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Direct observation of DNA condensation in a nano-cage by using a molecular ruler

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Abstract

In this report we studied structural compaction of a genomic DNA, an important mechanism for the chromatin condensation, in a nano-space of an anionic AOT (bis(2-ethylhexyl)sulfosuccinate) reverse micelles (RM). The homo-molecular picosecond-resolved fluorescence resonance energy transfer (FRET) studies on the DNA-bound acridine orange encapsulated in the RM indicate that the DNA in the nano-space is condensed and shows an insignificant dependency of the structural compaction of the DNA on the degree of hydration (w_0) of the microenvironment. The circular dichroism (CD) spectrum shows that condensed DNA assumes the psi-form.

1. Introduction

The understanding of the mechanism of encapsulation of DNA in very small volumes is important as the media control structure, function, dynamics and thermodynamics of the DNA molecule in the confined regions of space. These studies are also very relevant to understand important biological functions of DNA in nucleosome formation and chromatin condensation [1,2]. During the past decade, attempts have been made to learn about DNA-condensation by using various condensing agents including reverse micelles [3–8]. Reverse micelles (RM) are tiny aqueous droplets surrounded and stabilized by a monolayer of surfactant molecules, and dispersed in water immiscible organic solvent [9–12]. The degree of hydration (w_0) of a RM is the ratio of concentration of water to that of the surfactant. RM at present are well known as common organic media to perform biocatalysis. They have been associated to the idea of a 'Nano-Cage' or 'Micro-Reactor' [10–12], where enzyme can be sheltered and protected from the detrimental effects of solvents. RM have also been used as medium of controlled hybridization [7].

In this work we have used homo-molecular (self-quenching) fluorescence resonance energy transfer (FRET) technique to assess distances between probe molecules (acridine orange; AO) attached with a genomic DNA (from salmon testes) before and after encapsulation of the DNA in anionic (bis(2-ethylhexyl)sulfosuccinate; AOT) RM. We have also carried out the circular dichroism (CD) study of the DNA in buffer solution and DNA in the RM. Particular emphasis has been given to the DNA packaged in reverse micelles as a model for condensed form of DNA. AO is known to interact with the DNA with two different mechanisms [13]; one is intercalation and other is electrostatic binding. The technique of FRET is frequently used for deducing intermolecular distances in the proteins and DNA ([14] and references therein) and well known as 'molecular ruler'. By observing the picosecond to nanosecond dynamics of nonradiative energy transfer of the AO–DNA complex in buffer and in the RM, we elucidate change in distance between two AO molecules

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upon encapsulation of the DNA in the RM. These observations clearly indicate the signature of condensed form of the DNA in the nano-space. For comparison, we also studied AO inside the RM without the DNA. In order to unravel the change in local geometrical restriction on the physical motions of the probe we have studied picosecond resolved polarization-analyzed anisotropy of AO in the RM without and with the DNA.

2. Experimental methodologies

Acridine orange (AO), AOT, isooctane and DNA (from salmon testes, sodium salt) were purchased from Sigma-Aldrich and used as received. All aqueous sample solutions were prepared in phosphate buffer (0.1 M, pH 7). The procedure for making DNA aqueous solution and to measure its concentration is similar to that of reference [15]. The ratio of absorption at 260 and 280 nm for the DNA solutions (450 μM base pair (bp)) gave a value 1.84, in accord with the limit of 1.8–1.9 for highly purified preparation of the DNA [15,16]. A known concentrated AO solution was added drop-wise to the DNA solutions with continuous stirring for 2 h to achieve various ratios of concentrations of the probe AO to that of DNA bp in the AO–DNA complex. In order to impregnate the RM with AO-labeled DNA, AO–DNA complex was solubilized in the AOT-isooctane solutions by direct injection techniques. For example, 36 μL of aqueous solution was injected to 2 ml of 100 mM AOT in isooctane to achieve the RM at $w_0 = 10$.

