THE HETEROGENEITY OF THE NON-HISTONE CHROMATIN PROTEINS FROM MOUSE TISSUES

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1. Introduction

Recent investigations in a number of laboratories have indicated that the specificity of the restriction of the DNA template in chromatin is associated with the non-histone fraction [1–5]. Since this fraction consists largely of protein it is obviously of interest to isolate and characteristise these proteins from a number of tissues.

The present communication describes the characterisation of non-histone proteins obtained from several mouse tissues by chromatography of salt-urea chromatins dissociated by salt and urea on hydroxyapatite (HAP). Electrophoresis in sodium dodecyl sulphate (SDS)–acylamide gels shows these proteins to be heterogeneous and most of high molecular weight. The non-histone proteins of kidney, liver and spleen appear to be generally similar.

2. Materials and methods

2.1. *Preparation of chromatins*

All procedures were carried out at 4° unless otherwise stated. With the exception of spleen, which was always used immediately, mouse tissues were usually frozen at −20° after excision. Nuclei were prepared by homogenising up to 25 g of tissue in 0.025 M citric acid [6]. After being washed three times in the same citric acid solution by homogenisation and centrifugation the pellet of nuclei was homogenised in approximately 10 volumes of 0.14 M NaCl, followed by centrifugation at 1000 *g* for 10 min. This extraction was repeated and then the pellet was treated twice in a similar manner using approximately 10 volumes of 0.1 M tris-HCl, pH 7.5. Both of these solutions contained 0.005 M sodium bisulphite to inhibit proteolytic activity [7]. Analysis of such chromatins showed DNA, RNA and protein to be in the proportions 1:0.04:1.5–2.2, respectively.

2.2. *Chromatography on hydroxyapatite*

The chromatin preparation was homogenised in approximately 10 volumes of 2 M NaCl–5 M urea–0.001 M sodium phosphate, pH 6.8. After centrifugation at 15,000 *g* for 15 min the pellet was homogenised in a similar volume of the same solution. The two extracts were pooled and dialysed overnight against the same solution. The chromatin solution was sonicated for two periods of 15 sec each using an MSE Ultrasonic Power Unit at 1.5 mA and then centrifuged at 15,000 *g* for 15 min to remove any small insoluble residue.

Hydroxyapatite was prepared according to the method of Bernardi [8]. Columns were packed, equilibrated and run at room temperature in 2 mM NaCl–5 M urea–0.001 M phosphate, pH 6.8. For analytical experiments 12–17 ml (A_{260} = 3–9) of chromatin solution were applied to a 20 × 1 cm column of hydroxyapatite. For preparative purposes 30–80 ml (A_{260} = 10–20) of chromatin solutions were similarly applied to 50 × 1.6 cm columns. The column was then eluted with the equilibration buffer at room temperature (fig. 1). After the unretained protein (fraction 1) had been eluted, a second protein fraction was removed by treating the hydroxyapatite with 2 M NaCl–5 M urea–0.05 M sodium phosphate, pH 6.8 (fraction 2). The column was then usually treated with 2 M NaCl–5 M urea–0.5 M phosphate, pH 6.8 to remove nucleic acids (fraction 3) and regenera-

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tion was completed by washing the column with the equilibration buffer.

2.3. *Electrophoresis in SDS-acrylamide gels*

The fractions obtained from the HAP columns were dialysed extensively against 0.1% SDS at room temperature. After freeze drying the SDS proteins were dissolved in 8 M urea to give a concentration of approximately 0.5 mg/ml. After dialysis at room temperature against 8 M urea–1% SDS–1% metcaptoethanol–0.01 M sodium phosphate, pH 7, the solutions were incubated for 3 hr at 37° to complete dissociation followed by dialysis overnight against the same buffer except that it contained 0.1% SDS. Prior to electrophoresis up to 200 μl of each solution containing 50–100 μg of protein were mixed with 3 μl of bromophenol blue marker dye and 5 μl of mercaptoethanol [9] and stored at room temperature for 1 hr.

