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Note

Quantitative analysis of non-histone chromosomal proteins HMG 14 and HMG 17 by polyacrylamide gel electrophoresis

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The high mobility group (HMG) non-histone chromatin proteins[1] are currently the subject of intensive investigation as a result of their proposed association with transcribed regions of the eukaryote genome[2–4]. Procedures involving limited digestion of nuclei with micrococcal nuclease have been described for isolating nucleosomes enriched in transcribed sequences[5,6]. We have been analysing the HMG protein composition of such nucleosomes in chicken erythrocytes and have developed a quantitative technique for the purpose. The method involves a comparison of the HMG protein content relative to that of the nucleosomal histone H4 on polyacrylamide disc gels, and should have general application.

EXPERIMENTAL

Nuclei were prepared from chicken erythrocytes as previously described[7]. The preparation of salt-soluble and salt-insoluble monomer nucleosomes will be published elsewhere[8]. Proteins were extracted from solutions of nuclei or nucleosomes which had been dissolved at DNA concentrations of 0.1–0.3 mg/ml. After addition of sodium dodecyl sulphate to 0.5% and sodium perchlorate to 0.25 *M*, the suspensions were shaken with 0.5 volumes of phenol–cresol–hydroxyquinoline solution[9] and centrifuged. The phenol phase was made 0.5 *M* hydrochloric acid and six volumes of acetone were added. After leaving at −20°C overnight, the protein precipitate was washed twice with acetone–0.1 *M* hydrochloric acid (6:1) and twice with acetone, then dried under a vacuum. (DNA can be recovered from the aqueous phase by extraction with isoamyl alcohol–chloroform (1:24) and ethanol precipitation.)

Proteins were dissolved in sample solvent (9 *M* urea, 0.9 *M* acetic acid, 1% β-mercaptoethanol) and loaded onto cylindrical polyacrylamide gels (10 cm × 7 mm I.D.). Gel composition was as described by Panyim and Chalkley[10] except that concentrations of 20% acrylamide, 0.3% bisacrylamide and 2.5 *M* urea were used. Electrophoresis was for 3½ h at 2.5 mA/gel. Gels were stained overnight in 0.2% procion navy, 7% acetic acid, and destained in 40% ethanol at 55°C. The gels were scanned at 580 nm in a Gilford-linear transport device. Scans were photocopied, and peak areas determined by weighing the paper cut-outs.

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The protein content of phenol extracted nuclear protein was determined by amino acid analysis[11].

RESULTS AND DISCUSSION

We have been developing methods for purifying nucleosomes which contain non-histone proteins HMG 14 and HMG 17, since these nucleosomes may be derived from transcriptionally active chromatin[2–4]. We wished to measure the quantities of HMG 14 and HMG 17 in these nucleosomes, in other nucleosomal fractions, and also in the unfractionated nucleus, in order to quantify the degree of enrichment of HMG 14 and HMG 17 in transcriptionally active nucleosomes. This we have done by measuring HMG 14(17)/histone H4 ratios in the proteins extracted from the nucleosomes and from chicken erythrocyte nuclei.

This analysis has been quantified by electrophoresis of phenol-extracted proteins on acid–urea polyacrylamide gels, followed by staining and scanning the gels. Since the HMG proteins amount to only about 3% (w/w) of the histones in nuclei[12], two loadings of samples from unfractionated nuclei (or from nucleosomes only moderately enriched in HMG proteins) are required. Histone H4 is scanned at a low loading (5–25 μg) and HMG 14 and 17 at a higher loading (50–250 μg). Each pair of gels is scanned at the same scale expansion on the chart recorder, the scans are photocopied, and the cut-outs of the peaks are weighed. The ratio of the peak areas (weights) of HMG 14 to histone H4 and of HMG 17 to H4 in the various nucleosome fractions is compared to the ratio obtained in total nuclear protein. (The ratios of HMG 14 and HMG 17 to H4 in total nuclear protein represents a 1.0-fold enrichment.)

Scans of nuclear protein at low and high loadings are shown in Fig. 1A and B. Fig. 1C is a scan of a nucleosomal fraction highly enriched in HMG proteins. In the latter case it is clearly unnecessary to employ the higher loading as well. Table I shows the ratios of HMG/H4 peak areas (expressed as a percentage) for nuclear protein and two nucleosomal fractions. Enrichments are calculated from the average of two determinations.

