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THE PROTEINS OF THE CONTENT OF THE SECRETORY GRANULES OF THE RAT PAROTID GLAND

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SUMMARY

The proteins of the secretory granules of the rat parotid gland were characterized by sodium dodecylsulfate gel electrophoresis, by chromatography of [^{3}H]proline-labeled proteins on DEAE-cellulose and by amino acid analysis.

Sodium dodecylsulfate gel electrophoresis of the secretory granule content showed five principal proteins and a limited number of minor components. Only two of the principal bands could be identified as known secretory enzymes of the parotid gland. One was identified as the α-amylase and one as deoxyribonuclease. Peroxidase and ribonuclease form minor portions of the secretory proteins.

The other three major proteins constitute, together, about 60 % by weight, of the secretory granule content proteins. Of these, one which represents more than 30 % of the total granule protein was found to contain uniquely high amounts of leucine residues (21 mole%). Another one of these principal proteins was relatively rich in cysteine residues (7 mole%).

The fifth principal protein was found to contain high amounts of proline (28 mole%) glutamic acid (17 mole%) and glycine (18 mole%) residues. Its amino acid composition was very similar to that of the proline-rich proteins that were previously shown to be present in the membrane isolated from these granules. This protein, however, differed from the "membranous" proline-rich proteins by several criteria.

Two minor glycoproteins of the secretory granule content were also found to be rich in proline residues (37 mole%). As with the other proline-rich proteins of the granule, they contained no sulphur-containing amino acids, stained faintly pink with Coomassie Blue and were underestimated by the Lowry method. They differ however, from all the other proline-rich proteins of the granule by having a significantly higher content of threonine, less glycine (9 mole%) and much less glutamic acid (3 mole%).

Of the principal proteins, only the deoxyribonuclease and the half-cystine-rich proteins were positively stained by periodic acid Schiff staining.

The possible functions of the leucine-rich, the half cystine-rich and the various proline-rich proteins are discussed.

INTRODUCTION

The parotid gland and its secretory granules have been widely used for studying the mechanisms of the intracellular transport and secretion of secretory proteins [1–5]. The secretory granules have been shown to serve as the intracellular pool in which the proteins are accumulated and concentrated and from which they are secreted after fusion of the secretory granule membrane with the plasma membrane [3, 5, 6].

The enzymes amylase and deoxyribonuclease were shown, in previous studies [6, 7] to be major components of the proteinaceous content of the rat parotid secretory granules. However, by comparing the specific activities of the enzymes in the whole secretory granule lysates to those of the purified enzymes, the amount of both could be estimated to form no more than 50 %, by weight, of the total [6, 7].

Most of the rest of the protein content has not been identified with any known enzyme. There is no proteinase [6] or lipase [8] activity in the secretory granule of the parotid gland. The phosphatase activity which is exhibited by the granule [6] is localized exclusively in the membranes which are prepared from that fraction (ref. 9 and Wallach, D., unpublished). Peroxidase was shown by histochemical studies to be present in the secretory granules of the rat parotid gland [10]. As shown in this paper, this enzyme and the ribonuclease which was shown to be present in these granules [11] represent a tiny portion of the granule protein.

In a previous communication we showed that the membrane of the parotid secretory granule contains several proline-rich proteins and that these can be specifically extracted from the membrane by treatment with salt solution [12]. From the radioactivity pattern of the secretory granule content proteins on acrylamide gels it appeared that some of the content proteins are also enriched in proline residues [12]. It was therefore of interest to identify these proteins and to determine their relationship to the proline-rich proteins of the membrane. The membranous proline-rich proteins were shown to be underestimated by the Lowry method for protein determination and to be faintly stained on acrylamide gels by Coomassie Blue [12]. Several proteins with similar amino acid composition which were isolated from human parotid saliva [13–18] are similarly underestimated by the Lowry method [13, 15] and faintly stained on acrylamide gels by Coomassie Blue and Amido Black [13, 19]. Therefore, in order to identify the proline-rich proteins, we prelabeled the granule proteins in vivo by [^{3}H]proline and identified the proline-rich proteins after fractionation on DEAE-cellulose column, by their high specific radioactivity. The other principal proteins of the content of the granule were also identified and characterized by sodium dodecylsulfate gel electrophoresis and amino acid analysis.

