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HIGH MOBILITY GROUP NON-HISTONE CHROMOSOMAL PROTEINS FROM CHICKEN ERYTHROCYTES

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Summary

The high mobility group non-histone chromosomal proteins have been isolated from chicken erythrocytes and analysed by polyacrylamide gel electrophoresis, amino acid analyses and isoelectric focusing. The results show that four HMG proteins, similar to HMG 1, 2, 14 and 17 in thymus, are present in inactive chicken erythrocyte nuclei, indicating a possible structural role rather than involvement in chromatin transcription.

Introduction

Although the high mobility group (HMG) non-histone proteins are now a well-characterized group of chromosomal proteins, their functions remain unknown (see ref. 1 for review on HMG proteins). The four main proteins in mammals, HMG 1, 2, 14 and 17, appear to be present in all the tissues so far studied (1,2) and we have also found that these proteins are associated with isolated nucleosomes (3). One possibility is that they might be attached to that sub-group of nucleosomes that are associated with actively transcribing genes. If this is so, then they should not be found in transcriptionally inactive chromatin and therefore, in order to investigate this possibility, we have carried out an analysis on the presence of HMG proteins in the transcriptionally inactive chicken erythrocyte. Previous studies have found very little (4) or no HMG proteins at all (5) in these cells which has supported the idea that these proteins may be attached to transcribing chromatin. However, HMG proteins are easily lost from chromatin and we have therefore re-examined this question by isolating erythrocyte nuclei in the presence of

TCA = Trichloroacetic acid

HMG = High mobility group

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proteolytic inhibitors and avoiding the use of heparin or trisodium citrate, which may inadvertently dissociate HMG proteins from the chromatin during collection of blood. In this paper we show that, taking these precautions, HMG proteins, similar to those found in calf thymus, are present in comparable quantities in chicken erythrocyte nuclei.

MATERIALS AND METHODS

Preparation of chicken erythrocyte nuclei and isolation of HMG proteins

All procedures were carried out at 4°C and all buffers contained 0.5 mM phenylmethylsulphonyl-fluoride as a proteolytic inhibitor.

Chicken blood was collected in an equal volume of cold 75 mM NaCl, 25 mM EDTA (pH 7.5), and the nuclei were prepared as follows:

The blood was filtered through two layers of surgical gauze and the cells were sedimented at 2000 x g for 15 mins. The supernatant, together with the upper layer (buffy coat) containing white blood cells were discarded and the erythrocytes were washed twice more in the same manner. The erythroid cells were lysed by stirring for 30 mins. in 0.2% saponin in 0.34 M sucrose in TMKC buffer (10 mM tris-HCl - pH 7.4, 5 mM $MgCl_2$, 2 mM KCl, 1 mM CaCl) at 4°C and then pelleted by centrifugation at 16,000 x g for 20 min. The pellet was washed twice with 0.34 M sucrose in TMKC buffer, then suspended in 2 M sucrose in TMKC buffer and centrifuged for 1 h at 30,000 x g. The nuclei were washed with TMKC buffer and HMG proteins were extracted three times with 0.35 M NaCl pH 7.0 followed by acetone precipitation as described previously (6). The weight of HMG proteins recovered per gram of nuclear DNA was 22 mg.

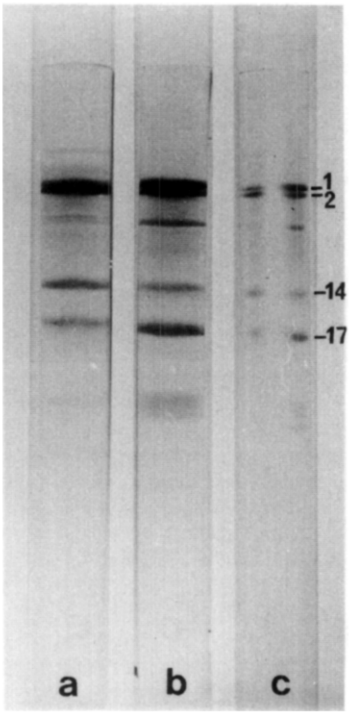
Fractionation of chicken erythrocyte HMG proteins and analytical methods

HMG proteins were fractionated on CM Sephadex C25 column (2.5 x 30 cm) as described (2) except that a shallower linear salt-gradient (400 ml 0.1 M NaCl in borate buffer - 400 ml 1.25 M NaCl in the same buffer) was employed. The fractions were pooled and collected by acetone precipitation (6).

