The involvement of histone H1^o in chromatin structure

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Received 30 November 1984; Accepted 18 March 1985

ABSTRACT

Micrococcal nuclease digestion and light scattering are used to compare native chromatin with various histone H1^o contents. The experimental data show that the higher the H1^o content, the greater the ability to form compact structures with increasing ionic strength, and the lower the DNA accessibility to micrococcal nuclease. On the contrary, reconstituted samples from H1-depleted chromatin and pure individual H1 fractions behave in such a way that samples reconstituted with pure H1^o give rise to a looser structure, more accessible to nuclease than samples reconstituted with H1-1.

This contradiction suggests that the effect of H1^o on chromatin structure must originate from the interaction of this histone with other components in native chromatin among which other histone H1 subfractions are good candidates.

INTRODUCTION

H1^o, a histone of the H1 family, is regulated during development (1,2) and it has been suggested that H1^o could be implicated in cellular differentiation (2).

Like H1, H1^o is located on the linker region of the nucleosome (1). We have found, as previously reported, that H1^o is associated with an increased resistance of chromatin to micrococcal nuclease digestion (3). This result suggested that H1^o could be associated with, or responsible for, a more compact conformation of chromatin. In the present report, we have studied chromatin samples from newborn mouse liver, adult rat liver and adult mouse liver having respectively 3%, 7% and 21% of H1^o relative to total H1. We have compared their resistance to micrococcal nuclease, and their ability to condense by a light scattering study (4, Girardet J.L. and Roche J., in preparation). We found an increased resistance to the nuclease associated with a greater amount of H1^o. Light scattering studies showed that the greater the amount of H1^o, the easier the chromatin can take up compact conformation with increasing ionic strength.

However, the three chromatin samples differ not only in their H1^o content, but also in the concomitant variation of the other H1 subfractions, as well as in their non histone proteins (NHP) content. In order to obtain the "pure" effect of each H1 subfraction on chromatin structure, we decided to compare chromatin samples reconstituted with the various individual H1 fractions. Unexpectedly, H1-1 was found more efficient than H1^o in inducing the compaction of chromatin. These results suggest that H1^o is not responsible alone for a particular conformation of chromatin.

MATERIALS AND METHODS

1. Preparation and characterization of native chromatin

Nuclei were prepared as described (5) from the liver of newborn mouse, adult mouse and adult rat. Long chromatin fragments were obtained by gentle digestion of nuclei by micrococcal DNase : 20 units DNase/10⁸ nuclei for 90 s. at 37°C (6). High order oligomers were further purified on a 5-30 % isokinetic sucrose gradient in 10 mM Tris pH 7.4, 0.2 mM EDTA, 0.5 mM PMSF (PMSF : phenyl methyl sulfonyl fluoride). After centrifugation for 3 h. at 270,000 g at 4°C, fractions containing oligomers higher than 20 nucleosomes were collected and dialysed overnight against 1 mM TEA buffer pH 7.4, 1 mM NaCl, 0.5 mM PMSF for further micrococcal nuclease digestion or against 1 mM phosphate buffer, pH 7.4, 1 mM NaCl, 0.2 mM EDTA, 0.5 mM PMSF for physical measurements.

The protein content of the various samples was extracted as described (3) and analysed by electrophoresis in an SDS polyacrylamide gel (7). The nucleosome repeat length of DNA was measured as described (8).

2. Kinetics of micrococcal nuclease digestion of chromatin

Long chromatin fragments (2A₂₆₀/ml) were digested with micrococcal nuclease in 1 mM TEA pH 7.4, 1 mM NaCl, 0.1 mM CaCl₂, 0.5 mM PMSF. Digestion was performed at 4°C with 40 units DNase/A₂₆₀. The reaction was stopped by 1 mM EDTA. DNA was analyzed after treatment of the samples with proteinase K and SDS (9) on horizontal 1,5 % agarose gel (10) in 40 mM Tris-acetate pH 7.8, 20 mM NaCl and 2 mM EDTA.

3. Chromatin reconstitution

Chromatin was reconstituted from H1 depleted chromatin and the various histone H1 fractions as described in details elsewhere (8).

4. Light scattering experiments

Light scattering experiments were performed with a home-made spectrophotometer (Dupont Y., in preparation) and the variations of the

intensity of scattered light were measured at $\approx 90^\circ$ to the incident monochromatic beam ($\lambda = 365 \text{ nm}$; bandwidth $\approx 1 \text{ nm}$).

