THE METHIONINE-CONTAINING SUBFRACTION OF F1 HISTONE FROM RAT TISSUES

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1. Introduction

Kinkade [1] found that the extraction of nuclei by aqueous trichloroacetic acid isolates lysine-rich histones, which could be fractionated into 5 peaks using an Amberlite IRC-50 column. In a comparison of these fractions from different tissues of calf, chicken, cat and rat, it was shown that in addition to the quantitative and qualitative tissue and species differences of these lysine-rich histone, one of the 5 lysine-rich histones from rat tissues contained methionine (0.4 mol/100 mol of total amino acids). All other lysine-rich histones were methionine-free. Methionine has also been found in one of the peptides released during partial digestion of lysine-rich histones from rat [2]. Both results suggest that a rat-specific histone may exist. However, it was recently found that the extraction of lysine-rich histones by 5% perchloric acid from nuclei of rat liver and ascites cells gives a mixture of acid-soluble proteins, 'crude F1 histone', which, in addition to F1 histone, contains several methionine-rich non-histone proteins [3]. Contaminating proteins of 'crude F1' from ascite cells have 7.5 mol of methionine per 100 mol of total amino acid. Nevertheless, the remaining F1 histone after elimination of these methionine-rich non-histone proteins on a Sephadex column still had 0.4 mol of methionine/100 mol of amino acid. However we have subsequently been informed (personal communication from L. Stocken) that further purification of F1 with G75 Sephadex reduces the methionine in F1 beyond the sensitivity of the amino acid analyser (lower than 0.1%).

It is necessary to resolve the question of whether the methionine-containing protein in F1 type histone from rat is a minor lysine-rich histone subfraction, or a contamination. To check this we used two methods: first, measurement of the comparative incorporation of [^{35}S] methionine and [^{3}H] leucine into F1 histone from rat thymus and liver purified by Biogel chromatography and dialysis with comparative determination of turnover rate of [^{35}S] methionine and [^{3}H] leucine labeled fractions (non-histone proteins of nuclei have about 2 mol% of leucine [3], F1 histone about 5 mol%). Second, we used cyanogen bromide [4] to destroy methionine within electrophoretically homogeneous F1 histone from rat thymus chromatin and compared the electrophoretic profiles before and after the reaction with cyanogen bromide. Both methods revealed the existence of methionine containing F1 subfraction in rat histones.

2. Materials and methods

The nuclei were isolated from rat thymus and liver [5]. Chromatin was obtained by blending the nuclei three times with 0.025 M EDTA in 0.074 M NaCl, pH 7.5, and then washing three times with 0.35 M NaCl to remove the soluble non-histone proteins, some of which closely resemble histones [6]. (These 0.35 M NaCl soluble nuclear proteins have been identified as impurities in histone fractions [7] after direct acid extraction of nuclei, and it was also shown that they could combine with F1 histone and polylysine [3]). Histone F1 was extracted from chromatin with 5% (w/v) $HClO_4$ and precipitated and purified by method no. 1 of Johns [8]. The next stage of purification was made by use of Biogel P-60 exclusion chromatography [9]. Quantitative determination of histones were made by the method of Lowry et al. [10], using commercial histone as a standard.

The cyanogen bromide reaction was followed according to the method of Gross and Witkop [4]. Polyacrylamide electrophoresis was carried out by the high resolution method developed by Panyim and Calkley [11] especially for histones. In our case, a slight modification of the conditions of electrophoresis was made (50 V was applied for 17 hr). The histones in the gels were stained with Coomassie Brilliant Blue [12]. Gels were scanned with a Unicam SP 1809 scanning densitometer with an SP 1800 spectrophotometer at a speed of 5 cm/sec.

In the experiment with [^{35}S] methionine and [^{3}H] leucine the high specific activity aminoacids (from Amersham Radiochemical Centre) were administred intraperitoneally (500 μCi of [^{35}S] methionine and 600 μCi of [^{3}H]leucine to each of 10 Sprague Dawley rats, aged eleven weeks). Rats were used for determination of radioactivity 20 hr and two weeks after administration of labeled amino acids. Turnover rates of histones are much slower than other nuclear non-histone proteins and therefore the decay of radioactivity of methionine-containing subfraction should be different from the decay of leucine-containing protein, if methionine is incorporated in non-histone contamination. The separate measurements of ^{35}S

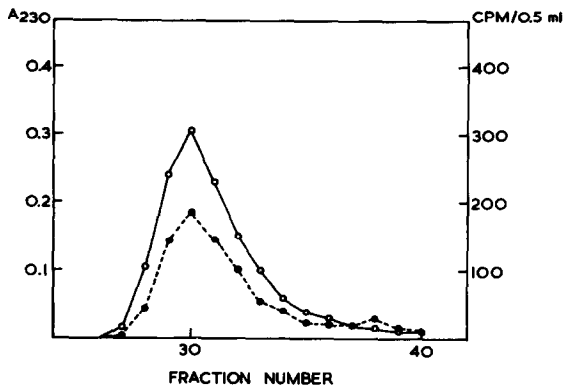

Fig.1. The concentration (——) and ^{35}S-radioactivity (– – –) of rat thymus F1 histone elution profile from Biogel P-60 column (150 × 1.5 cm). Flow rate 3 ml per hr. 1 ml fractions were collected, and 0.5 ml of each fraction was used for radioactivity measurements.