METHODS AND MATERIALS

Isolation of secretory granules from rat parotid gland, isolation of the secretory granule membrane and content, the extraction of the proline-rich proteins from the secretory granule membrane and the sodium dodecylsulfate gel electrophoresis technique were as previously described [12]. Labeled granules were isolated 2 h after intravenous injection of 50 μCi of [^{3}H]proline (0.5 Ci/mmol) in 0.6 ml of 0.15 M NaCl, to each rat.

Fractionation of the secretory proteins on a DEAE-cellulose column

Lyophilised secretory granule content proteins were solubilized in 5 mM sodium phosphate buffer, pH 8.0, and equilibrated with that buffer by dialysis (12 h at 4 °C). The concentration of the protein solution after dialysis was 36 mg/ml. A sample of 1.4 ml of the solution was loaded on a column (1 × 40 cm) packed with DEAE-cellulose which was preequilibrated with the above potassium phosphate buffer. The proteins were eluted from the column with 75 ml of the 5 mM buffer followed by two linear gradients (300 ml 5–35 mM; 300 ml 35–200 mM) of the same buffer. The flow rate was kept at 30 ml/h. 90 fractions of 2 ml were collected followed by 130 fractions of 4 ml. The entire procedure was performed at 4 °C.

Better separation between Proteins C_{11}, C_{12} and C_8 (see Fig. 2 for the nomenclature of the proteins) was achieved by using an initial buffer concentration of 3 mM. Proteins C_{11} and C_{12} were eluted with the 3 mM buffer and Protein C_8 with a 3–15 mM buffer gradient.

The sample of the Protein C_5 that was subjected to amino acid analysis was further purified from Protein C_3 (α-amylase) as follows: A sample of 800 μg of the isolated C_5 protein in 0.25 ml 40 mM potassium phosphate buffer, pH 8.0, was applied to a small DEAE-cellulose column (0.9 × 2.5 cm) which had been equilibrated with the above buffer. The column was washed with 60 ml of the 40 mM buffer (30 ml/h) and then the C_5 protein was eluted by 2.5 ml of 150 mM of that phosphate buffer. The concentration of contaminating amylase was decreased from 91 units/mg protein to 6 units/mg protein. The remaining amylase may be calculated to form 0.4 %, by weight, of the total protein.

Analytical methods

Protein was estimated by the Lowry reagent [20] and by determination of organic nitrogen with the Nessler reagent after ashing according to the method of Kjeldhal [21, 22]. To measure the radioactivity liquid samples of 1 ml were mixed with 12 ml toluene/Triton X-100 mixture [12] and counted in Packard liquid scintillation counter Model 3320. The salt concentration in the fractions eluted from the DEAE-cellulose was estimated by measuring the conductivity of the solutions.

Amylase was determined according to Bernfeld [23]. DNAase was assayed by measuring the formation of acid-soluble deoxypentose compounds with dephenylamine reagent [24]. The conditions of incubation were as previously described [7].

RNAase was determined by a modification of the method of Kunitz [25, 26]. The assayed sample was incubated with 1 mg of RNA in 0.2 ml of assay mixture which contained magnesium acetate (5 mM), sodium acetate (50 mM) and imidazole (30 mM) and was brought to pH 6.7 by acetic acid. The incubation was carried out for 40 min at 37 °C. The reaction was stopped by the addition of 50 μl of uranyl reagent (0.75 % (w/v) uranyl acetate in 25 % (w/v) perchloric acid). The tubes were centrifuged (5 min, 1600 × g at 4 °C) and the supernatant was diluted to a volume of 3 ml. The absorbance of the supernatant at 260 nm was determined using cuvettes with a light path of 1 cm. A unit of ribonuclease activity is defined as the amount of enzyme which releases acid-soluble material in the amount equivalent to 1.0 absorbance unit under the above described conditions.

Assay of the secretory granule peroxidase based on peroxidation of iodide [27] or *O*-dianisidine [28] gave positive qualitative results but were insufficiently sensitive.

