PAROTID α-AMILASE ACTIVITY: A POSSIBLE ROLE FOR PROLINE-RICH PROTEINS

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

*Unité n° 16 de l'INSERM Place de Verdun, 59045 Lille Cédex, France, and *Institut de Recherches sur le Cancer de Lille et Unité n° 124 de l'INSERM B.P. 3567, 59020 Lille Cédex, France*

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1. Introduction

Human salivary amylase exists in multiple forms, some of which are glycosylated [1,2]. The glycosylated part appears to be constant with different donors and with various origin: parotid and submandibular glands [3].

In this paper we investigate the nature of two peaks with α-amylase activity, which were obtained during the purification of the main components of human parotid saliva: α-amylase, proline-rich glycoproteins and proteins (PRP). Previous investigations had shown that this kind of molecules consisted of about 64% of prolyproline II helical structure, 29% of β-turns and 7% of unordered structure [4,5]. That these peculair proteins may modify or protect the α-amylase activity is discussed.

2. Materials and methods

2.1. *Isolation of α-amylase*

Human parotid saliva was collected from a single donor as previously described [4]. α-Amylase was purified by gel-filtration chromatography.

2.1.1. Chromatography of parotid saliva on Sephadex G-200

300 mg of parotid saliva were applied to a column of Sephadex G-200 (4.5 × 70 cm). The column was equilibrated with 0.1 M sodium phosphate 0.2 M NaCl buffer, pH 8.0 and 5 ml fractions were collected. The effluent was monitored at 278 and 230 nm. Carbohydrate containing components were located by means of orcinol–sulphuric acid reagent [6]. Each fraction from Sephadex G-200 were studied for chemical composition or further purified.

2.1.2. Fractionation of α-amylase on Sephadex G-100

Fraction 4 (Sephadex G-200) was subsequently loaded into a column of Sephadex G-100 (2.5 × 100 cm). The column was equilibrated with 0.1 M sodium phosphate, 0.2 M NaCl buffer, pH 8.0. The elution profile was determined by 230 and 278 nm ultra violet absorption, orcinol–sulphuric acid reaction and amylase activity measurements.

2.2. *Amylase assay*

Amylase activity was measured by the method of Bernfeld [7]. One unit of amylase was defined as the amount of enzyme which hydrolyzed 5 mg of starch per 15 min at 37°C.

2.3. *Chemical composition*

Amino acid analysis was performed with a Technicon amino acid analyzer after 5.6 N HCl hydrolysis for 24 h at 100°C [6]. Carbohydrate composition was determined by quantitative gas–liquid chromatography [8].

2.4. *Circular dichroism measurements*

The circular dichroism was measured with a Jobin–Yvon dichrograph R. J. Mark III in 0.01 cm pathlength at an absorbance less than 1.5. The samples (FIIa, FIIb, FIIc) were dissolved in 10 mM sodium phosphate buffer and 0.1 M NaF. The pH was 6.45. The protein

concentration of the solutions was determined by the Lowry method [9] and was always about 1 mg/ml. The ellipticity [Θ] was expressed as mean residual molar ellipticity [Θ] in degrees. $cm^2 \cdot decimole^{-1}$. The mean residue weight was 100 for FIIc, 105 for FIIb and 109 for FIIa. The ellipticity curves were constructed from at least five spectra. Even if the protein is glycosylated the CD spectrum is characteristic of the protein moiety; indeed, we have seen in a previous paper [5] that the contribution of the glycan chains to the ellipticity in the 180–250 nm domain is always less than 2% and therefore could be neglected.

3. Results

3.1. *Purification of parotid α-amylase*

Sephadex G-200 chromatography (fig.1a) resulted in five fractions numbered in order of elution from the column. Fraction 1 was a minor peak. Fractions 2 and 3 corresponded to proline-rich glycoproteins as previously demonstrated [5]. Fraction 4 exhibited α-amylase activity and was further purified on Sephadex G-100 column (fig.1b). Two peaks were separated. The second FII appeared to be heterogeneous: three fractions FIIa, FIIb, FIIc were pooled according to their α-amylase activity and to their relative absorption at 278 and 230 nm.