Electrophoresis was carried out in a system similar to that described by Laemmli [10] which consists of introducing SDS to the double gel/discontinuous buffer system of Davis [11]. In these experiments the gels contained 15% acrylamide and 4 M urea. After running for 2 hr at 2 mA/gel, the gels were fixed in methanol–20% acetic acid (1:1), stained with 1% naphthalene black in 7% acetic acid and destained in methanol–acetic acid–water (300:70:630). The gels were then scanned using a Joyce Loebl UV scanner.

2.4. *Gel filtration on agarose*

10 mg of mouse liver chromatin Fraction 2 proteins were obtained in a volume of 3.2 ml of 8 M urea–0.1% SDS–1% mercaptoethanol–0.01 M phosphate, pH 7 as described above. This sample was applied to a 75 × 2.2 cm column of Sepharose 4B run in the same solution at room temperature. Protein was detected in the eluate by turbidity in 1.1 M trichloroacetic acid (TCA) and the type of protein in each 3 ml fraction was monitored by electrophoresis in SDS-gels as described above. Fractions were then pooled to form 5 different molecular weight groups (fig. 2B, 1–5).

2.5. *Amino acid analysis*

HAP fractions were dialysed extensively against 1 mM HCl at 4° and freeze-dried. Protein was recovered from SDS complexes by precipitation with TCA at a final concentration of 20%. After storage overnight at 4° the precipitate was washed successively with 5% TCA, 1% TCA and acetone, and then dried. The equivalent of 1 mg of protein was hydrolysed in vacuo at 110° for 18 hr in 6 N HCl. Quantitative amino acid analysis was then carried out using the Technicon TSM analyser.

2.6. *General analyses*

RNA was estimated according to the method of Fleck and Begg [12], DNA by the diphenylamine procedure [13] and protein by the biuret method of Itzhaki and Gill [14].

3. Results and discussion

Recovery of chromatin protein from the HAP columns was usually at least 70%, e.g. 63% and 22% of mouse liver chromatin proteins were recovered in fractions 1 and 2 respectively. Analysis of fractions 1 and 2 are given in table 1. Both contain small amounts of RNA. Amino acid analysis of the proteins of fraction 1 was very similar to that of whole histone [15]. That these proteins were histones was confirmed by electrophoresis of desalted preparations according to the method of Panyim and Chalkley [16].

Amino acid analysis of fraction 2 showed that the proteins eluted by 0.05 M phosphate were acidic since they had an acidic/basic amino acid ratio in excess of 1. Although small amounts of protein were sometimes detected by electrophoresis in SDS-gels, fraction 3 consisted mostly of DNA whose recovery from the columns was essentially complete.

For the purpose of this discussion we shall refer to the fraction 2 proteins as chromatin non-histone proteins. Although practically all the histones were recovered in fraction 1, the recovery of the non-histone proteins was less, e.g. 50% in the mouse liver experiment quoted above. Such proteins, however, appeared representative of the non-histone proteins since the electrophoresis patterns were very similar to those given by another method, viz. solubilization by SDS of chromatin from which histones had been removed by acid [17, 18]. Fig. 2 (a, b, c) shows the results of electrophoresis of these proteins from mouse kidney, liver and spleen respectively in SDS-acrylamide gels. In all cases the non-histone proteins are shown to be quite heterogeneous. Since the SDS-electro-

Table 1

Analyses to HAP column fractions. Amino acid analyses are given as moles %. No corrections have been made for hydrolytic losses, nor were amide groups determined. Protein and nucleic acids were determined as described in the text.

