HISTONES ISOLATED FROM HUMAN PAROTID FLUID

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Received November 27,1972

SUMMARY

Histones were isolated from human parotid saliva by ion exchange chromatography on Bio-Rex 70. The amino acid compositions of the purified histones indicated the presence of three predominant types, ranging from 35 to 65 per cent in basic amino acid residues. All three histones were rich in histidine which constituted nearly half of the basic amino acid residues. The polyacrylamide disc electrophoretic patterns also indicated presence of three major histone bands, and thus supported the chromatographic elution pattern and the amino acid profiles of the histones.

INTRODUCTION

Basic molecules in human parotid fluid have been previously observed by various investigators in both electrophoretic and chromatographic separations (1,2,3). These unknown basic proteins have not been previously isolated in pure form and chemically characterized. Proteins of similar size and charge have been isolated from either animal or plant eukaryotic cells and chemically characterized. Cationic proteins from bovine spinal cord (4), erythrocytes (5), rainbow trout testis (6), and pea buds (7,8) have been separated from purified chromatin by acid extraction and chromatography. These proteins have been referred to as histones, and were shown in the rainbow trout testis to be replaced by protamines during spermatogenesis (6). Marked differences as well as striking similarities in amino acid profile have been noted between histones from above-mentioned sources and calf thymus histones used as the standard of reference. The present communication describes a chromatographic technique for the isolation of histones from a cell-free, glandular secretion.

MATERIALS AND METHODS

Protein concentration in eluates of chromatographic separations was monitored as ultraviolet light absorption at the dual wavelengths of 230 nm and 280 nm. Lysozyme was assayed by measuring the rate of lysis of Micrococcus lysodeikticus cells according to the method of Shugar (9). Peroxidase was assayed according to a method outlined in the "Worthington Enzymes and Enzyme Reagents" manual.* Amino acid analyses were performed on a Beckman-Spinco Model 120B amino acid analyzer following hydrolysis of histone samples for 22 hours in 6 N HCl, under vacuum, at 110°C. Polyacrylamide disc electrophoresis was performed according to the method of Ornstein and Davies as modified by Reisfeld, Lewis and Williams (10).

Experimental - Saliva Collection and Treatment

Human parotid saliva was collected from male donors by indirect cannulation of the right parotid gland, using sour lemon drops to stimulate the secretion. The pH of a pooled parotid saliva sample (300 ml) was adjusted to 6.7 by the addition of glacial acetic acid.

Adsorption of Basic Proteins on Bio-Rex 70

Four ml of Bio-Rex 70 resin, previously equilibrated with 0.1 M phosphate buffer (pH 6.7) were added to the parotid saliva sample (at pH 6.7) and stirred for 30 minutes at room temperature.

Bio-Rex 70 Column Chromatography

The four ml of Bio-Rex 70 resin carrying the adsorbed basic proteins were quantitatively transferred to the top of a Bio-Rex 70 column (0.9 x 20 cm) previously equilibrated with 0.1 M phosphate buffer (pH 6.7 starting buffer). Elution was carried out at room temperature and 100 fractions were collected. Elution was resumed with 0.25 M phosphate buffer (pH 6.7), and 200 fractions were collected. A final buffer

*Worthington Biochemical Corporation, Freehold, New Jersey.

change using 0.3 M phosphate buffer (pH 8.0) was made and 100 fractions collected. The flow rate was maintained at 30 ml/hr and 5 ml fractions collected throughout the experiment.

Isolation of Parotid Fluid Histones

Eluted fractions from Bio-Rex 70 which lay under a peak eluted with 0.3 M phosphate buffer (pH 8.0) were pooled separately and rendered phosphate-free by ultrafiltration with 0.3 M NH_4HCO_3 (brought to pH 8.5 with conc. NH_4OH) in a Diaflo cell equipped with a UM-2 membrane at 4°C.* At least six cell volumes of 0.3 M NH_4HCO_3 were required to achieve a complete buffer exchange. The volume in the final Diaflo filtration step was reduced to approximately 5 ml and lyophylized. The fractioned material was relyophylized several times with distilled water to remove the volatile buffer.

RESULTS

Figure 1 shows a typical elution pattern of parotid fluid basic proteins from Bio-Rex 70. Glycoproteins were eluted by the first 0.1 M phosphate buffer (pH 6.7). Peroxidases were eluted in two fractions with the second 0.25 M phosphate buffer (pH 6.7) as determined by enzyme assay. Lysozyme was also eluted in the second fraction of the 0.25 M phosphate buffer (pH 6.7).

