 M citrate-phosphate buffer (pH 6.0), containing three protease inhibitors, i.e., pepstatin (1 μg), phenylmethylsulfonyl fluoride (10 mM) and iodoacetic acid (10 mM), at 37°C, for appropriate periods (0, 1.5, 3, 5 and 8 h). In the control experiment, *N*-acetylglucosamine (5 nmol) was used instead of glycoprotein substrates. The oligosaccharide released was reduced by the method of Takasaki and Kobata [12], using 5 μl NaB^3H_4 (150 nmol, 62.5 μCi, The Radiochemical Centre, U.K.) in 30 μl of the reaction mixture, adjusted to pH 8.0 with 1 M NaOH, at room temperature for 4 h. The reaction was terminated by the addition of 500 μl acetic acid and evaporated to dryness. The evaporation with 200 μl 6% acetic acid was repeated five times. The reaction mixture was passed through a column (0.5 × 2.5 cm) of Amberlite IR-120(H^+) and the effluent was evaporated to dryness. Borate was removed by evaporation with 1 ml methanol (five times). The residue was dissolved in a small amount of water, applied to Whatman No. 1 paper, and was subjected to paper chromatography for 2 days at 20—21°C. Radioactivity on paper strips was determined in the toluene scintillation fluid, with a Packard Tri-Carb liquid scintillation spectrometer 3380. The total radioactivity of all oligosaccharide alcohols of the paper chromatogram was expressed as mol oligosaccharide released per mol glycoprotein.

Analytical methods

Activities of the exoglycosidases were determined by using the corresponding *p*-nitrophenyl glycosides as described by Muramatsu and Egami [13]. Protease activity was assayed by measuring digested protein using the method of Lowry et al. [14], after precipitating protein with trichloroacetic acid as described by Schwabe [15]. In addition, protease activity was detected by polyacrylamide disc-gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) according to the method of Weber and Osborn [16] with a slight

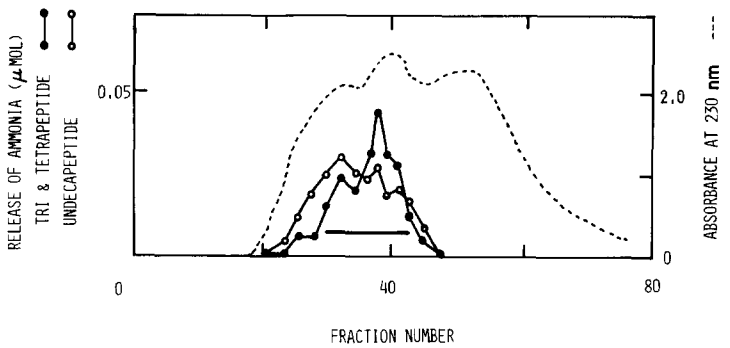


Fig. 1. Sephadex G-200 column chromatography of the glycopeptidase. The crude extract of almond emulsin was applied to a column of Sephadex G-200. The release of ammonia from bromelain glycopeptides was determined. The chromatographic conditions and enzyme assay are described in the Materials and Methods. The enzyme fractions indicated by a bar were pooled. ●———●, tri- and tetrapeptide substrates; ○———○, undecapeptide.

modification [5]. Descending paper chromatography was performed with the solvent system, ethylacetate/pyridine/water(12 : 5 : 4). The buffer for electrophoresis was pyridine/acetic acid/water(5 : 0.2 : 95 v/v), pH 6.5.

Enzyme purification

Almond emulsin (β-glucosidase) was obtained from Sigma Chemical Co. All steps were carried out below 4°C.

Step 1. Sephadex G-200 column chromatography. Almond emulsin (500 mg) was dissolved in 2 ml 0.01 M acetate buffer (pH 5.0)/0.1 M NaCl. The crude extract was applied to a column of Sephadex G-200 (2.5 X 86 cm) equilibrated with 0.01 M acetate buffer (pH 5.0)/0.1 M NaCl and eluted with

