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The Isolation, Characterization and Partial Sequences of the Chicken Erythrocyte Non-Histone Chromosomal Proteins HMG14 and HMG17

COMPARISON WITH THE HOMOLOGOUS CALF THYMUS PROTEINS

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Non-histone chromosomal proteins HMG14 and HMG17 were isolated from chicken erythrocyte nuclei. The proteins were characterized by amino acid analysis and by *N*-terminal sequence analyses. Comparison with the corresponding data for the calf thymus proteins shows that 11% of the residues in HMG14 protein and 5% of the residues in HMG17 protein differ between the two species. Proteins HMG14 and HMG17 therefore do not appear to exhibit the evolutionary stability shown by the nucleosome core histones. Detailed evidence for the amino acid sequence data has been deposited as Supplementary Publication SUP 50101 (4 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies may be obtained on the terms given in *Biochem. J.* (1978) **169**, 5.

Chromatin contains a group of non-histone proteins called the high-mobility-group (HMG) proteins (Johns *et al.*, 1975). There are four main HMG proteins in calf thymus, HMG1, HMG2, HMG14 and HMG17 proteins, which have all been shown to be present in isolated nucleosomes (Goodwin *et al.*, 1977). The presence of HMG proteins in a variety of organisms and tissues, including avian erythrocytes (Rabbani *et al.*, 1978; Sterner *et al.*, 1978), trout testis (Watson *et al.*, 1977) and wheat and yeast (Spiker *et al.*, 1978), implies a widespread occurrence in eukaryotic nuclei. The quantities of these proteins in the cell (about 10^6 molecules/nucleus) suggests that they may have a structural role. Partial amino acid sequences for calf thymus HMG1 and HMG2 proteins (Walker *et al.*, 1977*a*, 1979*b*) and the complete sequences of calf thymus HMG14 (Walker *et al.*, 1979*a*) and HMG17 proteins (Walker *et al.*, 1977*b*) have been determined. HMG14 protein is 100 amino acids long and HMG17 protein is 89 residues long. Previous work in our laboratory has shown that four proteins similar to calf thymus HMG1, HMG2, HMG14 and HMG17 proteins are present in chicken erythrocyte nuclei (Rabbani *et al.*, 1978; Mathew *et al.*, 1979), and recent results have suggested that one or both of HMG14 and HMG17 proteins are responsible for conferring a deoxyribonuclease-I-sensitive structure on the globin gene in chicken erythrocyte chromatin (Weisbrod & Weintraub, 1979). We now report the isolation, characterization and *N*-terminal sequences for the chicken erythrocyte HMG14 and HMG17 proteins, and compare them with the corresponding calf thymus proteins.

Abbreviation used: HMG protein, high-mobility-group protein.

Materials and Methods

Proteins were isolated by using a modification of our previously reported method (Mathew *et al.*, 1979). All procedures were carried out at 4°C and all buffers contained 0.5mM-phenylmethanesulphonyl fluoride as a proteolytic inhibitor. Chicken blood (2 litres) from freshly killed chickens was added to an equal volume of cold 75mM-NaCl/25mM-EDTA, pH 7.5, the mixture was filtered through a sieve to remove any blood clots, and the cells were sedimented at 5000*g* for 15min. The supernatants, 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 then lysed by stirring for 1h in 0.2% saponin in 0.25M-sucrose/10mM-$MgCl_2$, and then pelleted by centrifugation at 30000*g* for 30min. After one further wash in 0.25M-sucrose/10mM-$MgCl_2$, the pelleted nuclei were extracted twice with 400ml of 5% (v/v)

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$HClO_4$, with centrifuging at 5000*g* for 10min after each extraction. The combined extracts were filtered through a glass-fibre filter (GF/B), then acidified to 0.3M-HCl, and protein was collected by precipitation with 6vol. of acetone; 1.8g of protein was recovered.

