GLYCOSYLATED PROLINE-RICH PEPTIDE OF HUMAN PAROTID SALIVA. CIRCULAR DICHROISM STUDY

J. P. AUBERT*, A. BOERSMA**, M. H. LOUCHEUX-LEFEBVRE*, P. DEGAND** and G. BISERTE**

* *Institut de Recherches sur le Cancer de Lille et Unité n° 124 de l' INSERM - B. P. 3567, 59020, Lille Cédex, France*

** *Unité n° 16 de l'INSERM Place de Verdun, 59045, Lille Cédex, France*

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1. Introduction

Previous studies have demonstrated the presence in human parotid saliva of proline-rich proteins and glycoproteins [1–5]. Interest in these components is related to their high affinity to hydroxyapatite minerals [6,7]. Thus it is possible to speculate that they may play a role in mineralization processes of enamel surfaces.

The characterization of unglycosylated proline-rich proteins of low molecular weight allow to consider that these components are metabolic precursors during the biosynthesis of the glycoprotein. Therefore they would represent the polypeptide moiety of the proline-rich glycoprotein used for the circular dichroism study presented in this work.

On the other hand, several investigations reported on the protein content of secretory granule membranes [8,9]. These preparations have a similar amino acid composition to the proline-rich proteins isolated from human parotid saliva. It is tempting to suggest that these proteins may be involved in the secretion process of serous cells.

2. Materials and methods

2.1. *Purification of the proline-rich proteins*

Collection of parotid saliva. Parotid saliva was collected from a single donor with the aid of the Lashley appartus for periods of 30 min and lemon gum stimulus. Cellular debris, separated by centrifugation (5000 rev/min for 10 min) was discarded. Saliva after dialysis was lyophilized.

Chromatography of parotid saliva on Sephadex. 300 mg of parotid saliva were applied to a column (70 X 4.5 cm) of Sephadex G 200. The column was washed with 0.1 M phosphate, 0.2 M sodium chloride buffer pH 8.0 and 5 ml fractions were collected. Ninhydrin reaction and ultraviolet absorption at 278 and 230 nm were measured. Carbohydrate-containing components were located by means of orcinol-sulphuric acid reagent [10].

Purification of the proline-rich proteins. Each fraction eluted from Sephadex G 200 was concentrated on UM_2 amicon membrane and lyophilized. Fraction 2 was analyzed for its chemical composition. Fraction 3 was further applied to a Biogel P_{10} column (100 X 2.5 cm) using as eluant distilled water. The excluded peak was then chromatographed on Biogel P_{100} washed by 0.1 N acetic acid. The 230 nm ultraviolet absorption profile showed 5 fractions.

2.2. *Analytical procedures*

Protein samples were hydrolyzed in evacuated and sealed tubes for 24 hr in 5.6 N HCl at 100°C. The hydrolysate was examined using a Technicon amino acid analyzer [11]. Carbohydrate composition was determined by gas-liquid chromatography with a Perkin-Elmer 900 analyzer, as described by Reinhold [12].

2.3. *Circular dichroism measurements*

The circular dichroism (CD) was measured with a dichrograph Jouan III in 0.01 cm pathlength at an absorbance less than 1.5 optical density unit. The proteins were dissolved in 10 mM sodium phosphate buffer and 0.1 M NaF. The pH was 6.45. The protein

concentrations of the solutions were determined by the Lowry method [13] and were always about 1 mg/ml. The ellipticity was expressed as mean residual molar ellipticity [Θ] in degrees · cm^2 · $decimole^{-1}$. The mean residue weight was 100. The ellipticity curves were constructed from at least five spectra.

3. Results

3.1. *Purification, amino acid and carbohydrate analysis of proline-rich glycoproteins*

Sephadex G 200 chromatography resulted in the isolation of four fractions, numbered in order of elution from the column (fig.1). Fraction 2 corresponded to the elution of a 36 000 mol. wt proline-rich glycoprotein. Fraction 3 was separated with subsequent chromatography on Biogel P_{10} and Biogel P_{100}. The elution profiles showed a molecular weight heterogeneity. Fraction 4 exhibited α-amylase activity.