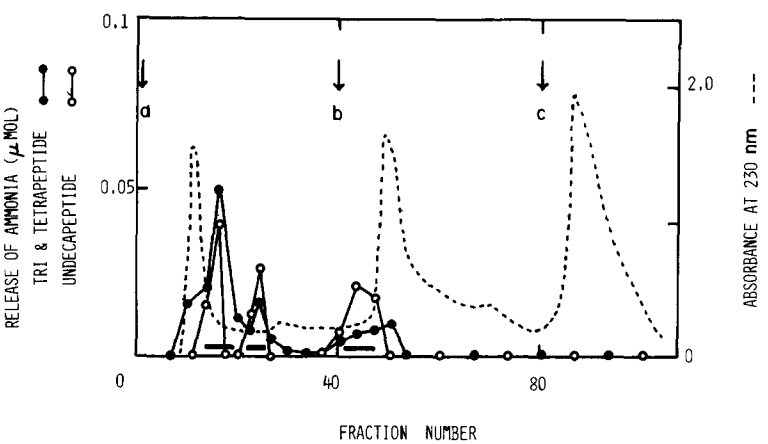


Fig. 2. DE-52 cellulose chromatography of the glycopeptidase. Enzyme fraction from the previous step (Fig. 1) was chromatographed on a DE-52 cellulose column. The chromatographic conditions are described in the Materials and Methods. The enzyme fractions indicated by bars were individually pooled. a, 0.01 M Tris-HCl (pH 8.5)/0.04 M NaCl; b, 0.01 M Tris-HCl (pH 8.5)/0.09 M NaCl; c, 0.01 M Tris-HCl (pH 8.5)/0.14 M NaCl. ●———●, tri- and tetrapeptides as substrates; ○———○, undecapeptide.

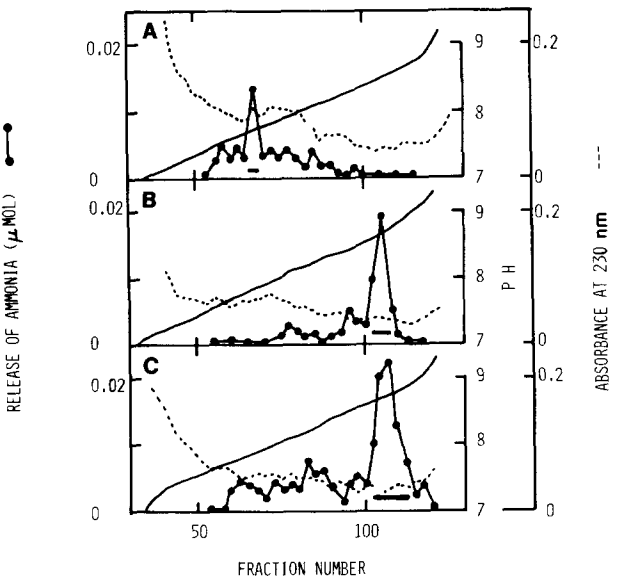


Fig. 3. Isoelectric focusing of glycopeptidase Groups A, B and C. Enzyme fraction (Groups A, B and C) obtained from the previous step (Fig. 2) was applied to a column. Isoelectricfocusing was carried out as described in the Materials and Methods. Enzyme was assayed by using undecapeptide as a substrate. Enzyme fractions indicated by a bar were pooled. A, Group A; B, Group B; C, Group C.

the same buffer. Fractions of 6 ml were collected (Fig. 1). The major active fractions were pooled and concentrated.

Step 2. Chromatography of DE-52 cellulose. The enzyme solution was applied to a column of DE-52 cellulose (2 X 32 cm) equilibrated with 0.01 M Tris-HCl buffer (pH 8.5)/0.04 M NaCl. The column was eluted with a step elution which consisted of 200 ml each of 0.01 M Tris-HCl (pH 8.5)/0.04 M NaCl; 0.01 M Tris-HCl (pH 8.5)/0.09 M NaCl and 0.01 M Tris-HCl (pH 8.5)/ 0.14 M NaCl. Fractions of 5 ml were collected (Fig. 2). Fractions for the three active components were named Groups A, B and C.

Step 3. Isoelectric focusing. The enzyme fraction was placed in the middle of the isoelectric focusing LKB column (110 ml), containing the sucrose gradient from 0 to 50% with a pH gradient from 7 to 9 prepared with Ampholite. Isoelectricfocusing was carried out at 4° C for 48 h with a final potential at 800 V. Fractions of 1 ml were collected. Their absorbance at 230 nm and pH values were measured. Active fractions were pooled and concentrated (Fig. 3).

