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Study of DNA Accessibility in the Condensed Chromatin Structures by Resonance Energy Transfer

R. Labarbe,[1] S. Flock,[1] P. Colson,[1] and C. Houssier[1]

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The linker DNA accessibility of chicken erythrocyte chromatin was studied by diffusion-enhanced resonance energy transfer (DERET). The 4"-{9"'-[((4-carboxy-3-hydroxyphenyl)-acetatamido)-3"',6"',9"'-(triacetyl)-3",6"',9"'-triazanonamido]-2",6"-diazanonyl}-4,5',8-trimethyl psoralen–terbium complex was photocovalently bound to linker DNA and transferred its energy to fluorescein free in solution or bound on proteins of different sizes. We observed a diminution of linker DNA accessibility in chromatin as the protein size increased. Free fluorescein and proteins (up to a molecular weight of 24,000) labeled with fluorescein isothiocyanate (FITC) showed no variation in linker accessibility as chromatin condensation from 10- to 30-nm fibers was induced by an increase in ionic strength. We can conclude from these observations that linker DNA is located on the outside of the condensed chromatin fiber or, alternatively, that small proteins are free to diffuse toward an inside-located linker DNA, even in the condensed state of chromatin, possibly through the central cavity of the solenoïd model.

KEY WORDS: Chromatin; condensation; energy transfer; terbium; psoralen.

INTRODUCTION

Chromatin can adopt different conformations as the ionic strength changes [1,2]. At a low salt concentration, chromatin has a bead-on-a-string form, called a 10-nm fiber. When the salt concentration increases, it condenses into a 30-nm fiber, which could be partly responsible for long-term gene repression *in vivo* [3–5]. It would be interesting to measure the accessibility of different molecules to DNA integrated into chromatin in both condensed and uncondensed forms, to determine whether the proteins are prevented from approaching DNA in the condensed state.

DERET (diffusion-enhanced resonance energy transfer) is a promising method to investigate in this process [6,7]. Förster showed that a fluorescent donor can transfer its energy to an acceptor molecule via dipole–dipole interactions [8]. The static rate constant k_2 for this process is

$$k_2 = 8.71 \cdot 10^{23} (J \kappa^2 n^{-4} \phi \tau_0^{-1} r^{-6}) \qquad (1)$$

where J is the overlap integral; κ, an orientation factor; n, the refractive index of the solution; ϕ and τ_0, the quantum yield and lifetime of the donor (without acceptor); and r, the distance between donor and acceptor. If the donor lifetime is sufficiently long, the acceptor molecule has sufficient time to diffuse toward the donor and so the transfer efficiency increases: This is the DERET situation [9]. Then the rate constant k_2 is related to the donor lifetime τ (in the presence of acceptor) by

$$\frac{1}{\tau} = \frac{1}{\tau_0} + k_2[A] \qquad (2)$$

where $[A]$ is the acceptor concentration and τ_0 the lifetime in the absence of acceptor. It is possible to relate the rate constant k_2 to the minimal approach distance

[1] Laboratoire de Chimie Macromoléculaire et Chimie Physique, Institut de Chimie (B6), Université de Liège, B-4000 Liège, Belgium.

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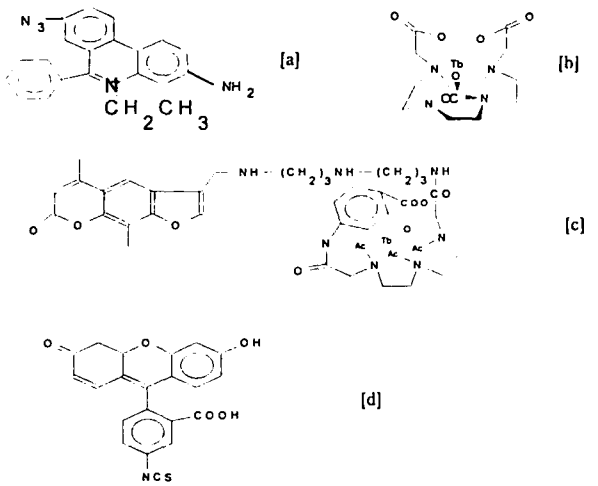

Fig. 1. Molecule of (a) ethidium-8-monoazide, (b) TbDO3A, (c) psoralen–Tb, and (d) FITC.

between the donor and the acceptor molecules. Integration of Eq. (1) for all possible orientations and distances between a donor bound to a cylinder (DNA) of radius R_c and a spherical acceptor of radius R_a leads to [10]

$$k_2 = 1.486 \cdot \frac{J \kappa^2 \phi n^{-4} 8.8 \cdot 10^{17}}{\tau_0 a^3} \qquad (3)$$

where a is the minimal approach distance ($a = R_c + R_a$)

Binding one probe molecule to chromatin DNA and another to a protein allowed us to measure any accessibility changes of the protein to DNA during chromatin condensation. It is necessary to bind the probe covalently to DNA to prevent any exclusion of the probe from DNA during the condensation process.