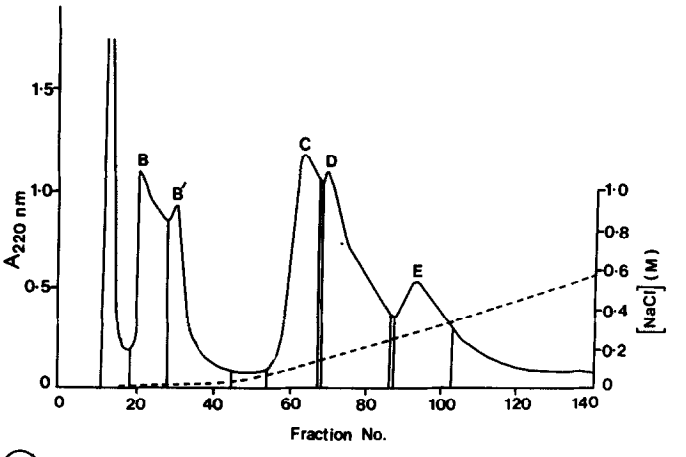
Fractions D and E (Fig. 2) were further purified by TCA precipitation. Fractions were dissolved in distilled water (1 mg/ml) and the impurities were precipitated at final concentrations of 12% TCA (fraction D) and 18% TCA (fraction E) by adding 100% TCA (w/v) and centrifuging at 1800 x g for 10 min. The supernatants were acidified to 0.2 N H_2SO_4 and 6 vol. of acetone added. The precipitates were collected as described before (6).

Polyacrylamide gel electrophoresis of proteins was carried out as described previously using 20% polyacrylamide gels at low pH (7). Proteins were reduced with 40% mercaptoacetic acid (8) for 20 h prior to electrophoresis.

Isoelectric focusing, N-terminal and total amino acid analyses were carried out as described previously (6,8).



(1)



(2)

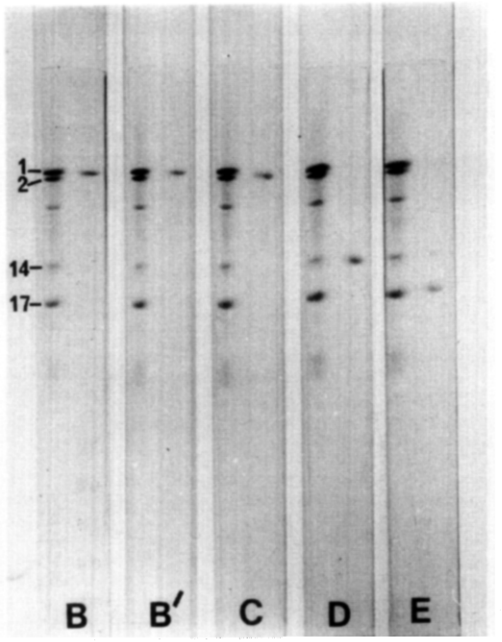
Figure 1: Polyacrylamide gel electrophoresis of

(a) chicken erythrocyte total HMG proteins
(b) pig thymus total HMG proteins
(c) comparative polyacrylamide gel electrophoresis of chicken erythrocyte HMG protein (left) and pig thymus HMG protein (right)

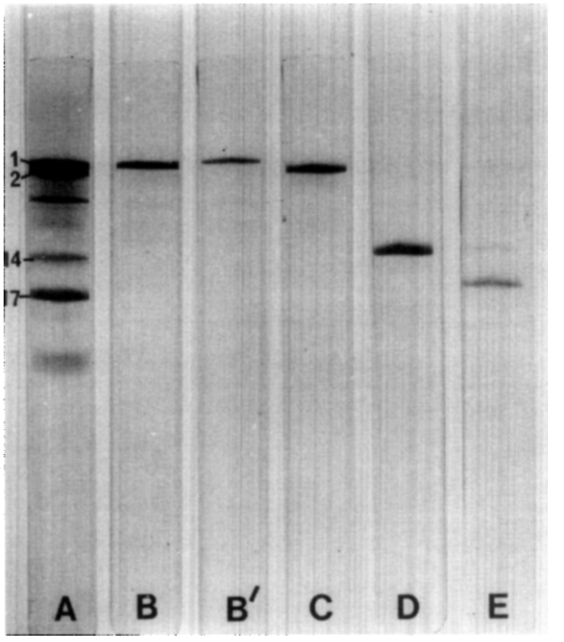
Figure 2: CM-Sephadex ion-exchange column chromatography of chicken erythrocyte HMG proteins. 5 ml fractions collected. (———) absorbance at 220 nm (------) NaCl concentration.

