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Studies on LM Protein Appearing in Submandibular Glands of Isoproterenol-treated Rats. II. Physicochemical Properties

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Some physicochemical properties of the purified LM protein isolated from submandibular saliva of IPR-treated rats were studied. The molecular weight of the LM protein was estimated to be 12000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and its isoelectric point was 4.75. The sugar content of the protein was estimated to be 1.62% and the calcium content was 1.07 mol/mol of the protein. Phosphorus and magnesium were not detectable. Amino acid analysis revealed that the protein cotained relatively large amounts of aspartic acid (asparagine) (14.8%), glutamic acid (glutamine) (14.8%) and serine (11.6%), and small amounts of proline (1.9%) and glycine (5.4%). A part of the amino acid sequence from the N-terminal was determined; the N-terminal was proline, followed by five hydrophobic amino acids. These results clearly indicate that the LM protein isolated from submandibular glands of IPR-treated rats is different from proline-rich proteins previously isolated by others from parotid glands of IPR-treated rats.

Keywords——rat submandibular glands; isoproterenol; hypertrophy and hyperplasia; saliva proteins; LM protein; dansyl-Edman degradation; isoelectric focusing; amino acid sequence

Isoproterenol (IPR), a β-adrenergic drug, is known to accelerate protein secretion from and to stimulate macromolecule synthesis in the salivary glands of rats.[2–6] Acidic and basic proline-rich proteins, which increase dramatically in the parotid glands of IPR-treated rats, have been characterized by several workers.[7–9] The molecular weights of basic proline-rich proteins ranged from 15000 to 18000.[8] On the other hand, Menaker *et al.*[9] reported the appearance of a large mobile (LM) band in the saliva of IPR-treated rats as determined by polyacrylamide gel electrophoresis. We have previously shown[10] that this LM protein is synthesized in rat submandibular glands upon chronic administration of IPR and is secreted through the action of β-adrenergic receptors. This paper describes some properties of the LM protein, which is different from the proline-rich proteins partially characterized by others.[7–9,11–13]

Materials and Methods

Materials——LM protein was purified as described previously.[10]

Molecular Weight Determination——Electrophoresis was carried out in 15% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) by the method of Dunker and Rueckert.[14] Bovine serum albumin (MW 66000) (Sigma Chemical), ovalbumin (MW 46000), aldolase (MW 40000), chymotrypsinogen (MW 25700) (Boehringer Mannheim), lysozyme (MW 14400) (Seikagaku Kogyo Co., Tokyo) and insulin (MW 5700) (Fluka AG) were used as marker proteins. After electrophoresis, the gels were stained with 0.1% Coomassie brilliant blue R-250 as described by Weber and Osborn.[15] The mobilities of the LM protein and insulin were measured without preincubation with 2-mercaptoethanol.

Isoelectric Focusing——The isoelectric point of the LM protein was measured with an LKB Model Multiphor electrofocusing apparatus.[16] Filter paper moistened with LM protein solution was placed on the polyacrylamide gel plate (PAG-plate: pH ranges 3.5 to 9.5, LKB). During the operation, the voltage was gradually elevated to 1120 V over 2 hr. After electrophoresis, the pH gradient on the gel was measured at 1 cm intervals and the isoelectric point of the LM protein was estimated.

Amino Acid Analysis——LM protein was hydrolyzed at $110° \pm 2°$ with 6 N HCl, and analyzed with an amino acid analyzer (JEOL model 6 AH). Values obtained from samples hydrolyzed for 24, 36 and 48 hr

were corrected by extrapolation to zero time of hydrolysis.[17] The amount of tryptophan was determined by the method of Edelhoch.[18]

Determination of the Amino-terminal Residue——The amino-terminal residue was determined by dansyl-Edman degradation following the procedure of Gray and Hartley.[19,20] Standard dansylated amino acids were prepared according to Weiner *et al.*[21] After dansylation of the LM protein, the reaction product was hydrolyzed, and spotted on polyamide layer sheet (5×5 cm) (Seikagaku Kogyo Co., Tokyo). Two-dimensional thin-layer chromatography was carried out as follows: the plates were first developed with (a) 1.5% formic acid in water. For the second dimension, the plates were first developed with (b) benzene–acetic acid (9: 1, v/v), then air-dried and developed again with (c) ethyl acetate–acetic acid–methanol (20: 1: 1, v/v/v). The spots of dansylated amino acids were detected under ultraviolet irradiation (254 nm).

