Distribution of the H1 Histone Subfractions in Syrian Hamster Chromatin Fractions

Hanna Modrzejewska, Grażyna Gałązka, Janusz Szemraj, and Henryk Panusz

Department of Biochemistry, Institute of Physiology and Biochemistry, School of Medicine in Łódź, 6 Lindleya St., 90–131 Łódź, Poland

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Chromatin from two Syrian hamster tissues: the Kirkman-Robbins hepatoma and the liver, has been separated into soluble (S) and insoluble (P) fractions. Both fractions contain the complete set of five main histones but differ in respect of H1 subfractions. The hepatoma chromatin is known to contain an unusual H1 subfraction, H1 slow [12, 13], probably identical with a similar subfration present in hamster testes. The content of H1 slow in total H1 histone has been estimated for total, S and P chromatin from hamster hepatoma. The values 20.9 ± 7.2, 13.8 ± 1.8 and 26.8 ± 4.2%, respectively, were obtained.

Introduction

Besides the known evolutionary variance of the H1 histones, their tissue dependent differences became a well established fact. The H1 family is composed of several subfractions characteristic for the given species. The sets of subfractions, however, used for the construction of chromatin of various tissues, show both quantitative and qualitative differences. All H1 subfractions, as well as their close relative, the H5 histone, possess a common molecular structure [1–3]. Individual subfractions differ mainly in aminoacid number and composition of the N-terminal domain [4]. Some less striking differences are also observed in the C-terminal domain [5]. N-terminal and C-terminal domains comprise all loci of phosphorylation, the crucial modification of H1 histones. The plurality of H1 subfractions seems to have its source in the plurality of H1 genes [6]. Thus, regulation at the level of transcription of particular H1 genes seems to be one of the most probable mechanisms of chromatin reconstruction in differentiation.

Among the mammalian H1 subfractions, two kinds are easily distinguished by their specific behaviour during electrophoresis in acid-urea polyacrylamide gels: $H1^0$, of elevated mobility, and several subfractions of decreased mobility in this medium. The last ones have been discovered in rodent testes [7–10] and other animals [11], and in transplantable tumors of Syrian hamster, where they were called H1 slow [12, 13].

Almost no data are available concerning the distribution of the H1 subfractions in fractionated chromatin [14]. This lack of information may be due to the easiness of translocation of H1 molecules during preparative procedures [15]. The method used in this work seems to partially avoid the danger of errors caused by translocation during chromatin fractionation: the solution used for isolation and fractionation of chromatin seems to stabilize its higher order structure [16]. This allowed us to check whether there are differences in the content of H1 slow among the chromatin fractions of Syrian hamster hepatoma.

Materials and Methods

The transplantable hamster hepatoma, derived from the line originally induced by Kirkman and Robbins [17], was obtained at the 447 passage from the Department of Pathological Anatomy of the Medical School in Wrocław and further passaged in our department. The neoplastic material was collected on the eighth day after transplantation of the tumor. Immediately after removal of the neoplastic tissue from the animal, nuclei were isolated according to Fenske *et al.* [18]. The protease inhibitor – PMSF, was added to all solutions up to the concentration of 5 mM. Chromatin was separated into its soluble (S) and insoluble (P) fractions by extraction by means of 0.1 M $(NH_4)_2SO_4$ [18].

Reprint requests to Dr. H. Modrzejewska.

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Total histones from nuclei, chromatin P and chromatin S were isolated as follows: isolated nuclei and chromatin P were extracted twice with 6 volumes of 0.25 M HCl. Total histones were precipitated by adding 100% w/v trichloroacetic acid (TCA) solution up to the final concentration of 20% and leaving the samples to precipitate overnight (0 °C). The samples were centrifuged, TCA was extracted from the precipitates with acetone-HCl, the sediments were washed with acetone and dried under vacuum.

Soluble chromatin S had to be dialized against 0.14 NaCl + 5 mM PMSF in order to remove $MnCl_2$ and $(NH_4)_2SO_4$ before HCl extraction. 10 M HCl was added up to the final concentration of 0.25 M and histones were isolated as described above.

H1 histone subfractions were separated by polyacrylamide gel electrophoresis acc. to Panyim and Chalkley [19]. 15 and 25% arcylamide gel slabs containing 2.5 M urea, 30 cm long were run for 72 h at 4 °C and 210 V.

The gels were stained with Amido-Black 10B. After destaining, the gels were photographed and densitometric patterns of the gel fragments containing H1 subfractions were obtained using the Joyce-Loebl microdensitometer. The ratios of H1 slow to the sum of the H1 subfractions were estimated by calculating the ratios of the areas under the proper peaks. We found this procedure to be sufficiently precise and reproducible for an estimation of differences in the relative content of H1 slow among chromatin fractions. The ratio of chromatin S to chromatin P was estimated by measuring A_{260}.

Results and Discussion

The lack of information on the localization of H1 subfractions within various chromatin domains is mainly due to the methodical obstacles causing the danger of erroneous results. H1 are the weakest bound histones in the chromatin frame. They are known to easily and rapidly translocate [15]. In the method described by Lindigkeit *et al.* [18], fractionation of chromatin is probably based on the fact that chromatin S represents such areas which either contain higher activities of autolytic endonucleases or their structure makes them especially susceptible toward such enzymes. Chromatin P contains all the highly repetitive sequences of satellite DNAs, a feature characteristic for constitutive heterochromatin [20]. The rapidity of extraction of chromatin by ammonium sulfate may not leave enough time for the H1 histones to translocate, which allows to define the difference in H1 subfractions contents.

