Journal of Chromatography, 173 (1979) 373–378

CHROM. 11,743

FRACTIONATION OF HISTONES ON A METAL ION EQUILIBRATED CATION EXCHANGER

I. CHROMATOGRAPHIC PROFILES ON AN AMBERLITE IR-120 (Al^{3+}) COLUMN

A. M. DIWAN and P. N. JOSHI
Department of Chemistry, University of Poona, Pune 411 007 (India)
(First received October 10th, 1978; revised manuscript received January 16th, 1979)

SUMMARY

Amberlite IR-120, a polystyrene sulphonate type of cation exchanger, equilibrated with Al^{3+} ions, has been employed for the fractionation of whole histone. This adsorbent permits the quantitative and reproducible recovery of whole histone in six fractions.

INTRODUCTION

The fractions of whole histone are closely similar in size and charge, and the chromatographic techniques available for their separation are not adequate for their resolution[1–4]. Further, these techniques are subject to operational limitations and irreversible binding. For these reasons, methods based on alternative properties are desirable for the separation of histones. This paper deals with histone fractionation on a cation-exchange column.

EXPERIMENTAL AND RESULTS

Whole histone was isolated from buffalo liver by acid extraction. The whole histone was characterized by high-resolution acrylamide gel electrophoresis as described by Paniym and Chalkley[5]. The electrophoretic pattern of buffalo liver histones was comparable with those of rat liver and calf thymus histones (Fig. 1A). The absence of non-histone proteins was ascertained by gel electrophoresis at alkaline pH. The absence of DNA and RNA was ascertained by colorimetric methods[5]. The whole histone was 98% pure as protein, with bovine serum albumin as the standard protein.

An Amberlite IR-120 (Al^{3+}) column was prepared from Amberlite IR-120 (Na^+) by treatment with Al^{3+} ions as described by Shankar and Joshi[7]. Acetate buffer of pH 4.0 (50 m*M*) was prepared according to Gomori[8]. The aluminium ions were determined by Sandell's procedure[9]. The basic amino acids were analysed according to Moore and Stein[10].

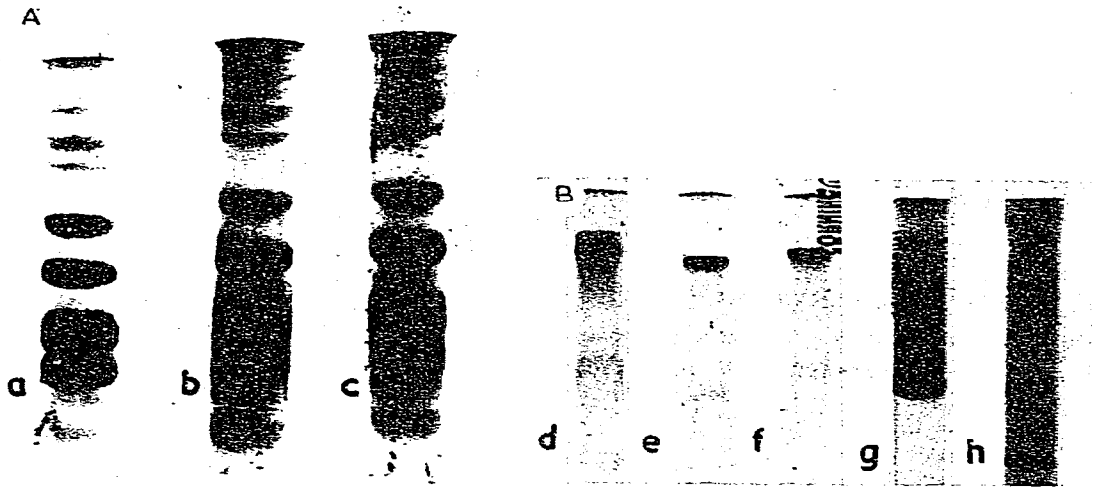

Fig. 1. (A) Gel electrophoretic patterns of (a) rat liver, (b) calf thymus and (c) buffalo liver whole histone. Electrophoresis was carried out according to Paniym and Chalkley[5]. (B) SDS gel electrophoretic pattern of fractions from the IR-120 (Al^{3+}) column. (d)–(h): fraction I–V, respectively.

Typical chromatographic profiles of whole histone

Buffalo liver whole histone was dissolved in acetate buffer (pH 4.0) and the solution was applied to the column containing 5 g of IR-120 (Al^{3+}), having 6 mequiv. of Al^{3+} previously equilibrated with the above buffer. The solution was allowed to percolate through the column. The column was operated at a flow-rate of 30 ml/h at 25°. The effluent was collected and the column was washed with four bed volumes of the buffer to remove loosely retained species of histones.

