THE PRESENCE OF β-MANNOSIDIC LINKAGE IN ACIDIC GLYCOPEPTIDE FROM PORCINE THYROGLOBULIN

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SUMMARY

The mannose residue in $(Man)_1(GlcNAc)_2$-Asn obtained by a Smith degradation of the acidic glycopeptide from porcine thyroglobulin was found to be insusceptible to α-mannosidase. This residue was hydrolyzed, however, by purified β-mannosidase. After β-mannosidase treatment, the resulting $(GlcNAc)_2$-Asn was compared with synthetic glycosyl-asparagine derivatives. From these experiments, the core structure of the acidic glycopeptide was proposed to be β-Man-(1 → 3 or 4)-β-GlcNAc-(1 → 4)-GlcNAc-Asn.

The presence of a β-Man-$(GlcNAc)_2$-Asn* sequence has been reported in the core region of a number of glycoproteins from a variety of sources (1-6). Up to the present, however, the occurrence of β-mannosidic linkage has been found only in the neutral carbohydrate chain, which consists of mannose and N-acetylglucosamine, of the glycoproteins. In this communication, we will report the presence of the β-mannosidic linkage also in the acidic carbohydrate chain, which contains sialic acid, of porcine thyroglobulin.

MATERIALS AND METHODS

The acidic glycopeptide (glycopeptide B) of porcine thyroglobulin was prepared according to the procedure previously described (7). This glycopeptide contains sialic acid, galac-

* The abbreviation for GlcNAc-Asn in the glycosyl-asparagine derivatives refers to : 2-acetamido-N-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine.

tose, mannose, N-acetylglucosamine and fucose in the molar ratio of 2:3:3:5:1, and its tentative structure has been proposed (7). α-Mannosidase, β-mannosidase and β-N-acetylglucosaminidase were purified from the liver of Turbo cornutus as described by Muramatsu and Egami (8), and Iijima et al. (9). Each purified enzyme used in this study was found to be virtually devoid of other glycosidase activity. One unit of enzyme activity was defined as the amount of enzyme which could liberate 1.0 μmole of p-nitrophenol from the appropriate p-nitrophenyl glycosides in 1 min. Enzymatically released reducing sugars were determined by the ferricyanide method of Park and Johnson (10). Quantitative determination of individual neutral sugar was carried out by gas-liquid chromatography according to the method of Matsumoto and Osawa (11). Hexosamine was determined by the method of Belcher et al. (12). N-Acetylglucosamine released by β-N-acetylglucosaminidase was measured by the method of Reissig et al. (13). Sequential Smith degradation was carried out according to the procedure described by Arima and Spiro (14). Isolation of $(GlcNAc)_2$-Asn from the glycopeptide B was carried out by β-mannosidase treatment followed by exhaustive pronase digestion of the first Smith degradation product of the glycopeptide. Dansylation (1-dimethylaminonaphthalene-5-sulfonylation) of N-(4-L-aspartyl)-glycosylamine derivatives were performed according to the method of Tsuruo et al. (15), and the dansylated products were purified by thin layer chromatography.

RESULTS AND DISCUSSION

We reported previously the isolation of a glycopeptide containing mannose, N-acetylglucosamine and fucose in the molar ratio of 1:2:1 from the glycopeptide B of porcine thyroglobulin

TABLE I. Effect of Sequential Periodate Oxidation on Desialized Glycopeptide B.

Component	Untreated	After Smith Degradation	
		First	Second
		residues/glycopeptide	
Fucose	1.0	0	0
Galactose	3.1	0	0
Mannose	3.0	1.3	0.4
N-acetylglucosamine	5.0	2.0	2.0

Glycopeptide B (1.0 μmole) was incubated at 4° in the dark with 20 μmole of sodium metaperiodate in 0.05 M acetate buffer, pH 4.0. After 48 hours, 40 μmole of ethylenglycol were added to react with excess periodate (60 min at 25°). Then, $NaBH_4$ (8 mg) was added and the solution was kept in the dark for 16 hours at 4°. The oxidized and borohydride reduced glycopeptides were desalted by adsorption on Dowex X 2, 200-400 mesh (H^+) and subsequent elution with 1.5 N NH_4OH. The ammonia was removed by lyophilization. In each degradation the oxidized, reduced and desalted glycopeptides were hydrolyzed in 0.05 N H_2SO_4 at 80° for 30 min.

by sequential enzymic degradation (7). The hydrolysis of this glycopeptide with α-mannosidase or β-N-acetylglucosaminidase did not release reducing sugars, whereas the slow release of mannose was observed by the action of β-mannosidase. We, therefore, tentatively assumed that the innermost mannose residue of the glycopeptide B had a β-mannosidic linkage, and that the presence of the fucose residue possibly prevented the complete removal of the mannose residue by the enzyme.

