THERMAL DENATURATION AND CIRCULAR DICHROISM PROPERTIES OF SHEARED AND UNSHEARED CHROMATINS

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1. Introduction

Nicolini et al. [1] recently reported that the circular dichroism spectrum of chromatin solubilized by strong shear forces differs markedly from the circular dichroism spectrum (corrected for differential scattering) of 'unsheared' chromatin prepared simply by suspending a swollen chromatin gel. Their results suggest that chromatin solubilized by shearing may not be appropriate to use for physical measurements. Unfortunately the huge size of the chromatin fragments in the 'unsheared' preparation of Nicolini et al. [1] seriously complicates study of some physical properties (such as circular dichroism) and makes hydrodynamic investigations virtually impossible.

Noll et al. [2] introduced the use of a short exposure of nuclei to micrococcal nuclease as an alternative to shear solubilization. This procedure avoids subjecting a chromatin gel to shear forces and has the additional benefit of solubilizing only chromatin. One disadvantage of the nuclease procedure is that the DNA molecules in the released chromatin fragments vary widely in the molecular weight [2]. This variation would greatly complicate hydrodynamic studies of chromatin as well as fractionation schemes based on chromatography or sedimentation. Strong shear forces can be used to reduce the size heterogeneity of DNA samples, but Noll et al. [2] reported that shearing nuclease-released chromatin results in a deterioration of the electrophoretic pattern of DNA isolated after more extensive digestion of the chromatin with micrococcal nuclease and interpreted this result to indicate that histone-DNA interactions of chromatin were seriously altered by the shearing. Clearly, if shearing of nuclease-released chromatin irreversibly alters the binding of histones to DNA, one should avoid shearing altogether.

We have examined the thermal denaturation and circular dichroism properties of rat liver chromatin solubilized by the nuclease treatment of Noll et al. [2] and of nuclease-released chromatin sheared in a Sorvall Omnimixer and subjected to sonication to determine whether or not alterations in histone–DNA interactions are caused by exposing nuclease-released chromatin to strong shear forces. In addition, the circular dichroism properties of nuclease-released chromatin are compared to those of chromatins that had been solubilized by shearing.

2. Experimental

We isolated nuclei from thawed rat liver (Pel Freeze Biologicals) using the method of Blobel and Potter [3]. To inhibit a protease that is associated with chromatin [4,5], we added phenylmethane sulfonyl fluoride to the homogenized liver. Chromatin was released from the purified nuclei precisely as outlined by Noll et al. [2] except that we used 5 units of micrococcal nuclease (Worthington) per ml rather than 15 units/ml. We obtained release of 60–70% of the A_{260} units in the nuclei, which is similar to the 75% release obtained by

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Noll et al. [2]. A portion of the chromatin released by the nuclease treatment was sheared at a concentration of about 0.7 mg DNA/ml for 2 min at full speed in a Sorvall Omnimixer – the conditions Noll et al. [2] used in studies of the effects of shearing. Some of the sheared chromatin was sonicated at a setting of 5 on a Branson Sonifier equipped with a microtip. The sonication was carried out in eight 15 s periods, and after each period the sample was cooled by immersion in an ice–water mixture. These sonication conditions, which we used in earlier studies [6] to reduce the average molecular weight of chromatin that had been solubilized by shearing in a Waring blender, result in an average molecular weight of chromatin DNA of about 500 000 [7]. The chromatin samples had A_{320}/A_{260} of 0.01.

Circular dichroism measurements were conducted on samples with A_{260} of about 1.2 using a Cary 60 spectropolarimeter with a Model 6001 circular dichroism attachment. The thermal denaturation of chromatin samples with A_{260} of about 1 was followed by making absorbance measurements at 260 nm with a Gilford Model 250 spectrophotometer as the temperature of water circulating through the jacketed cell holder was increased (5°C every 10 min).

To judge qualitatively the effect of shearing and of sonication on the molecular weight of chromatin fragments, we measured outflow times of the samples (0.67 mg DNA/ml) at 21°C in an Ostwald viscometer (Cannon) that had an outflow time for solvent of 286.6 s. Twenty hours before the measurements we adjusted the chromatin samples, which were in 0.2 mM EDTA, pH 7.0, to 1% sodium dodecyl sulfate to denature the chromatin proteins and prevent them from interacting with DNA. Because the intrinsic viscosities of DNA molecules are much greater than those of denatured proteins, the outflow times are determined almost entirely by the molecular weight distributions of the DNA in the samples. The outflow times, relative to that of the solvent, were 1.68, 1.59, and 1.22, respectively, for nuclease-released chromatin, nuclease-released and sheared chromatin, and nuclease-released chromatin that had been sheared and then sonicated. These measurements unambiguously demonstrate that the shear forces generated by the Omnimixer and sonicator were sufficient to produce decreases in the average molecular weights of the DNA in the chromatin fragments.