Amino Acid Sequence Analysis——The amino acid sequence of the LM protein was determined by using an automatic sequence analyzer (JAS-47K).[22]

Other Analytical Methods——Total phosphorus was determined by the method of Miyazaki and Takemura.[23] Calcium and magnesium contents were measured by the method of Dawes,[24] with a Shimadzu model MAF-1 autoabsorption spectrometer. Sugar content was determined by the phenol–H_2SO_4 method of Dubois *et al.*[25] with glucose as a standard. Protein was determined by the method of Lowry *et al.*[26] with bovine serum albumin as a standard.

Results and Discussion

The molecular weight of purified LM protein was determined by SDS-polyacrylamide gel electrophoresis to be 12000 from the plots shown in Fig. 1. This value agreed fairly well with the behavior on Sephadex G-75 gel filtration (distribution coefficient 0.49).[10] Thus, the molecular weight of the LM protein is smaller than those of most submandibular saliva proteins,[27] and is also smaller than those of acidic proline-rich protein (24500) and basic proline-rich proteins (15000—18000).[7] The isoelectric point of the LM protein was determined to be 4.75 by isoelectrofocusing. In the electrofocusing, the protein gave a single protein band, confirming its homogeneity (data not shown).

Table I shows the amino acid composition of the LM protein. The presence of a relatively large amount of aspartic acid (asparagine), serine and glutamic acid (glutamine) are characteristic features of the LM protein. On the assumption that the molecular weight of LM protein is 12000, the number of residues is calculated to be 98. These data clearly show that the LM

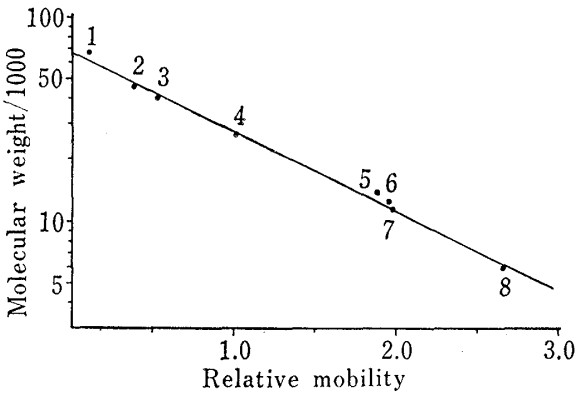

Fig. 1. Calibration of Molecular Weight by SDS-polyacrylamide Gel Electrophoresis

1, bovine serum albumin; 2, ovalbumin; 3, aldolase; 4, chymotrypsinogen; 5, lysozyme; 6, cytocrome C; 7, LM protein; 8, insulin.

Table I. Amino Acid Composition of LM Protein

Amino acid	LM Protein	Acidic proline-rich protein[a]
	mol/100 mol	
Lys	7.6	2.3
His	3.8	5.0
Arg	1.0	2.7
Asx	14.8	11.9
Thr	2.5	1.2
Ser	11.6	4.7
Glx	14.8	19.2
Pro	1.9	29.5
Gly	5.4	17.1
Ala	4.2	2.0
Cys	1.6	0
Val	9.6	1.1
Met	1.0	0
Ileu	4.0	0.4
Leu	7.2	1.4
Tyr	3.1	0.6
Phe	4.8	0.4
Trp	1.0	—

a) Results reported by Fernandez-Søresen and Carlson.[7]

protein is different from a proline-rich peptide (57 residues)[11] and calcium binding proline-rich phosphoprotein (106 residues)[12] isolated from human parotid saliva. The amino-terminal sequence of the LM protein was partially determined. As shown in Fig. 2, dansyl-OH, dansyl-NH_2 and dansyl-proline were detected on the polyamide layer sheet, indicating that the N-terminal amino acid is proline. The amino acid sequence determined by Edman degradation is shown in Fig. 3. Among the N-terminal twently-eight residues of the LM protein, the 14th, 16th, 21st and 26th amino acids could not be identified by gas chromatography or by thin layer chromatography. These amino acid residues may be basic amino acids or sulfur-containing amino acids. The 27th tyrosine and 28th aspartic acid are not certain, because only rather faint spots were detected on the thin layer plates. After twenty-eight steps of Edman degradation, twenty-three residues were identified as phenylthiohydantoin amino acids. It is noteworthy that the six amino acids from the N-terminal have side chains of hydrophobic nature.