Amino acid	HAP F1	HAP F2	HAP F2 group 1	HAP F2 group 2	HAP F2 group 3	HAP F2 group 4	HAP F2 group 5
Asp	5.4	9.2	9.4	9.7	9.2	9.2	7.9
Thr	5.1	4.9	4.9	5.2	5.0	5.2	4.9
Ser	5.5	6.1	6.3	6.2	6.3	5.5	6.9
Glu	8.3	13.5	13.4	14.0	13.0	12.3	10.9
Pro	5.1	5.5	5.4	4.9	5.0	5.2	6.9
Gly	10.7	8.0	8.5	8.0	8.0	8.0	9.9
Ala	11.8	7.4	8.1	8.0	8.0	7.4	6.9
Cys	0.6	0.6	0.9	1.0	0.8	1.2	2.0
Val	6.0	6.1	6.3	6.4	6.7	7.1	6.9
Met	1.1	1.8	2.2	2.2	2.1	2.4	2.0
Ile	4.3	4.3	4.9	4.8	5.0	4.9	3.0
Leu	7.4	8.0	9.0	9.1	8.8	8.9	7.9
Tyr	2.3	3.7	3.6	3.0	3.4	3.1	4.0
Phe	1.9	4.9	4.5	3.8	4.6	4.3	4.9
Lys	13.5	6.7	5.8	6.4	6.3	6.7	5.9
His	2.2	3.1	2.2	2.1	2.5	2.8	2.0
Arg	8.8	6.7	4.5	5.2	5.0	5.8	6.9
Acidic/basic	0.6	1.4	1.8	1.7	1.6	1.4	1.3
DNA	0	0					
Protein-RNA	1:0.02	1:0.03					

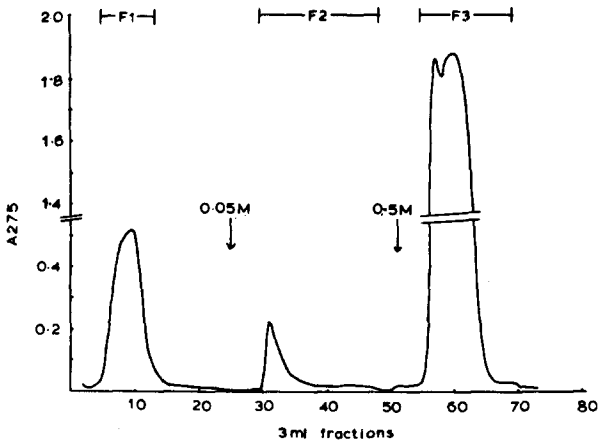

Fig. 1. Chromatography of 17 ml mouse spleen chromatin solution (A_{260} = 9) on 20 × 1 cm column of HAP. The concentration of phosphate in the eluant was changed to 0.05 M and 0.5 M where indicated. The flow rate was maintained at 9 ml/hr. Fractions 1–3 were collected by pooling the designated volumes.

phoresis system separates proteins on the basis of differences in molecular weight [19], this indicates that they range considerably in size. This is in marked contrast to the histones, which consist of five main components of molecular weight range 12,000–20,000 [20] which apart from histone F_1 run as a few fast moving bands in SDS-acrylamide gels (fig. 2d).

The patterns given by kidney, liver and spleen proteins are very similar; they all contain predominantly high molecular weight proteins together with a discrete number of smaller proteins. Mouse liver non-histone proteins treated with SDS were seperated into five groups by gel filtration through Sepharose (fig. 2B). Amino acid analysis (table 1) shows that both the high and low molecular weight proteins of groups 1–4 and 5 respectively were acidic, indicating that the latter were not residual histones.

The limited heterogeneity of the non-histone proteins of a number of tissues has also recently been described by Elgin and Bonner [18]. The complexity of the non-histone proteins has also been described

using both conventional [21, 22] and SDS electrophoresis systems [18, 23, 24]. Other experiments carried out in this laboratory indicate that the electrophoretic patterns of non-histone proteins from kidney, liver and brain of other species are very similar to those of the mouse.

The similarity of non-histone proteins from different organs at first seems at variance with evidence implicating them as organ-specific restrictors of chromatin templates [1–5]. However, some of them are almost certainly proteins common to all chromatins, e.g. enzymes such as RNA polymerase. Moreover, it is reasonable to predict that specificity-determining proteins might conform to specific size classes, although the primary structures of the proteins might differ. Hence, techniques for demonstrating specific binding properties may be needed to distinghuish them [25, 26].

