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CHARACTERIZATION OF A GLYCOPEPTIDE FROM PATHOLOGICAL HUMAN IgM WITH AN UNUSUAL OLIGOSACCHARIDE CORE

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SUMMARY

A glycopeptide from human pathological IgM has been prepared and characterized. It contains an unusual oligosaccharide core with only one N-acetylglucosamine carrying six mannosyl residues, and linked to the asparagine. The involvement of sugar has been determined by use of α and β mannosidases and endoglycosidase digestions. The amino acid sequence has been elucidated and from the obtained data it could be located on the C terminal portion of the IgM Du heavy chain at the Asn 563.

This glycopeptide is characterized by the absence of a N-N-diacetylchitobiose unit in the oligosaccharide moiety and its condensed structure is proposed :

Tyr - Asn - Val - Ser
|
GlcNAc
| β
Man
| α
(Man)$_5$

INTRODUCTION

IgM are glycoproteins which contain a high sugar content included between 7 to 15 % (1). The presence of two types of oligosaccharide chain in immunoglobulin is well documented : simple carbohydrate moieties with only mannose and N-acetylglucosamine and complex carbohydrate moieties with in addition galactose, fucose and sialic acid (2, 3).

On the basis of structural studies, it has been suggested that the oligosaccharide moiety of plasma and perhaps also that of membrane glycoproteins is derived from a common structural core consisting of : Man_3-GlcNac-GlcNac-Asn (4).

Most of the glycopeptides so far investigated seem to be in agreement with this, but an exception is described here with the isolation of a simple glycopeptide which contains a single N-acetylglucosamine residue in its oligosaccharide chain.

EXPERIMENTAL PROCEDURE

IgM was prepared from the plasma of a patient with Waldenström macroglobulinemia as previously reported (2), and checked for homogeneity by immunoelectropho-

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resis, acrylamide gel electrophoresis and analytical ultracentrifugation. 17 glycopeptides were prepared from IgM Du as described earlier (5).
The amino acid composition was determined in a Beckman Unichrom analyser after a 6N HCl hydrolysis for 7 and 24 hours in sealed, evacuated tubes. Both mannose and N-acetylglucosamine content were determined by gaz liquid chromatography according to Chambers and Clamp Method (6). Glucosamine was also measured by Beckman analyser after several conditions of hydrolysis : with 6N HCl at 100 °C for 3, 6 and 24 hours and 3N HCl at 100 °C for 4 hours. Mannose and glucosamine contents were also determined using colorimetric methods (7 , 8). The alkaline treatment of IgM Du glycopeptides was performed as described by Tanaka et al. (9). An estimate of the molecular weight of the glycopeptide was made by gel filtration according to Bhatti and Clamp (10).
The amino acid sequence has been determined using a combination of the Edman Procedure (11) and Dansyl derivatives (12) and carboxypeptidase A hydrolysis during 24 hours at 37 °C. The liberated amino acids have been directly identified by amino acid analyser.
In order to elucidate the mannose linkages, α-mannosidase from Jack bean was assayed according to Li and Li (13). The remaining glycopeptide was submitted to β-mannosidase digestion (14). The liberated mannosyl residues were determined by direct analysis of an aliquot of the hydrolysate in a sugar autoanalyzer using orcinol method.
A β-endo-N-acetylglucosaminidase preparation from Clostridium perfringens was assayed at pH 5.5 as previously reported (15) on labelled glycopeptide. The digestion was monitored by radioscanning (Packard Radioscanner) after paper electrophoresis.
[^{14}C] acetyl-labelled glycopeptide was prepared by acetylation with [^{14}C] acetic anhydride as reported by Kaplan and Schlamowitz (16).