Nuclei isolated from hamster hepatoma and liver, when tested by microscopic evaluation for pyronine positive contaminations, appeared sufficiently pure. The ratio of soluble chromatin S to total chromatin was considerably stable. The amount of isolated chromatin S, expressed as percentage of total chromatin of hamster hepatoma and liver, is given in Table I.

Total histones were isolated from nuclei and from both soluble (S) and insoluble (P) chromatin fractions of hamster hepatoma and liver. Their electrophoretic analysis on acid-urea gels has shown that all five main histone fractions are present and that the ratios of H1 to H4, as judged by comparing densitometric areas, are almost identical for all materials tested. Histone H4 was selected as datum fraction for being the most conservative core histone [21]. The results suggest that the molecules never get lost into solution. The H1 histone subfractions were separated by urea-polyacrylamide gel electrophoresis using different gel concentrations. The best separations of H1 slow from the remaining H1 subfractions were obtained in 25% polyacrylamide gels.

The densitograms of H1 subfractions, separated by electrophoresis of total histones isolated from whole nuclei, chromatin S and chromatin P fractions of hamster hepatoma, are shown in Fig. 1. As indicated in Fig. 1, H1 slow was present in extracts from whole hepatoma chromatin as well as from both chromatin P and S. Quantitative analysis of the relative amounts of individual subfractions revealed remarkable differences between chromatin P and S. The ratios of the H1 slow subfractions to the total H1 histone in hamster hepatoma chromatin

Table I. The amount of isolated chromatin S, expressed in percentage of total chromatin. Mean values from 5 experiments, each performed on 8–12 animals, are given.

Tissue	% of chromatin S
Kirkman-Robbins hepatoma	23.4 ± 5.9
Liver of tumor-bearing animals	26.3 ± 7.1
Liver of healthy animals	24.6 ± 6.1

Table II. The relative contents of the H1 slow in Kirkman-Robbins hepatoma of Syrian hamster, expressed as % of total H1 histone.

Chromatin source	Prep. no	[%]
Chromatin from total nuclei	1	12.4
	2	28.8
	3	17.8
	4	24.5
	$\bar{x}$	20.9 ± 7.2
Chromatin P	1	24.3
	2	30.2
	3	25.8
	$\bar{x}$	26.8 ± 4.2
Chromatin S	1	11.3
	2	14.6
	3	13.8
	4	13.4
	$\bar{x}$	13.8 ± 1.8

fractions are presented in Table II. Even though the observed differences between chromatins S and P in their content of H1 slow may not be exactly those existing in the living cells, they seem sufficient for stating that H1 slow is preferentially accumulated in chromatin areas more resistant to autolytic degradation. A similar observation was made by Gorka and Lawrence who found the H1 subfraction associated with the nuclease-resistant fraction in chromatin [14]. A similar analysis was performed on liver chromatin isolated from control animals. It proved that H1 slow is absent from this tissue. If, however, liver was taken from the tumor-bearing animals, considerable but fluctuating amounts of H1 slow were detected. The presence of H1 slow in chromatin from livers of tumor-bearing animals may be explained by accumulation of neoplastic cells of this fast growing and highly malignant tumor in the liver vessel net. This fact has been confirmed by histopathologic examination (data not shown). H1 slow was also found in embryos and SV-40 induced tumors but it was absent from regenerating and normal liver [13]. A similar subfraction has been found in Syrian hamster testes. The appearance of H1 slow in hepatoma and its absence from liver may be responsible for differences in the structure of these chromatins, especially in insoluble chromatin P, which is more resistant to endonuclease action.

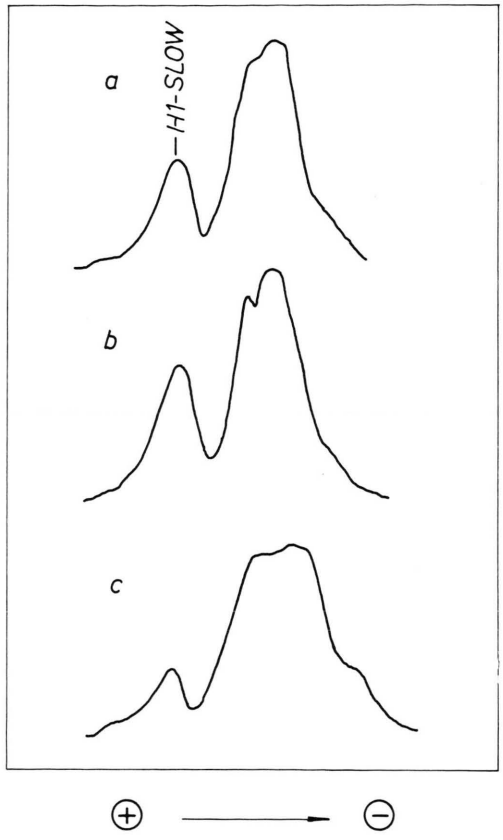

Fig. 1. Comparison of electrophoretic densitograms of H1 histone subfractions. A. H1 from total nuclei. B. H1 from chromatin P. C. H1 from chromatin S.

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