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Dynamics of DNA in Chromatin and DNA Binding Mode to Core Protein

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We have studied the dynamics of DNA in nucleosome core particles and in the linker region of chromatin using nanosecond fluorescence anisotropy decay measurements of intercalated ethidium. DNA in the core undergoes torsional motions to the same extent as the linker DNA in extended chromatin. We therefore concluded that the binding of DNA to the histone octamer is relatively weak or limited to a few points; stretches of at least several tens of base pairs exist which can move as freely as DNA in solution.

It has been recently recognized that B-form DNA in solution is a rather flexible molecule (*1–3*) and undergoes several kinds of thermal motion. By measuring the nanosecond fluorescence anisotropy decay of ethidium bromide, that is intercalated in DNA, one can estimate how actively DNA undergoes internal motions, of which the main one is known to be torsional motion (*3*, *4*), and determine whether the molecule is flexible or resistant against twisting deformation. Using this technique, we have investigated the extent of the torsional motion of DNA in the nucleosome core particles and of linker DNA in chromatin.

DNA (Mw 2 million–4 million) was isolated from calf thymus following the method of Kay *et al.* (*5*). Preparation of calf thymus nucleosome cores and long chromatin has been described previously (*6*, *7*). The intactness of DNA or histones in our samples was checked by polyacrylamide gel electrophoresis. Nanosecond fluorescence depolarization measurements were performed with a single photon counting apparatus (*8*). In the present work, we used 520 nm components of pulsed light as an excitation source, and emission above 560 nm was collected. From the measured decay curves it was seen that the anisotropy decay is as follows, $r(t)=(I_{//}(t)-I_{\perp}(t))/(I_{//}(t)+2I_{\perp}(t))$ ($I_{//}(t)$, $I_{\perp}(t)$; the fluorescence intensities of the parallel and perpendicular components, respectively, at time t) were calculated. All measurements were performed in buffer solutions (5 mM or 1 mM Tris, 0.2 mM EDTA, pH 7.5) at 20°C. Samples with no added dyes gave negligible signals.

Abbreviation: bps, base pairs.

Figure 1 shows the fluorescence anisotropy decays of ethidium bromide which is intercalated in free DNA in solution (dotted line) and in DNA in nucleosome cores (solid line). The dye to DNA ratio, P/D ([phosphates]/[dye]), was kept below 2,000, so that energy transfer between intercalated dyes should be negligible. As reported earlier (*3*), Barkley's equation (*4*), whose form is exp $(-t^{1/2})$ (dashed line), can be fitted very well to the decay curve of DNA in solution (dotted line). The two decay curves (dotted line and solid line) are somewhat similar, but the solid line accurately reflects the anisotropy decay of DNA in the core. The possibility that some free DNA remained in the nucleosome core sample can be ruled out because even in the presence of 1 mM Ca^{2+} and microccocal nuclease the anisotropy decay did not change. Under the same conditions, the anisotropy decay of free DNA in solution became very fast, due to the appearance of short fragments of DNA by nuclease cutting.

In Fig. 1 the anisotropy decay of ethidium bromide in nucleosome cores deviates largely from the single exponential decay in the time range of 0–20 ns and this suggests that DNA in nucleosome cores still undergoes restricted torsional motions to some extent. If DNA is firmly fixed to the core proteins, the anisotropy of an intercalated dye should decay approximately single-exponentially with a rotational correlation time of 150–220 ns, which reflects the rotation of a whole core. The ratio of the largest and smallest components of the rotational correlation times, for such an oblate ellipsoid, as a core particle with an axial ratio of about 2 : 1, will be 1.15 (*9*). The observed decay must therefore be almost single-exponential, and the initial decay (0–20 ns) is probably caused by the internal motion of core DNA.

