INTERACTION OF ANTIMANNAN WITH GLYCOPEPTIDES

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SUMMARY: Taka amylase A glycopeptide (TA-GP) strongly inhibited the interaction of antimannan (antibodies directed towards mannan from Saccharomyces cerevisiae) with yeast mannan, whereas ovalbumin glycopeptide (OA-GP) did so only poorly. We inferred that this is due to the strong reactivity of antimannan with terminal trimannosides composed of Manα1→2Man or Manα1→3Man linkages which occur in mannan and TA-GP. In contrast, TA-GP and OA-GP were nearly equally reactive with concanavalin A having the ability to interact with terminal mannose and 2-O-mannose residues which occur abundantly in these glycopeptides. Thus, antimannan should be useful as a probe for characterizing glycoproteins from extracellular fluids or cellular membranes.

Glycoproteins with carbohydrate moieties linked N-glycosidically to polypeptide moieties contain mannose as a normal component and occur widely in nature, as in extracellular fluids and cellular membranes (1). As a probe to characterize the carbohydrate moieties of these glycoproteins, antibodies directed towards the carbohydrate moieties should be useful. These carbohydrate moieties, however, have been regarded as poorly antigenic although they interact with some lectins (2). Serum antibodies produced towards the glycoproteins are usually specific to the polypeptide moieties (3).

Yeast mannans from various genetic types of yeasts have different structures and produce antibodies (antimannan) of different specificities. Mannan from Saccharomyces cerevisiae produces antibodies directed towards the oligomannoside branches with Manα1→2Man or Manα1→3Man linkages rather than towards the back bone with purely Manα1→6Man linkage (4). Inhibition studies of this particular antimannan - mannan reaction using various mannosides have shown that tetra- or trimannosides, composed of Manα1→2Man or Manα1→3Man linkages representing the terminal structures of the antigenic mannan, inhibit the reaction strongly whereas such dimannosides as Manα1→2Man or Manα1→3Man are about 10-fold poorer inhibitors (4, 5).

Structures analogous to these oligomannosides have been suggested to occur in some of the glycoproteins with oligosaccharide chains composed of N-acetylglucosamine and mannose. Taka amylase A and ovalbumin are glycoproteins of this type. The presence of the terminal Manα1→2Manα1→3Man structure has been es-

tablished for the former (6). Ovalbumin is heterogeneous with respect to its carbohydrate moiety (7), the trimannoside structure having been shown to occur in only one of five different oligosaccharide chains (8).

This paper reports that antimannan strongly interacted with Taka amylase A glycopeptide, but only poorly with ovalbumin glycopeptide prepared by digesting these glycoproteins with pronase, whereas these glycopeptides were nearly equally reactive with concanavalin A.

MATERIALS AND METHODS

Antimannan sera were prepared by injecting heat-killed yeast cells into the ear veins of male rabbits weighing about 3 kg. Injection was performed twice a week for a period of 10 weeks. The yeast used, Saccharomyces cerevisiae, was a commercially available Baker's yeast purchased from the Oriental Yeast Co., Tokyo. Mannan from this yeast was provided by Dr. S. Suzuki of the Tohoku College of Pharmacy. Concanavalin A was purchased from Sigma Chemical Co., U. S. A.

A portion of the Taka amylase A glycopeptide (TA-GP) was given by Dr. H. Yamaguchi of Osaka Prefectural University. TA-GP was also prepared in our laboratory according to the method of Yamaguchi et al. (9). Ovalbumin glycopeptide (OA-GP) was prepared by the method of Yamashina and Makino (10). Note that OA-GP stands for an unfractionated glycopeptide mixture from ovalbumin.

[^{3}H]-Acetylated glycopeptides were prepared according to the method of Kaplan and Schlamowitz (11) with [^{3}H]-acetic anhydride. The [^{3}H]-acetic anhydride (100 mCi/mmole) was purchased from the Radiochemical Centre, Amersham, England. Before use, [^{3}H]-acetic anhydride was diluted with 24 volumes of cold acetic anhydride. The specific activities of the [^{3}H]-acetylated TA-GP and OA-GP were 1.41×10^6 and 1.52×10^6 dpm/μmole, respectively.

