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A Comparison of the High Mobility Group Non-Histone Chromatin Protein HMG 2 in Chicken Thymus and Erythrocytes

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Summary

The high mobility group non-histone chromatin protein HMG 2 has been isolated from chicken thymus and erythrocytes by CM-Sephadex chromatography. The subfractions of this protein in the two tissues were resolved into two peaks, HMG 2a and 2b. These two fractions were characterised by polyacrylamide gel electrophoresis, isoelectric focusing, amino acid analysis and peptide maps. The analyses reveal that there are quantitative rather than qualitative differences in the subfractions of HMG 2 in the two tissues.

Introduction

Chromatin contains a group of non-histone proteins called the high mobility group proteins which, although well-characterized, are of unknown function (see ref. 1 for a review). There are four main HMG proteins in calf thymus, HMG 1, 2, 14 and 17, all of which have been isolated in pure form (2-4). The presence of HMG proteins in a variety of organisms and tissues including avian erythrocytes (5,6), trout testis (7), wheat and yeast(8) implies a wide-spread occurrence in eukaryotic nuclei. The quantities of these proteins in the cell (about 10^6 molecules per nucleus) suggests that they may have a structural role.

Previous work in our laboratory has shown that four proteins similar to calf thymus HMG 1, 2, 14 and 17 are present in chicken erythrocyte nuclei (5). Further studies showed that the protein HMG 2 from chicken erythrocytes migrated as two bands on SDS polyacrylamide gels and that the HMG 2 peak on a CM Sephadex ion-exchange column could be split into two by using a shallow

Abbreviations

PCA : Perchloric acid HMG : High mobility group

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salt gradient (A. Rabbani and G. Goodwin, unpublished result). These observations, coupled with the known microheterogeneity of calf thymus HMG 2 (9) and a recent report of the isolation of an erythrocyte specific HMG protein from duck which is similar to HMG 2 (6), prompted us to extend our studies on chicken HMG 2. We report here a comparative structural analysis of the HMG 2 proteins isolated from chicken erythrocyte and thymus tissue.

Materials and Methods

Isolation of HMG proteins from chicken tissues

Chicken blood was collected in an equal volume of cold 75 mM NaCl, 25 mM EDTA (pH 7.5) containing 0.5 mM phenylmethylsulphonyl fluoride as proteolytic inhibitor. After centrifugation at 2000 x g for 20 minutes the sediments were extracted with 5% perchloric acid and histones H1 and H5 separated from the HMG proteins by fractional acetone precipitation (1). Chicken thymus tissue was also extracted directly with 5% PCA and histone H1 and the HMG proteins collected in the same way. The HMG proteins were then fractionated on a CM Sephadex C25 column (2.5 x 16 cm) as described (5) except that a shallower linear salt gradient (400 ml 0.1 M NaCl in borate buffer - 400 ml 0.6 M NaCl in the same buffer) was employed. The fractions were pooled and collected by acetone precipitation (5).

Analysis of HMG proteins

Amino acid analysis, N-terminal amino acid analysis, isoelectric focusing (10) and one-dimensional peptide maps (11) were carried out as described. Proteins were separated by electrophoresis on 15% polyacrylamide gels in 0.1% SDS according to Laemmli (12).

Results

In our previous studies of the HMG proteins from chicken erythrocytes (5) the proteins were fractionated by CM Sephadex ion-exchange chromatography, and HMG 2, which eluted as a single peak between HMG 1 and HMG 14, migrated as a single band on acid polyacrylamide gels. However, the HMG 2 was shown to consist of at least four subfractions having isoelectric points between pH 7 and 9. In subsequent studies, a shallowing of the salt gradient used in the chromatography split the HMG 2 into two peaks. This is reminiscent of the behaviour of calf thymus HMG 2 which is split by high resolution chromatography into four subfractions with different isoelectric points (9). Figure 1 (top) shows the separation of chicken erythrocyte HMG proteins by CM Sephadex chromatography with HMG 2 splitting into two peaks, HMG 2a and