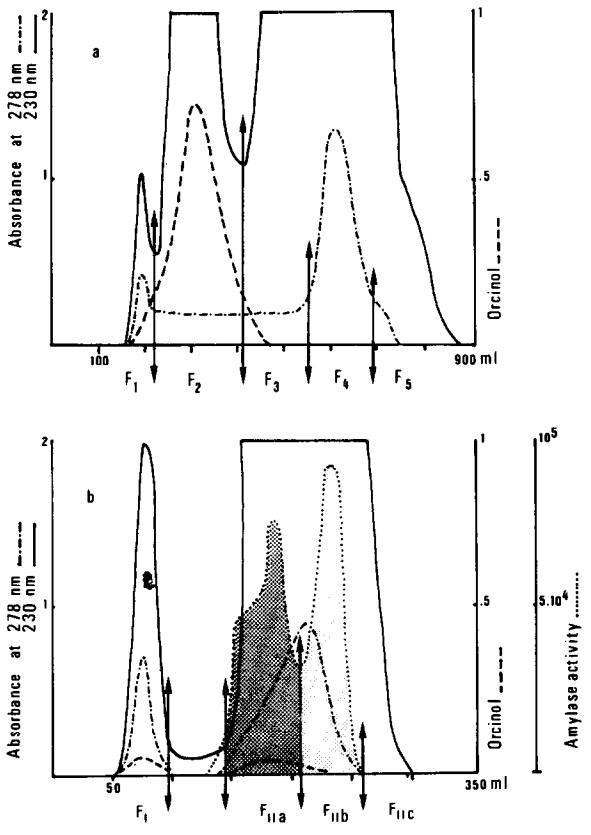

Fig.1. (a) Elution diagram of human parotid saliva on Sephadex G-200 column. (b) gel filtration chromatography of G-200 F_4 on Sephadex G-100.

3.2. *Chemical analysis*

Amino acid and carbohydrate analysis were performed in fractions FIIa, FIIb and FIIc (Sephadex G-100) and in fraction F_2 (Sephadex G-200) (see table 1).

The two fractions FIIa and FIIb which exhibited α-amylase activity had different amino acid compositions. FIIa composition was very close to that of α-amylase preparations obtained by other workers [1,10]. FIIc was rich in three amino acid residues, proline, glycine and glutamic acid and/or glutamine).

Table 1
Amino acid composition of α-amylase fractions and proline-rich glycoprotein or protein of human parotid saliva

Amino acids	A	B	C	D
Aspartic acid	6.75	13.36	10.62	9.50
Threonine	1.81	4.76	3.16	0.90
Serine	7.89	8.19	8.02	6.48
Glutamic acid	15.60	7.12	13.03	22.43
Proline	28.78	6.52	17.11	26.55
Glycine	22.58	13.56	16.44	18.37
Alanine	2.13	6.13	4.39	2.77
Half-cystine	0	1.42	0.87	0
Valine	1.14	6.13	4.16	2.77
Methionine	0	1.67	0.61	0
Isoleucine	0.59	3.93	2.54	0.82
Leucine	1.51	5.21	3.71	2.83
Tyrosine	0.48	4.18	2.66	0.53
Phenylalanine	0.50	4.54	3.05	0.83
Lysine	4.77	5.00	4.48	3.59
Histidine	1.14	2.71	1.50	0.47
Arginine	4.14	5.54	3.67	1.17

(A) F_2 Sephadex G-200: Proline-rich glycoprotein
(B) FIIa Sephadex G-100: α-amylase
(C) FIIb Sephadex G-100: α-amylase-PRP
(D) FIIc Sephadex G-100: PRP

(Values expressed as residues/100 amino acid residues)

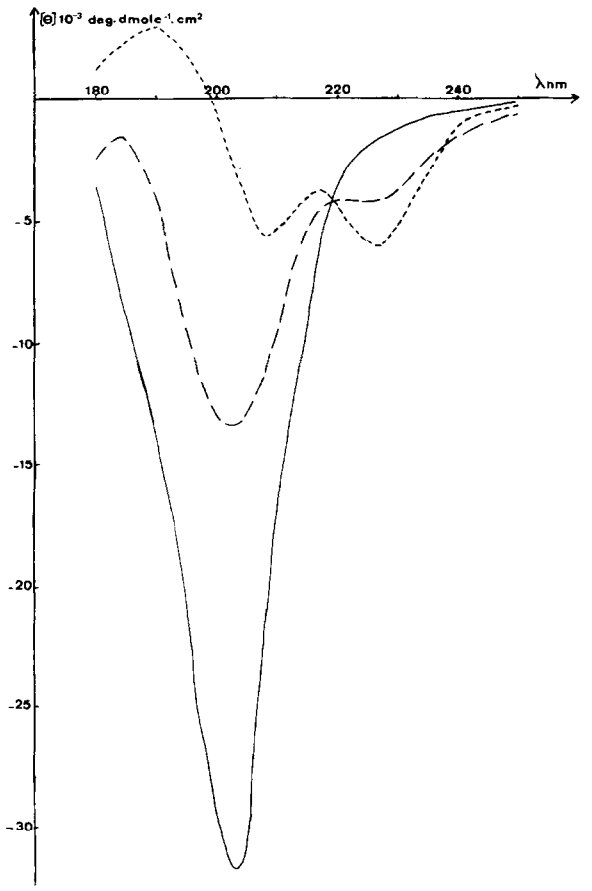

Fig.2. Experimental dichroic spectra of FIIa (—·—) FIIb (– – –) and FIIc (——) fractions (Sephadex G-100).