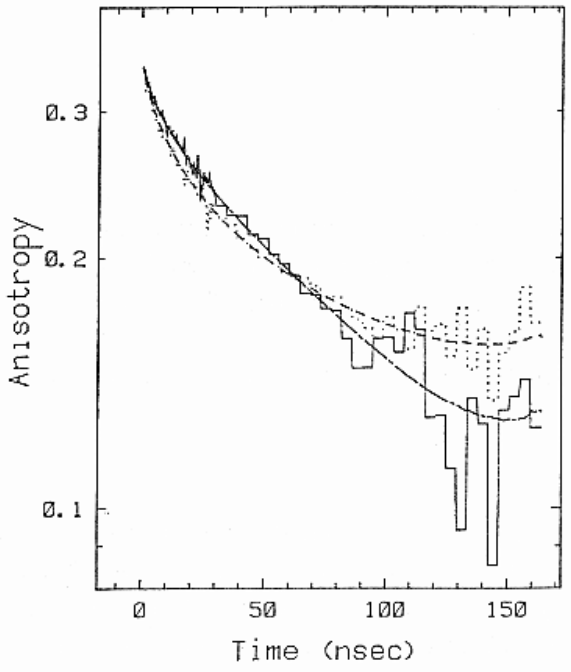

Fig. 1. Anisotropy decay curves of the ethidium intercalated in the DNA of nucleosome core particles (solid line) and DNA (dotted line). The ordinate of this figure is in log scale. The buffer used was 5 mM Tris, 0.2 mM EDTA. pH 7.5, and sample concentrations were about 0.1 mg/ml. The P/D ([phosphates of DNA]/[dyes]) value is 2,000–3,000. The anisotropy values, after 30 ns, were averaged over 10 channels; 1 ns equals 2.32 channels. The dashed line is the least-square fitting curve of Barkley's formula to the decay of DNA in solution. The chain line in this figure represents the result of a least-square fit of Eq. 3 to the observed decay of DNA in nucleosome cores. The calculated correlation time of the rotation of a whole core, ϕ_3, was about 170 ns.

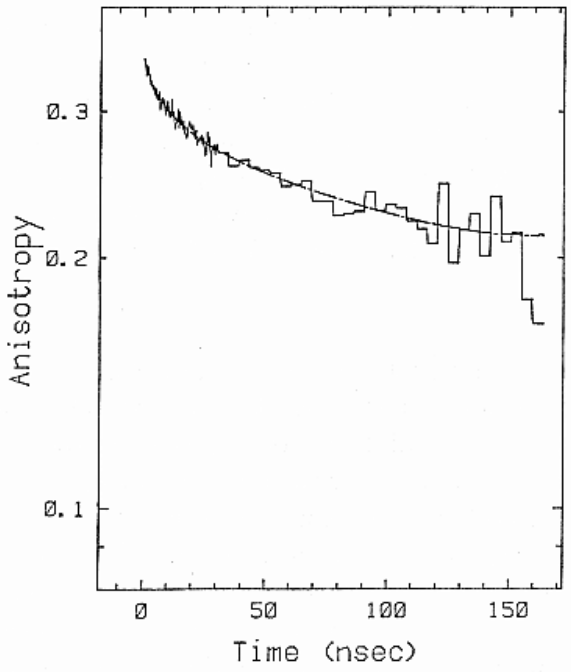

Fig. 2. Anisotropy decay curves of ethidium intercalated in DNA in chromatin (solid line). The ordinate of this figure is in log scale. The buffer used was 1 mM Tris, 0.2 mM EDTA pH 7.5. Concentrations of samples were about 0.2 mg/ml. The P/D value was 3,000. In this experiment after 30 ns, the anisotropy values were averaged over 10 channels. The chain line is the least-square fit of Eq. 3 to the observed decays. The effective correlation time, ϕ_3, for the rotation of whole linker DNA in chromatin was about 450 ns.

Figure 2 shows the fluorescence anisotropy decay of ethidium bromide intercalated in chromatin DNA. The ratio of the dye to chromatin DNA was also very low, and for the concentration of the dye used, ethidium is known to intercalate exclusively into the linker region of chromatin (*10*, *11*). In Fig. 2 the solid line represents the anisotropy decay of chromatin in 1 mM Tris, 0.2 mM EDTA, pH 7.5; under these low ionic strength conditions the chromatin is known to take an extended form (*12*). The form of the decay curves arises from two motions. One is the internal motion of the linker DNA, which is mainly manifested in the time range of 0–20 ns. The other is the movement of a nucleosome in chromatin, whose correlation time would be expected to be in the order of several hundreds of ns, and would be a predominant cause for the decay in the time range above 50 ns.