Inhibition studies of the precipitation reaction of antimannan with mannan using the glycopeptides were carried out at an equivalence zone of the reaction. Thus, 0.1 ml of antimannan serum and 0.5 ml of a 0.01 M phosphate buffer - 0.15 M NaCl, pH 8.0, solution of glycopeptides were mixed, and after incubation at 37° for 2 hr, 0.5 ml of a 0.01 % solution of mannan in the buffer - saline was added. Incubation was continued first at 37° for 2 hr, then at 4° for 24 hr. The precipitate formed was collected by centrifugation, washed with 1 ml of the buffer - saline twice, then dissolved in 1 ml of 1 % Na_2CO_3. Absorbance of the solution was measured at 280 nm. Similar studies of the precipitation reaction of concanavalin A with mannan at an equivalence zone of the reaction were carried out in 0.01 M Tris-HCl buffer - 0.15 M NaCl, pH 8.0, containing 1.5×10^{-4} M $CaCl_2$ and 5×10^{-4} M $MgCl_2$. One half ml of a 0.1 % solution of concanavalin A and 0.5 ml of a glycopeptide solution were mixed and incubated at 37° for 30 min. Then, 0.5 ml of a 0.01 % solution of mannan was added, and the whole was incubated first at 37° for 30 min, then at 4° for 1 hr. The precipitate was treated as for the antimannan - mannan reaction.

Binding of antimannan with the glycopeptides was determined in 0.01 M phosphate buffer - 0.15 M NaCl, pH 8.0, essentially according to Farr (12). Thus, 0.1 ml of antimannan serum, 0.2 ml of a [^{3}H]-acetylated glycopeptide solution corresponding to 2 μg mannose and 0.1 ml of the buffer - saline were mixed. After incubation at 37° for 1 hr, then at 4° for 24 hr, 0.4 ml of a saturated $(NH_4)SO_4$ solution, adjusted to pH 8.0, was added. After incubation at 4° for 30 min, the precipitate formed was collected by centrifugation, washed with 1 ml of a half saturated $(NH_4)_2SO_4$ solution, adjusted to pH 8.0, twice, then dissolved in 1 ml of water. To determine radioactivity, this solution was mixed with a toluene - Triton X-100 scintillation mixture. Counting was carried out with a Beckman, model LS-100, scintillation counter.

Binding of concanavalin A with the glycopeptides was determined in 0.01 M Tris-HCl buffer - 0.15 M NaCl, pH 8.0, containing 1.5×10^{-4} M $CaCl_2$ and 5×10^{-4} M $MgCl_2$. One half ml of a 0.1 % solution of concanavalin A and 0.5 ml of a [^{3}H]-acetylated glycopeptide solution corresponding to 2 μg mannose were mixed.

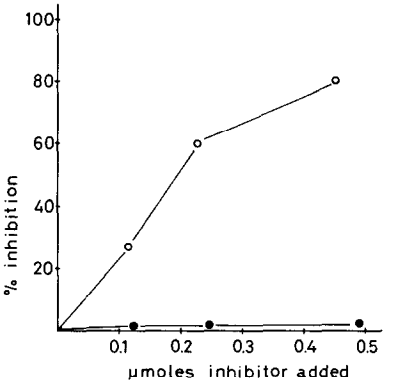

Fig. 1. Inhibition of the precipitation of antimannan with mannan by Taka amylase A glycopeptide (o) and ovalbumin glycopeptide (●)

After incubation at 37° for 1 hr, the solution was applied to a column of Bio-Gel P-100 (1.2 X 28 cm) equilibrated and eluted with the buffer - saline to separate the bound and free glycopeptides. The radioactivity of each fraction was determined using the procedure described above.

RESULTS AND DISCUSSION

Of the glycopeptides tested, only TA-GP strongly inhibited the precipitation of antimannan with mannan, as shown in Fig. 1. The inhibitory power of TA-GP was nearly equivalent to that of the oligomannosides representing the antigenic determinants of the mannan, as shown in Table 1. This cross reaction is consistent with the presence of the terminal Manα1→2Manα1→3Man structure in TA-GP (6).

Of the five glycopeptides with different oligosaccharides constituting OA-GP, three have N-acetylglucosamine at both internal and terminal positions (7). Although the oligomannoside structures of these glycopeptides are not yet known, it is likely that the terminal N-acetylglucosamine residue masks oligomannosides with possible inhibitory power. Structures of the other two glycopeptides have recently been determined by Tai et al. (8), and only one has the terminal Manα1→2Manα1→3Man structure. Since this glycopeptide comprises about 10 % of the total on a molar basis (7), conceivably OA-GP is a weak inhibitor for the antimannan - mannan reaction.