Therefore, peroxidase was estimated by following the increase in the absorbance at 470 nm during peroxidation of guaiacol. The assay system contained: 50 mM potassium phosphate buffer, pH 7.4; 0.4 % (v/v) guaiacol and 0.001 % hydrogen peroxide. The assay was carried out in cuvettes with a light path of 1 cm in a final volume of 1.2 ml at 25 °C in a Gilford recording spectrophotometer Model 2000. Sodium azide (1 mM) completely inhibited the enzyme action. A unit of enzyme is defined as the amount which causes an increase of 1 absorbance unit per min.

Hydrolysis of protein samples for amino acid analysis was done in 6 M HCl at 110 °C for 22 h in ampules sealed under vacuum. Oxidation of the cysteine residues of Protein C_7 was done with performic acid as described by Moore [29]. The analysis was done with a Beckman 120B amino acid analyser.

The samples of Proteins C_3, C_7 and C_8 that were used for the analyses did not show in gel electrophoresis any sign of contaminating bands even when 20 μg of each protein were applied on the gel. The sample of Protein C_5 showed a fine band of the contaminating Protein C_3 (see above) and the sample of Proteins $C_{11}+C_{12}$ contained small amounts of Protein C_1. We could detect bands of as low as 0.1 μg protein by the staining method used.

Materials

Deoxyribonucleic acid (calf thymus) was purchased from Sigma, DEAE-cellulose (microgranular Cat. No. 24521) from Whatman, Guaiacol from Mallinckrodt and hydrogen peroxide from Merck. RNA from yeast (Schwarz-Mann) was purified by precipitation with 20 % $HClO_4$ [30].

RESULTS

The pattern of the secretory granule content proteins in sodium dodecylsulfate gel electrophoresis

The pattern of the secretory granule content proteins in sodium dodecylsulfate gel electrophoresis is shown in Figs 1A and 2. Five principal bands are seen to constitute most of the protein. They are designated Proteins C_3, C_5, C_6, C_7 and C_8 (Fig. 2). Of these major bands, only Proteins C_6 and C_7 are stained by the periodic acid Schiff procedure. Of the minor bands that are indicated in Fig. 2, Proteins C_1, C_2, C_3' and C_4 are also stained by the periodic acid Schiff procedure.

The fractionation of the granule proteins on the DEAE-cellulose column revealed two additional proteins: Proteins C_{11} and C_{12}. These two proteins form very diffuse bands after staining with Coomassie Blue or by the peroidic acid Schiff procedure. Due to their overlap with the principal glycoprotein bands, Proteins C_6 and C_7, it is difficult to identify them in the pattern of the whole secretory granule content.

While most of the bands are stained blue by Coomassie Blue, the band comprising Protein C_5 as well as Protein C_{11} are stained in a faint reddish colour. The proline-rich proteins which are found in the membranes isolated from these granules are stained a similar colour [12].

Fractionation of the secretory granule content proteins on a DEAE-cellulose column