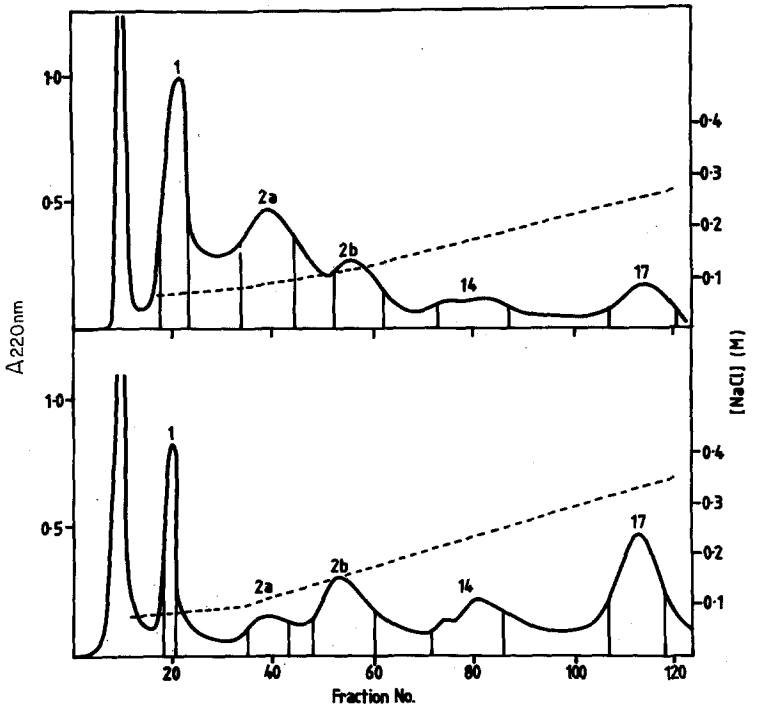

Figure 1: CM-Sephadex ion-exchange column chromatography of chicken HMG proteins. 5 ml fractions collected.
(———) Absorbance at 220 nM, (-----) NaCl concentration.
Top: chicken erythrocyte HMG proteins
Bottom: chicken thymus HMG proteins.

HMG 2b. Chromatography of chicken thymus HMG proteins gives a similar elution profile (Fig. 1; bottom) but the proportions of the 2a and 2b peaks are different.

The HMG 2 peaks from both tissues have been analysed by isoelectric focusing (Fig. 2). Erythrocyte HMG 2 has 4-5 subfractions which have been partially separated in the two peaks, 2a and 2b; the more acidic subfractions elute in peak 2a whilst the more basic subfractions predominate in peak 2b. This is also true for chicken thymus HMG 2. It should be noted that the chicken HMG 2 subfractions focus in similar positions to those from calf thymus HMG 2 except that the calf pattern contains an additional, more acidic subfraction.

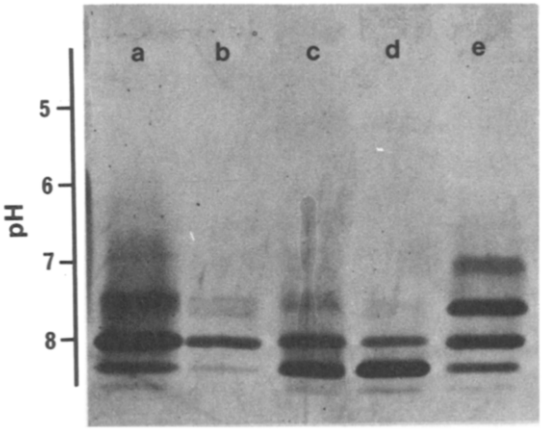

Figure 2: Isoelectric focusing of HMG proteins (a) chicken erythrocyte HMG 2a (b) chicken thymus HMG 2a (c) chicken erythrocyte HMG 2b (d) chicken thymus HMG 2b (e) calf thymus HMG 2.

The proteins isolated from the peaks have also been characterized by SDS polyacrylamide gel electrophoresis (Fig. 3). This shows that HMG 2a migrates just ahead of HMG 2b, and that the corresponding fractions from the two tissues have the same mobility. The most significant point, however, is that while the HMG patterns from the two tissues are qualitatively similar, they show clear quantitative differences. In chicken thymus (Fig. 3c) HMG 2b is a major component and 2a is a minor band, but in erythrocytes (Fig. 3e) the reverse is true. We do not think that HMG 2a is a degradation product of 2b since a small-scale preparation of chicken erythrocyte HMGs from a freshly killed animal gave the same result (not shown). The possibility of chicken thymus being contaminated with blood is ruled out by an examination of the histones extracted from the two tissues with perchloric acid. Histones H1 and H5 are obtained from erythrocytes (Fig. 4d) but only histone H1 was present in thymus tissue (Fig. 4b). The chicken thymus HMG preparation (Fig. 4a) does have a band running between histone H5 and HMG 1 which may be related to the histone $H1^{o}$ reported by Panyim and Chalkley (13). We have also seen a band in this position in PCA extracts of rabbit thymus nucleosomes (14).