RESULTS

The glycopeptide has been prepared and tested for homogeneity as previously reported (2). Its amino acid and carbohydrate compositions are reported in Table I. No galactose nor galactosamine residues were found as generally in the O-glycosidic linkage, the amino acid composition was not affected by alkaline treatment and the ratio : Glucosamine/Aspartic acid obtained from Composition is set to 1. Therefore the linkage between the oligosaccharide moiety and the peptide structure must be a N-glycosidic one. This glycopeptide has been obtained at a recovery rate of 16.4 % molar, of the total glycopeptides indicating that there is one glycopeptide of this type per IgM heavy chain (J. Jouanneau and R. Bourrillon unpublished result).
The estimated molecular weight is 1670 and agrees with the calculated one 1430, on the basis of the composition with aspartic acid being set to 1.
The amino acid sequence studies have shown that the first dansylation step liberated only a DNS-Tyr. The following step yielded nothing suggesting that Asn linked to oligosaccharide moiety is the second aminoacyl residue. Ser was the major amino acid liberated by carboxypeptidase. Thus the peptidic sequence appeared to be :

Tyr - Asn - Val - Ser.

The glycopeptide is completely hydrolysed by α (82 % of the total mannose) and then β (95 % of the total mannose)-mannosidases. These results indicating that

TABLE I

Amino acid and carbohydrate composition of the glycopeptide.

Asp	1
Ser	0.6
Val	0.6
Tyr	0.5
GlcNAc	1
Man	6

The results are expressed with aspartic acid residue being set to 1.

the first mannose residue to be attached on glucosamine is a β one while the others are of α conformation.

The [^{14}C] acetylated glycopeptide was submitted to β endo-N-acetylglucosaminidase preparation from Clostridium perfringens. The action of the enzyme has been monitored after electrophoresis of the digest. The unhydrolysed glycopeptide does not move from the origin, while the remaining labelled one after enzymic treatment does. The described specificity of this enzyme is to split the osidic linkage between the two N-acetylglucosamine of the N-N-diacetylchitobiose unit (15). The unlabelled oligosaccharide chain liberated by the enzyme from the [^{14}C]-glycopeptide was eluted from the origin of the electrophoretic run and the remaining [^{14}C]-glycopeptides peaks from their zones. The observed difference between the two peaks is due to a slight peptide heterogeneity resulting from the papain digestion performed on IgM for preparing the glycopeptides.

The carbohydrate content and the molar ratio Asn/$GlNH_2$ have been determinated on each fraction. The fraction eluted from the origin contains only mannose and the other one Aspartic acid and glucosamine with a ratio set to 1. The obtained results have been determinant to assume that this glycopeptide does contain only one N-acetylglucosamine (Table II). From the data available a general structure of this IgM glycopeptide may be proposed :

$$(Man)_5 \overset{\alpha}{-} Man \overset{\beta}{-} GlcNAc - \begin{matrix} Tyr \\ \uparrow \\ Asn \\ \uparrow \\ Val \\ \uparrow \\ Ser \end{matrix}$$

DISCUSSION

The evidency of a pathologic IgM glycopeptide with only one N-acetylglucosamine in the core oligosaccharide structure is reported in this paper ; its determined structure is : $(Man)_5 \overset{\alpha}{-} Man \overset{\beta}{-} GlcNAc$ - Asn. It has been possible

TABLE II

Analysis of compounds obtained after endoglycosidase digestion.

	Asp/$GlcNH_2$	$GlcNH_2$	Mannose
Origin	- [a]	0	5.6
[^{14}C] remaining	0.8	1	0
glycopeptides	1.3	1.2	0

[a] no glucosamine neither aspartic acid were detected.

The results are expressed with Asp residue being set to 1.

to eliminate definitively the alternative possibility of two aspartic residues and consequently one N-N-diacetylchitobiosyl unit for two reasons : the calculated M.W. should have been 3300 whereas determinated value is 1670, and in that case the endoglycosidase should have produced a remaining labelled glycopeptide with Asn/$GlcNH_2$ = 2. From the data here available two particularly interesting findings emerge : 1) the existence of a plasma protein with a single N-acetylglucosamine in a sugar chain, which is significant in view of glycoprotein biosynthesis process ; 2) the β endo-N-acetylglucosaminidase preparation appears not to be exclusively specific for the N-N-diacetylchitobiosyl unit, unless this may result of another endoglycosidase specificity.

