A GROUP OF H1 HISTONE SATELLITE ACID-SOLUBLE NON-HISTONE CHROMATIN PROTEINS

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1. Introduction

Non-histone proteins of chromatin (NHP) have been under intensive research during last years because of their possible role in the regulation of genetic transcription. Several methods used to isolate NHP from chromatin are based on differences in their ability to bind with DNA. NHP that are are loosely bound to chromatin can be dissociated with solutions of NaCl up to 0.35 M, which leave histones unaffected [1,2]. So-called residual NHP tightly bound with DNA can be extracted by use of stronger solubilisation technique, after removal of histones by different acid solutions [3]. In both groups of non-histone chromatin proteins some basic acid-soluble proteins exist as well, the best studied are among the so-called 'high mobility group' of NHP that can be separated from the 0.35 M NaCl extracts of chromatin [1,2].

It is possible, however, that among the many NHP there are some which have almost identical binding capacity to DNA as histones and at the same time are basic and acid-soluble. These proteins have not attracted much attention because they are generally extracted during studies of histones and considered as 'non-histone contamination'. Methods exist to purify histones from these contaminations and it was found earlier that the P-60 Biogel column chromatography as well as other chromatographic methods can be efficiently used for high purification of the H1 histone and its subfractions and other histones [4–6]. The very high purification of individual proteins even from extremely minor contaminations is of crucial importance to study, for example, the fidelity of protein biosynthesis and search for misincorporation of labeled amino acids into proteins which do not normally contain certain amino acids. H1 histone from mouse tissues contains neither methionine, nor cysteine and we tried to use it to study error frequency in protein biosynthesis [7]. In the course of this work, where comparatively large amounts of the H1 histone must be fractionated by P-60 Biogel chromatography and individual elution subfractions collected for different measurements, we suggested that a very minor contamination might be present at the beginning of the H1 elution curve. In the current work we have identified this contamination as a specific group of acid-soluble 'low mobility' proteins different from the 'low mobility' group of NHP found [1,2] among NHP extracted by 0.35 M NaCl and precipitated by 2% trichloracetic acid.

2. Materials and methods

The experiments were carried out with 4 groups of rats (Sprague-Dawley strain, 6 male rats in each separate experiment) and 10 groups of mice (Mill Hill CBA strain, ~50 male mice in each group). The nuclei were isolated from rat and mouse liver and spleen by homogenization of tissues in a glycerol–Tris–KCl–$MgCl_2$ medium at –20°C according to [8] with the modifications introduced in [9]. Additional repeated homogenizations of nuclei with Triton solutions recommended in [10] eliminates the chromatin bound proteases and prevent the degradation of chromatin proteins. In all experiments however, Trasylol (Bayer UK Ltd) an inhibitor of a wide variety of proteases, was added to all solutions and also during the initial homogenization. The purity of the nuclear pellet was checked by the fluorescent microscopy acridin orange

method [11]. Other analytical procedures and polyacrylamide gel electrophoresis of histones and non-histone proteins were as in [5,12] with H1 histone from rat and mouse tissues, with special care to check the level of purification at the end of every step. Extraction of chromatin by 0.35 M NaCl to eliminate NHP loosely bound to chromatin was repeated 3 times before the extraction of the H1 histone. Extraction of the H1 histone group by 5% perchloric acid already results in their selective purification [13]. H1 histone subfractions were separated and further purified by P-60 Biogel chromatography [14]. A P-60 Biogel column (90 X 1.6 cm) with a flowrate of ~2 ml/h usually produced a rather sharp peak of H1 histone well separated from the H1° fraction (fig.1,2). H1° histone was originally identified and analysed in [15] where it was found to be a usual component of chromatin in non-proliferating calf tissues. The same H1° fraction was later found in mouse and rat liver chromatin [5,16,18]. Some variations of polyacylamide disc electrophoresis were able to divide rat liver H1 histones into 3 subfractions [17]. We have classified subfraction 3 as H1°°. SDS–polyacrylamide gel electrophoresis also separates H1 histone from liver chromatin into 3 fractions [18], but in this work they were classified as H1-1, H1-2 and H1-3.

