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Molecular recognition of genomic DNA in a condensate with a model surfactant for potential gene-delivery applications

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ABSTRACT

The functionality of a gene carrying nucleic acid in an artificial gene-delivery system is important for the overall efficiency of the vehicle in vivo. Here, we have studied a well-known artificial gene-delivery system, which is a condensate of calf thymus DNA (CT-DNA) with a model cationic surfactant cetyltrimethylammonium bromide (CTAB) to investigate the molecular recognition of the genomic DNA in the condensate. While dynamic light scattering (DLS) and circular dichroism (CD) reveal structural aspects of the condensate and the constituting DNA respectively, picosecond resolved polarization gated spectroscopy and Förster resonance energy transfer (FRET) reveal molecular recognition of the genomic DNA in the condensate. We have considered ethidium bromide (EB) and crystal violet (CV), which are well known DNA-binding agents through intercalative (specific) and electrostatic (non-specific) interactions, respectively, as model ligands for the molecular recognition studies. A fluorescent cationic surfactant, Nonyl Acridine Orange (NAO) is considered to be a mimic of CTAB in the condensate. The polarization gated fluorescence of NAO at various temperatures has been used to investigate the local microviscosity of the condensate. The excellent spectral overlap of NAO emission and the absorption spectra of both EB and CV allow us to investigate FRET-distances of the ligands with respect to NAO in the condensate at various temperatures and thermal stability of ligand-binding of the genomic DNA. The thermodynamic properties of the molecular recognition have also been explored using Van't Hoff equation. We have also extended our studies to molecular recognition of the genomic DNA in the condensate as dried thin films. This has important implications for its application in bioelectronics.

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1. Introduction

The search for harmless synthetic vectors that allow an efficient delivery of genes for the treatment of genetic and acquired diseases leads to intense research activities and a wealth of literature in the last two decades [1–5]. Circumventing limitations associated with the viral vectors, e.g. packaging DNA with particular size, immunogenicity and mutagenicity, were the main motives of those studies. DNA condensates (complexes) with cationic surfactants/lipids are considered to be efficient candidates for gene-delivery (transfection) applications [6,7]. Among other obvious requirements for achieving efficient transfection, the most important factor is that the interaction of DNA with the vectors should yield a nanometer size close to that of viruses [4,8–10]. This requirement is closely related to the fact that the critical size limit for endocytosis is 150 nm and the condensate is expected to escape from the blood vessel if its size is beyond a limit [4,8]. Another important factor is the intactness of the duly hydrated B-form of the gene carrying DNA in the synthetic vector [5,11]. While the above important considerations limit the efficiency of cationic polymers and cationic lipids, [12,13] cationic detergent cetyltrimethylammonium bromide (CTAB) is found to condense DNA into discrete particles containing even single nucleic acid molecule [14–16]. One of the notable properties of CTAB is the discrete first order phase transition of DNA between elongated coils and collapsed globules [15] well below the critical micellar concentration (CMC) of the surfactant and the formation of aggregates [15]. The interesting structure of the CTAB–DNA condensate is thought to result from the interaction of the negatively charged DNA phosphate groups with cationic surfactants and a further stabilization by the hydrophobic tails of the CTAB molecules [17].

Despite the unique feature of the CTAB–DNA condensate, the surfactant CTAB is found to be poorly efficient in transfection *in vitro* [18,19]. In order to study the sole role of CTAB in the stabilization of the CTAB–DNA condensate, in non-polar solvents rather than polar water media are the choice. The high solubility of the complex makes the contribution of parents (DNA and/or CTAB) inconclusive in the stability of the condensate in aqueous conditions. It was also concluded that due to higher solubility of the condensate, the complex in the cells is thought to induce fast release of CTAB revealing detergent related toxicity [4]. Earlier it has been shown that DNA-surfactant complexes are soluble

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in low-polarity organic solvents [3,20–22]. In one of these reports the structural properties of a genomic DNA upon complexation with CTAB in non-aqueous solvents of different degrees of polarity have been studied in details [3]. By using UV-vis, circular dichroism (CD) spectroscopy and fluorescence microscopy, the study has concluded that DNA–CTAB condensate dissociates into their initial components at concentrations of 40–60% (*v*/v) for ethanol or 30–50% (*v*/v) for 2-propanol, while conserving the double-stranded structure of the native DNA. Several other recent studies [23,24] unravel structural aspects of the genomic DNA in the condensate. However, the molecular recognition properties (both specific; intercalation, and non-specific; Coulombic) of the gene carrying DNA in the condensate are sparsely covered in the literature. In our earlier reports, the DNA-binding drugs Hoechest 33258 (non-specific) [25] and ethidium bromide (specific) [26] appear to be promising DNA-probes in the condensate. Ethidium bromide (EB) intercalates into the genomic DNA and provides the structural details of DNA even when it is in condensed form in a self-assembled reverse micellar nanocage. On the other hand, Hoechest 33258, a well-known DNA minor groove binder, provides dynamical and structural information of the condensed DNA either bound to nucleic acid binding protein or CTAB surfactant [27]. It is important to note that the functional properties of gene carrying DNA (molecular recognition) in the condensate will dictate the overall activity of the gene after delivery in the target cells. A detailed investigation on the structural and functional properties (intercalation and electrostatic binding) of a genomic DNA (calf thymus; CT-DNA) in its condensate form in a non-aqueous solvent (butanol), where stabilization of the condensate is essentially governed by the hydrophobic tails of the CTAB surfactant, is the main motive of our present studies.