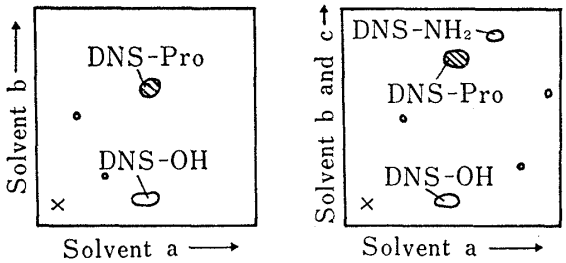

Fig. 2. Chromatography of the Dansylated Amino Acid Obtained after Edman Degradation of LM Protein (see "Materials and Methods")

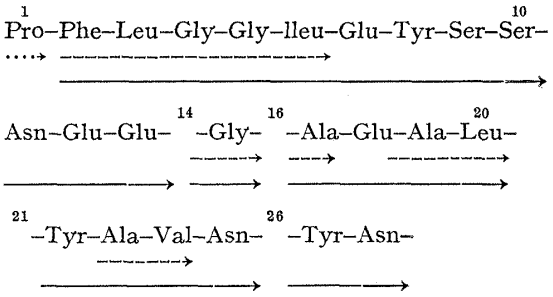

Fig. 3. Sequence Study of LM Protein

Symbols used are as follows; ····→, the residues identified as dansylated amino acids by polyamide layer chromatography (see "Materials and Methods"); ——→, the residues identified as phenylthiohydantoin amino acids by thin layer chromatography on silica gel plates with xylene-isopropanol (7: 2, v/v); - - - →, the residues identified as phenylthiohydantoin amino acids by gas chromatography (JGS 20K-FP gas chrchromatograph; JEOL Ltd., Tokyo) using a silicone SE-30 coated Gaschrom Q column.

In contrast to the LM protein, a proline-rich protein has many –Gly–Pro–Gly– repeating units.[11] The calcium binding proline-rich phosphoprotein contained many negatively charged residues in the N-terminal part and proline residues in the C-terminal part of the molecule.[12]

The contents of phosphorus, calcium and magnesium in the LM protein were determined (Table II). No significant amounts of phosphorus or magnesium was detected in the LM protein. However, the protein contained 1.07 mol of calcium per mol of 12000 molecular weight protein. Unlike the LM protein, the acidic proline-rich protein[8] and the calcium binding proline-rich phosphoprotein[12] contained two atoms of phosphorus per mol of protein.

Table II. Phosphorus, Calcium and Magnesium Contents of LM Protein

Phosphorus was measured by the method of Miyazaki and Takemura.[23] Calcium and magnesium were measured by the method of Dawes.[24]

	LM Protein	Acidic proline-rich protein[a]
	mol/mol protein	
Phosphorus	0	2.00
Calcium	1.07	0.05
Magnesium	0.12	0.10

a) Results reported by Muenzer *et al.*[8]

The sugar content of the LM protein was estimated to be 1.62% based on the calibration curve for glucose, corresponding to one residue/mol of protein. The acidic proline-rich protein is a glycoprotein containing 3.5 mol of galactose and 10 mol of galactosamine.[8]

In spite of many differences in physicochemical properties, the LM protein and proline-rich protein are both exocrine proteins which cause hyperplasia and hypertrophy of the salivary glands. The proline-rich proteins are thought to be involved in pellice formation on teeth, since they bind calcium and bind to the surface enamel of teeth.[11–13] However, the physiological role and biochemical properties of the LM protein have not yet been clarified.

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References and Notes

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