Histones were eluted in the last buffer, 0.3 M phosphate pH 8.0 (Figure 1). Three distinct peaks could be identified by inspection of the chromatogram. The amino acid profiles of the three histone peaks are summarized in Table 1. The number of residues per 100 residues for each amino acid in the table is expressed as the average of two experiments. These average values were within less than one residue of the paired values arrived at in the two separate experiments. Table 1 also

*Diaflo ultrafiltration cells and membranes are products of the Amicon Corporation, Lexington, Mass., U.S.A.

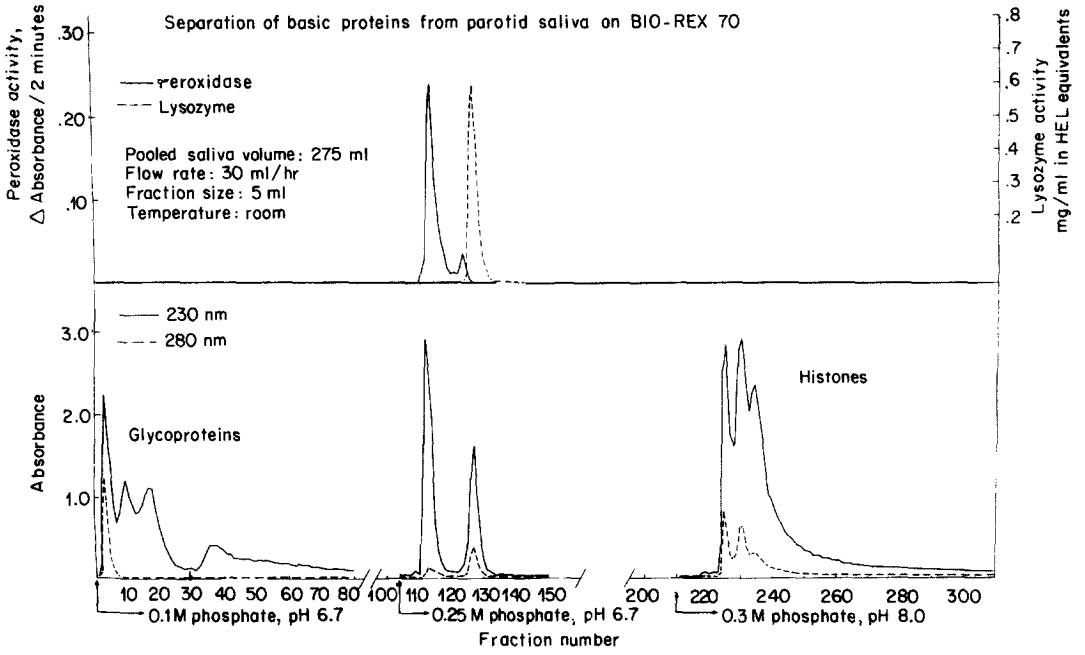

Fig. 1. Elution pattern of parotid fluid basic proteins from Bio-Rex 70. Column dimensions were 0.9 x 20 cm and a stepwise gradient elution technique was used. Glycoproteins eluted with 0.1 M phosphate buffer (pH 6.7). Peroxidase(s) and lysozyme eluted with 0.25 M phosphate buffer (pH 6.7). Parotid fluid histones were eluted in the last buffer, 0.3 M phosphate pH 8.0. Top half of chart depicts peroxidase and lysozyme activities in the eluates. Hen egg white lysozyme (HEL) was used as standard for lysozyme activity.

shows basic amino acids gradually increased with ascending order of the histones. Histidine made up approximately 50 per cent of the basic amino acids, the remainder being divided almost equally between lysine and arginine in all three histones isolated.

Polyacrylamide disc electrophoretic patterns of the three histone peaks showed three major bands (Figure 2). Migration was towards the cathode from top to bottom. The histone bands migrated progressively faster towards the cathode in the same order as they eluted from Bio-Rex 70 as revealed in the chromatography.

DISCUSSION

The existence of three predominant types of histones in human

TABLE I

AMINO ACID PROFILES OF PAROTID SALIVA HISTONES

BIO-REX 70 COLUMN SEPARATIONS

	PEAK #1		PEAK #2		PEAK #3	
	*	**	*	**	*	**
LYSINE	8.4	22.8	13.3	25.6	16.9	27.3
HISTIDINE	17.8	48.4	25.4	48.9	31.9	51.6
ARGININE	10.6	28.8	13.2	25.4	13.0	21.0
Total	36.8	100	51.9	100	61.8	100
ASPARTIC ACID	12.3		11.1		6.1	
THREONINE	0.4		0		0	
SERINE	7.9		8.6		7.8	
GLUTAMIC ACID	8.8		3.7		4.0	
PROLINE	4.2		0.1		0	
GLYCINE	8.8		6.3		7.2	
ALANINE	0.7		2.8		2.6	
ISOLEUCINE	0.2		0		0	
LEUCINE	2.9		2.6		0.4	
TYROSINE	9.2		10.1		7.4	
PHENYLALANINE	7.3		3.0		2.8	
TRYPTOPHAN***	present		present		present	