3. Results and discussion

We found in [12] that mouse chromatin has a higher proportion of H1° histone than rat chromatin. This is clearly seen not only on polyacylamide gels, but on the elution curves during P-60 Biogel fractionation (fig.1,2). The H1 group of histones extracted from mouse liver chromatin forms 2 well-visible peaks, while H1° histone from rat liver chromatin forms only a shoulder on the right side of the main H1 histone peak At the left side of the main H1 histone elution curve a very small shoulder is also formed (fig.1,2). Although we had paid little attention to this part of the P-60 Biogel elution profile, when in our work on the fidelity of mouse H1 histone synthesis [7] we measured the radioactivity of each elution fraction with [^{35}S]methionine, a slight increase of specific radioactivity at the very beginning of the H1 histone elution curve was found. This led us to collect separately the material from different parts of the P-60 Biogel curve and to dialyze, lyophilize and study them by polyacrylamide gel electrophoresis. H1 collected from the main part of the peak is free of contaminations (fig.1,2). H1° histone is highly contaminated by H1 because both peaks overlap. The small shoulder at the left part of the H1 histone curve has in both cases a rather specific contamination by non-histone proteins

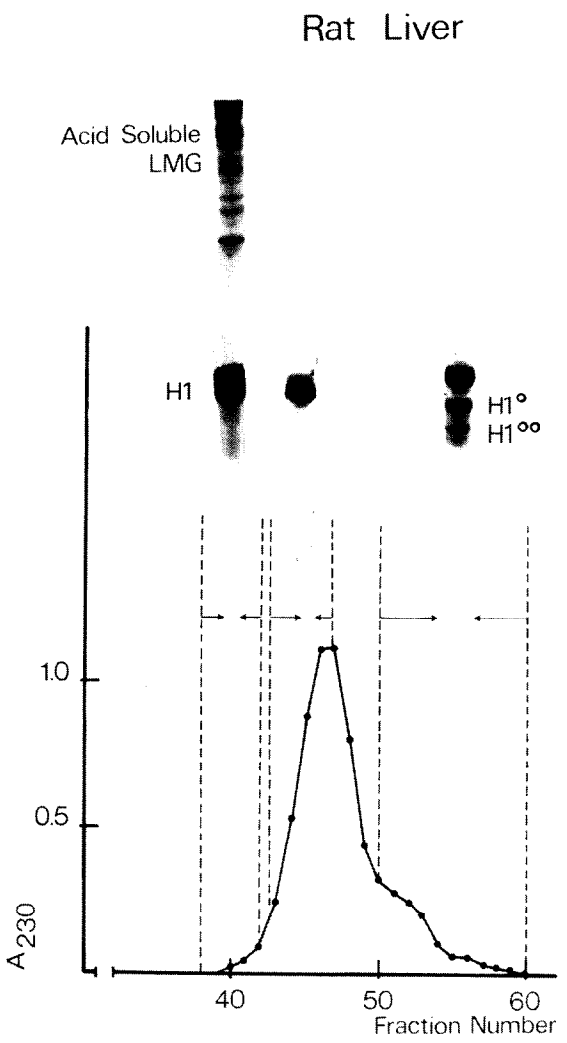

Fig.1. P-60 Biogel column chromatography elution profile of the H1 group of histones from rat liver chromatin and the polyacrylamide gel electrophoretic pattern of proteins from different elution fractions. The presence of LMG NHP is visible on the gel which represents fractions 38–42. The total yield from these fractions was electrophoresed on a single polyacylamide gel. In other fractions, ~20 μg protein were used for each electrophoresis. However, when more protein was applied from fractions 43–47 and 50–60 and the gels were overloaded, no LMG bands of NHP were visible.