The incident light was applied to a cuvette filled with the sample under study and saline solution was added to the solution, using an Hamilton syringe, in such a way as to avoid any perturbation of the optical path of the light. A photomultiplier gave the variations of the intensity of the scattered light with an experimental error around 1% (Girardet, J.L., and Roche, J., in preparation).

RESULTS

1. Native chromatin

a. Characterization of the samples.

A major difference between the three native chromatin samples prepared from liver resides in their protein content as shown in Fig. 1. Although the total amount of H1 relative to H4 is constant for the three samples, there is a considerable variation in the relative amount of the different H1 subfractions. Quantitative estimations from scanning the gels are given in Table I.

There is no major quantitative difference in the non histone protein (NHP) content of adult mouse liver and adult rat liver chromatin (which contain respectively 21% and 7% of H1 $^\circ$), whereas almost all the NHP present in these samples is absent from newborn mouse liver chromatin (which contains 3% of H1 $^\circ$, i.e. a value on the order of that found for adult rat liver).

Fig.1. Protein electrophoresis in SDS-15% polyacrylamide gel of liver chromatin: 1- adult rat ; 2- newborn mouse ; 3- adult mouse.

TABLE I : Characteristics of H1 content in the different chromatin samples.

Liver chromatin long fragments	: H1 : : H4 :	Total H1 = 100%		
		H1-1	H1-2	H1°
Adult rat	: .94 :	69	24	7
Newborn mouse	: .88 :	60	37	3
Adult mouse	: .92 :	67	12	21

The value in % is determined by optical determination of the concentration of the bound dye after scanning the gel. The amount of histone H1 (total H1) is expressed relative to histone H4. The amount of each H1 subfraction (H1-1, H1-2, H1°) is expressed relative to total H1.

The comparison of the various chromatin samples showed no differences in the value of their nucleosome spacing (196 ± 3 base pairs) as well as in their melting profiles which were monophasic, as described for rat liver (11) with a T_m of $82 \pm 0.5^\circ\text{C}$ and $35\% \pm 1\%$ relative hyperchromicity. These results show that there is no gross structural difference between these samples, such as the amount of unprotected DNA or the stability of DNA helix against thermal denaturation.

b. Nuclease digestion.

The kinetics of micrococcal nuclease digestion of chromatin samples having different amounts of histone H1° were studied under the conditions described in Materials and Methods. The results of such experiments are shown in Fig. 2.

Clearly the ability of micrococcal nuclease to digest chromatin is greater for chromatin which contains less H1°. A quantitative estimation of this effect was made by comparing the amounts of (Mono + Di)nucleosomes formed after 90 minutes digestion, related to the total DNA present on each sample. For rat liver, newborn mouse liver and adult mouse liver chromatin, these amounts are in the ratio 2.3, 2.1 and 1, respectively. This result confirms our previous observation that H1° is associated with an increased resistance of chromatin to micrococcal nuclease digestion (3).

c. Light scattering study.

Light scattering studies were performed with the liver chromatin samples. It has been shown recently (4, Girardet, J.L., and Roche, J., in preparation) that variations in the intensity of scattered light at 90° as a function of ionic strength are representative of conformational changes in chromatin

Fig.2. Micrococcal nuclease digestion of liver chromatin samples
Chromatin samples ($A_{260}=2$) were digested in 1 mM TEA, pH 7.4, 1 mM NaCl, 0.1 mM $CaCl_2$, 0.5 mM PMSF with micrococcal nuclease (40 units DNase/ A_{260}) at 4°C for 0, 5, 10, 15, 30, 60, 90 min. (slots from left to right). After treatment with proteinase K and SDS, DNA was analyzed on 1.5 % agarose gel. M : Hae III digest of RF x 174 and Hind III digest of Lambda DNA used as size markers. The length of the fragments is indicated in kilobase pairs.

structure, namely of the formation of a superstructure in chromatin. Measurements have been made at ionic strengths where particle aggregation does not occur as tested by centrifugation of the samples, i.e. below 80 mM NaCl for native samples.