This fraction was devoided of carbohydrate. Thus, this PRP was very similar to the glycoprotein characterized by chromatography on Sephadex G-200 (F_2).

Amino-acid composition of FIIb was reminding of PRP but was found to contain α-amylase activity and thus might be considered as a complex between α-amylase and PRP. FIIa and FIIb contained less than 2% carbohydrate.

3.3. *Enzymatic activity*

α-Amylase activity of the PR glycoprotein (F_2 Sephadex G-200) and fractions FIIa, FIIb, FIIc (Sephadex G-100) was measured. The enzymatic activity was related to dry weight powder. The results are presented in table 2.

Table 2
α-Amylase activity according to the method of Bernfeld

F_2 Sephadex G-200	363 U/mg
FIIa Sephadex G-100	8722 U/mg
FIIb Sephadex G-100	15023 U/mg
FIIc Sephadex G-100	1612 U/mg

Results expressed as units per mg of dry weight powder.

Proline-rich proteins (FIIc) were added to α-amylase preparation (FIIa). The amylase/PRP ratios (w/w) were 1/1; 1/2; 1/4; 1/8; 1/16; 1/32. As shown fig.3, the presence of PRP increased α-amylase activity.

3.4. *Circular dichroism study*

Non-glycosylated PRP (FIIc Sephadex G-100), fractions FIIa and FIIb were studied and the corresponding CD spectra between 180 and 250 nm are shown in fig.2. The shape of the fraction FIIc spectrum reminds of the one obtained for PRP [5]. Indeed a strong negative band centered at 203 nm with an ellipticity [Θ] max: $31 \cdot 10^3$ is observed.

The FIIa CD spectrum is very different from that of FIIc. More especially, it presents two negative bands at 227 and 208 nm and a positive band at 190 nm with ellipticities of −5900, −5600 and 3000 respectively. It does not correspond to one definite

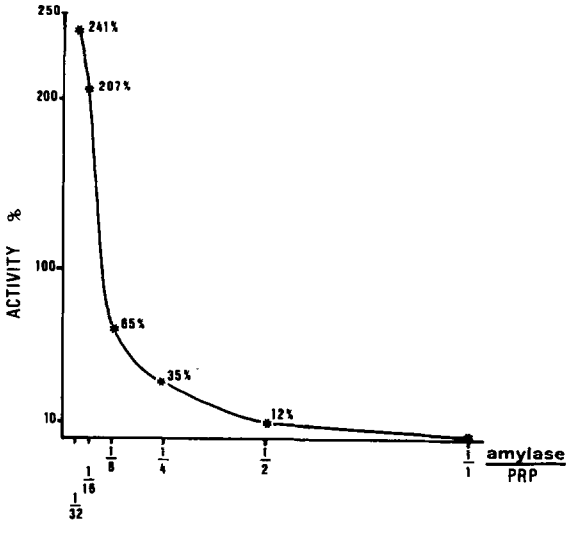

Fig.3. Effect of PRP on α-amylase activity.

structure or a combination of the usual three structures found in proteins (i.e. α-helical, β-sheets and unordered structures). Since this fraction FIIa exhibits α-amylase activity and presents an amino acid composition similar to α-amylase, the spectrum should correpond to amylase. The CD band centered at 227 nm does not exactly correspond to the position of the $n \rightarrow \pi^*$ transition of the α-helical form. We do think that such a band is due to a weak proportion of the helical form in the α-amylase. Moreover, the solution presented a light turbidity when we recorded the spectrum. Urry [10] showed that such a turbidity induced a disortion of the CD spectrum, more especially a shift to high values of wavelength was observed. Therefore, in our case the band at 227 nm could correspond to the $n \rightarrow \pi^*$ transition generally observed at 222 nm.

Finally, the FIIb CD spectrum is intermediate between the spectra of FIIa and FIIc. A weak band is observed at 227 nm and a more important at 203 nm. This is in excellent agreement with the enzymatic activity (Sephadex G-100) and chemical studies.

4. Discussion

When setting our results three comments had to be made:

(1) α-amylase in human parotid saliva *could be separated in two forms* and it should be noticed that if one of the two (FIIa) might be considered according to amino acid analysis as a pure amylase preparation, the second one (FIIb) appeared with the same criteria and with circular dichroism spectra as a complex of α-amylase and PRP. The chemical and physical results led us to consider that α-amylase was present in low quantity in FIIb fraction.

(2) The significative difference of amylase activity between FIIa and FIIb may be related to interactions between α-amylase and proline-rich proteins. PRP addition to α-amylase increase the specific activity of this enzyme (fig.3).

(3) The biological role of the proline-rich proteins and glycoproteins had not yet been defined. An hypothetical function of these PRP might be also to protect the enzyme during the secretion process of the parotid gland. As reported by Amsterdam, the PRP are present as structural units of the zymogen granule membranes [11].

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