The fractionation pattern of secretory granule content proteins prelabeled in

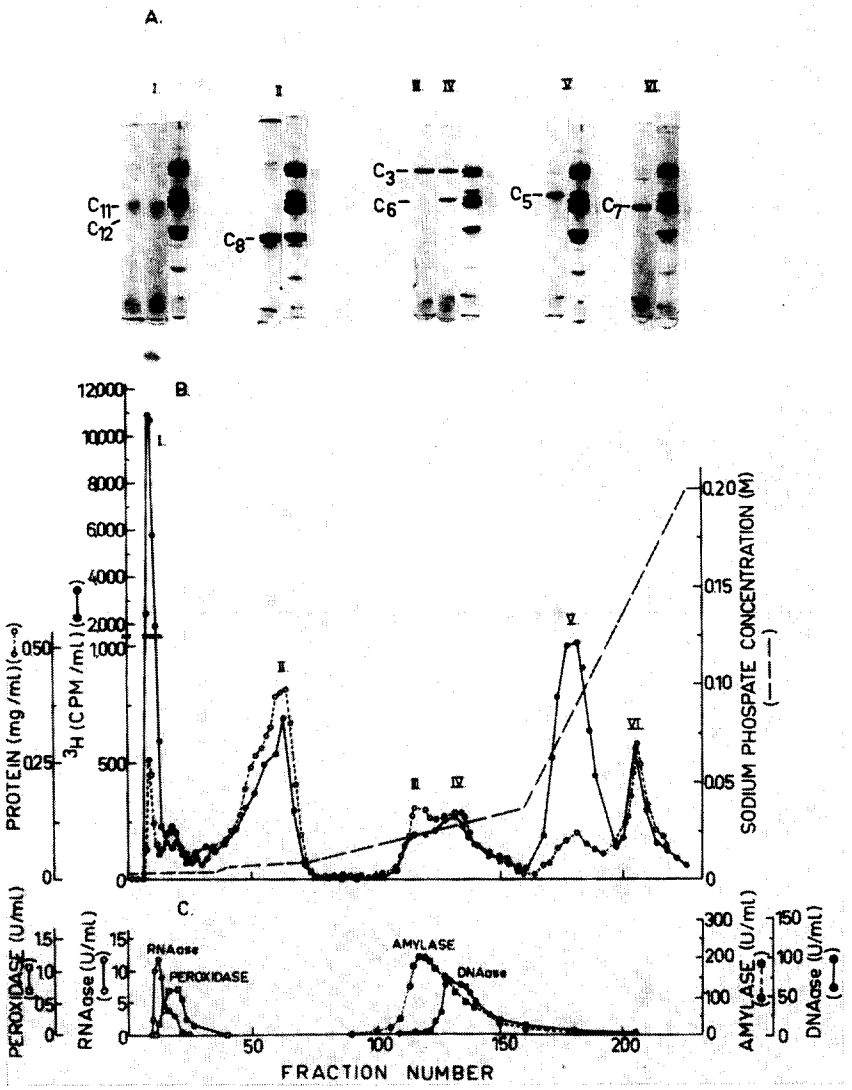

Fig. 1. Fractionation of [^{3}H]proline-labeled secretory granule content proteins on a DEAE-cellulose column. (A) Comparison of the pattern of the column-eluted proteins to that of the total secretory granule content proteins (sodium dodecylsulfate polyacrylamide gel electrophoresis). (B) Tritium label, protein concentration and buffer concentration in the different fractions. (C) Enzymatic properties of the fractions. The chromatographic procedures and the various assays were as described in Methods and Materials. Before application to the acrylamide gel, the fraction (or several combined fractions) were dialized against large volumes of water for 5 h and concentrated by lyophylisation. The amount of proteins which were applied on the gels were: I. Fraction 8, 3 μg; Fraction 9, 3 μg. II. Fractions 55–65, 10μg, III. Fractions 116–120, 3 μg. IV. Fractions 132–136, 3 μg. V. Fractions 172–176, 3 μg. VI. Fractions 208–209, 3 μg. The total secretory granule content was run in parallel. The amounts which were applied on the gels were: 50 μg in I, II and V; 30 μg in VI; and 10 μg in III and IV. The high concentration of buffer in Fractions 217–226 interfered with the protein determination.