Results

Purification of glycopeptidase

The glycopeptidase from almond emulsin was partially purified by two chromatographic procedures using Sephadex G-200 and DE-52 cellulose and by isoelectric focusing. Three distinct fractions of the enzyme were obtained by DE-52 cellulose chromatography; they were termed glycopeptidase Groups A, B and C. Group C corresponds to the enzyme reported previously [1,2]. It was detected with tri- and tetrapeptides from stem bromelain as substrates. Groups

TABLE I

PURIFICATION OF GLYCOPEPTIDASE GROUPS A, B AND C

Enzymes were assayed by using undecapeptide as a substrate. The amount of protein was measured by the method of Lowry et al. [14] using ovalbumin as a standard.

Steps	Total protein (mg)			Specific activity (munits/mg protein)			Recovery (%)			Purification (-fold)		
Crude extract		460			0.631			100			1	
Sephadex G-200		274			0.729			68.7			1.16	
	A	B	C	A	B	C	A	B	C	A	B	C
DE-52 cellulose	24.5	0.62	5.3	2.69	50.6	19.0	22.6	10.8	35.0	4.26	80.2	30.1
Isoelectric focusing	1.42	0.44	0.78	19.0	85.5	62.3	9.2	12.9	16.7	30.1	136	98.8

A and B, however, were scarcely detected with the tri- and tetrapeptides. Thus, we determined their activities by using undecapeptide as a substrate. The purification factors of glycopeptidase Groups A, B and C were 30-, 136- and 99-fold, respectively, when undecapeptide from stem bromelain was used as a substrate (Table I). The final preparation was free from activities of α-mannosidase, β-xylosidase, α-fucosidase, β-*N*-acetylglucosaminidase, β-galactosidase and protease (cf. Discussion).

Properties of the glycopeptidases

The isoelectric points of Groups A, B and C were 7.7, 8.6 and 8.7, respectively (Fig. 3). Glycopeptidase Group A showed a broad pH optimum at pH 6.0 and that of Groups B and C at pH 5.0 (Fig. 4). Activities of the glycopeptidase Groups A, B and C were not significantly inhibited by various compounds tested: i.e., 10 mM each of cations (Mg^{2+}, Ca^{2+} and Mn^{2+}), 10 mM of thiol inhibitors (iodoacetic acid, iodoacetamide and *N*-ethylmaleimide), 25 μg/ml of actinomycete protease inhibitors (leupeptins, chymostatin and pepstatin), 10 mM of other compounds (EDTA, L-cysteine and phenylmethylsulfonyl fluoride) and 10 mg/ml of γ-D-gluconolactone. The K_m values of Groups A, B and C for stem bromelain undecapeptide were 2.0, 2.3 and 4.0 mM, respectively. The *V* values of Groups A, B and C were 19.0, 85.5 and 62.3 nmol/mg of protein/min, respectively, toward the substrate.

Action of Groups A, B and C on stem bromelain glycopeptides

The three groups of glycopeptidase were compared with regard to substrate

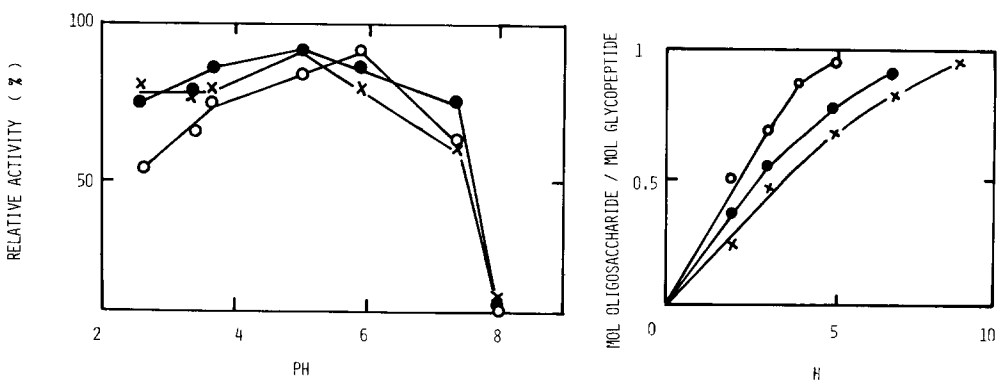


Fig. 4. Effect of pH on glycopeptidase activity. Activity was determined in 10 mM substrate concentration and 0.1 M citrate-phosphate buffer at pH 2.7—7.4 and in 0.1 M Tris(hydroxymethyl)aminomethane buffer at pH 7.2—9.0. Undecapeptide was used for glycopeptidase Groups A and B as a substrate and tri- and tetrapeptides for Group C as described in the Materials and Methods. Relative activity is shown in percentage of maximum activity in each group of enzyme. o———o, Group A; •———•, Group B; x———x, Group C.