In the present work, we have synthesized a condensate of CT-DNA and CTAB in butanol. While dynamic light scattering (DLS) studies confirm the size of the condensate in the solution to be less than 100 nm, CD spectroscopy reveals the intactness of B-form structure of the genomic DNA in the condensate. Picosecond resolved fluorescence of a well-known DNA intercalator EB to the nucleic acid in the condensate clearly shows some degree of perturbation of the intercalative binding of the genomic DNA in the condensate compared to that in aqueous solution. We have measured the temperature dependent binding constant of the EB with the genomic DNA in the condensate in butanol and compared with that in aqueous solution. A detailed analysis of thermodynamical parameters from the Van't Hoff plots reveals that the EB-binding to the genomic DNA in the condensate is significantly different from that of the DNA in aqueous solution. In order to probe the interaction of CTAB in the condensate, we have used Nonyl Acridine Orange (NAO) as model cationic surfactant with a fluorescence acridine moiety [28,29]. Picosecond resolved polarization gated anisotropy of NAO in the condensate shows a hydrodynamic rotation of the surfactant in the condensate and reveals an activation energy for the viscous flow [30,31]. Strong spectral overlap of NAO emission and absorption spectrum of intercalating EB also offers the opportunity to measure the distance between cationic surfactant and the intercalator (EB) in the condensate with molecular precision employing Förster resonance energy transfer (FRET) strategy. We have also used FRET between NAO and another electrostatic DNA binder, crystal violet (CV) in the condensate. The constructed probability distribution functions of FRET distance between NAO and either EB (intercalator) or CV (electrostatic binder) at various temperatures in the condensate unravel the efficacy of molecular recognition of the genomic DNA by small ligands. Our studies are expected to find relevance in the investigation of functionality of DNA molecules in condensate for potential gene-delivery application.

2. Materials and Methods

2.1. Chemicals

Calf thymus DNA (CT-DNA), Nonyl Acridine Orange, ethidium bromide (EB) and crystal violet (CV) were purchased from sigma-Aldrich (Saint Louis, USA) and were used as received. Cetyltrimethylammonium bromide (CTAB) and butanol were obtained from Spectrochem (Mumbai, INDIA) chemicals and Merck (Mumbai, INDIA), respectively. The aqueous solution of genomic DNA was prepared in phosphate buffer (50 mM, pH 7). In the present study the concentration of base-pair of the genomic DNA is considered as an overall concentration of DNA. The DNA concentration was determined by absorption spectroscopy, considering the molar extinction coefficient of DNA bases to be equal to 6600 M^{-1} cm^{-1} at 260 nm and found to be 6 mM [14].

2.2. Experimental Details

The steady state absorption and emission spectra were measured with Shimadzu UV-2600 spectrophotometer and Jobin Yvon fluorolog fluorimeter, respectively. CD spectra were recorded by JASCO-810 spectropolarimeter. Hydrodynamic diameter which were obtained from dynamic light scattering was measured by using Nano S Malvern instrument employing a 4 mW He–Ne laser ($\lambda = 632.8$ nm) and equipped with a thermostated sample chamber. All the scattered photons were collected at a 173° scattering angle at T = 298 K. The hydrodynamic diameter (d_h) of the particles is estimated from the intensity auto correlation function of the time-dependent-fluctuation in intensity. The diameter, d_h is define as, $d_h = {}^{k_B T}/_{3\pi\eta D}$, where k_B is the Boltzmann constant, T is the absolute temperature, η is the viscosity and D is the translation diffusion coefficient.

All the picosecond resolved fluorescence transients were measured by using commercially available time-correlated single-photon counting (TCSPC) setup with MCP-PMT from Edinburgh instrument, U.K. (instrument response function (IRF) of ~90 ps) using a 409 nm excitation laser source. The sample temperature was maintained by a controller from Julabo (Model: F32). Details of the time resolved fluorescence setup have been depicted in our previous reports [25,32]. To estimate the FRET efficiency of energy donor (D) to the different acceptors (A) and hence to determine the distance of the FRET pair (D–A), we have followed the methodology described elsewhere [33–35]. In brief, D–A distance, r, can be calculated from the equation, $r^6 = [R_0^6(1-E)]/E$, where E is the energy transfer efficiency between donor and acceptor, and R_0 is Förster distance.

For the fluorescence anisotropy measurements, the emission polarizer was adjusted to be parallel and perpendicular to that of the excitation and collected the fluorescence transients $I_{para}(t)$ and $I_{perp}(t)$, respectively. The anisotropy is defined as, $r(t) = {}^{(I_{para} - G*I_{per})}/_{(I_{para} + 2*G*I_{per})}$. The magnitude of G, the grating factor of emission monochromator of the TCSPC system, could be found using a long tail matching technique. The rotational relaxation time, τ_{rot} of the fluorescent probe is related to the local microviscosity, η_m, experienced by the probe molecules through the Stokes–Einstein–Debye equation: [32] $\tau_{rot} = {}^{\eta_m V_h}/_{K_B T}$ where K_B is the Boltzmann constant, T is the temperature, and V_h is the hydrodynamic volume of the probe.

Distance distribution function, P(r) were calculated using nonlinear least-squares fitting procedure by using SCIENTIST software to the following function $P(r) = \{1/[\sigma - (2\pi)^{1/2}]\} \exp\{-{}^1/_2[(\bar{r} - r)/\sigma]^2\}$, where $\bar{r}$ is the mean of the Gaussian with standard deviation of σ and r is the donor acceptor distance. Detailed theory and calculation of the distance distribution could be found elsewhere [36].

2.3. Preparation of DNA–CTAB Complex

The stock solution of the calf thymus DNA was diluted with the potassium phosphate buffer (50 mM, pH 7) so that the final concentration of the genomic DNA in nucleotide unit was 3 mM. To this solution, equimolar ratio of CTAB surfactant was mixed gently with continuous stirring. The resulting precipitate was separated and washed several times with the buffer solution. After that the precipitate was lyophilized

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