Under such conditions one can define two parameters :

- i) $(I_1 - I_0)$, the difference of intensity of scattered light between the compact form (observed at 20 mM NaCl) and the highly flexible chain (observed at 0 mM NaCl) ;
- ii) $\Delta I/\Delta\mu$, which is the slope of the linear part of the curve $I=f(\mu)$ related to the rate of compaction of the solenoid. Both parameters reflect the ability of chromatin to undergo conformational changes (formation and compaction of the solenoid).

The variations of the intensity of the scattered light as a function of ionic strength for the three liver chromatin samples are shown in Fig.3. Clearly the higher the H1° content, the easier the compaction of chromatin with increasing ionic strength. Table II shows the values of $(I_1 - I_0)$ and $\Delta I/\Delta\mu$ calculated from these curves.

The results described seem to suggest that both an increased resistance to micrococcal nuclease and a better ability of chromatin to

Fig.3. Dependence of the intensity of the scattered light versus ionic strength of liver chromatins, in 1 mM phosphate buffer pH 7.4, 0.2 mM EDTA, 0.5 mM PMSF. ● newborn mouse ; ■ adult mouse ; ▼ adult rat

undergo conformational change under ionic strength variations are correlated with a greater amount of histone H1°.

2. Reconstituted samples

To further substantiate this point we have studied reconstituted rat liver chromatin samples obtained by adding purified fractions of H1-1 and

TABLE II : Light scattering parameters for the chromatin samples.

Liver chromatin	:	$I_1 - I_0$	:	$\Delta I / \Delta \mu$
Adult rat	:	1 ± 0.05	:	1 ± 0.05
Adult mouse	:	1.2 ± 0.05	:	1.4 ± 0.1
Newborn mouse	:	0.9 ± 0.05	:	0.93 ± 0.05

The values of $(I_1 - I_0)$ and $\Delta I / \Delta \mu$, as defined in the text, are normalized to 1 for rat chromatin. I_1 is determined at 20 mM NaCl.

Fig.4. Dependence of the intensity of the scattered light versus ionic strength for various reconstituted samples, in 1 mM phosphate buffer, pH = 7.4, 0.2 mM EDTA, 0.5 mM PMSF, ▼ - one H1-1 per nucleosome ; ● - one H1° per nucleosome ; ▲ - two H1-1 per nucleosome ; ○ - two H1° per nucleosome ; □ - native chromatin (rat liver) ; ■ - H1-depleted chromatin.

H1° to H1 depleted chromatin. The biochemical characterization of such samples has been described in detail elsewhere (8).

For one molecule of added histone H1 per nucleosome, the variation of the intensity of scattered light as a function of ionic strength for reconstituted chromatin samples is as shown in Fig. 4. The corresponding values of $(I_1 - I_0)$, between 0 and 20 mM NaCl, and $\Delta I/\Delta \mu$ below 80 mM NaCl, are recorded in Table III. It can be concluded from these data that a chromatin sample reconstituted with H1-1 alone has a better ability to undergo compaction than a chromatin sample reconstituted with H1° alone.

TABLE III : Light scattering parameters for samples reconstituted from rat liver chromatin.

Samples	$I_1 - I_0$	$\Delta I / \Delta \mu$
Rat liver chromatin	1 ± 0.05	1 ± 0.05
H1-depleted chromatin	/	0.6 ± 0.05
Reconstituted samples :		
. 1H1° per nucleosome	0.8 ± 0.05	0.8 ± 0.1
. 1H1-1 per nucleosome	1 ± 0.05	1.1 ± 0.1
. 2H1° per nucleosome	1.5 ± 0.1	2.1 ± 0.2
. 2H1-1 per nucleosome	2.6 ± 0.2	4.6 ± 0.5

The values of $(I_1 - I_0)$ and $\Delta I / \Delta \mu$, as defined in the text, are normalized to 1 for rat liver chromatin. I_1 is determined at 20 mM NaCl.

When 2 molecules of either H1-1 or H1° were added in the reconstitution process, a similar behavior was observed although aggregation occurred earlier ; consequently $\Delta I / \Delta \mu$ was measured below 40 mM NaCl.

Since the substrate used in reconstitution may have an influence on the properties under investigation, we have also used H1-depleted adult mouse liver chromatin as a starting material in the reconstitution procedure. Light scattering parameters obtained from samples reconstituted with one molecule per nucleosome of either H1-1 or H1° are given in Table IV

TABLE IV : Light scattering parameters for samples reconstituted from adult mouse liver chromatin.