Steady-state absorption and emission were measured with Shimadzu Model UV-2450 spectrophotometer and Jobin Yvon Model Fluoromax-3 fluorimeter, respectively. CD was performed with a model J-600 Spectropolarimeter (Jasco) using 1 cm path-length quartz cell. All transients were taken by using commercially available picosecond-resolved time correlated single photon counting (TCSPC) setup from IBH, UK (excitation at 405 nm; instrument response function (IRF) ~90 ps). The time constants of the transients were obtained (uncertainty ± 10%) through numerical fitting using Scientist™ software. Detail experimental procedures including time-resolved anisotropy ($r(t)$) measurement could be found in our previous works [17,18]. In order to estimate fluorescence energy transfer efficiency of the donor (AO) and hence to determine distances of donor–acceptor pairs, we followed methodology described in [14, Chapter 13]. Here, the relative orientation of the transition dipoles (κ^2) of the donor and acceptor in space is assumed to be 2/3. As discussed in reference [14], the choice of the κ^2 value is not crucial in the estimation of distance (r) by using FRET technique. For example, variation of κ^2 value from 1 to 4 results in only 26% change in r. The implicit value of the refractive index of the sample solutions is 1.4. The quantum yield (Q_D) of AO–DNA in aqueous buffer is found to be 0.21. In the RM, Q_D of DNA-bound AO depends on degree of hydration (w_0); it varies from 0.12 ($w_0 = 5$) to 0.21 ($w_0 = 15$).

3. Results and discussions

As shown in Fig. 1a, upon complexation with the DNA, AO shows a red shift (10 nm) in absorption and

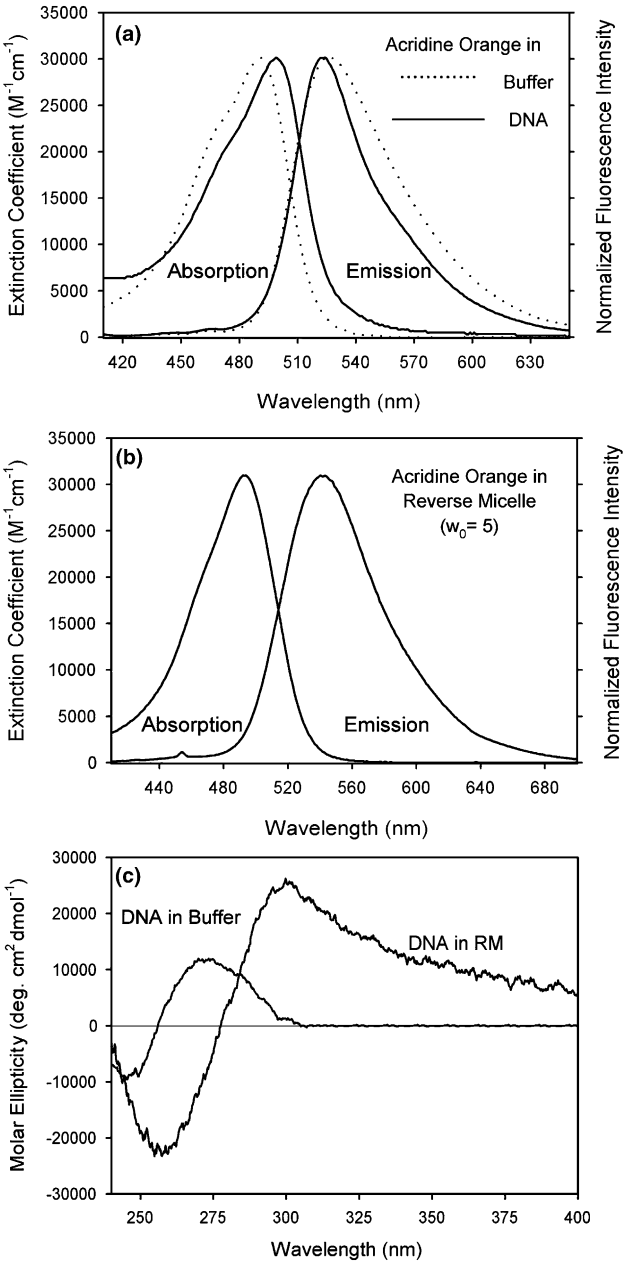

Fig. 1. (a) Absorption and emission spectra of AO–DNA complex (solid line) and free AO (dotted line) in buffer. (b) Absorption and emission spectra of AO in the RM at $w_0 = 5$. (c) CD spectra of DNA in buffer and in the 50 mM AOT/isooctane RM ($w_0 = 10$) at 27 °C. 1 cm path-length quartz cell used.