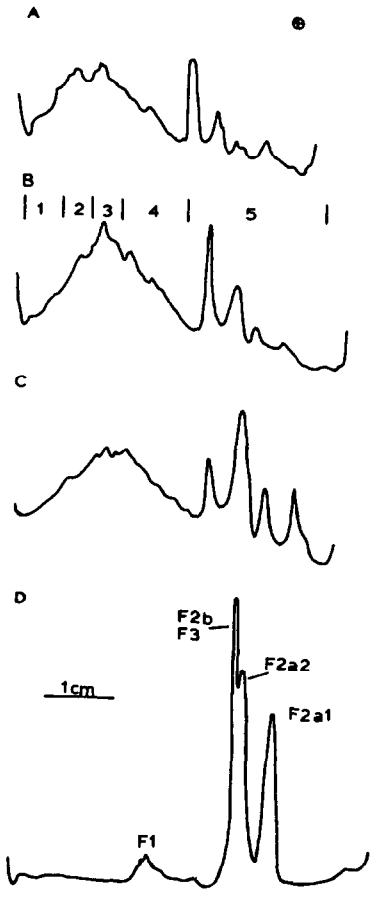

Fig. 2. SDS–polyacrylamide gel patterns. (A) mouse kidney non-histone proteins; (B) mouse liver non-histone proteins; (C) mouse spleen non-histone proteins; (D) calf thymus histones. Mouse liver SDS–proteins were fractionated on Sepharose 4B to yield fractions whose major components were those of groups 1–5 indicated in B. 15% acrylamide containing 4 M urea, tris glycinate system, 0.1% SDS in samples, gels and electrode buffer.

Acknowledgements

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References

[1] J.Paul and R.S.Gilmour, J. Mol. Biol. 34 (1968) 305.
[2] I.Bekhor, G.M.Kung and J.Bonner, J. Mol. Biol. 39 (1969) 351.
[3] R.S.Gilmour and J.Paul, J. Mol. Biol. 40 (1969) 137.
[4] R.C.C.Huang and P.C.Huang, J. Mol. Biol. 39 (1969) 365.
[5] R.S.Gilmour and J.Paul, FEBS Letters 9 (1970) 242.
[6] J.Paul and R.S.Gilmour, J. Mol. Biol. 16 (1966) 242.
[7] S.Panyim, R.H.Jensen and R.Chalkley, Biochim. Biophys. Acta 160 (1968) 252.
[8] G.Bernardi, Biochim. Biophys. Acta 174 (1969) 423.
[9] K.Weber and M.Osborn, J. Biol. Chem. 244 (1969) 4406.
[10] U.K.Laemmli, Nature 227 (1970) 680.
[11] B.J.Davis, Ann. N.Y. Acad. Sci., 121 (1964) 404.
[12] A.Fleck and D.Begg, Biochim. Biophys. Acta 108 (1954) 333.
[13] K.Burton, Biochem. J. 62 (1956) 315.
[14] R.F.Itzhaki and D.M.Gill, Anal. Biochem. 2 (1964) 401.
[15] A.J.McGillivray and J.P.P.V.Monjardino, Biochem. J. 107 (1968) 797.
[16] S.Panyim and R.Chalkley, Arch. Biochem. Biophys. 130 (1969) 337.
[17] K.Marushige, D.Brutlag and J.Bonner, Biochemistry 7 (1968) 3149.
[18] S.C.R.Elgin and J.Bonner, Biochemistry 2 (1970) 4440.
[19] A.L.Shapiro, E.Vinuela and J.V.Maizel, Biochim. Biophys. Res. Commun. 28 (1967) 815.
[20] E.W.Johns, in: Ciba Foundation Symposium: Homeostatic Regulators, eds. G.E.W.Wolstenholme and J. Knight (Churchill, London, 1969) p. 128.
[21] N.C.Kashtraba and T.Y.Wang, Intern. J. Biochem. 1 (1970) 327.

[22] J.Loeb and C.Creuzet, FEBS Letters 5 (1969) 37.
[23] K.R.Shelton and V.G.Allfrey, Nature 228 (1970) 132.
[24] C.S.Teng and T.H.Hamilton, Biochem. Biophys. Res. Commun. 40 (1970) 1231.
[25] L.J.Kleinsmith, J.Herdema and A.Carroll, Nature 226 (1970) 1025.
[26] C.T.Teng, C.S.Teng and V.G.Allfrey, Biochem. Biophys. Res. Commun. 41 (1970) 690.