From these experiments, we found that the internal motion of DNA, whose main component will be torsion, still occurs in a nucleosome core particle and in the linker region of chromatin. In order to characterize these internal motions, we separated the anisotropy decay curve caused only by internal motion of DNA ($r_i(t)$) from that caused by the rotation of a whole core or the movement of a nucleosome in chromatin ($r_t(t)$). The observed decay curve can be represented as follows,

$$r(t)=r_i(t)\times r_t(t) \qquad (1)$$

For $r_i(t)$, one must use the equation for the anisotropy decay of DNA with both ends fixed, however, there is not yet an exact equation for the decay in such a case, so in this work we assume the anisotropy decay to approximately follow the expression:

$$r_i(t)=r_0(a_1\exp(-(t/\phi_1)^{1/2})+a_2) \qquad (2)$$

where a_1, a_2, and ϕ_1 are the parameters that characterize the internal motion. In this expression, the term $\exp(-t^{1/2})$ and the constants a_1 and a_2 were inferred from Barkley's formula (*4*) and the anisotropy decay of restricted motion (*13*), respectively. In order to simplify the calculation, we assume that the rotation of a whole core and the movement of a nucleosome in chromatin are isotropic motions. The observed decays should then follow the equation:

$$r(t)=r_0(a_1\exp(-(t/\phi_1)^{1/2})+a_2)\exp(-t/\phi_3) \qquad (3)$$

In this equation, ϕ_3 is the rotational correlation time of a whole core or of a nucleosome in chromatin. We separated the decay curves due to the internal motion by fitting Eq. 3 to the observed decay curves of DNA in nucleosome core particles and of the linker DNA in extended chromatin. In the calculations the excitation pulse width had to be allowed for by deconvolution procedures. The results of this treatment, that is the decays due to the restricted torsional motion of DNA, are shown in Fig. 3. We feel that the approximation of Eq. 2 is reasonable, because the curves of the least-square calculation of $r(t)$ (chain lines in Figs. 1 and 2) well fit the observed decay curves.

On comparison of decay curves of the internal motion of DNA in Fig. 3, we found that the internal motion of core DNA rather resembles that of linker DNA of chromatin in the extended form. Slopes and r_∞/r_0 values of the calculated decay curves will depend on both the length and the rigidity of DNA. The length and the stiffness of movable DNA for a core and the linker may therefore be similar. In the case of linker DNA, the movable unit length must be at least 30 bps, because the linker DNA of about 30 bps was observed to be free from the core region in a nuclease digestion study (*14*).

The conclusion that DNA in the core has a movable region of as long as several tens of bps may be surprising. However, by a image recon-

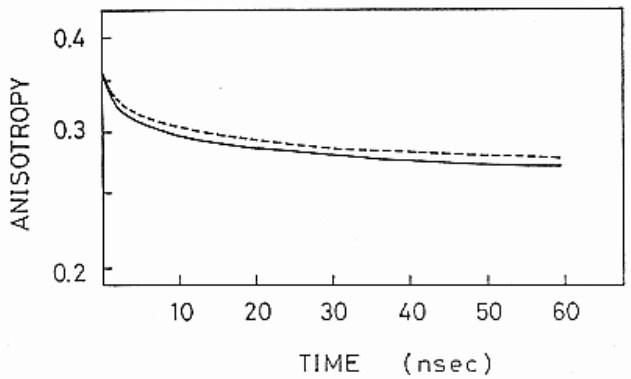

Fig. 3. Calculated anisotropy decays, $r_i(t)$, of intercalated ethidium due to the internal motion of DNA only in core particles (solid line) and of linker DNA in extended chromatin (dashed line). These curves were obtained by fitting Eq. 3 to the observed decays (Figs. 1 and 2), calculating parameters in this equation, and depicting the curve following Eq. 2.

struction study of the histone octamer and X-ray diffraction studies of the nucleosome core, Klug *et al.* (*15*, *16*) suggested that DNA in nucleosome cores will not be rigidly fixed to the histone core all around, but fixed at a few points and remaining portions will be in relatively free or in flexible states. Our results strongly support their suggestions. In our experiments it was demonstrated that DNA in the core particle has considerable freedom and flexibility. Until now we thought that transcriptionally active chromatin takes on a structure similar to the nucleosome core (*17*), and considering our results, it is possible that slight conformational changes, which may be caused by chemical modifications or the binding of some kinds of non-histone proteins, will be sufficient to turn the inactive form of chromatin into the active form.

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