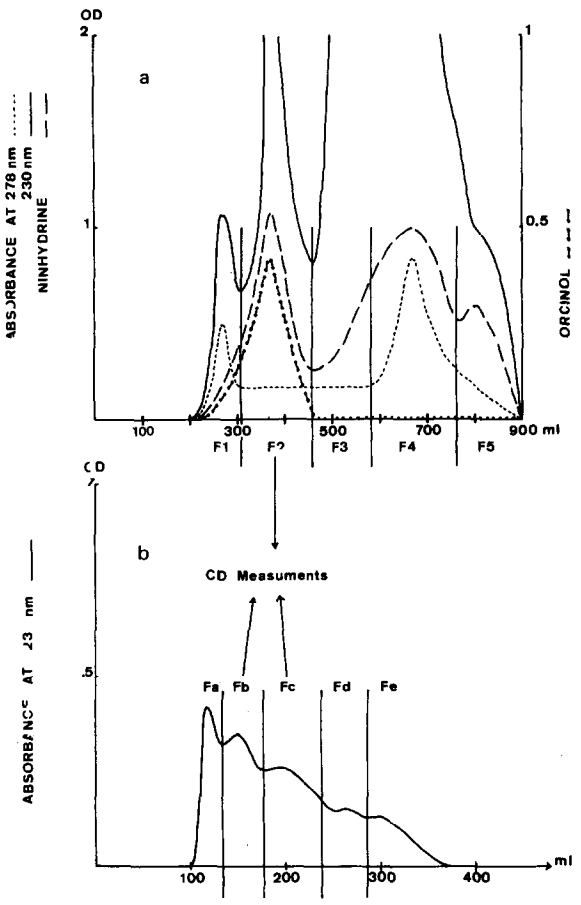

Fig.1. (a) Sephadex G 200 chromatography of the whole human parotid aliva. (b) Biogel P 100 chromatography of Sephadex G 200 fraction 3.

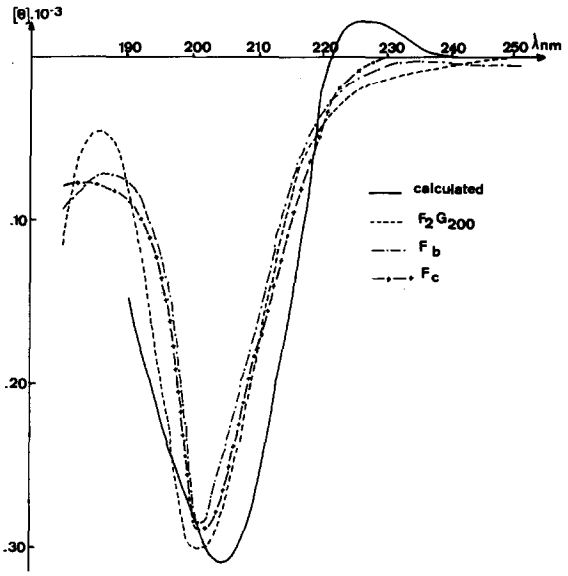

Fig.2. Experimental dichroic spectra of glycoproteins F_2 G 200, F_b, F_c and calculated spectra corresponding to the following composition : 28.6% β-turns, 64.3% PPII, and 7.1% unordered structure.

Amino acid composition of the different fractions obtained on Biogel P_{100} are presented in table 1. Proline, glycine and glutamic acid or glutamine were the amino-acid residues in greatest concentration. carbohydrate analysis showed the presence of fucose, galactose, mannose, N-acetylglucosamine (table 2).

3.2. *Circular dichroism study*

The dichroic spectra were recorded for the fractions F_2 G 200, F_b and F_c. Fucose, mannose, galactose and glucose which show an absorption band below 180 nm do not overlap with the amide chromophore bands. However for amino sugars the first band is around 205 nm. On the basis of optical activity of *N*-acetyl glucosamine and *N*-acetyl galactosamine and assuming simple additivity of contributions, its effect would be less than 2% at 205 nm.

Table 1
Amino acid composition of the human parotid proline-rich glycoproteins
(values expressed as residues/100 amino acid residues)

	F_2 Sephadex G 200	Biogel P 100[a]				
		F_a	F_b	F_c	F_d	F_e
Asp	5.43	7.71	5.79	5.72	6.61	12.99
Thr	0.96	3.96	1.72	1.89	1.70	1.75
Ser	5.37	7.95	5.99	7.40	5.61	8.20
Glu	20.51	16.15	18.73	17.90	20.42	23.03
Pro	35.63	20.81	30.96	32.02	31.55	20.07
Gly	16.46	15.61	20.41	20.14	18.38	14.75
Ala	0.93	4.27	1.79	2.28	1.87	2.31
1/2 Cys	—	—	—	—	—	—
Val	0.96	3.15	1.08	0.93	1.29	4.07
Ile	0.52	1.96	0.66	0.53	0.79	1.80
Leu	1.03	3.61	1.05	0.98	1.55	3.82
Tyr	0.41	2.46	0.97	0.85	0.58	0.56
Phe	0.48	1.71	0.41	0.46	0.34	1.24
Lys	5.05	5.08	5.07	4.16	4.52	2.32
His	1.12	2.10	1.98	1.27	1.56	0.80
Arg	5.14	3.46	3.40	3.48	3.25	2.30

[a] F_a, F_b, F_c, F_d, F_e represent the different fractions issued from Biogel P 100 chromatography of F_2 Sephadex G 200.