Fig. 5. Hydrolysis of glycopeptides with the glycopeptidase Group C. Glycopeptides (0.3 μmol) were incubated with glycopeptidase Group C (0.5 munit) in 30 μl 0.1 M citrate-phosphate buffer (pH 5.0), at 37° C. At the indicated times the oligosaccharides released were determined by the orcinol-sulfuric acid reaction after paper electrophoresis at pH 6.5, as described previously [2]. The extent of hydrolysis was expressed in mol oligosaccharide released per mol glycopeptide substrate. The glycopeptides used were as follows. o———o, Ala-Arg-Val-Pro-Arg-Asn-Asn(Man_{2-3}, Xyl_1, Fuc_1, $GlcNAc_2$)-Glu-Ser-Ser-Met, from stem bromelain; x———x, Glu-Glu-Lys-Tyr-Asn(Man_5, $GlcNAc_3$)-Leu-Thr-Ser-Val, from ovalbumin; •———•, Gly-Leu-Ile-His-Asn(Man_3, $Hexosamine_{5-6}$)-Arg, from ovotransferrin.

TABLE II

A COMPARISON OF THE RATE OF HYDROLYSIS OF STEM BROMELAIN GLYCOPEPTIDES WITH THE GLYCOPEPTIDASE GROUPS A, B AND C

In Expt. nos. 1—4, four kinds of glycopeptides with different lengths of peptide chain (0.3 μmol) were hydrolyzed with glycopeptidase (0.5 munit) in 30 μl 0.1 M citrate-phosphate buffer (pH 5.2), at 37°C for 3 h. The oligosaccharides released were determined by the orcinol-sulfuric acid reaction after paper electrophoresis. In Expt. no. 5, stem bromelain (5 nmol) was hydrolyzed with glycopeptidase (0.2 munit) in 20 μl 0.2 M citrate-phosphate buffer (pH 6.0), at 37°C for 3 h. The amount of oligosaccharide released was determined by radioisotopic procedure of NaB^3H_4 reduction followed by paper chromatography as shown in Fig. 6A. The radioactivity of the resulting oligosaccharide alcohols from stem bromelain was compared with that obtained from undecapeptide (5 nmol) in the same procedure (Expt. no. 4). Details are described in the Materials and Methods. Relative activity is shown in percentage of maximum activity in each group of enzyme. Relative activity among three groups of enzyme is not shown. n.d., not determined.

Expt. no.	Number of amino acids in glycopeptides	Relative activity (%)		
		Group A	Group B	Group C
1	3 and 4	38.4	49.0	100
2	5	44.0	n.d.	n.d.
3	6	37.3	67.4	70.4
4	11	100	100	89.0
5	ca. 230	33.5	0	0

specificity with stem bromelain glycopeptides that had varied lengths of peptide chains (Table II). Groups A and B hydrolyzed undecapeptide more easily than tri- and tetrapeptides; whereas the latter substrates were most rapidly hydrolyzed with Group C. Group A hydrolyzed intact stem bromelain. However, neither Group B nor Group C could act on the glycoprotein. As mentioned above, each enzyme showed characteristic specificity with respect to the chain length of the peptide moieties; Group A prefered the longer peptide and Group C the shorter ones.

Hydrolysis of glycopeptides by Group C

We investigated the action of glycopeptidase Group C on three kinds of glycopeptides prepared from stem bromelain, ovalbumin and ovotransferrin. The enzyme hydrolyzed almost completely the glycopeptides from stem bromelain, ovotransferrin and ovalbumin glycopeptides (Fig. 5).

Hydrolysis of glycoproteins with Group A

Glycopeptidase Group A hydrolyzed not only stem bromelain but also several glycoproteins such as ovalbumin, Taka-amylase A and desialylated human transferrin. The oligosaccharide released from glycoprotein was reduced with NaB^3H_4 and was analyzed by paper chromatography. Fig. 6 shows a typical paper chromatogram of radioactive oligosaccharide alcohols prepared from stem bromelain and Taka-amylase A. No smaller peptides were detected in all four kinds of glycoprotein reaction mixtures by the assay of released peptides as described in Materials and Methods. The minimum amount of digested protein detectable by this method was 1 μg. In addition, no fragmentation of the glycoproteins was noted by a polyacrylamide gel electrophoresis of the