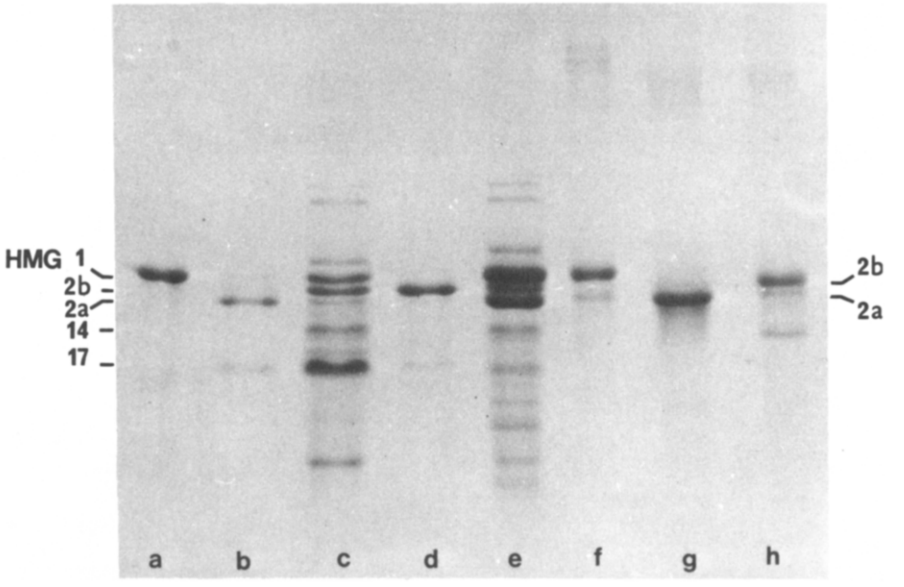

Figure 3: SDS polyacrylamide gel electrophoresis of chicken HMG proteins. Chicken thymus (a) HMG 1, (b) HMG 2a, (c) total, (d) HMG 2b. Chicken erythrocyte (e) total, (f) HMG 1, (g) HMG 2a, (h) HMG 2b.

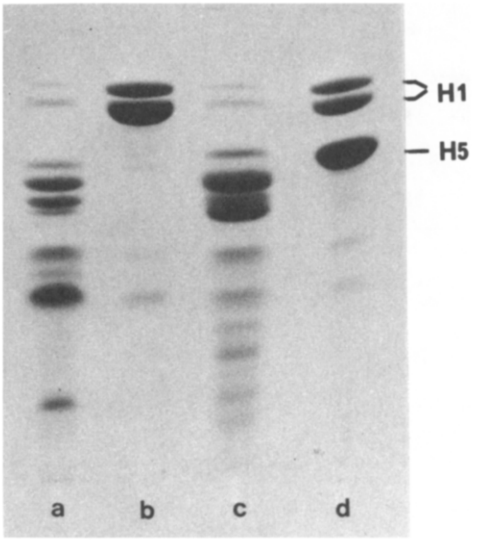

Figure 4: SDS polyacrylamide gel electrophoresis of chicken histone and HMG proteins extracted with perchloric acid. (a) chicken thymus HMG (b) chicken thymus histone (c) chicken erythrocyte HMG (d) chicken erythrocyte histone.

Table 1 Amino acid analyses (moles %) of chicken erythrocyte and thymus HMG proteins

	Ch E HMG 2a	Ch T HMG 2a	Ch E HMG 2b	Ch T HMG 2b
Asp.	12.1	11.4	9.8	8.3
Thr.	2.3	3.3	2.0	2.3
Ser.	5.3	6.4	6.6	7.1
Glu.	16.4	14.7	18.8	18.5
Pro.	7.2	8.0	7.8	8.4
Gly.	6.7	7.8	6.7	7.6
Ala.	9.2	11.4	9.8	10.2
Val.	3.4	3.2	2.5	2.1
Cys.	0.8	0.7	0.7	0.7
Met.	1.7	1.3	1.5	1.5
Ile.	1.5	1.3	1.4	1.4
Leu.	2.0	2.1	2.1	2.1
Tyr.	2.7	1.9	2.4	2.6
Phe.	4.9	3.4	3.5	3.0
Lys.	19.6	18.6	18.5	18.0
His.	0.7	1.0	1.5	1.7
Arg.	3.6	3.5	4.5	4.4
N-Terminal Amino acid	Ala+Gly	Ala+Gly	Gly	Gly