blue shift (7 nm) in the emission spectra. The emission peaks for AO in buffer and AO in AO–DNA complex were obtained at 522 and 529 nm, respectively. In the emission spectrum the line width (full width at half maximum) is reduced and fluorescence yield is increased significantly. From the spectra (Fig. 1a) it is evident that the spectral overlap of AO bound to the DNA is larger than that of AO in free buffer, which indicates efficient energy transfer in the former case. Fig. 1b shows absorption and emission spectra of AO in the RM ($w_0 = 5$) without the DNA. A significantly large spectral overlap is also evident from the spectra. Fig. 1c shows the CD spectra of DNA in buffer and encapsulated in the RM ($w_0 = 10$) indicative of condensed form of DNA in the latter medium (see below).

As shown in Figs. 2a and 3a, fluorescence transients of AO were fitted (solid lines) to sum of exponentials convoluted with the instrument response function (IRF). Upon complexation with the DNA, the lifetime of AO increases (longer time constant is 6.1 ns) considerably compared to that in buffer (1.86 ns). The evidence

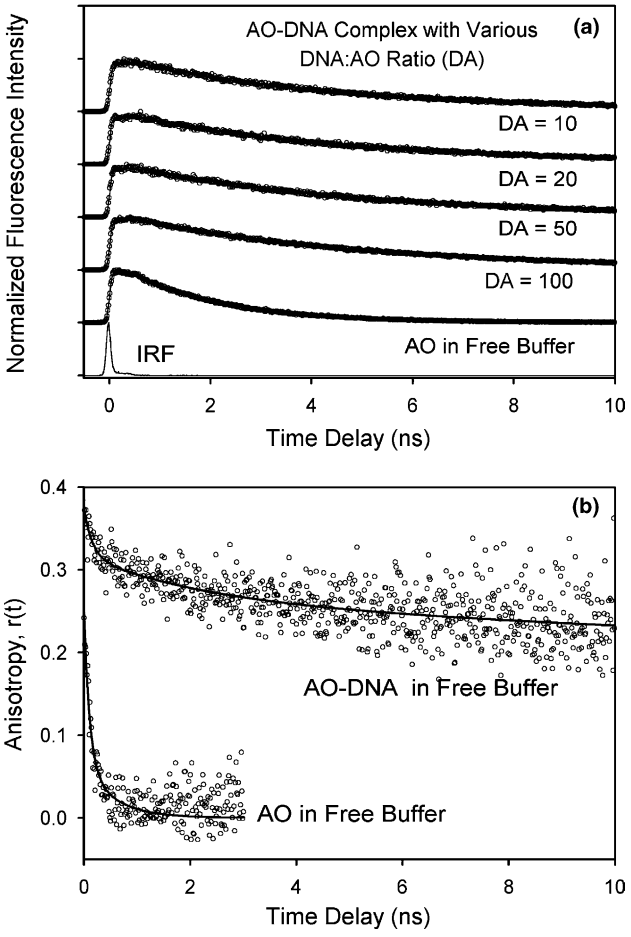

Fig. 2. (a) Time-resolved transients of AO–DNA complex with various DNA:AO ratio (DA). The instrument response function (IRF) is also shown for comparison. (b) Time-resolved anisotropies of AO and AO–DNA complex in buffer are shown.