The adsorbed histone was then eluted with a discontinuous gradient of ethanol–hydrochloric acid. Fractions of 10 ml were collected and assayed for protein content by the method of Lowry *et al.*[11] or by measuring the UV absorption at 280 nm.

The percentages of total histone adsorbed and eluted are given in Table I. Fig. 2 shows a typical profile of histone on the IR-120 (Al^{3+}) column.

TABLE I

CHROMATOGRAPHIC PROFILES OF BUFFALO LIVER AND RAT LIVER WHOLE HISTONE ON AN IR-120 (Al^{3+}) COLUMN

Retention (%)	*Elution by ethanol–hydrochloric acid (%)*						*Total elution of fractions I–VI (%)*
	I	*II*	*III*	*IV*	*V*	*VI*	
100	17.1	18.12	14.89	18.12	19.28	12.46	100
Molarity of acid in 50% ethanol	0.1	0.3	0.5	0.6	0.7	1.0	

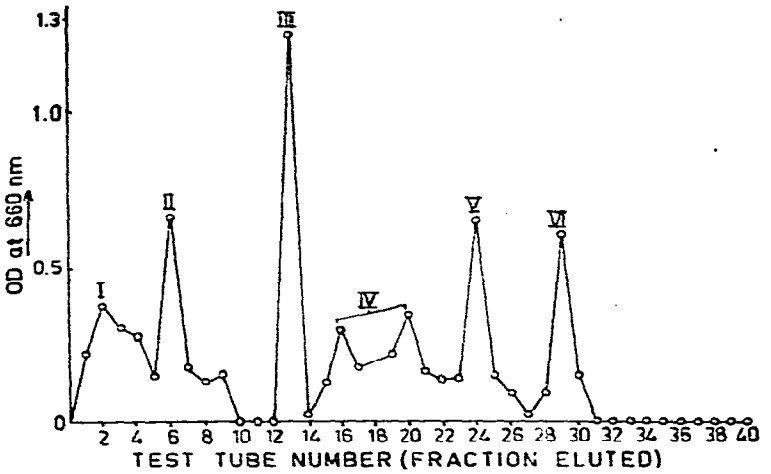

Fig. 2. Typical chromatographic elution profiles of buffalo liver whole histone on an IR-120 (Al^{3+}) column.

Re-chromatography

The behaviour of a fraction when subjected to re-chromatography is an important aspect of any chromatographic technique. Reproducible re-chromatographic behaviour can be regarded as confirming the homogeneity of the material eluted in a specific fraction.

Fraction I obtained by elution with 0.1 *M* hydrochloric acid–ethanol was re-chromatographed on a fresh IR-120 (Al^{3+}) column. The percentages adsorbed and eluted are given in Table II. Fig. 3 depicts the elution profile.

TABLE II

RE-CHROMATOGRAPHY OF FRACTION 1 ON AN IR-120 (Al^{3+}) COLUMN

Retention (%)	*Acid concentration in hydrochloric acid–ethanol eluting agent (M)*	*Elution (%)*
100	0.1	60
	0.3	10
	1.0	10
	Total:	80

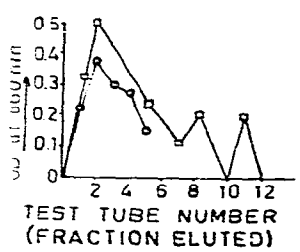

Fig. 3. Re-chromatography of fraction F_1 on an IR-120 (Al^{3+}) column. ○, Chromatography; □, re-chromatography.

Characterization of fractions

The histone fractions obtained from the IR-120 (Al^{3+}) column were characterized by basic amino acid analysis and by gel electrophoresis.

Amino acid analysis. The appropriate fractions from the IR-120 (Al^{3+}) column were concentrated and hydrolysed with 6 *N* hydrochloric acid. The fractions were evaporated to remove excess of acid and then diluted with buffer of pH 5.28. The amino acids were analysed on a 15-cm column essentially according to Moore and Stein[16]. Amino acids such as arginine, histidine and tyrosine were determined by specific colour reactions[12], the results being expressed as moles of individual amino acid per 100 moles of amino acids (Table III).