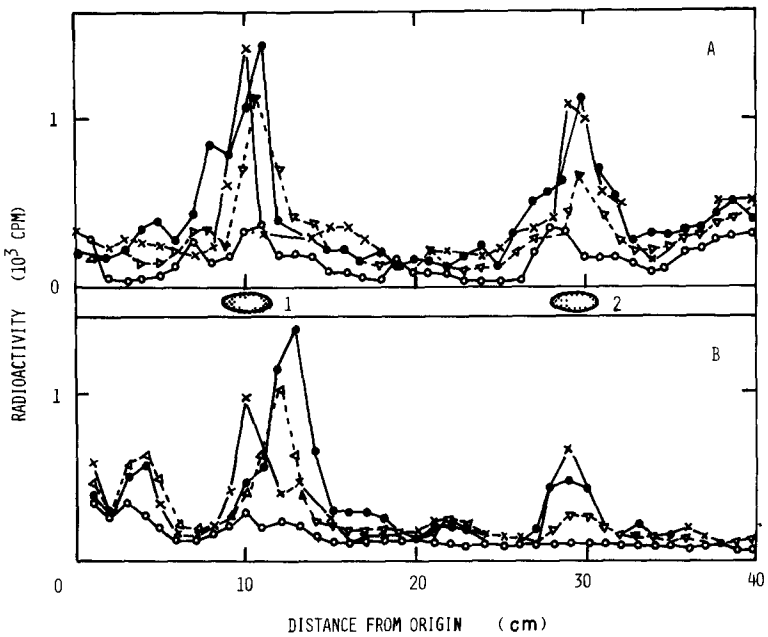
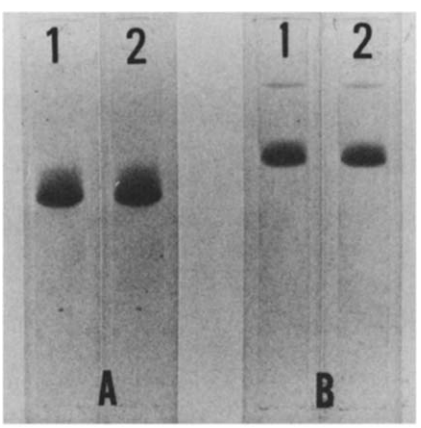


Fig. 6. Paper chromatogram of the radioactive oligosaccharide alcohols obtained from glycoproteins. Glycoprotein (5 nmol) was incubated with glycopeptidase Group A (0.2 munit) in 20 μl 0.2 M citrate-phosphate buffer (pH 6.0), containing three kinds of protease inhibitors at 37°C. At the indicated time (0, 3, 5 and 8 h), the oligosaccharides released from glycoprotein were reduced with NaB^3H_4 and analyzed by paper chromatography as described in the Materials and Methods. Positions of standard markers from sten bromelain glycopeptides [4] are shown in the middle of the figure. 1, heptasaccharide alcohol; 2, hexasaccharide alcohol. A, stem bromelain; B, Taka-amylase A. incubation time, ○———○, 0 h; △———△, 3 h; x———x, 5 h; ●———●, 8 h.



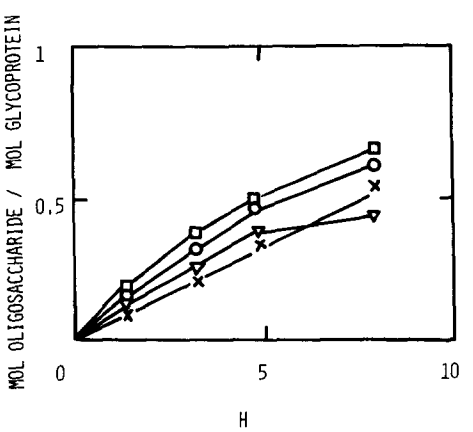


Fig. 7. SDS-polyacrylamide gel electrophoresis of glycopeptidase Group A digestion of stem bromelain and ovalbumin. The conditions are described in the Materials and Methods. Approx. 20 μg protein were subjected to 10% SDS-polyacrylamide gel electrophoresis. Migration was from top to the bottom. A, stem bromelain; B, ovalbumin; 1, presence of Group A; 2, absence of Group A.