RESULTS AND DISCUSSION

Chicken erythrocyte nuclei were prepared in the presence of phenylmethylsulphonyl-fluoride and the HMG proteins isolated by 0.35 M NaCl extraction. Low mobility group proteins were precipitated by 2% TCA and the HMG proteins were recovered from the supernatant by acetone precipitation (6). The polyacrylamide gel electrophoresis pattern of the protein thus obtained was compared with pig thymus total HMG protein and is shown in Fig. 1. It is



(3)



(4)

Figure 3: Comparative polyacrylamide gel electrophoresis of fractions obtained from ion exchange column chromatography. All fractions were reduced with 40% mercaptoacetic acid. The left side of each gel is pig thymus total HMG, and the right are fractions B, B′, C, and fractions D and E after further purification of the major components by TCA precipitation as described.

Figure 4: Polyacrylamide gel electrophoresis of fractions after reduction with 40% mercaptoacetic acid.

A, pig thymus HMG proteins; B-E, CM-Sephadex fractions as in Fig. 3.

apparent that chicken erythrocyte has four proteins with similar mobilities to the thymus HMG proteins 1, 2, 14 and 17. (Histone H1 is also present in these preparations and runs between HMG 2 and HMG 14.) The quantity of the chicken erythrocyte proteins recovered (2.2% by weight of the DNA) is somewhat lower than is found for thymus (3% by weight of the DNA). On the basis of their similar electrophoretic mobilities to the thymus proteins, we have numbered the erythrocyte proteins 1, 2, 14 and 17 also. In order to justify this we have fractionated these four components using procedures developed

for the isolation of calf thymus HMG proteins (2,6) and have characterised the purified (or partially purified) components by amino acid analyses and isoelectric focusing. Fig. 2 shows the elution profile of the CM-Sephadex ion-exchange chromatography of the erythrocyte HMG proteins. Figs. 3 and 4 show the polyacrylamide gel electrophoretic analyses of the peaks, B, B′, C, and peaks D and E after further purification of the major components by fractional TCA precipitation as described in Materials and Methods. Peaks B and B′ are both erythrocyte HMG 1. Thymus HMG 1 also occasionally elutes as two peaks (6), the reason for this is not known, but it is probably due to protein modification. Peak C is protein HMG 2. Peak D from the column contained mainly HMG 14 with some HMG 2 contamination which could be removed by precipitation with 12% TCA. Fraction E is mostly HMG 17 plus some HMG 14 and HMG 2. The HMG 2 and some of the HMG 14 could be removed by precipitation with 18% TCA but a little HMG 14 remains in the supernatant with HMG 17 and is seen as a faint band in Figs. 3 and 4. The total amino acid analyses and N-terminal amino acid analyses of the four proteins thus obtained are given in Table 1. For comparison, the amino acid analyses of thymus HMG 1, 2, 14 and 17 are also given. It can be seen that all four proteins are typical HMG proteins with high contents of acidic and basic residues. The amino acid analyses of the chicken erythrocyte HMG 1 and HMG 2 are very similar to the calf thymus HMG 1 and HMG 2 analyses and they have the same N-terminal, glycine. The chicken erythrocyte proteins HMG 14 and 17 again resemble the thymus HMG 14 and 17 in having little or no aromatic residues, having quite high values of proline, alanine and glycine, and having N-terminal proline. However, there are some differences in the amino acid contents between the two erythrocyte proteins and their thymus counterparts which can be seen in Table 1. It is also apparent that the two smaller, more basic HMG proteins of chicken erythrocytes are more closely related to one another than the two in thymus. It is interesting to note that a similar protein, histone H6, has been isolated from trout testis (9) which has

