THE PEPTIDE MOIETY OF THE REGION CARRYING THE CARBOHYDRATE CHAINS OF THE HUMAN PAROTID GLYCOPROTEIN

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1. Introduction

Previous investigations on the composition of human parotid saliva have shown that two main components are present: α-amylase and proline-rich glycoprotein [1–3]. We have already reported the fractionation of individual samples of parotid saliva according to their carbohydrate content and described the isolation and chemical composition of a parotid glycoprotein [2]. This molecule has a mol. wt of 36 000. Its approximate composition is 60 per cent protein and 40 per cent carbohydrate [4].

Subsequently several proline-rich proteins which did not contain carbohydrate were isolated and characterized [5,6].

The biological role of the glycoprotein has not yet been clearly defined, but it is suspected that the proline-rich proteins may be involved as structural subunits in the zymogen granule membranes [7].

In this paper, we investigate the nature of the carbohydrate peptide linkage and present evidence that one mannose and two *N*-acetyl-glucosamine residues are situated in the proximal portion of carbohydrate chains.

2. Materials and methods

2.1. *Collection of parotid saliva*

Samples of stimulated parotid saliva were collected from two donors with the aid of the Lashley apparatus for periods of 30 min. The cellular debris in the samples were separated by centrifugation (5000 rev/min for 10 min) and discarded. Saliva was then dialyzed against distilled water and lyophilized.

2.2. *Isolation of the proline-rich glycoprotein*

100 mg of lyophilized saliva were applied to a column of Sephadex G 200 (4.5 × 70 cm) equilibrated with 0.01 M sodium phosphate, 0.2 M NaCl buffer pH 8.0. Effluent was monitored at 278 nm. Carbohydrate was determined by orcinol–sulfuric acid reaction [8]. The orcinol fraction F_2 was dialyzed against distilled water and lyophilized.

2.3. *Papain-pronase digestion of the proline-rich glycoprotein (F_2 Sephadex G 200)*

100 mg of lyophilized fraction was suspended in 10 ml 0.01 M, sodium phosphate buffer, pH 6.5 and incubated with papain (0.1 mg/ml) at 37°C for 24 hr. Calcium chloride was added to give a final concentration of 0.01 M. Pronase digestion (0.25 mg/ml) was carried out for 24 hr at 37°C and pH 7.5. A second step of pronase digestion was performed for an additional 24 hr under the same conditions.

2.4. *Gel-filtration of the papain-pronase peptides*

The peptide solution was subsequently loaded into a column of Sephadex G 50 (2.3 × 100 cm). The column was eluted with distilled water in 5 ml fractions whose absorbancy at 230 nm was determined. Peptidic content was determined by ninhydrin reaction. The carbohydrate-containing peptides were located by means of orcinol sulphuric acid reagent. The fraction corresponding to the glycopeptide region was combined and lyophilized.

2.5. *Chemical composition*

Amino acid analysis of glycoprotein and glycopeptides was carried out on a Technicon analyzer after hydrolysis of the samples with 5.6 M HCl for 24 hr at

110°C in tubes sealed under vacuum [8]. The carbohydrate composition was determined by gasliquid chromatography Perkin-Elmer 900 analyzer, as described by Reinhold [9].

2.6. *Edman degradation and NH_2-terminal residue analysis*

The amino acid sequence was determined by three methods of Edman degradation:

– Edman procedure coupled with dansylation as described by Gray [10]. Dansyl amino acids were identified by thin-layer chromatography according to Woods and Wang [11].

– Edman degradation and identification of PTH-amino acid derivatives by thin-layer chromatography.

– Determination of residual amino acids after each step of Edman degradation.

2.7. *Periodate oxidation*

Oxidation of the glycopeptide was carried out with sodium metaperiodate (final concentration 0.01 M) at pH 4.0 at 4°C in the dark. The ratio of periodate to glycopeptide was approximatively 2 : 1 (w/w). Periodate consumption was followed by monitoring absorbance at 310 nm. After periodate oxidation for a minimum at 9 hr, the samples were treated with sodium borohydride. The solution of treated glycopeptides was then hydrolysed with H_2SO_4 at pH 1.0 for 24 hr at room temperature. This material was then applied to a column of Sephadex G 15 (1.5 X 150 cm) and eluted with water. The orcinol reacting fraction, excluded from the gel, was lyophilized.

3. Results and discussion

The elution pattern of parotid saliva obtained by gel-filtration on Sephadex G 200 in shown in fig.1. α-amylase (F_3) was located by absorbance at 278 nm and parotid glycoprotein (F_2) by orcinol–sulphuric acid reaction. Amino acid and carbohydrate compositions are presented in tables 1 and 2. Proline, glycine and glutamic acid (or/and glutamine) were the amino acid residues present in greatest concentration: approx. 80 per cent. They were followed by lysine, arginine (10 per cent) and by aspartic acid and serine. The protein content (46 per cent by dry weight) was calculated from amino acid analysis.