Ch T = chicken thymus Ch E = chicken erythrocyte

The amino acid compositions and N-terminal amino acids of the fractions HMG 2a and 2b from erythrocyte and thymus are given in Table 1. They are all typical HMG proteins, with high contents of lysine and the acidic amino acids. Fraction 2a is significantly different from 2b in both tissues, having a higher aspartic acid and lower glutamic acid content in each case. It is also distinguished by having two N-terminal amino acids, a major alanine and a minor glycine. Furthermore its amino acid analysis is

very similar to that of the protein 'HMG-E' isolated from duck erythrocytes, which also has an alanine N-terminal amino acid (6). The fact that chicken thymus HMG 2a and 2b are contaminated with HMG 17 (Fig. 3b and d) and chicken erythrocyte HMG 2b with HMG 14 (Fig. 3h) does not materially affect the results because both HMG 14 and 17 from erythrocytes (5) and thymus (C. Mathew, unpublished) have proline N-terminals. The lower glutamic acid content of chicken thymus HMG 2a may be related to this contamination since HMG 17 proteins from all tissues studied so far, including chicken erythrocytes, have glutamic acid contents of 10.5 - 12.5 moles % (1). The glycine and alanine N-terminals of HMG 2a prompted us to examine the N-terminals of the four main subfractions of calf thymus HMG 2, which we isolated by CM Cellulose chromatography as previously described (9). All four subfractions had a single N-terminal amino acid, which was glycine in each case.

One-dimensional peptide maps of the HMG 2 fractions have been produced by thin-layer chromatography of tryptic digests, followed by arginine staining of the peptides. The HMG 2a fractions (Figs. 5a and b) are structurally similar to HMG 2b (Figs. 5c and d) and both HMG 2a and 2b have similarities with calf thymus HMG 2 (Fig. 5e).

Discussion

Several conclusions may be drawn from the results of this study. Firstly, we have shown that the protein HMG 2 from chicken thymus and erythrocytes can be resolved by chromatography into two fractions, which we have called HMG 2a and 2b. The HMG 2a fraction predominates in erythrocytes while the reverse is true for the thymus. Both HMG 2a and 2b are structurally similar to calf thymus HMG 2 as judged by their amino acid analyses, peptide maps and microheterogeneity on isoelectric focusing gels. Furthermore, the HMG 2a fraction is analogous to the protein 'HMG-E' isolated by Sterner *et al.* (6) from duck erythrocytes, but in the chicken it is not erythrocyte specific.

The clear difference between the HMG 2 protein content of chicken thymus and erythrocyte is particularly interesting in view of the changes in

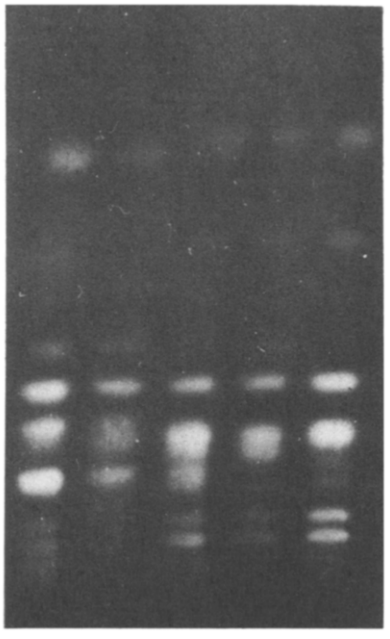

Figure 5: One-dimensional peptide maps of HMG proteins (a) chicken erythrocyte HMG 2a (b) chicken thymus HMG 2a (c) chicken erythrocyte HMG 2b (d) chicken thymus HMG 2b (e) calf thymus HMG 2.

the structure and composition of chromatin which accompany erythropoiesis in the chick. Weintraub has shown that the concentration of the red cell specific histone H5 increases during erythropoiesis and that this is correlated with an increase in the nucleosome repeat length (15). It will be interesting to see whether a similar increase in concentration is observed for the fraction HMG 2a, since this would suggest a possible correlation between the proportions of fractions HMG 2a and 2b and the transcriptional activity of the chicken genome.

Acknowledgements

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