We have also investigated the staining reponse of histone H4, HMG 14 and HMG 17, since it was important to ensure that this was linear in the range that was applied to the gels. Amounts of 4–40 μg of phenol-extracted nuclear protein were

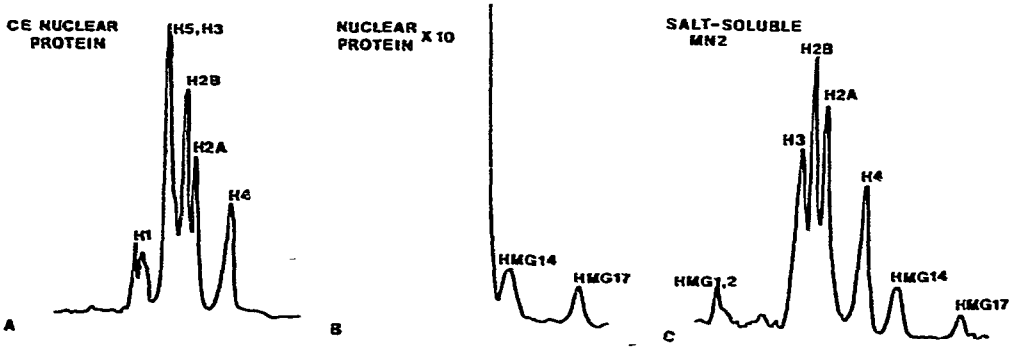

Fig. 1. Gel scans of (A) chicken erythrocyte nuclear protein (20 μg), (B) chicken erythrocyte nuclear protein (200 μg), (C) salt-soluble monomer nucleosome protein (20 μg).

TABLE I

HMG/H4 PEAK AREAS FOR NUCLEAR PROTEIN AND TWO NUCLEOSOMAL FRACTIONS

	Total nuclear protein	*Salt insoluble monomer*	*Salt soluble monomer*
HMG 14/H4 (%)	2.57 ± 0.08	3.7 ± 0.1	12.45 ± 0.1
HMG 14 enrichment	1.0	1.4	4.8
HMG 17/H4 (%)	1.37 ± 0.17	1.9 ± 0.1	4.2 ± 0.15
HMG 17 enrichment	1.0	1.4	3.1

electrophoresed, and the gels stained and scanned as described above. The peak areas of the histone H4 band have been plotted against weight of nuclear protein loaded (Fig. 2). Each point is the average of two determinations. The protein content of the sample was determined by amino acid analysis.

It can be seen that the curve is linear up to a weight of total nuclear protein of about 25 μg after which it levels off abruptly. It follows that calculations of enrichments based on scanning the H4 band from a nuclear protein loading of more than 25 μg will be erroneous. If the protein content of the sample cannot be determined, the area of the H4 peak may be compared to that produced from a 25 μg loading of a nuclear protein standard.

The latter approach has been used to establish the linearity of the staining response of proteins HMG 14 and HMG 17. Increasing amounts of a mixture of these proteins were loaded (in duplicate) onto gels such that their peak areas covered the same range as the HMG 14 and 17 areas encountered in the samples loaded in Fig. 1B and C. Fig. 3 shows that the relationship between peak area and protein load is linear for both HMG 14 and HMG 17 in the range employed.

This rather straightforward technique should be suitable for quantifying

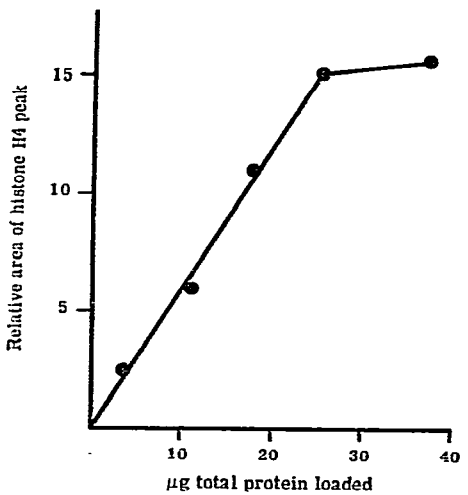

Fig. 2. Procion navy uptake by histone H4. The abscissa indicates the amounts of total nuclear protein extract loaded onto the gels. The ordinate gives the amounts of stain absorbed as measured by weighing cut-outs of the histone H4 peaks from the 580 nm scan of the gels.

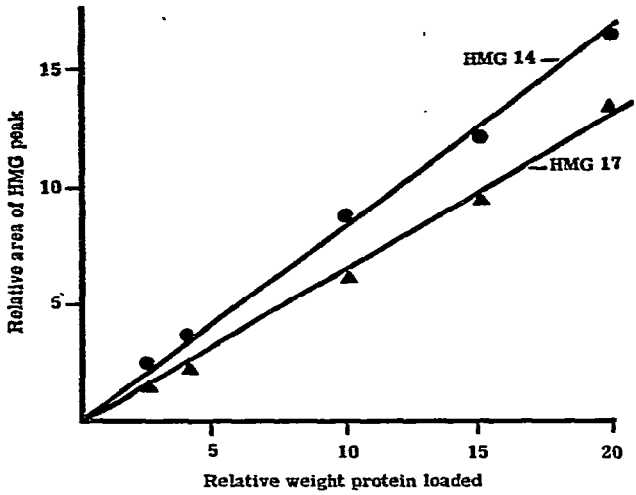

Fig. 3. Procion navy uptake by HMG 14 and HMG 17. The abscissa indicates increasing amounts of the HMG 14 and HMG 17 mixture loaded onto the gels (arbitrary units). The ordinate shows the amount of stain absorbed by each of the proteins as measured by weighing the paper cut-outs of the HMG 14 and HMG 17 peaks.

enrichments or depletions of HMG proteins (or other non-histone proteins) in chromatin fractions, provided that the staining response of the proteins being studied is linear.

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