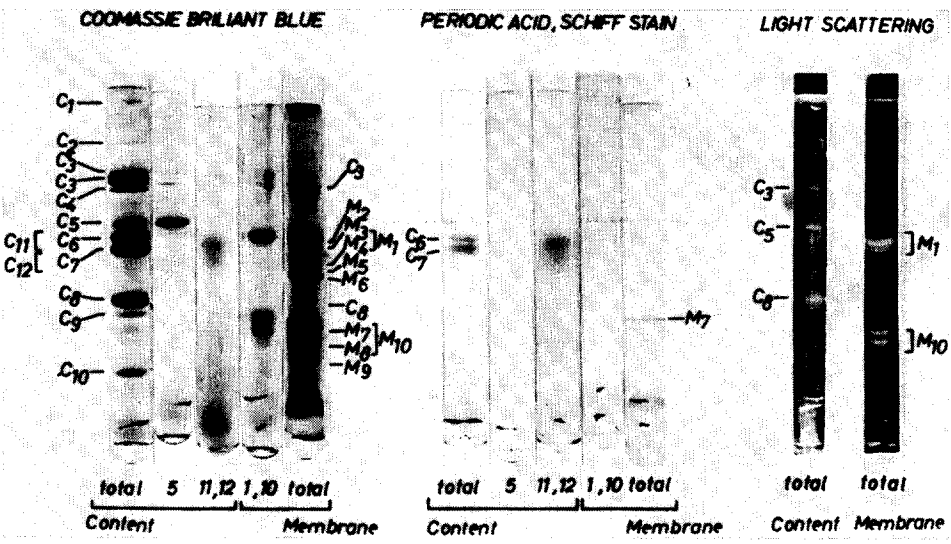

Fig. 2. The pattern of the proline-rich proteins in sodium dodecylsulfate gel electrophoresis. Staining was with Coomassie Brilliant Blue and with periodic acid Schiff procedure. Light scattering was from non-stained gels which were fixed in 50 % trichloroacetic acid/20 % sulfosalicylic acid solution [12]. The fractions of the secretory granule content (Proteins C_5, C_{11} and C_{12}) are the same as those for which the electrophoretic pattern is given in Fig. 1. Proteins M_1 and M_{10} were extracted from the membrane preparation as previously described [12]. The amounts of proteins which were applied on the gel for staining with Coomassie Blue and for light scattering were: Secretory granule content, 50 μg; Proteins C_5, C_{11} and C_{12}, 3 μg; Proteins M_1+M_{10}, 11 μg; secretory granule membrane, 50 μg. For the periodic acid Schiff procedure: 100 μg of the secretory granule content and secretory granule membrane proteins, 10 μg of Proteins C_5, $C_{11}+C_{12}$, and 11 μg of Proteins M_1+M_{10} were applied on the gel. Because of the low contrast of the photograph of the periodic acid Schiff procedure, the band comprising Protein M_7 was retouched with a soft pencil.

vivo with [^{3}H]proline is shown in Fig. 1. The properties of the isolated proteins are given in Table I.

Peak I and Peak V. These two peaks are more heavily labeled with proline than the others (Fig. 1B and Table I). They are expected, therefore, to contain proline-rich proteins. By sodium dodecylsulfate gel electrophoresis Peak V is shown to contain Protein C_5 (Fig. 1A).

Peak I contains several proteins. The fractions with the highest amount of radioactivity contain the glycoproteins Protein C_{11} and Protein C_{12} and very low amounts of Protein C_1 (Fig. 1A). The three polypeptides do not seem to belong to a single complex protein molecule. Protein C_{11} is eluted from the DEAE-cellulose a little ahead of Protein C_{12} (Fig. 1A) and Protein C_1 can be separated from Proteins C_{11} and C_{12} on a Sephadex G-200 column (results not shown).

Though the RNAase and the peroxidase are eluted in 5 mM buffer, their elution pattern does not correlate to that of the proline-rich proteins Proteins C_{11} and C_{12}. The label reaches a maximum in Fraction 9, the elution of RNAase begins in Fraction 10 and the elution of peroxidase begins in Fraction 12.

The RNAase and the peroxidase constitute only a small fraction of the granule protein (Figs 1B and 1C). Protein C_{10} seems to be the RNAase. It appears in the frac-

TABLE I

CHARACTERISTICS OF THE SECRETORY PROTEINS ELUTED FROM THE DEAE-CELLULOSE COLUMN

The data which are illustrated in Fig. 1B served for estimation of the amount of protein in each peak. The amount of Protein C_3 (amylase) was calculated by dividing the total activity of eluted amylase by the specific activity of amylase in Fractions 116–120 (which contained pure amylase). The amount of Protein C_6 (DNAase) was calculated by subtracting the total amount of Protein C_3 from that of the total protein in Peaks III and IV. The molecular weights of the proteins were estimated by sodium dodecylsulfate gel electrophoresis using standards of known molecular weight (see Fig. 2 in ref. 12). The amount of nitrogen per mg of "Lowry" protein and the specific radioactivities were determined on the dialyzed samples which were analysed by gel electrophoresis (Fig. 1).