Interaction of the glycopeptides with concanavalin A was thus examined. As shown in Fig. 2, both TA-GP and OA-GP strongly inhibited, to a similar extent, the concanavalin A - mannan reaction. The concentration of TA-GP required to cause 50 % inhibition of the concanavalin A - mannan reaction was about 5-fold lower than that required to inhibit the antimannan - mannan reaction. These findings are consistent with the specificity of concanavalin A interacting with various saccharides. Goldstein and his colleagues (13, 14) and Kornfeld and

Table 1 : Inhibition by various mannosides of the precipitation of mannan by antimannan

Inhibitor	μmoles to give 50 % inhibition
TA-GP	0.190
OA-GP	(13 %)[a]
Manα1→3Manα1→2Manα1→2Man	0.083[b]
Manα1→2Manα1→2Man	0.250[b]
Manα1→3Manα1→2Man	
Manα1→3Man	1.67[b]
Manα1→2Man	4.20[b]
Manα1→6Man	12.0[b]

[a]Percent of inhibition caused by 1.0 μmole of OA-GP, [b]Data from Ref. 5.

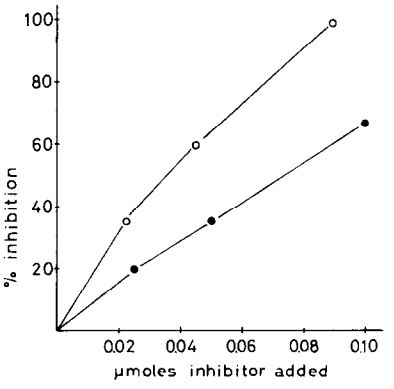

Fig. 2. Inhibition of the precipitation of concanavalin A with mannan by Taka amylase A glycopeptide (o) and ovalbumin glycopeptide (●).

Ferris (15) have shown that concanavalin A interacts with oligosaccharides which possess either terminal mannose or internal 2-O-mannose (mannose substituted on the C-2 hydroxyl group) residues. TA-GP possesses three terminal mannose and one 2-O-mannose residues. Of the ovalbumin glycopeptides whose structures have been established by Tai et al. (8), one has three terminal mannose residues and the other three terminal and one 2-O-mannose residues. The other three ovalbumin glycopeptides with unknown structures may also have inhibitory ability since the presence of terminal N-acetylglucosamine linked β1, 2 to the underlying mannose

increases the ability of the glycopeptides to interact with concanavalin A (15, 16).

Table 2 shows the binding of the glycopeptides with antimannan and concanavalin A. As anticipated, antimannan binds only to TA-GP with no measurable binding to OA-GP. Concanavalin A showed the ability to bind to both TA-GP and OA-GP with nearly equal potency. Since the amount of antimannan and that of concanavalin A used in the binding experiments were the same (based on their potency to bind to mannan), the much lower value for the TA-GP binding to antimannan compared to the binding to concanavalin A seems to indicate that the affinity of TA-GP to antimannan is lower than that to concanavalin A, unless the antimannan serum contains some interfering substance.

Table 2 : Binding of ^{3}H-acetylated glycopeptides to antimannan and concanavalin A

	TA-GP	OA-GP
Antimannan	2.4	≒ 0
Concanavalin A	70.0	61.0

Values are expressed as percentages of the bound glycopeptides.

Results reported here demonstrate clearly that glycopeptides having oligomannosides in their carbohydrate moieties interact quite differently with antimannan whereas they interact similarly with concanavalin A. In view of the antigenic specificity of antimannan, the presence of terminal tetra- or trimannosides composed of either Manα1→2Man or Manα1→3Man in the carbohydrate moiety seems to be a necessary factor for glycopeptides to interact with antimannan. Glycoproteins with carbohydrate moieties containing oligomannoside structures have been shown to occur in such cellular membranes as the endoplasmic reticula, the mitochondrial or nuclear membranes of rat liver and the plasma membranes of ascites hepatoma cells (17 - 19). Antimannan would be useful in characterizing these membrane glycoproteins. In a preliminary study of the glycopeptides from the mitochondrial membranes of rat liver, we found that some glycopeptides composed of N-acetylglucosamine and mannose interacted with antimannan.

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