The CD spectra obtained for F_2 G 200, F_b and F_c between 180 and 250 nm (fig.3) are identical and reminiscent of a polyproline II (PPII) helical structure spectrum. Indeed, it is a basically non-conservative spectrum with a strong negative band centered at 202 nm and an ellipticity $[\Theta]_{max}$ = 29 800, 28 400 and 28 800 for F_2 G_{200}, F_b and F_c respectively. An another extrema is observed at about 185 nm with a corresponding negative value of the ellipticity. It is difficult to decide if this band exists or not in the PPII helical structure spectrum because a corresponding experimental dichroic spectrum below 188 nm could not be found. The calculated spectrum [14] exhibits however a small negative band at this wave-

Table 2
Carbohydrate composition of human parotid glycoproteins
(values expressed as μ moles/g)

	Sephadex G 200	Biogel P 100		
		F_a	F_b	F_c
Fucose	341	207	463	408
Galactose	305	250	417	405
Mannose	339	228	450	433
N-Ac. glucosamine	389	312	538	511

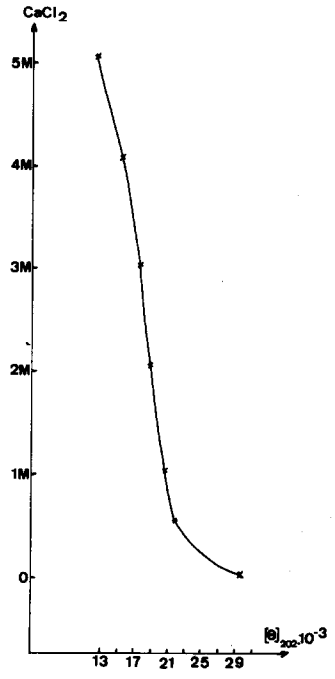

Fig.3. Variation of the ellipticity at 202 nm against 6 M $CaCl_2$ added in aqueous solution of glycoproteins.

length. The weak dichroic band around 225 nm which is generally observed in the PPII spectra is not seen in our experimental spectra. We will discuss this point in part 4.

4. Discussion

The main difference between the three experimental spectra and the PPII spectra concerns the small positive band near 225 nm which is never seen for glycoproteins. Even though this band is characteristic of the PPII helical structure [14], we do not think that its loss would correspond to a disorganising of the helical structure. We believe however that the isolated proteins do not necessarily contain both PPII helical structures and unordered forms. Other unidentified structures may well exist and explain the shape and the experimental dichroic spectra.

Indeed, it is well established that an important fraction of the residues of globular proteins can be involved in turn regions [15]. Aspartic acid, glycine, serine, threonine and proline are especially abundant in these turns [15,16]. Moreover in these folds, the β-turn conformation has been recognized as an important element : such a structure, constituted by four peptide residues allows the peptide chain to fold back on itself and a hydrogen bond is formed between the carbonyl group of residue i and the peptide hydrogen of residue i + 3 [17]. Various types on β-turns exist.

Recently, the calculations of the circular dichroism corresponding to β-turn conformations have been carried out by Woody [18] : the predicted CD curves of the β-turn resembles the so called β-II type curve described by Fasman and Potter [19]. In other words, this spectrum is similar to that of the β-structure poly-L-lysine [20] but with its extrema red-shifted by 5–10 nm. Let us now consider the protein composition given in table 1. Important quantities of residues found in turn regions, that is aspartic acid, serine and of course, glycine and proline are present.

Therefore we have tried to fit the experimental spectrum with calculated spectra obtained by the linear combination of PPII helical structure (from Ronish and Krimm) [14], the β-turn spectrum (taking the so-called type I class B spectrum from Woody) [18] and the unordered spectrum (Chen and Yang) [21]. The results are given on fig.3 where one can see that an excellent agreement exists between the experimental spectra of the three studied fractions (F_2 Sephadex 200, F_b and F_c Biogel P_{100}) and the calculated spectra corresponding to 64.3% of PPII structure, 28.6% of β-turn and 7.1% of unordered structure. This result explains that the small positive band at 225 nm is not observed in the proteins : this is due to the fact that, in this region, the β-turn exhibits a negative band.

A weak discrepancy exists between the ellipticity at the maximum of the dichroic spectra of both experimental and calculated curves. Therefore, we have tried to observe if a transformation to an unordered chain conformation may occur in presence of concentrated (1 M, 2 M, 3 M, 4 M, 5 M) $CaCl_2$ [22]. Unfortunately $CaCl_2$ is not sufficiently transparent in the far ultraviolet to permit measurements below 200 nm. Therefore, the variation ellipticity at 202 nm was followed at a function of $CaCl_2$ added : it is the same for the three glycoproteins (fig.4), and is in excellent agreement with the existence of a PPII structure.

Moreover, an interesting point to discuss is the heterogeneity of glycosylated proline-rich polypeptides. As reported by Hay [23], components with a mol. wt of 6000 and 12 000 can be isolated from human parotid saliva. It is suggested that they may be involved in the biosynthesis of this proline-rich glycoprotein with larger molecular weight. The hypothesis of a polymerization and glycosylation of these elementary structures might be suggested in the biosynthesis of the larger molecular weight proline-rich glycoproteins and in the maturation processes of zymogen granules.

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