TABLE III

AMINO ACID CONTENT OF CHROMATOGRAPHIC FRACTIONS FROM AN IR-120 (Al^{3+}) COLUMN

Fraction	*Lysine**	*Arginine**	*Lys/Arg*	*Phenylalanine**	*Tyrosine**	*Histidine**	*Tentative identification of fractions*
I	28.3	1.7	16.6	0.4	0.3	0	F_1
II	16.9	6.3	2.5	1.4	3.7	2.2	F_{2b}
III	12.5	9.2	1.4	1.6	2.2	2.8	F_{2a2}
IV	9.6	13.8	0.7	2.2	3.4	1.9	F_{2a1}
V	10.1	13.6	0.9	2.0	2.1	2.3	F_3
VI	10.0	10.2	1.0	2.1	2.5	2.0	Aggregate

* Expressed as total moles of individual amino acid per 100 mole of total amino acid.

Electrophoresis. The histone fractions obtained were subjected to polyacrylamide gel electrophoresis using 15% acrylamide in 6.25 *N* urea according to Paniym and Chalkley[5]. Electrophoresis in 7.5% acrylamide was carried out according to the method of Wray and Stubblefield[13]. However, in all instances low mobilities and hence poor resolutions were observed for the IR-120 (Al^{3+}) fractions. Even the method of John and Forrester[14] did not give satisfactory results. Histones are eluted as histone–Al^{3+} complexes from IR-120 Al^{3+} column and these complexes could not be resolved satisfactorily under various conditions of electrophoresis. Therefore, sodium dodecyl sulphate (SDS) electrophoresis was carried out according to Shapiro *et al.*[15]. The order of mobility of the fractions was $5 > 4 = 3 > 2 > 1$ (Fig. 1B). This only indicates that fraction I has the highest molecular weight and fraction V the lowest molecular weight. As the histone fraction of highest molecular weight is the very lysine-rich histone F_1, it can be concluded that very lysine-rich histone elutes first from the IR-120 (Al^{3+}) column, and this conclusion is supported by the results of amino acid analyses.

DISCUSSION

The failure of the IR-120 (Na^+) column to adsorb histone and the large increase in retention on the IR-120 (Al^{3+}) column show that interaction on the IR-120 (Al^{3+}) is the basis of adsorption. Dissociation of the resin Al^{3+}–histone complex and its subsequent removal could be the basis for resolution by elution.

Reagents such as sodium chloride, sodium fluoride, sodium citrate, sodium tartrate, hydrochloric acid alone or ethanol alone fail to elute significant amounts of histones from the IR-120 (Al^{3+}) column. Acid–ethanol was the only eluting agent that was successful. Different fractions of IR-120 (Al^{3+}) contain various concentrations of Al^{3+} ions, and this may indicate that different fractions have different binding capacities with immobilized Al^{3+}. A pH of 4.0 was selected for adsorption mainly because (1) the maximal concentration of Al^{3+} was found to be achieved at pH 4.0 and (2) the tendency for aggregation is minimal at pH 4.0 and increases with increasing pH. Low ionic strength buffers were employed in order to minimize the tendency for aggregation.

As indicated in Table III, the lysine to arginine ratio decreases with increasing elution volume. The tentative identifications of the first five IR-120 (Al^{3+}) fractions are F_1, F_{2b}, F_{2a2}, F_{2a1} and F_3, respectively. The sixth fraction was a unique fraction, in which the lysine content was approximately equal to the arginine content. This fraction might have resulted from aggregation. The fractionation on IR-120 (Al^{3+}) seems to be qualitatively related to the amino acid composition. The first fraction had the highest lysine content, which gradually decreased until the fourth fraction and for the fifth and sixth fractions remained virtually constant. On the other hand, the arginine content was low for the first fraction and gradually increased until the fourth fraction.

The order of elution from Amberlite IRC-50 for whole histone, using a gradient of guanidium chloride, is F_1, F_{2a2}, F_{2b}, F_3 and F_{2a1}. This is slightly different from the order on IR-120 (Al^{3+}). The results also agree with those on Amberlite IRC-50, in which lysine-rich histones are eluted earlier than arginine-rich histone. Another advantage of the IR-120 (Al^{3+}) column is that all of the fractions are clearly separated.