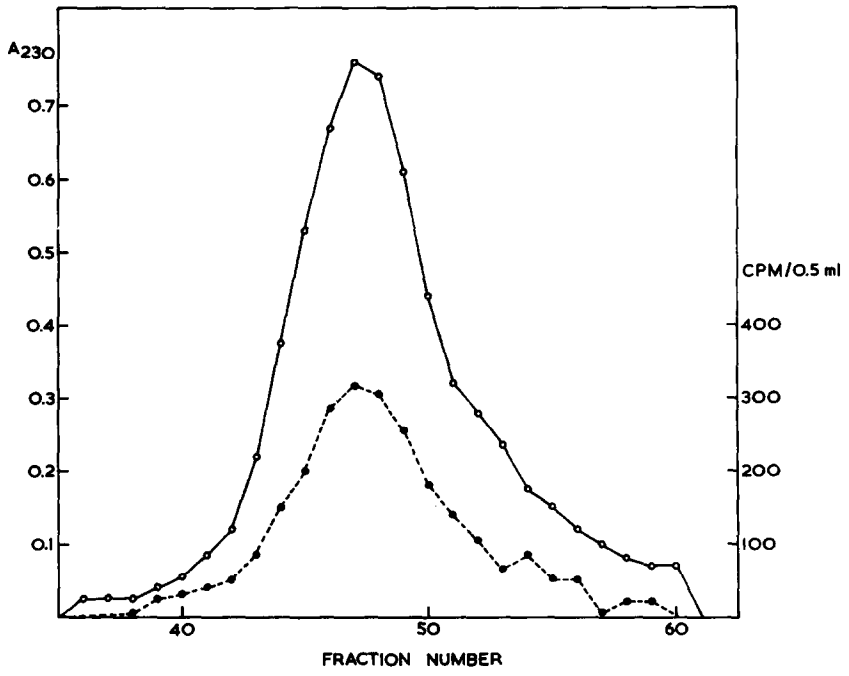

Fig.2. The concentration (——) and ^{35}S-radioactivity (– – –) of rat liver F1 histone elution profile from Biogel P-60. Other conditions are the same as in fig.1.

and ^{3}H radioactivity of proteins were made with the Packard Tri-Carb liquid scintillation spectrometer.

3. Results

Moderately lysine-rich and arginine-rich histones (F2a1, F2a2, F2b and F3) have one methionine residue per molecule [13], and turnover rates of these in rat liver measured with [^{14}C]lysine [14] do not differ significantly, but F1 histone usually had a slightly higher rate of lysine incorporation. In our experiment with incorporation of [^{35}S]methionine into histones, F1 histone precipitated from 5% $HClO_4$, before Biogel chromatography, had lower specific activity than the arginine-rich histones, but the incorporation of [^{35}S]methionine into F1 histone was quite clear. The specific activity of F3 histone from rat liver was about 13 000 cmp/mg, whereas the activity of F1 histone was about 3800 cpm/mg. This might indicate that methionine-containing fraction, or contamination, is not higher than 20–25% of total F1 histone. After Biogel chromatography, the specific activity of F1 histone was reduced by about 60% for F1 from thymus and 30% for F1 histone from rat liver. This suggests that there might be contamination by other methionine-containing proteins, but figs. 1 and 2 show clearly that in the F1 histone Biogel elution the curves of the protein concentration and radioactivity are almost parallel. These results indicate that rat F1 histone may have a methionine-containing subfraction of the same molecular weight. This suggestion was strongly supported by the observation that the decay of ^{35}S-activity and ^{3}H-activity during