EXPERIMENTAL

Samples

Chicken erythrocytes chromatin samples were prepared using standard procedures outlined in the literature [11]. Ethidium-8-monoazide (Fig. 1a) from Molecular Probes Inc. was equilibrated with chromatin for 1 h prior to photoirradiation with an Hamamatsu xenon lamp (Model 1.2569) during 4 h at 0°C. During the irradiation, the sample was in a glass test tube. The terbium–DO3A complex (Fig. 1b) was kindly synthetized by the laboratory of Professor Desreux (University of Liège) [12]. The psoralen–terbium complex (Fig. 1c) was synthetized by the Oser reaction [13,14] with some modifications. The complex was equilibrated for 1 h with chromatin prior to irradiation at 366 nm during 4 h at 0°C. The 366-nm wavelength was isolated from a Hamamatsu xenon lamp (Model 1.2569) by a blue filter that removed part of the lamp emission above 455 nm and below 340 nm. Fluorescein isothiocyanate (Fig. 1d) was purchased from Sigma and bound to protein by the method described in Ref. 15.

Fluorescence Measurements

The fluorescence lifetimes were measured with an Edinburgh photocounting instrument, Model 199S, equipped with a microsecond xenon flashlamp, Model 199XF. Excitation and emission wavelengths were set at 457 and 490 nm, respectively. The lifetimes were calculated by the least-squares method using software provided by the IBH Company.

RESULTS AND DISCUSSION

Transfer from TbDO3A to Ethidium Azide–Chromatin

TbDO3A (Fig. 1b) is a stable complex in solution: No free Tb^{3+}, which could cause DNA damages, was detected. The fluorescence emission spectrum is very similar to that of free $TbCl_3$. The luminescence lifetime is 1.3 ms and the quantum yield is 27%. The complex has a small size compared to proteins, so it can be considered as a representative of the solvent accessibility to DNA.

Ethidium-8-monoazide (Fig. 1a) intercalates into linker DNA in chromatin and can be photochemically bound to the base pairs. Electric and circular dichroism spectra of chromatin with bound ethidium azide showed that the probe induced no significant perturbation of the chromatin structure. The overlap integral with the Tb^{3+} luminescence spectrum is $2.51 \cdot 10^{-14}$ cm³/mol.

The Tb^{3+} luminescence lifetime in the case of transfer to fully accessible free ethidium azide in solution is 1.13 ms. This lifetime is 1.30 ms for transfer to chromatin-bound ethidium azide; i.e., the same lifetime as for Tb^{3+} donor in the absence of acceptor. This means that when azide is bound to chromatin, the energy transfer does not occur. Furthermore, no lifetime variation was observed during chromatin condensation induced by Na^+, K^+, Mg^{2+}, Ca^{2+}, spermine, and spermidine (Fig. 2).

Since TbDO3A is a very small molecule, it is very likely that it will have access to linker DNA, particularly in uncondensed chromatin at a low ionic strength. The

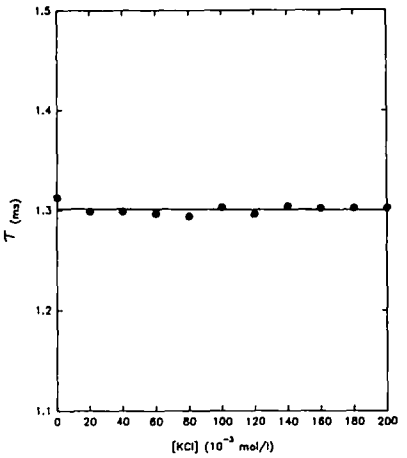

Fig. 2. Fluorescence lifetime of TbDO3A (10^{-4} *M*) in the case of resonance energy transfer to ethidium azide (10^{-5} *M*) bound to chromatin ($7 \cdot 10^{-4}$ *M*) as a function of KCl concentration. Measurements were done in 1 m*M* sodium cacodylate at pH 6.5. Excitation wavelength, 457 nm; emission wavelength, 490 nm.

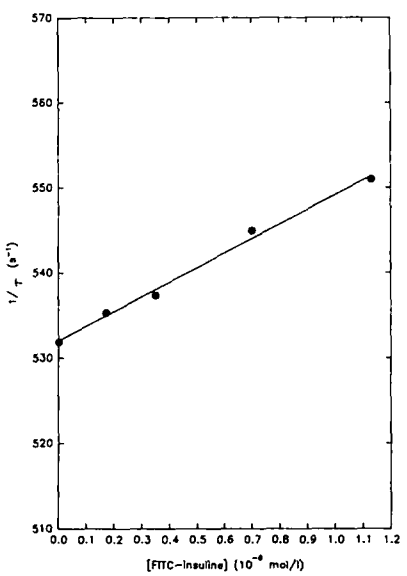

Fig. 3. Reciprocal of fluorescence lifetime of psoralen–terbium (10^{-5} *M*) bound to chromatin (10^{-3} *M*) with transfer to FITC bound to protein as a function of FITC concentration. Measurements were done in 1 m*M* sodium cacodylate at pH 6.5. Excitation wavelength, 457 nm; emission wavelength, 490 nm.

absence of transfer is probably due to a screening of the ethidium chromophore by the base pairs when it is intercalated. We thus designed a new probe that will not be protected by the base pairs and will be able to transfer its energy.