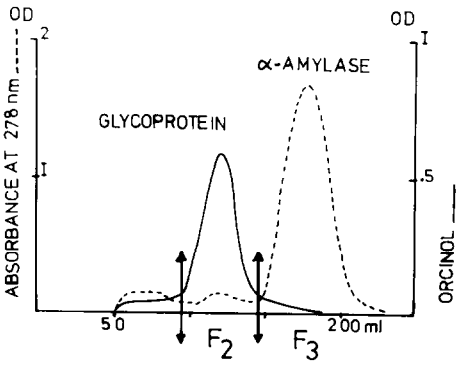

Fig.1. Fractionation of human parotid saliva on Sephadex G 200.

Table 1
Amino acid compositions of the parotid glycoprotein and papain-pronase glycopeptide.
(Values expressed as residues/100 amino acid residues)

Amino acids	Native glycoprotein (F_2 Sephadex G 200)	Papain-pronase glycopeptide (F_3 Sephadex G 50)
Aspartic acid	5.52	22.23
Threonine	1.54	
Serine	6.56	19.36
Glutamic acid	22.41	29.20
Proline	26.85	
Glycine	17.68	29.21
Alanine	1.95	
Cystine	0	
Valine	1.87	
Methionine	0	
Iso-leucine	1.18	
Leucine	1.98	
Tyrosine	0.90	
Phenylalanine	0.80	
Lysine	4.91	
Histidine	1.22	
Arginine	4.63	

Edman degradation of the papain-pronase glycopeptide
(Values expressed as molar ratios of amino-acids)

		1st step	2nd step	3rd step
Gly ne	1.0	0.5	0.2	0.2
A artic acid	0.8	0.9	0.4	0.2
utamic acid	1.0	1.1	1.4	0.6
Serine	0.7	0.7	0.7	0.7

Table 2
Carbohydrate composition of the parotid glycoprotein and papain-pronase glycopeptide.
(Values expressed as μmoles/g dry weight material)

Sugars	Native glycoprotein (F_2 Sephadex G 200)	Glycopeptide (F_3 Sephadex G 50)	Glycopeptide after periodate oxidation
Sialic acid	29	n.d.	n.d.
Fucose	347	1061	0
Galactose	322	644	0
Mannose	372	894	605 (1)
N-Ac.-glucosamine	488	1192	1176 (2)
N-Ac.-galactosamine	0	0	0

n.d.: not determined.

Carbohydrate chains (28 per cent by dry weight) were heterosaccharidic units with galactose, mannose, *N*-acetyl glucosamine and fucose. Neuraminic acid was present in low concentration. Minor variations in the carbohydrate content and in the amino acid moiety were observed from one donor to another.

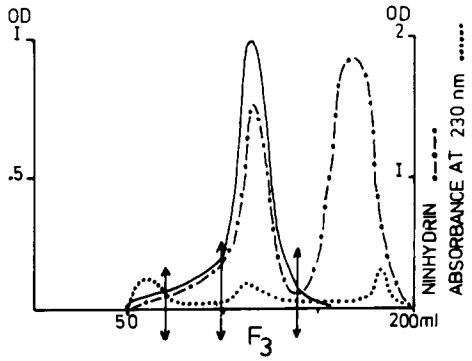

Fig.2. Chromatography of papain-pronase digest on Sephadex G 50.

Fig.2 presents the elution profile of the papaine-pronase digest from a Sephadex G 50 filtration column. The eluate was pooled into four fractions and lyophilized. Fraction 3 corresponding to the carbohydrate containing peptide region was subjected to amino acid and carbohydrate analysis (see tables 1 and 2).

This glycopeptide fraction was found to be homogeneous, giving a single amino acid, glycine, on N-terminal analysis. The molar ratios (see table 1) of the amino acids were not integral numbers, especially for serine partially degraded during 5.6 N HCl hydrolysis. Nevertheless, sequencing by Edman techniques was carried out.

The results summarized on table 3 indicate that the carbohydrate bound amino acid residue could only be determined by comparing the data obtained with

Table 3
Amino-acid sequence of the Sephadex G 50 glycopeptide

Edman-dansylation (TLC)	Edman-PTH-amino acid derivatives (TLC)	Edman-amino acid composition
Gly	Gly	Gly
As(x)	no Asp/Asn	As(x)
Gl(x)	Gln	Gl(x)
Ser	Ser	

Thus, the amino-acid sequence was identified as:

Gly–Asn–Gln–Ser
|
Carbohydrate

three methods of Edman degradation. The PTH carbohydrate linked asparagine did not migrate on thin layer chromatography. That this technique failed to give a result for one of the amino acid residues demonstrates that this residue was glycosylated.

It can be seen that only one single type of glycopeptide has been found and studied. Its mol. wt would be approx. 2600 based on the chemical composition and assuming a single carbohydrate unit. Carbohydrate analysis of the same preparation after periodate oxidation showed three moles sugar per mole of aspartic acid. The results (table 2) indicate a molar ratio of mannose to *N*-acetylglucosamine: 1 : 2.

Our results support the concept of the presence in the human parotid saliva glycoprotein of a single type of carbohydrate–peptide linkage with only one amino acid sequence. The occurrence of serine in the vicinity of a carbohydrate linked asparagine has already been described in several glycoproteins [12].

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