On comparison of the results on IR-120 (Al^{3+}) with those obtained by CM-cellulose chromatography as described by Senshu and Iwai[3] using ethanol–formic acid for elution, it can be seen that in the latter instance arginine-rich histones are eluted first, followed by moderately lysine-rich histones and finally very lysine-rich histones. The order of elution of whole histone from CM-cellulose corresponded to their extractability from nucleoprotein with ethanol–hydrochloric acid and the reverse of their extractability with aqueous acids. Here it was concluded that the interaction of histone fractions with CM-cellulose seems to be similar to the interaction with deoxyribonucleoprotein. Using similar systems, with IR-120 (Al^{3+}) lysine-rich histones are eluted first, followed by arginine-rich histones. The order is therefore different from that in CM-cellulose chromatography and may indicate a difference in the mechanism of operation of IR-120 (Al^{3+}) and CM-cellulose as far as histones are concerned.

The reported amino acids analysis suggests that fraction I is very lysine-rich histone which is heterogeneous. The known chromatographic systems are unable to resolve whole histone into individual fractions or into sub-fractions in one operation and therefore the sub-fractionation that occurs during re-chromatography is not totally unexpected. During re-chromatography, in the absence of other fractions, further resolution seems possible. In fact, chemically fractionated histones are known to be better suited for the resolution of histones by chromatographic means. Therefore, sub-fractionation during re-chromatography of F_1 is to be expected.

The validity of the chromatographic method for achieving satisfactory resolutions on IR-120 (Al^{3+}) of proteins[16], enzymes[17] and RNA[7] has been demonstrated

by several workers. During re-chromatography, as reported in Table II, fraction I was resolved into three peaks. Most of the elution occurred with 0.1 *M* hydrochloric acid–ethanol reagent, which is also the eluting agent for the fraction during the earlier elution. The other two peaks are very small in comparison with the major peak.

Quantitative elution and satisfactory re-chromatography suggest that the fractionation procedure is reliable. IR-120 (Al^{3+}) fractionates histones according to amino acid composition and to some extent according to molecular weight.

CONCLUSIONS

These studies were aimed mainly at examining the chromatographic behaviour of histones. It can be stated that the described fractionation procedure does not appear to be of the conventional ion-exchange chromatographic type because Al^{3+} ions are also involved in the adsorption.

The investigation has shown that the use of an IR-120 (Al^{3+}) column is a promising technique for the fractionation of whole histone. Further, IR-120 (Al^{3+}) is reasonably stable at elevated temperatures and even at high salt concentrations and the technique offers a simple, reproducible and inexpensive system for the fractionation of proteins. In addition, certain factors such as concentration of protein, flow-rate and ageing of histones do not have significant effects on the chromatographic behaviour of histones on the IR-120 (Al^{3+}) column.

ACKNOWLEDGEMENT

The authors are grateful to Dr. H. J. Arnikar, Senior Professor, former Head of the Department of Chemistry, University of Poona, for providing laboratory facilities.

REFERENCES

1 J. M. Luck, P. S. Ramussen, K. Satake and A. N. Tvsetikov, *J. Biol. Chem.*, 233 (1958) 1407.
2 H. J. Cruft, *Biochim. Biophys. Acta*, 54 (1961) 611.
3 T. Senshu and K. Iwai, *J. Biochem. (Tokyo)*, 67 (1970) 473.
4 J. Mizon, H. M. Mayne-D'Haultfoeuille, C. C. Mizon, P. Sautire and G. Biserte, *FEBS Lett.*, 47 (1974) 125.
5 S. Paniym and R. Chalkley, *Arch. Biochem. Biophys.*, 130 (1969) 125.
6 G. Ashwell, *Methods Enzymol.*, 3 (1957) 73.
7 V. Shankar and P. N. Joshi, *J. Chromatogr.*, 90 (1974) 99.
8 G. Gomori, *Methods Enzymol.*, 1 (1955) 138.
9 E. B. Sandell, *Colorimetric Determination of Traces of Metals*, Interscience, New York, 1950, p. 146.
10 S. Moore and W. H. Stein, *J. Biol. Chem.*, 176 (1948) 367.
11 O. H. Lowry, N. J. Rosenbrough, A. L. Fary and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
12 J. L. Bailey, *Techniques in Protein Chemistry*, Elsevier, Amsterdam, London, New York, 1967, p. 17.
13 W. Wray and E. Stubblefield, *Anal. Biochem.*, 38 (1970) 454.
14 E. W. John and S. Forrester, *J. Chromatogr.*, 55 (1971) 429.
15 A. L. Shapiro, E. Vinuela and J. V. Maizel, *Biochem. Biophys. Res. Commun.*, 28 (1967) 815.
16 P. G. Batliwala, *Ph.D. Thesis*, University of Poona, Pune, 1972.
17 A. S. Sane, M. V. Hegde and P. N. Joshi, *J. Chromatogr.*, 168 (1979) 495.