Fig. 8. Hydrolysis of glycoproteins with the glycopeptidase Group A. Glycoproteins (5 nmol) were hydrolyzed at 37°C with the glycopeptidase Group A (0.2 munit) in 20 μl 0.2 M citrate-phosphate buffer (pH 6.0), containing three kinds of protease inhibitors. At the indicated intervals the oligosaccharides released were analyzed as shown in Fig. 6. The extent of hydrolysis was expressed by mol oligosaccharide released per mol glycoprotein substrate. The glycoproteins used were as follows. ○———○, stem bromelain of M_r 26 000 (Man_{2-3}, Xyl_1, Fuc_1, $GlcNAc_2$) [5]; x———x, ovalbumin of M_r 48 000 (Man_5, $GlcNAc_3$); □———□, Taka-amylase A of M_r 51 000 (Man_5, $GlcNAc_2$) [16], △———△, desialylated human transferrin of M_r 76 000 (Gal_2, Man_3, $GlcNAc_4$).

reaction mixture (Fig. 7). These results confirmed that the oligosaccharides were indeed released from glycoproteins, and not from glycopeptides produced by possible contamination of proteases in the enzyme preparation. The time course of hydrolysis of several glycoproteins, including stem bromelain, ovalbumin, Takaamylase A and desialylated transferrin, are shown in Fig. 8. Since intact transferrin was scarcely hydrolyzed by the enzyme, sialic acid may have hindered the enzymatic action.

Discussion

Asparagine-linked oligosaccharides of stem bromelain, ovotransferrin and ovalbumin glycopeptides were quantitatively released by digestion with almond glycopeptidase. The isolation of peptide-free oligosaccharides is essential for the structural analysis of carbohydrate moieties. In this respect, almond glycopeptidase is an excellent tool to isolate intact oligosaccharide moieties from glycopeptides. The glycopeptidase which we reported previously [2] corresponds to glycopeptidase Group C. Using the longer glycopeptide from stem bromelain Ala-Arg-Val-Pro-Arg-Asn-Asn^{os}-Glu-Ser-Ser-Met as a substrate, we detected new enzyme Groups A and B. These enzymes also seem to act on desialylated glycopeptides of so-called complex type (Takahashi, N. and Nishibe, H., unpublished data).

Glycopeptidase Group A hydrolyzed intact glycoproteins in addition to glycopeptides with short peptide chain, though the rates of hydrolysis of the glycoproteins were slow. Intact glycoproteins and deglycosylated glycoproteins are readily separated by several procedures, especially by affinity chromatography of lectin-agarose. Thus, glycopeptidase Group A may be a valuable agent in examining the function of intact protein moieties in biochemical as well as biological study of glycoproteins.

As for the experimental procedure, the following three points deserve mentioning: (1) Almond glycopeptidase cleaves β-aspartylglycosylamine linkages in glycopeptides to yield equimolar amounts of oligosaccharide, peptide and ammonia [2]. The present method based on determining the released ammonia by spectrophotofluorimetry is simple and rapid, making the enzyme purification easier. The rates of the release, however, differ among the types of the substrates. Orcinol-sulfuric acid reaction which determines the oligosaccharide released [2] was alternatively employed for the quantitative analysis in the present experiment. The reaction is specific but rather tedious. In the previous paper, the glycopeptidase activity was assayed by determining the amount of released peptides with Autoanalyzer [2]. This procedure was suitable for the bromelain tri- and tetrapeptides used as standard substrates, but not for other peptides. (2) In the purification procedure, α-mannosidase was efficiently removed by Sephadex G-200 chromatography. The separation of three kinds of enzymes has been achieved by the use of DE-52 cellulose ion-exchanger (Fig. 2). Isoelectric focusing very effectively increased the specific activity of the enzyme. The three groups of the enzymes, however, still appeared to be heterogeneous. (3) The sort and amount of contaminating glycosidases in the purified final products depended on the lot of 'β-glucosidase' preparation. For example, glycopeptidase preparation from lot

39C-0488 was free from β-*N*-acetylglucosaminidase but that from lot 95C-0230 showed strong activity of β-*N*-acetylglucosaminidase. β-*N*-Acetylglucosaminidase however, can be removed by passing through a column of *p*-aminophenyl-1-thio-β-D-acetylglucosamine-Sepharose [17]. Although β-glucosidase activity was detected in all glycopeptidase preparations, this enzyme does not appear to affect the structural studies of glycopeptides because of the paucity of glucose residues in the oligosaccharide moieties.

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