In this study, we isolated a core glycopeptide, which contained only mannose and N-acetylglucosamine in the molar ratio of 1:2, from the desialized glycopeptide B by means of Smith degradation as shown in Table I. The mannose residue of this core glycopeptide was found to be resistant to hydrolysis by α-mannosidase, even after treatment with β-N-acetylglucosaminidase. The digestion with β-mannosidase, however, smoothly released 0.9 mole of mannose (Table II). This fact

TABLE II. Effect of Glycosidases on the First Smith Degradation Product of Desialized Glycopeptide B.

Glycosidase	Residues Released per Mole of Glycopeptide	
	Man	GlcNAc
1. α-mannosidase	0.2	---
2. β-N-acetylglucosaminidase	---	---
then α-mannosidase	0.2	---
3. β-mannosidase	0.88	---
then β-N-acetylglucosaminidase	---	0.84

The reaction mixture contained 3 μmole glycopeptide and enzyme in 0.1 M sodium acetate buffer, pH 4.0, containing 0.5 M NaCl. The tubes were incubated at 37° under a toluene atmosphere for 48 hours. The amount of enzyme added in each case was as follows: α-mannosidase 1.2 unit; β-mannosidase 0.7 unit; β-N-acetylglucosaminidase, 0.6 unit.

TABLE III. Comparison of Various Dansylated (DNS) Glycosyl-asparagine Derivatives by Paper Chromatography[a]

DNS Derivatives	$R_{DNS-Asn}$[b]
Asn	1.00
GlcNAc-Asn[c]	0.85
β-GlcNAc-(1 → 4)-GlcNAc-Asn[c]	0.74
β-GlcNAc-(1 → 6)-GlcNAc-Asn[c]	0.72
$(GlcNAc)_2$-Asn from Glycopeptide B	0.74

[a]Solvent System: 1-butanol-ethanol-water (4:1:5, v/v, upper phase).
[b]Dansylated asparagine.
[c]Kindly provided by Drs. E. Walker and R. W. Jeanloz.

confirmed the previous assumption that the innermost mannose residue of the glycopeptide B had a β-mannosidic linkage. In order to clarify the mode of linkage between subsequent N-acetylglucosamine residues, the $(GlcNAc)_2$-Asn obtained by the β-mannosidase treatment followed by exhaustive pronase digestion of the core glycopeptide was further hydrolyzed with β-N-acetylglucosaminidase, and the release of 0.8 mole of N-acetyl-glucosamine was observed as shown in Table II. Furthermore,

when the $(GlcNAc)_2$-Asn was dansylated and compared with dansylated synthetic glycosyl-asparagine derivatives by means of paper chromatography, a fluorescent spot corresponding to dansyl β-GlcNAc-(1 → 4)-GlcNAc-Asn was detected (Table III). These facts indicate that the linkage between N-acetylglucosamine residues is β 1 → 4. This conclusion was also supported by the fact that both N-acetylglucosamine residues were not destroyed by two successive Smith degradations of the glycopeptide B (Table I). We have, therefore, assumed the core structure of the glycopeptide B to be β-Man-(1 → 3 or 4)-β-GlcNAc-(1 → 4)-GlcNAc-Asn.

The β-mannosidic linkages in the neutral glycopeptides containing only mannose and N-acetylglucosamine from ribonuclease B (3), ovalbumin (1-6), α-amylase from Aspergillus oryzae (2,6) and stem bromelain (6) have been reported. The presence of β-mannosidic linkage also in the acidic glycopeptide such as the glycopeptide B in this communication is most interesting from the standpoint of biosynthetic scheme of natural glycoproteins.

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