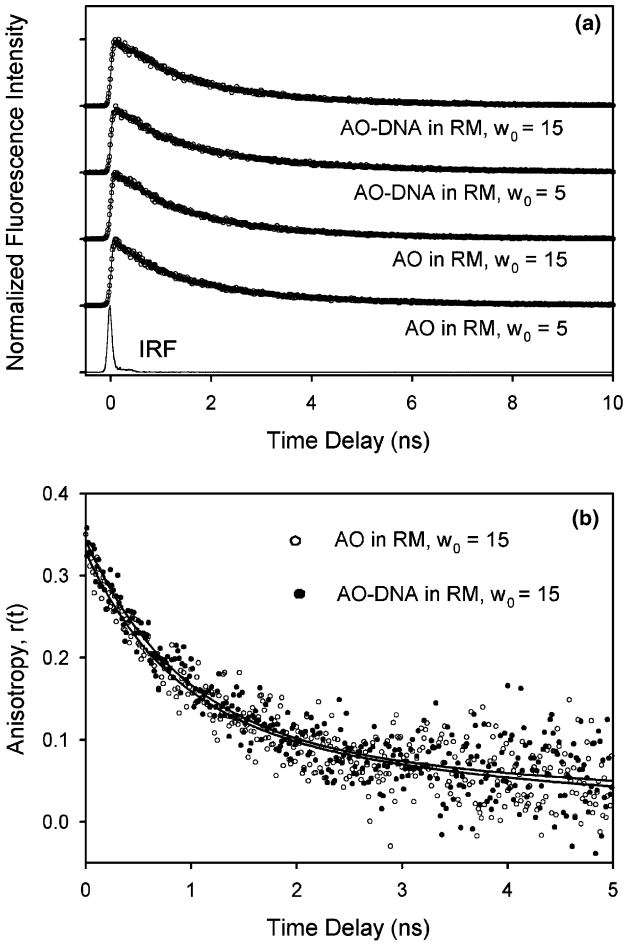

Fig. 3. (a) Time-resolved transients of AO in the RM with various degrees of hydration (w_0) in absence and presence of the DNA. The instrument response function (IRF) is also shown for comparison. (b) Time-resolved anisotropies of AO in the RM at $w_0 = 15$, in absence and presence of the DNA.

of formation of AO–DNA complex also comes from time-resolved anisotropy measurement. The anisotropy of AO–DNA complex is much slower than that of AO in free buffer (Fig. 2b). Relatively faster lifetime of free AO in buffer compared to that in the DNA could be due to self-aggregation of AO in the former medium [19]. The lifetime of AO in the DNA depends on the DNA:AO ratio (DA). Here, we consider concentration of base pairs of the DNA as overall concentration of the DNA. As shown in Fig. 2a the transients show two decay components with time constants 3.2–4.2 and 6 ns with a rise component of ~200 ps. Relatively faster time constants (3.2–4.2 ns) reveal lifetimes of AO molecules, which undergo FRET. The longer time constant (6 ns) is consistent with the lifetime of monomeric AO intercalated between the base pairs of DNA [19]. In these studies we regard 6 ns as τ_D, i.e. lifetime of donor AO in absence of acceptor AO. The rise components in the transients might be indicative of formation of charge transfer (CT) species. The possibility of such dynamical

processes (CT) is reported in the literatures [19–21]. Absence of the rise component in the blue end of the emission spectrum (488 nm) of AO in buffer supports the inference.

By using FRET techniques, we measured distances 45.2, 43.9, 40.6 and 40.9 Å between two DNA-attached AO molecules in the buffer solutions for DA values 100, 50, 20 and 10, respectively. The similarity in the distances for DA values from 10 to 100 indicates unavailability of AO binding sites in the DNA. In other words, addition of extra AO molecules in the DNA solution below DA = 100 is not appreciably helping AO to bind more into the DNA. The AO–DNA complex with DA = 10 was used here to study the structural change of the DNA upon encapsulation in the RM. The distance (40.9 Å) between two AO in the complex in buffer at DA = 10 is associated with 12 bp of DNA, on taking separation between two DNA base pairs to be 3.4 Å [22].

The transients of AO in the RM without and with the DNA show two decay components, as shown in Fig. 3a. No rise components were found in these systems, indicating absence of the CT dynamics in the microenvironments. In the RM at $w_0 = 5$ two decay components, 0.50 and 2.20 ns are associated with two average separations of 27.2 and 36.8 Å, respectively between AO molecules. Note that AO is completely insoluble in isooctane phase and expected to reside in the RM. The separation 27.2 Å might be reflective of distance between two AO molecules in the same RM (intra-micellar). The separation (27.2 Å) is larger than typical diameter (20 Å) of the aqueous pool of the RM at $w_0 = 5$ [9]. This anomaly could be due to the structural feature of AO, it has a positively charged (protonated imine) group and a hydrophobic moiety. If two AO molecules are located at diametrically opposite points at the interface of a RM ($w_0 = 5$) in such a way that the hydrophobic moiety was buried into the interface projecting their positively charged groups toward the negatively charged head groups of AOT (estimated diameter ~4 Å), the inter-AO distance would be larger than the diameter of the aqueous pool. The spatial distribution of similar organic dye molecules in RM and lipid bilayer is reported in the literatures [23,24]. Other separation of 36.8 Å might indicate the distance between two AO molecules in different RM (inter-micellar). In the case of RM at $w_0 = 15$ (decay time constants 1.36 and 3.10 ns), the separations mentioned above (intra and inter-micellar) were estimated to be 33.0 and 41.0 Å, respectively. The intra-micellar distance (33.0 Å) is larger than that in the RM at $w_0 = 5$ and shorter than estimated diameter (60 Å) of the aqueous pool of the RM at $w_0 = 15$.