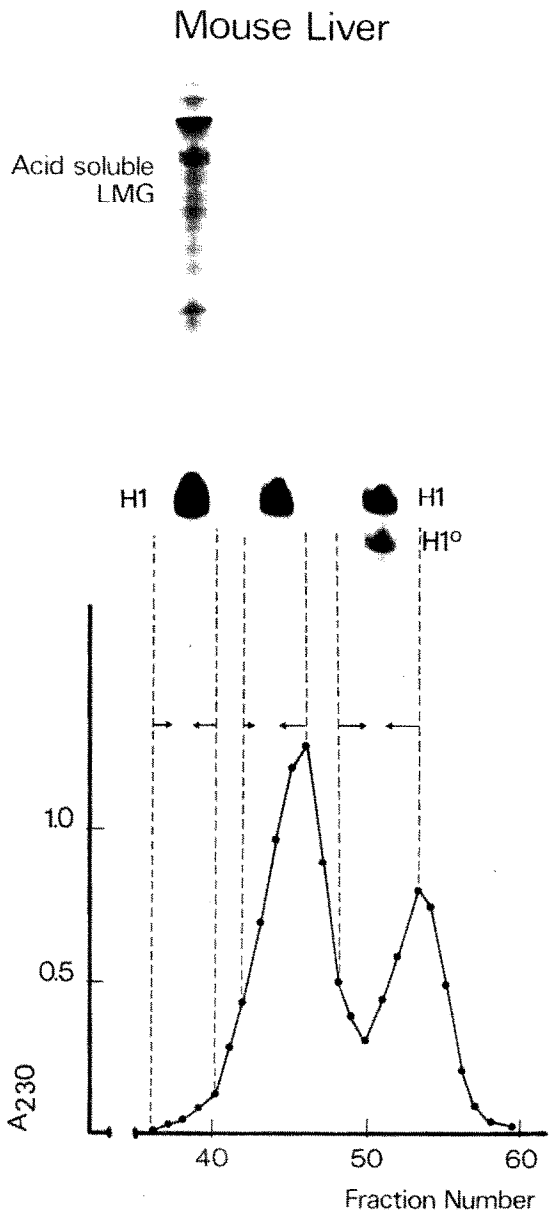

Fig.2. P-60 Biogel elution profile of H1 histones from mouse liver chromatin and the electrophoretic pattern of proteins from different elution fractions. Other conditions are the same as during the analysis shown on fig.1.

which have low mobility during polyacrylamide gel electrophoresis. This is a very minor contamination of the total H1 histone extracted from chromatin. As a rule, a well-visible and differentiated pattern of individual protein bands in this low mobility group (LMG) could be obtained when the total yield of proteins of this small shoulder was used for a single electrophoresis. For liver histone this means the accumulation of material from 50 mice for a single polyacrylamide gel. For spleen chromatin the results shown on fig.3 were obtained when the material was accumulated from 200 mice. From several analyses of this type it was possible to make only an approximate evaluation that LMG of NHP contaminating histone H1 constitute <1–2% of the total protein eluted from the P-60 Biogel. These acid-soluble LMG proteins have higher molecular weights than histones and it was natural to suspect that this group might not be specific, but represent a part of the LMG of

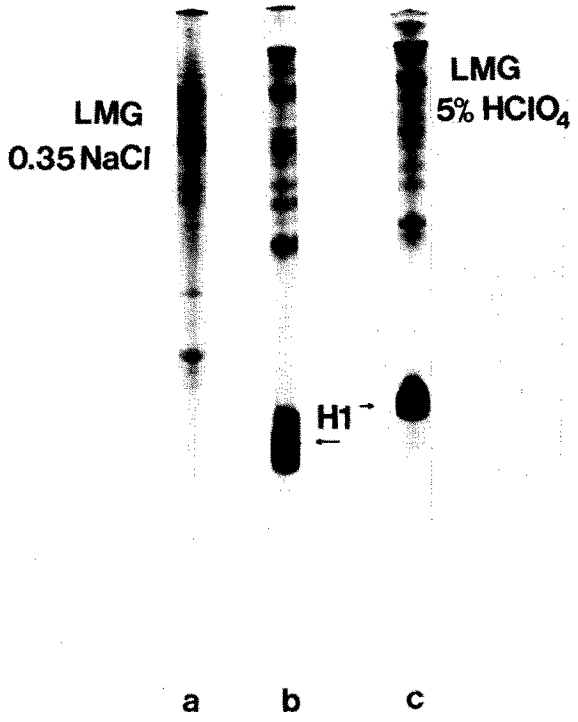

Fig.3. (a) The polyacrylamide gel electrophoretic pattern of LMG proteins from 0.35 M NaCl extracts of mouse liver chromatin. The proteins were isolated by the method in [1,2]. (b) Acid-soluble LMG proteins contaminating H1 histone isolated from mouse spleen chromatin. (c) Acid-soluble LMG proteins contaminating H1 histone isolated from mouse liver chromatin. In (b,c) the total yield of the small shoulder at the beginning of the H1 histone curve was applied for electrophoretic separation. The amount of H1 histone therefore in both cases is higher than usually recommended for polyacrylamide gel electrophoresis and the gels appear overloaded by H1 histone.

NHP which are present in 0.35 M NaCl extractions. However, the LMG proteins in 0.35 M NaCl extracts are not acid-soluble and can be recovered from 2% trichloracetic acid precipitates [1,2]. Precipitation by acid was the method used to separate HMG proteins (acid-soluble) from LMG. The LMG proteins which contaminate the H1 histone are soluble in perchloric acid, but they are also soluble in 2% trichloracetic acid. This difference in solubility indicates that the proteins described here are not a result of incomplete extraction of chromatin by 0.35 M NaCl. Nevertheless it was necessary to compare LMG proteins from 0.35 M NaCl extracts of mouse chromatin and LMG proteins which contaminate H1 histone. The results of this comparison are shown on fig.3. Differences in the electrophoretic pattern are obvious.

It is possible to suggest that the acid-soluble LMG proteins, which during analytical procedures behave like a satellite of H1 histone, could contain basic proteins and have similar binding affinity for DNA as H1. This does not indicate, however, that H1 histone and these LMG proteins are functionally related.

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