3. Results and discussion

Figure 1 shows the thermal denaturation profiles of the three types of chromatin. Because of interactions with histones, the thermal denaturation of DNA in chromatin occurs at much higher temperatures and over a much broader temperature range than the thermal denaturation of DNA that is free of protein. There are no experimentally significant differences among the profiles of fig.1, which indicates that shearing and sonication do not alter the thermal denaturation properties of the DNA in nuclease-released chromatin.

The optically active bands of the peptide bonds of proteins occur in the fal ultraviolet, whereas the double helix of DNA exhibits positive ellipticity from 265–300 nm that is strongly affected by the presence of bound histones [8]. Thus, from a circular dichroism

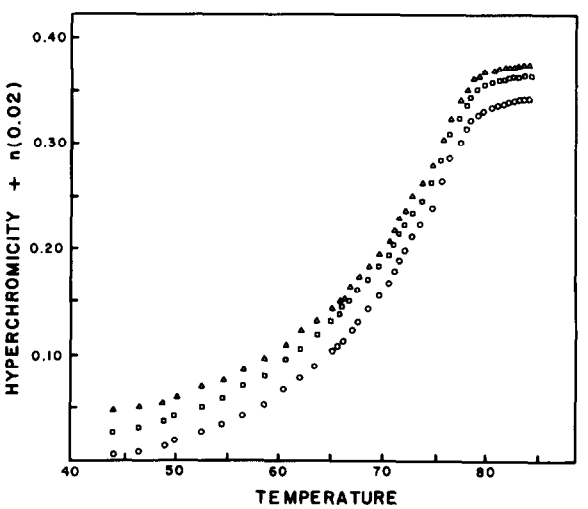

Fig.1. Thermal denaturation profiles of nuclease-released chromatin (○); nuclease-released and sheared chromatin (□); and nuclease-released chromatin that had been both sheared and sonicated (△). To facilitate comparison, the latter two profiles have been displaced by 0.02 and 0.04 on the ordinate axis. Thus, at any temperature, the hyperchromicity for a given sample is $Y - n(0.02)$, where Y is the ordinate value for that sample and n is 0 for nuclease-released chromatin, 1 for nuclease-released and sheared chromatin, and 2 for nuclease-released chromatin that had been both sheared and sonicated. The solvent was 0.25 mM EDTA, pH 7.0. Denaturation of the samples was followed simultaneously by using 3 compartments in the cell holder of the spectrophotometer.