Peak No.	Principal band on gel electrophoresis	Mol. wt	"Lowry" protein (% of total applied on the column)	μg Nitrogen/mg "Lowry" protein	Labeling by [^{3}H]proline (cpm/mg protein)	Staining by periodic acid schiff procedure	Other properties
I	C_{11}	approx. 30 000	1–2	340	15 000	+	Proline rich
	C_{12}	approx. 25 000					
II	C_8	20 000	33	120	1 000	−	Leucine rich
III	C_3	56 000	25	160	1 000	−	Amylase
IV	C_6	31 000	8	—	—	+	DNAase
V	C_5	37 000	10	280	8 000	−	Proline rich
VI	C_7	29 000	25	130	1 200	+	Relatively rich in half cystine

tions which show RNAase activity and runs on the acrylamide gel parallel to bovine pancreatic RNAase A (Sigma R-4875).

Estimation of the organic nitrogen in Peaks I and V (Table I) shows that the Lowry method underestimated the proteins quantities, as observed previously with the proline-rich proteins of the granules membrane [12].

Peak II. Of the six peaks, Peak II contains the highest amount of protein (Table I). It contained the principal protein, Protein C_8, and very low amounts of the minor protein, Protein C_9.

Peak III. The amylase is eluted in pure form in Peak III. In gel electrophoresis it appears as a single protein band (Protein C_3). When purified by precipitation by glycogen [31] the amylase preparate contains also the minor glycoprotein Protein C_3. The latter might be an isozyme of amylase. Such isozymes were indeed shown to be present in human parotid saliva [32].

Peak IV. Peak IV contains the glycoprotein DNAase which forms the band designated Protein C_6. Pancreatic DNAase (Worthington DPEF) runs on the acrylamide gel in parallel with this band and is also stained by the periodic acid Schiff procedure.

Peak VI. Peak VI contains the glycoprotein Protein C_7. In runs of the total secretory granule content protein and of the isolated C_7 protein, Protein C_7 sometimes appeared on the acrylamide gel as two closely located bands.

TABLE II

AMINO ACID COMPOSITION OF PROTEINS ELUTED FROM THE DEAE-CELLULOSE COLUMN

Amino acid	Protein C_8	Protein C_7	Protein C_3
	mol/100 mol of amino acids		
Pro	3.7	5.7	5.2
Gly	11.0	7.6	13.3
Glx	6.7	11.3	7.9
Asx	13.7	14.1	14.9
Arg	2.4	3.5	6.3
Lys	3.8	5.5	4.9
His	0.0	3.2	2.1
Thr	5.4	5.3	3.5
Ser	9.2	6.8	6.5
Ala	6.0	3.9	5.9
Val	9.5	6.9	7.3
Ileu	4.9	2.1	4.3
Leu	20.9	7.8	4.5
Cys	0.2	7.2*	1.1
Meth	0.2	1.6*	1.8
Tyr	0.4	5.5	3.9
Phe	2.1	3.7	5.3

* Determined after oxidation with performic acid [29]. In an analysis that was done without such oxidation the cysteine comprised 6.2 mole% and the methionine 1.0 mole% of the amino acids.

TABLE III

AMINO ACID COMPOSITION OF THE PROLINE-RICH PROTEINS

Amino acid	mol/100 mol of amino acids					
	Rat parotid secretory granules			Human parotid saliva		
	Proteins $C_{11}+C_{12}$*	Protein C_5	Proteins M_1+M_{10}**	ref. 15	ref. 17	ref. 13
Pro	36.5	28.3	35.6	35.3	33.6	27.1
Gly	9.2	17.6	22.8	20.3	20.5	22.0
Glx	3.2	17.3	22.2	19.4	20.9	19.4
Asx	8.4	7.0	2.4	5.3	6.7	7.6
Arg	4.6	4.1	5.8	4.3	4.9	4.7
Lys	0.2	3.7	2.7	4.7	5.0	1.7
His	3.6	2.9	0.6	1.6	0.9	2.5
Thr	6.5	1.5	1.1	0.6	0.4	1.2
Ser	4.4	6.3	2.3	4.8	5.2	4.3
Ala	5.1	3.2	1.1	1.2	0.5	1.0
Val	2.5	2.4	1.1	0.8	0.6	2.8
Ileu	3.3	1.0	0.6	0.5	0.3	2.1
Leu	0.3	2.4	1.3	1.1	0.7	2.7
Cys	0.0	0.0	0.0	—	—	0.0
Meth	0.0	0.0	0.3	0.1	0.0	0.0
Tyr	—***	1.2	0.3	0.3	0.0	0.0
Phe	—***	1.2	0.4	0.2	0.0	0.9

* Peak I.