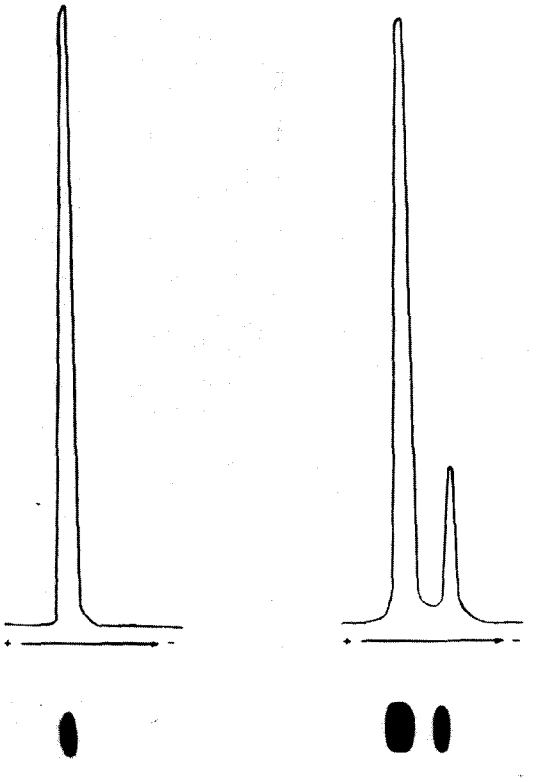

Fig.3. Polyacrylamide gel (15%) electrophoretograms and their photometric profiles of F1 histone from rat thymus purified by column chromatography on Biogel P-60, before (left, 20 μg) and after (right, 30 μg) reaction with cyanogen bromide.

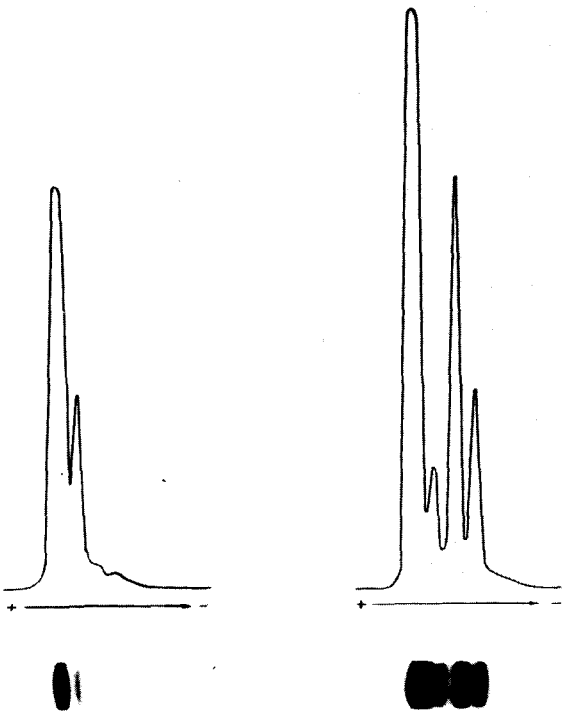

Fig.4. Polyacrylamide gel (15%) electrophoretograms of rat liver F1 histone purified by column chromatography on Biogel P-60 before (left, 20 μg) and after (right, 30 μg) reaction with cyanogen bromide.

two weeks was the same in F1 histone (drop of specific activity for [^{35}S]methionine-labeled protein to 48% and to 50% of initial activity for [^{3}H]leucine labeled histone). All other histones (F2a, F2b and F3) had different levels of decay, but parallel for ^{35}S and ^{3}H activity in each case. The maximal turnover rate was found for non-histone residue chromatin proteins which lost during two weeks 88% of ^{3}H activity and 87% of ^{35}S activity.

To be sure that F1 histone from rat is a mixture of methionine-free and methionine-containing fractions, we used the cyanogen bromide method to destroy the methionine within histone polypeptide chain. Results are shown in figs. 3 and 4. It is clear that splitting the methionine bond produces one new histone fraction from thymus F1 histone, and two new fractions from liver F1, which are easily separable from the methionine-free fractions. This picture indicates that the methionine in the polypeptide chain of F1 thymus histone is situated near the terminal part of the molecule. Before the cleavage of the methionine residue the thymus F1 histone was homogeneous. The cleavage produced one new fraction for thymus histone. The other part of the methionine-containing molecule is probably too small and was lost during electrophoresis or during destaining. F1 histone from liver was heterogeneous before the cyanogen bromide reaction and it produced two new fractions after selective methionine degradation.

4. Discussion

Different histones have rather different rates of changes during evolution, the lysine-rich histones being much more variable and heterogeneous in evolution than the arginine-rich types of histones [13,15]. Histone genes are clustered in the DNA in the form of reiterated DNA sequences, with several hundred copies of each in the cell of sea urchins [16–18]. If the redundancy of histone genes is similar for mammalian cells, which seems probable, then single genes in the DNA cluster coding the F1 histone could mutate independently from other genes of the same cluster. The rat-specific methionine-containing F1 histone subfraction could be a result of such mutations, which change the cluster of DNA sequences for F1 histone into a collection of non-identical repeats of the same molecular weight. It is also possible that the numbers of genes for different histones are different, and that F1 histone is represented in the genome by a smaller number of gene copies than other histones. This could be responsible for the higher rate of molecular evolution in comparison with the more conservative structure of protein coded by highly reiterated genes [19]. The tissue specificity of F1 histone revealed in this experiment will be discussed elsewhere, together with results using other tissues and animals of different age.

Acknowledgements

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