*Residues per 100 residues
**Percent of basic residues
***Tryptophan was present in all three histone peaks. It was not calculated since the analyses were performed on acid hydrolyzates.

parotid saliva were confirmed by chromatography, amino acid analysis and polyacrylamide disc electrophoresis. The amino acid profiles and the polyacrylamide disc electrophoretic patterns were consistent with the chromatographic behavior of the histones on Bio-Rex 70. Greater affinity for the resin was associated with higher content of basic amino acids and faster electrophoretic migration towards the cathode.

The origin of these histones in human parotid saliva is not well understood at the present time. It can be speculated however that their secretion results from the normal turnover of the striated duct cells or the acinar cells of the parotid gland. The biologic role of these histones is as equally uncertain as their origin. The histone

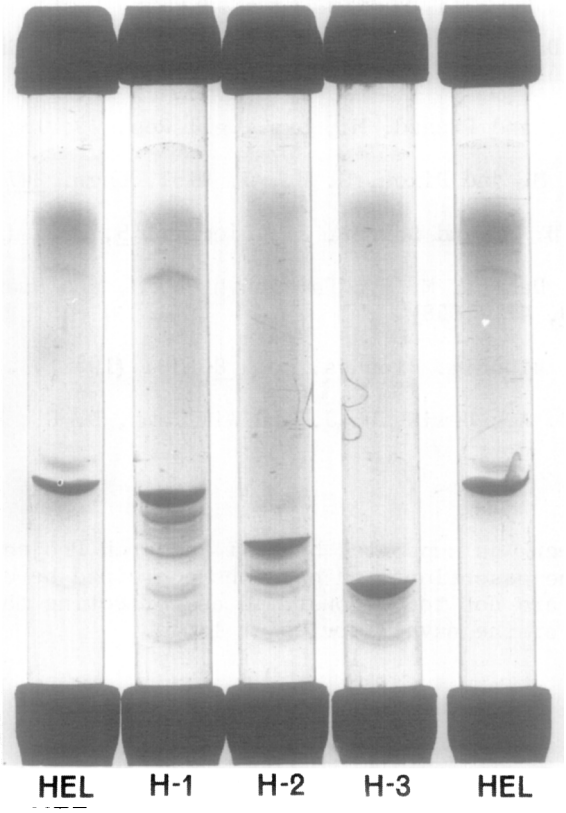

Fig. 2. Polyacrylamide disc electrophoretic patterns of the three parotid fluid histones isolated by ion exchange chromatography on Bio-Rex 70. Migration towards the cathode was from top to bottom. Thirty micrograms of hen egg white lysozyme (HEL) were used as markers. H-1, 106 ug, H-2, 20 ug, and H-3, 51 ug, the three histone peaks which showed progressively faster migration towards the cathode in the same order they were eluted from Bio-Rex 70.

molecules may serve as regulators of gene expression. A recently published article relates electrophoretic patterns to genetic polymorphism observed among different racial groups (3).

ACKNOWLEDGEMENTS

We wish to thank V. J. Berzinskas for the amino acid analyses and excellent technical assistance.

BIBLIOGRAPHY

1. Mandel, I. D., J. Dent. Res. 45, 634 (1966).
2. Bonilla, C. A. and Stringham, R. M., J. Chromatog. 50, 345 (1970).
3. Azen, E. A., Science 176, 673 (1972).

4. Nakao, A., Davis, W. J. and Einstein, E. R., Biochim. Biophys. Acta 130, 163 (1966).

5. Vendrely, R. and Picaud, M., Exp. Cell Res. 49, 13 (1968).

6. Sanders, M. M. and Dixon, G. H., J. Biol. Chem. 247, 851 (1972).

7. Fambrough, D. M. and Bonner, J., Biochem. 5, 2563 (1966).

8. Bonner, J., Dahmus, M. E., Fambrough, D. M. and Huang, R. C., Science 159, 47 (1968).

9. Shugar, D., Biochim. Biophys. Acta 8, 302 (1952).

10. Reisfeld, R. A., Lewis, U. J. and Williams, D. E., Nature 195, 281 (1962).

This research was supported by NMRI Research Project No. MR041.20. 02.6003A3IJ. The assertions and opinions contained herein are those of the authors and are not to be construed as reflecting the views of the Navy Department or the naval service at large.