** Extracted from the membrane by 0.15 M NaCl [12].

*** The large peaks of glucosamine and galactosamine partly overlapped with the tyrosine and with the phenylalanine. The tyrosine is roughly estimated to comprise 1–2 mole% and phenylalanine 4–5 mole% of the amino acids in the protein.

Amino acid composition of the secretory granule proteins

Tables II and III give the amino acid composition of the proteins which were isolated from the secretory granule content on a DEAE-cellulose column as described above.

The amino acid composition of Protein C_3 is almost identical to that which was reported for α-amylase from pig pancreas [33]. Protein C_8 contains a very high amount of leucine residues (21 mole%), a low amount of sulphur-containing amino acids, a low amount of tyrosine and no histidine. Protein C_7 contains a much higher amount of cysteine (7.2 mole%) than any of the proteins that were isolated from the secretory granule. About 21 residues of cysteine are calculated to be found in a single molecule of this protein (mol. wt 29 000).

As suggested by the pattern of labeling of the proteins, (Fig. 1B and Table I) the content of the proline in Protein C_5 and Proteins $C_{11}+C_{12}$ is much higher than in Proteins C_3, C_8 and C_7 (Tables II and III). The amino acid composition of Protein C_5 is very similar to that of the group of proline-rich proteins which were extracted from the membrane and to that of several proteins and glycoproteins which were isolated from human parotid saliva (Table III). All contain high amounts of proline, glutamic acid and glycine residues in a ratio of about 3 : 2 : 2. These three amino acids comprise 70–80 % of the amino acids in the molecule. All these proteins contain

no cysteine and are essentially devoid of methionine, tyrosine and phenylalanine residues.

Though the glycoproteins Proteins C_{11} and C_{12} are similar to the other proline-rich proteins by their high content of proline and by the lack of sulphur-containing amino acids, they differ markedly from the other proline-rich proteins in several respects. They contain significantly higher amounts of threonine, fewer glycine and much fewer glutamic acid residues. Large peaks which correspond to glucosamine and galactosamine appeared in the amino acid analysis of Proteins C_{11} and C_{12}. Since these amino sugars partly overlapped with the eluted tyrosine and phenylalanine we did not get an exact value for the quantity of these amino acids. From a rough approximation the content of phenylalanine in these proteins seems however, (Table III) to be much higher than in the other proline-rich proteins.

Since we did not separate Protein C_{11} from Protein C_{12} it is not possibly to say to what extent these two proteins differ from each other in their amino acid composition. It is difficult to assume, however, that the peculiarities in the amino acid composition of this fraction arise from the fact that one of the two proteins is proline low unless we assume that the other one contains much more than 37 % proline.

Comparison of the electrophoretic pattern of the different proline-rich proteins

The identification of proline-rich proteins in the content of the granule raises the question whether these proteins are identical to the proline-rich proteins which were previously shown to be attached to the membrane of the granule [12]. Comparing the electrophoretic pattern of the various proline-rich fractions, the proline-rich proteins of the content of the granule are found to differ from the membranous proline-rich proteins by the following criteria: None of the membranous proline-rich proteins are stained by the periodic acid Schiff procedure. They differ thus from the proline-rich glycoproteins of the content of the granule Proteins C_{11} and C_{12} (Fig. 2).

The relative mobility on the gel of the "membranous" proteins which form Band M_1 is similar to that of Protein C_5 (Fig. 2). But they differ from Protein C_5 by their staining properties. All the membranous proline-rich proteins are extracted from the acrylamide gel by the staining procedure [12]. Therefore, by staining in Coomassie Blue, they form very diffuse and faint bands which fade quickly and cannot be restained. Unlike the membranous proline-rich proteins, Protein C_5 is fixed to the gel by the staining procedure though it seems that it forms only weak interactions with the Coomassie Blue. Therefore, it forms a sharp band which fades slowly and can be restained. A temporary fixation of the membranous proteins is achieved in 50 % (w/v) trichloroacetic acid 20 % (v/v) sulfosalicylic acid solution. In this solution the fixed proteins can be visualized, without staining, as sharp light scattering bands (Fig. 2). This method was found to be relatively specific for the membranous proline-rich proteins. The proteins of the content of the granule, including the proline-rich proteins and the proline-low proteins of the membrane form, with this method, much fainter bands.