The transients (Fig. 3a) of AO–DNA complex in the RM show biexponential decay components, indicating two kinds separations between AO molecules as mentioned above. In the DNA included RM at $w_0 = 5$ (decay time constants 1.18 and 3.20 ns), we recovered these separations to be 31.3 and 40.5 Å, respectively. The distance, 31.3 Å between two AO molecules attached to the DNA included in the RM is definitely shorter than that in free buffer (40.9 Å). This observation clearly indicates that in the RM, DNA strand is bent and/or different DNA strands are coupled together in the nano-space revealing DNA condensation in the microenvironment. For $w_0 = 15$ (decay time constants 1.40 and 3.20 ns), the shorter separation (intra-micellar, 32.5 Å) is similar to that in $w_0 = 5$, which indicates that the structural compaction of the DNA is apparently unaffected by w_0 of the RM.

A recent structural study [8] on various types of DNA including one genomic DNA from herring testes, which is similar to the DNA used in our study packaged in AOT/isooctane RM ($w_0 = 18.5$) using circular dichroism (CD) spectroscopy and dynamic light scattering (DLS) techniques has confirmed that the RM induce the configuration of a condensed form of the DNA. The particular DNA structure showed an interesting and characteristic CD feature, the so-called psi spectrum, which was indicative of super-condensed form of the DNA. The study [8] also observed three population of aggregates with an average radius centered at 5, 100 and 1000 nm, respectively, all three containing DNA. In the case of 'empty' RM with $w_0 = 18.5$ (RM containing water, no DNA) an average dimension centered ~5 nm radius was found. It has been suggested that the very large components with relatively smaller percentage, which is not so stable over long time period, can be ascribed to a cluster of smaller micelles.

Our study is in agreement with the previous structural studies [8]. The CD spectrum of DNA encapsulated in RM ($w_0 = 10$) [intermediate between $w_0 = 5$ and $w_0 = 15$] shows a strong negative CD signal at 260 nm and positive molar ellipticity at longer wavelength which is characteristic feature of psi-form of DNA. This feature is not evident in the CD spectrum of DNA in buffer. Shortening of inter-probe distance in the DNA upon encapsulation in the RM (~31 Å) compared to that in the buffer (~41 Å) is clear indication of the DNA compaction in the RM. We also observed that the longer distances in the RM with DNA at $w_0 = 5$ and $w_0 = 15$ (40.5 Å for both the cases) are similar to those in the RM without the DNA. This separation could be indicative of the inter-micellar distance and/or inter-probe distance in a DNA strand entrapped in larger RM that may exist [8]. However, any conclusive comment requires further study. The time-resolved anisotropy studies of AO in the RM ($w_0 = 15$) without and with the DNA show similar temporal behavior as shown in Fig. 3b (time constants are 0.91 ns (70%) 7.2 ns (30%)). This observation is in agreement with the compartmentation of DNA (oligomolecular form) in the reverse micellar system. In this case, each oligomolecule (condensed DNA) is almost

dynamically independent and expectedly resembles time scales of motions of the RM.

4. Conclusion

Studies of homo-molecular fluorescence resonance energy transfer (FRET) of a probe acridine orange (AO) with the genomic DNA (from salmon testes) encapsulated in an anionic reverse micelle (RM) elucidate the nature of condensation in the RM and the effect of degree of hydration (size) of the RM on the compaction. These studies attempt to explore structural features of the DNA compaction in an aqueous nano-space of the RM, which is particularly important for the biological function of DNA in nucleosome formation and chromatin condensation.

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