The HMG proteins thus extracted were fractionated by ion-exchange chromatography on a CM-Sephadex C-25 column (2.5cm × 30cm) as described previously (Rabbani *et al.*, 1978) except that a shallower linear salt gradient (800ml of 0.1M-NaCl in borate buffer to 800ml of 0.6M-NaCl in the same buffer) was employed. The elution profile is shown in Fig. 1. The fractions were pooled as shown and collected by acetone precipitation (Rabbani *et al.*, 1978). The column flow rate was 60ml/h, and 10ml fractions were collected.

Automatic sequence determinations

N-Terminal sequence determinations were carried out on a Beckman 890C sequencer, by using the standard *NN*-dimethyl-*N*-allylamine buffer programme. The phenylthiohydantoin derivatives of the released amino acids were determined by both t.l.c. and back-hydrolysis to the free amino acids, with subsequent identification by amino acid analysis as described previously (Walker *et al.*, 1978). A 3mg portion of each of HMG14 and HMG17 proteins were used for sequence analysis.

Amino acid analysis

Total amino acids were measured by using a Rank–Hilger Chromaspek amino acid analyser. Samples were hydrolysed in 6M-HCl at 110°C for 24h. No corrections were made for hydrolytic losses.

Results and Discussion

HMG17 protein was eluted as a single peak (peak C, Fig. 1), whereas HMG14 protein was eluted as two poorly resolved peaks (peaks A and B, Fig. 1). Protein from both peaks A and B gave a single band running in the same position on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and had identical amino acid analyses. This splitting of the

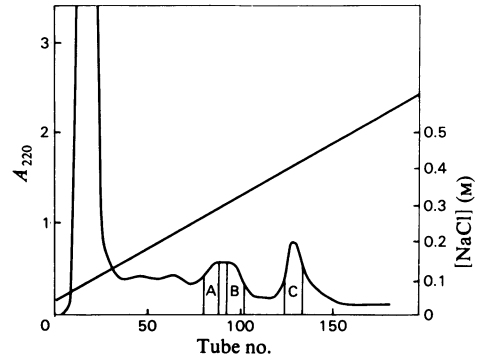

Fig. 1. *CM-Sephadex C-25 chromatography of chicken erythrocyte total HMG proteins*
For experimental details see the text.

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                                                    10
CT HMG14:  Pro-Lys-Arg-Lys-Val-Ser-Ser-Ala-Glu-Gly-Ala-Ala-Lys-Glu-Glu-Pro-Lys-Arg-Arg-

ChE HMG14: Pro-Lys-Arg-Lys-Ala-Pro- o -Ala-Glu-Gly-Glu-Ala-Lys-Glu-Glu-Pro-Lys-Arg-Arg-

                20
           Ser-Ala-Arg-Leu-Ser-Ala-Lys-Pro-Ala-Pro-

           Ser-Ala-Arg-Leu-Ser-Ala-Lys-( )-Ala-Pro-


                                                    10
CT HMG17:  Pro-Lys-Arg-Lys-Ala-Glu-Gly-Asp-Ala-Lys-Gly-Asp-Lys-Ala-Lys-Val-Lys-Asp-Glu-

ChE HMG17: Pro-Lys-Arg-Lys-Ala-Glu-Gly-Asp-Thr-Lys-Gly-Asp-Lys-Ala-Lys-Val-Lys-Asp-Glu-

                20                                  30
           Pro-Gln-Arg-Arg-Ser-Ala-Arg-Leu-Ser-Ala-Lys-Pro-Ala-Pro-Pro-Lys-Pro-Glu-Pro-

           Pro-Gln-Arg-Arg-Ser-Ala-Arg-Leu-Ser-Ala-Lys-Pro-Ala-Pro-Pro-Lys-Pro-Glu-Pro-
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Fig. 2. *Comparison of the N-terminal sequences of HMG14 and HMG17 proteins from chicken erythrocyte and calf thymus*
Key: CT, calf thymus; ChE, chicken erythrocyte.