DISCUSSION

Three secretory proteins, Proteins C_5, C_7 and C_8, which constitute together about half of the amount of the protein in the rat secretory granule, could not be identified with any of the enzymes as yet known to be present in the rat parotid saliva.

Some idea of their function might be hypothesized from their amino acid composition. The ratio of the hydrophilic to apolar amino acids in the C_8 protein is much lower than the ratio which was reported to be characteristic for soluble proteins, soluble lipoproteins or even membranous lipoproteins [35]. Yet, the solubility of this protein in water and the fact that it is readily extracted from the granules in aqueous media show that this molecule is quite hydrophilic. It seems possible therefore that this protein behaves as a detergent and interacts with hydrophobic components of the food in a way that makes it available to attack by digestive enzymes.

The saliva contains appreciable amounts of immunoglobulin A [36]. Of the four kinds of polypeptide chains which are known to be part of the secretory immunoglobulin A, only the J chain shows some similarities to a protein that was isolated from the secretory granule. Its molecular weight (23 000) is similar to that of Protein C_7. In addition the high amounts of glutamic acid (11 mole%) aspartic acid (15 mole %) and cysteine (5 mole%) in the J chain from human colostrum [37] are similar to those in Protein C_7. The amount of several amino acids in Protein C_7 (Glycine, Proline, Arginine, Isoleucine, Phenylalanine) is more similar to that which is found in rabbit colostral J chain [38]. The similarities between Protein C_7 and the J chain would justify further study.

The data presented indicate that Proteins C_5, C_{11}, C_{12} are not identical with proteins of Bands M_1 and M_{10} although they are all rich in proline. It is therefore possible that Proteins M_1 and M_{10} are not artifically absorbed by the membrane during isolation but are specifically attached. The proline-rich Proteins C_5, M_1 and M_{10} share many similarities with several proline-rich proteins which were isolated from human parotid and submaxillary saliva [13–19, 39]. The human salivary proline-rich proteins are supposed to take part in processes of remineralization of the teeth enamel [13, 14]. There is definitely a large difference between the pattern of proline-rich proteins in the human and in the rat parotid saliva. The glycosilated human proline-rich proteins comprise at least 35 % of the parotid salivary proteins and almost 100 % of the glycoproteins which are secreted from that gland [40, 15]. In the rat parotid all the proteins which are similar to the human proline-rich proteins are not glycosilated and the majority of the glycoproteins consist of the proline-low Proteins C_6 and C_7. In spite of these differences between the human and the rat proteins, the similarities in the amino acid composition of the various proline-rich proteins from the two sources seems to indicate that they all fulfill similar functions.

This does not seem to be the case with the proline-rich glycoproteins Proteins C_{11} and C_{12}. The amino acid composition of these proteins differs in many respects from that of all the salivary proline-rich proteins which have been, so far, studied. It is possible that the amino sugars that are found in these molecules are bound to the threonine residues which are found in these proteins in significantly higher amounts than in all the other proline-rich proteins. Such linkage of the carbohydrate units to threonine was shown in several proteins, including submaxillary glycoproteins [41–43].

The interaction of some of the proline-rich proteins with the membrane of the parotid secretory granule might be important for the stability of the granule [12]. Another way by which the secretory granule might be stabilized, is interaction of the proteins which are accumulated in the granule with each other. Such aggregation was shown for the secretory granules of the exocrine pancreas [44, 45]. The change in the

opacity of the secretory granule of the parotid gland during the transition from condensing vacuoles to mature secretory granules [3, 4] is indicative of the occurance of such aggregation also in the parotid gland. In the neurosecretory granules, neurophysine was shown to bind the accumulated peptides, possibly stabilizing the concentrated material inside the granule [46]. It seems likely that the function of one, or several of the proteins which are found in the rat parotid granule, is to interact with the other secretory proteins to form a stable complex inside the granule.

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