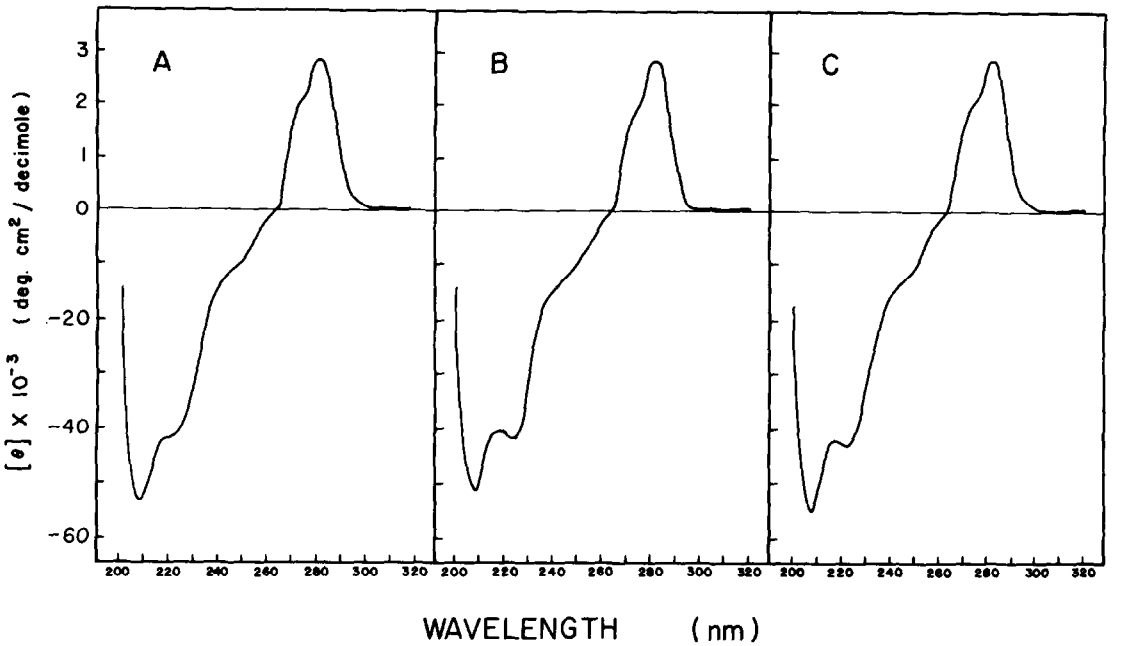

Fig.2. Circular dichroism spectra of nuclease-released chromatin (A), nuclease-released and sheared chromatin (B), and nuclease-released chromatin that had been both sheared and sonicated (C). The solvent was 0.25 mM EDTA, pH 7.0. Note the difference in scale on the ordinate axis for positive and negative ellipticities.

spectrum of chromatin, one can obtain insight into the conformations of both chromatin proteins and of DNA. As shown in fig.2, the spectra of our three chromatin preparations are identical within experimental error. This can be taken as evidence that the conformations of proteins and of DNA are substantially similar in nuclease-released chromatin that has and has not been subjected to strong shear forces.

Unfortunately it is not possible to specify the molecular geometries that result in a particular thermal denaturation profile or circular dichroism spectrum. These physical properties are, nevertheless, sensitive probes of changes in the interactions of histone and DNA, and the results presented in fig.1 and 2 would seem to be inconsistent with the proposal of Noll et al. [2] that, in shearing nuclease-released chromatin, the '... DNA may even be partly stripped from the histone core'.

The circular dichroism spectra in fig.2 differ in two respects from published spectra of chromatins that had been solubilized by shearing. First, the maximum DNA ellipticity of 2800 degrees cm^2 $dmol^{-1}$ is substantially lower than the maximum ellipticities (ranging from 3800–6000 degrees cm^2 $dmol^{-1}$) that several workers have reported for chromatins that were solubilized by shearing and exposed to EDTA in their purification [8–11]. (In comparing circular dichroism spectra of chromatins, it is essential to take into account the substantial increase that extensive exposure to EDTA causes in the DNA ellipticities [10,11].) Second, the shape of the spectra from 265–300 nm differs from the shapes of spectra of chromatins that had been solubilized by shearing, in that the ellipticity falls off much more rapidly as one approaches the crossover point at 264 nm. This is seen clearly by examining the ratio of the ellipticities at 270 and 282 nm. Such ratios, calculated from several published spectra, are: 0.9 [9,10] for thymus chromatins; 0.9 for erythrocyte chromatin [11]; and 1.2 [11] and 1.1 [8] for liver chromatins. A much lower ratio of 0.50 is obtained from the spectra in fig.2. The shape of our spectra bears a remarkable resemblance to solutions of protein-free DNA in sufficiently high salt concentrations to induce a shift from the B conformation toward the C conformation [12].

It is interesting to compare our circular dichroism

spectra with those of Nicolini et al. [1]. The workers found that 'unsheared' chromatin, obtained by simply suspending a chromatin gel, exhibited differential light scattering that resulted in positive ellipticities above 300 nm. In contrast, the nuclease-released chromatin we have used in our studies is essentially free from differential scattering effects as evidenced by the lack of ellipticity above 300 nm. When Nicolini et al. [1] estimated and subtracted the very considerable scattering contributions to the ellipticity, the resulting circular dichroism spectrum of 'unsheared' chromatin prepared from cultured cells arrested in G_1 phase had a maximum ellipticity of 2000 degrees cm^2 $dmol^{-1}$, a crossover point of 267 nm, and a ratio of ellipticities at 270 and 282 nm of 0.5. Although substantial assumptions were necessary in making the corrections for light scattering, their corrected spectrum resembles those in fig.2 much more closely than any previously published spectra.

Our circular dichroism spectra of nuclease-released chromatin and the studies by Nicolini et al. [1] on 'unsheared' chromatin suggest strongly that the circular dichroism properties of chromatin that has been solubilized by shearing differ significantly from the circular dichroism properties of 'native' chromatin. It therefore seems that at least for some types of physical studies, solubilizing chromatin by shearing is ill-advised. Since it is virtually impossible to make many types of physical measurements on molecules as huge as those in the 'unsheared' chromatin preparation of Nicolini et al. [1], using nuclease to solubilized chromatin would seem to be the method of choice.

Our results also demonstrate that strong shear forces do not affect the circular dichroism properties of nuclease-released chromatin. This is not entirely unexpected since the stress experienced in a shear gradient by the immense nucleoprotein molecules of a chromatin gel would be much greater than the stress experienced in the same shear gradient by the relatively small fragments of nuclease-released chromatin.

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