Table 1. *Comparison of the amino acid analyses of HMG14 and HMG17 proteins from chicken erythrocytes and calf thymus*

Key: ChE, chicken erythrocyte; CT, calf thymus. Values in parentheses for the calf thymus proteins are the total residues calculated from the amino acid sequence. The values for the chicken erythrocyte proteins are the total residues determined from the amino acid analysis. A hydrolytic loss of 10% has been assumed for serine residues.

	Amino acid composition (mol%)			
	HMG14 protein		HMG17 protein	
Amino acid	ChE	CT	ChE	CT
Asp	9.3 (9)	8.3 (8)	9.1 (8)	12.0 (11)
Thr	4.6 (5)	4.1 (4)	3.0 (3)	1.2 (1)
Ser	5.2 (6)	8.0 (9)	4.3 (5)	2.3 (2)
Glu	15.6 (16)	17.5 (18)	11.7 (10)	10.5 (9)
Pro	10.5 (10)	8.1 (7)	12.1 (11)	12.9 (11)
Gly	5.6 (6)	6.4 (6)	10.0 (9)	11.2 (10)
Ala	18.0 (18)	14.8 (16)	17.2 (15)	18.4 (16)
Cys	— (0)	— (0)	—	—
Val	0.3 (0)	4.0 (4)	2.2 (2)	2.0 (2)
Met	— (0)	— (0)	—	—
Ile	— (0)	— (0)	—	—
Leu	1.1 (1)	2.0 (2)	1.2 (1)	1.0 (1)
Tyr	0.2 (0)	— (0)	0.2 (0)	—
Phe	0.1 (0)	— (0)	0.1 (0)	—
His	1.1 (1)	— (0)	0.2 (0)	—
Lys	24.0 (24)	21.1 (21)	23.6 (22)	24.3 (22)
Arg	4.1 (4)	5.4 (5)	4.6 (4)	4.1 (4)

HMG14-protein peak is most probably due to side-chain modifications in the molecule affecting the behaviour of the protein on the ion-exchange resin. However, for the present work, only protein from peak A was used as the source of chicken erythrocyte HMG14 protein. Recoveries were 15 mg of HMG17 protein and a total of 15 mg of HMG14 protein from 2 litres of chicken blood.

Comparison of the amino acid analyses of chicken erythrocyte HMG14 protein with calf thymus HMG14 protein (Table 1) shows at least 11 amino acid changes between the two proteins. Of particular interest are the differences in the hydrophobic amino acids, where the four valine residues and one of the two leucine residues are absent from the chicken protein. Four differences between the two HMG14 proteins are apparent when the *N*-terminal sequences of the two proteins are compared (Fig. 2). There are three amino acid changes, Val↔Ala, Ser↔Pro and Ala↔Glu, and it is also necessary to introduce a deletion in the chicken erythrocyte sequence in order to maintain the sequence homology between the two proteins. Comparison of the amino acid analyses (Table 1) for HMG17 proteins shows there are at least five amino acid differences between the two molecules. One of these differences, Ala↔Thr, is evident in the *N*-terminal sequence (Fig. 2). There is therefore approximately 11% sequence variation in the HMG14-protein sequences from the two species and 5% sequence variation for HMG17 protein. Sequence variations of 5% and 11% represent considerable evolutionary stability when compared for example with the variation of 28% that exists between the sequences of α-globin from calf and chicken. However, this sequence variation is greater than that observed for the nucleosome core histones. The sequence of histone H3 differs in only one position in 135 residues between the chicken and calf proteins (Brandt & Von Holt, 1974), and the sequence of calf histone H2B differs from that of chicken histone H2B at four positions in 125 residues (Van Holden *et al.*, 1978). Comparable data (chicken versus calf) for histones H4 and H2A are not available, but it is known that the evolutionary rate of histone H3 is similar to that of histone H4, and that of histone H2B similar to that of histone H2A. It therefore appears that the HMG14 and HMG17 proteins do not exhibit the degree of sequence conservation that is known to exist for the four nucleosome core histones.

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