Table I. Transfer from Psoralen Tb^{3+} (10^{-6} *M*) Bound to Chromatin (10^{-4} *M*) to a Different Acceptor (10^{-6} *M*)[a]

Transfer to	Acceptor MW	Transfer constant, $k_2 \cdot 10^{-7}$ (s^{-1})	Minimal approach distance *a* (Å)
Fluorescein	332	2.7 ± −0.2	6.8
Insulin–FITC	6,000	1.71 ± −0.08	8.0
Trypsin–FITC	23,800	1.06 ± −0.05	9.4
Albumin–FITC	65,400	No transfer	

[a]Measurements done in 1 m*M* sodium cacodylate at pH 6.5. Excitation wavelength, 457 nm; emission wavelength, 490 nm.

Transfer from Tb Psoralen–Chromatin to FITC–Protein

The 4"-{9"'-[((4-carboxy-3-hydroxyphenol)-acetamido)-3"',6"',9"'-(triacetyl)-3",6"',9"'-triazanonamido]-2",6"-diazanonyl}-4,5',8-trimethyl psoralen–terbium complex (Fig. 1c) will avoid this screening problem. The psoralen moiety is intercalated in linker DNA at alternating AT-rich sites and can be covalently bound by photochemical reaction [16]. The Tb^{3+} complex moiety is kept away from the base pairs thanks to the diazanonyl linker. The fluorescence emission spectrum is similar to that of uncomplexed Tb^{3+}. The fluorescence lifetime is 1.88 ms and the quantum yield is 39%. Binding to chromatin causes very little structural changes, as evidenced by circular and electric dichroism.

The acceptor molecule is fluorescein isothiocyanate (FITC; Fig. 1d) covalently bound to a protein. The overlap integral between the two compounds is $1.53 \cdot 10^{-13}$ [cm^3/(mol/L)].

The transfer happened between the psoralen–terbium complex bound to chromatin and FITC-labeled proteins of increasing size: insulin, trypsin (inhibited by phenylmethanesulfonyl fluoride), and albumin (Fig. 3). These proteins are not naturally involved in DNA recognition so they should not interact with any specific DNA sequences. They are used just as a molecular size probe. The results are collected in Table I. The rate constant decreases when the acceptor size increases, indicating that smaller proteins can more easily access linker DNA than larger proteins can. Albumin shows no energy transfer at all.

Furthermore, when chromatin is condensed with Na^+, a decrease in the Tb^{3+} lifetime is observed, although we would expect an increase if the DNA accessibility decreased during condensation. In fact, this is the result of an increase in the overlap integral between FITC and Tb^{3+}, due to a hyperchromic effect on the FITC absorp-

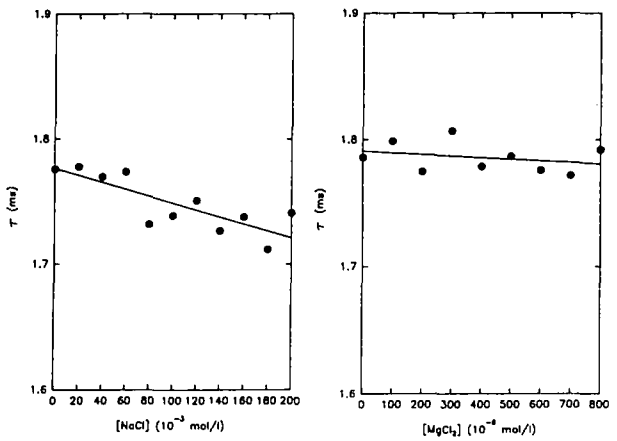

Fig. 4. Fluorescence lifetime of psoralen–Tb^{3+} ($2 \cdot 10^{-6}$ *M*) with transfer to FITC ($1.13 \cdot 10^{-6}$ *M*) bound to insulin ($1.2 \cdot 10^{-5}$ *M*) as a function of sodium and magnesium concentration. Measurements were done in 1 m*M* sodium cacodylate at pH 6.5. Excitation wavelength, 457 nm; emission wavelength, 490 nm.

tion spectra, as the [Na^+] increases. When the condensation is driven by Mg^{2+}, no lifetime variation is observed because the change in salt concentration is smaller (Fig. 4).

These results show that there is no variation of linker DNA accessibility to small proteins (at least up to a relative weight of 24,000) as chromatin condenses. This implies either that the linker is located on the outside of the chromatin fiber, so that it remains accessible in condensed chromatin, or that small proteins can diffuse freely inside the chromatin fiber, for example, through the central hole of the solenoid model. On the other hand, larger proteins are excluded from chromatin. We are now trying to determine the maximum size of a molecule that can have access to the linker and to follow the access ability of some gene regulatory proteins.

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