Samples	$I_1 - I_0$	$\Delta I / \Delta \mu$
Adult mouse liver chromatin	1.2 ± 0.05	1.4 ± 0.1
Reconstituted samples :		
. 1 H1° per nucleosome	0.8 ± 0.05	1 ± 0.1
. 1 H1-1 per nucleosome	1 ± 0.05	1.1 ± 0.1

The values of $(I_1 - I_0)$ and $\Delta I / \Delta \mu$ are normalized to be compared with Tables II and III.

and comparison with Table III shows that there is no significant difference with samples reconstituted from with H1-depleted rat liver chromatin.

The kinetics of micrococcal nuclease digestion were analyzed for both types of samples. As shown in a previous study (8), H1-1 was found more efficient than H1° in protecting DNA against micrococcal nuclease digestion. The amounts of (Mono + Di)nucleosomes after 90 minutes of digestion relative to the total DNA were in the ratio of 2.5, 1.1 and 1 for H1° reconstitutes, H1-1 reconstitutes and native adult mouse liver chromatin respectively.

DISCUSSION

We have found two apparently contradictory results. On one hand, native chromatin having a greater content in H1° is more efficiently protected against micrococcal nuclease digestion. This confirms our previous observation (3) that the first nucleosomes to be released in the course of digestion of rat liver nuclei by micrococcal nuclease contained very little H1°, whereas on increasing the digestion, the nucleosomes released contained an increased amount of H1°. It seems then, that H1° has a protective effect on chromatin against micrococcal nuclease action.

This property is correlated in all cases studied with the ability of chromatin to undergo conformational changes (solenoid formation and compaction) upon increasing the ionic strength of the medium. The higher the H1° content, the easier the formation of the solenoid.

On the other hand, when studying samples reconstituted with only one histone H1 fraction, we found that H1-1 confers upon this chromatin a greater protection against micrococcal nuclease digestion than H1°. Similarly, the ability to undergo solenoid formation was better with H1-1 than with H1° reconstitutes.

The first question that emerges is the fidelity of the reconstitution procedure. In the absence of any physiological test, the efficiency of chromatin reconstitution must be estimated by indirect, structural properties. Virtually all the methods in use for reconstitution procedures (12, 13, 14, 15) lead to apparently identical results.

In our hands, the repetitive unit of chromatin is restored, as well as its melting properties, circular dichroism spectrum ($\theta_{282\text{nm}}$) and its ability to undergo conformational changes upon increasing ionic strength, as studied by electron microscopy (8). In addition to that, the electrophoretic mobility of nucleosomes obtained after micrococcal nuclease digestion of reconstituted samples is the same as that of native nucleosomes, including

the slight differences originating from the influence of the individual histone H1 fractions. It seems thus reasonable to accept the method of reconstitution to compare the properties of the various histone H1 fractions.

The differences found between the various reconstitutes reflect differences in structural properties of the individual histones. The similarity of the properties observed with samples reconstituted from different substrates indicates that the residual chromatin is not responsible for such differences.

We must therefore consider that histone H1° does not act alone, in conferring to native chromatin its particular properties. Possible candidates include the non histone proteins, but we have already mentioned that there are no major differences in the NHP content of adult rat liver and adult mouse liver chromatins. These chromatin samples however show important differences in the properties we have studied. Hence, differences of NHP alone cannot explain the structural differences we have observed between the three native chromatins. Moreover, it has been demonstrated (16) that NHP have no detectable influence upon the formation of higher order chromatin structures ; they can only prevent the unravelling of nucleosomes at very low ionic strength.

The existence of possible interaction between H1° and other H1 fractions (17, 18, 19) which are not present in the reconstituted samples, made with only one pure histone fraction, may be the basis for further investigation. On the other hand, supranucleosomal structures, as described recently (20), involving H1 linking of adjacent nucleosomes, may have different properties depending on which histone fraction is concerned.

ACKNOWLEDGEMENTS

We are greatly indebted to H.B. OSBORNE (ER 199, CNRS) for fruitful discussions. We are grateful to D. ALIX for her participation in animal dissection.

This work was supported in part by grants from CNRS (ATP - Développement and ATP - Organisation et Expression du Génome dans les cellules eucaryotes) and DGRST (ACC 81.L.076).

- Claude GORKA and Jean-Jacques LAWRENCE are members of "Institut National de la Santé et de la Recherche Médicale".
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