The absence of chitobiosyl unit for complex oligosaccharide chain has been reported in few cases i.e. for a delipidated low density glycolipoprotein (18). The authors however did not discuss the possible origin of this structure. In fact, the presence of a single N-acetylglucosamine glycopeptide as described in this paper is difficult to explain in terms of lipophosphate dependant biosynthesis which until now, requires the necessity of a N-N-diacetylchitobiose unit. However one would have to speculate a biosynthesis process via a dolichol-P-P-GlcNAc - $(Man)_n$ intermediate. It has been reported recently that several sugar transfer by mean of dolichol must exist (19), otherwise this unusual structure could result of a direct transfer of N-acetylglucosamine to asparagine with requisite transferase, in the rough endothelium reticulum as reported for human serum glycoproteins (20). In that point of view, when it may be possible to find out how the stepwise reactions of oligosaccharide chain are regulated, it could be understood if such a glycopeptide is associated with pathologic disease and highly increased immunoglobulin biosynthesis.

The recovery of the one glucosamine glycopeptide is 16.4 % (% molar) of the total glycopeptides ; this value indicates the presence of one glycopeptide of this type per IgM heavy chain (J. Jouanneau and R. Bourrillon, unpublished results). Its determinated peptidic sequence : Tyr-Asn-Val-Ser, is the same as the sequence reported for IgM Ou heavy chain by Putnam et al. (17) and allows us to locate this oligosaccharide chain on the Asn 563 of the heavy chain.

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REFERENCES

1. Clamp J.R. and Johnson I. (1972) Glycoproteins : their composition, structure and function. Ed. by Gottschalk A.J. BBA Library Elsevier, Amsterdam.
2. Jouanneau J., Razafimahaleo E. and Bourrillon R. (1970) Eur. J. Biochem. 17, 72-77
3. Johnson I. and Clamp J.R. (1971) Biochem. J. 123, 739-742.
4. Hickman S., Kornfeld R., Osterland C.K. and Kornfeld S. (1972) J. Biol. Chem. 247, 2156-2163.
5. Jouanneau J. and Bourrillon R. (1975) Biochimie 57, 1203-1208.
6. Chambers R.E. and Clamp J.R. (1971) Biochem. J. 125, 1009-1018.
7. Lustig B. and Langer A. (1931) Biochem. Z. 242; 320-337.
8. Elson L.A. and Morgan W.T. (1933) Biochem. J. 27, 1827-1828.
9. Tanaka K., Bertolini H. and Pigman W. (1967) Biochem. Biophys. Res. Commun. 16, 404-410.
10. Bhatti T. and Clamp J.R. (1968) Biochim. Biophys. Acta 170, 206-208.
11. Edman P and Begg G. (1967) Eur. J. Biochem. 1, 80-91.
12. Gray W.R. (1967) Methods in Enzymology 11, 139-151.
13. Li Y.T. and Li S.C. (1972) Methods in Enzymology 28, 702-713.
14. Wan C.C., Muldrey J.E., Li S.C. and Li Y.T. (1976) J. Biol. Chem. 251, 4384-4388.
15. Chien S.F., Yevich S.J., Li S.C. and Li Y.T. (1975) Biochem. Biophys. Res. Commun. 65, 583-691.
16. Kaplan M. and Schlamowitz M. (1972) J. Immunochem. 9, 737-747.
17. Putnam F.W., Florent G., Paul C., Shinoda T. and Shimizu A. (1973) Science 182, 287-291.
18. Swaminathan N. and Aladjem F. (1976) Biochemistry 15, 1516-1522.
19. Parodi A. and Leloir L. (1979) Biochim. Biophys. Acta 559, 1-37.
20. Khalkali Z. and Marshall R.D. (1976) Carbohydrate Research 49, 455-473.