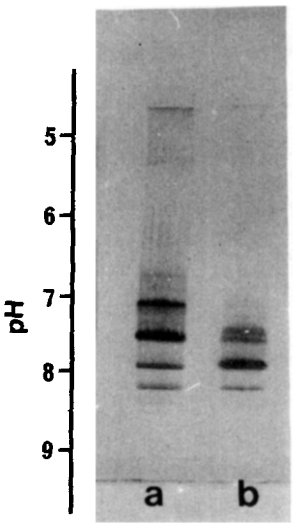


Figure 5: Isoelectric focusing pattern of

(a) calf thymus HMG 2
(b) chicken erythrocyte HMG 2

features in common with both thymus HMG 14 and 17 (1).

The similarity between the HMG 1 and HMG 2 proteins from thymus and erythrocytes is also shown in their isoelectric focusing patterns. Calf thymus HMG 1 has a complex smeared pattern of bands between pH 6 and 8 whilst HMG 2 shows a number of distinct bands between pH 7 and 9 (8). Chicken erythrocyte HMG 1 also has a smeared pattern on the pH 6 to 8 range (not shown) and erythrocyte HMG 2 has three of the four bands focusing in the same position as three of the thymus HMG 2 bands (Fig. 5).

In conclusion, this paper has shown that the inactive chicken erythrocyte nucleus has four HMG proteins which resemble the four HMG proteins found in calf thymus. This finding would tend to argue against the idea that HMG proteins might be only associated with actively transcribing genes. Whilst this paper was in preparation, a report appeared by Vidali et al. (10) in which a protein fraction containing proteins similar to HMG 1 and HMG 2 was shown to be present in duck erythrocytes and found to be present in

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

	C.T. HMG 1	Ch.E. HMG 1	C.T. HMG 2	Ch.E. HMG 2	C.T. HMG 14	Ch.E. HMG 14	C.T. HMG 17	Ch.E. HMG 17
Asp	10.7	11.5	9.3	12.9	8.1	11.2	12.0	10.5
Thr	2.5	2.7	2.7	2.5	4.2	4.2	1.2	3.1
Ser	5.0	5.8	7.4	5.7	7.8	5.1	2.3	4.1
Glu	18.1	18.1	17.5	15.3	17.1	13.2	10.5	12.5
Pro	7.0	5.1	8.9	6.1	8.5	10.2	12.9	10.1
Gly	5.3	5.9	6.5	7.2	6.5	6.6	11.2	9.4
Ala	9.0	8.7	8.1	10.0	14.5	18.6	18.4	16.5
Val	1.9	3.5	2.3	3.5	4.2	1.0	2.0	2.6
Cys	trace	0.9	trace	0.4	0.7	0.7	-	1.0
Met	1.5	0.5	0.4	0.4	-	0.3	-	-
Ile	1.8	1.8	1.3	1.7	0.5	-	-	-
Leu	2.2	2.7	2.0	2.3	2.0	1.0	1.0	1.2
Tyr	2.9	2.2	2.0	2.5	0.4	-	-	-
Phe	3.6	4.3	3.0	4.9	0.6	-	-	-
Lys	21.3	18.2	19.4	17.3	19.0	17.8	24.3	22.6
His	1.7	1.7	2.0	0.9	0.3	1.0	-	0.4
Arg	3.9	4.0	4.7	4.2	5.6	4.3	4.1	5.1
N-terminal amino acid	Gly	Gly	Gly	Gly	Pro	Pro	Pro	Pro

C.T. = Calf thymus Ch.E. = Chicken erythrocyte

nucleosomes, as we have found for thymus nucleosomes (3). In addition, they presented evidence that these two proteins are associated with DNAse I-susceptible regions in erythrocyte chromatin which suggested that these proteins are attached to 'active' or potentially active chromatin regions (10).

However, this does not appear to be the case in thymus and liver since DNAse I digestion releases only a small proportion of HMG 1 and 2 (1) and we are therefore inclined to believe that HMG proteins are not involved in transcription but rather in a more general structural role like the histones.

Acknowledgements

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