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Interaction of surfactants with DNA. Role of hydrophobicity and surface charge on intercalation and DNA melting

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Abstract

A probe, 9-(anthrylmethyl)trimethylammonium chloride, **1**, was prepared. **1** binds to calf-thymus DNA or *Escherichia coli* genomic DNA with high affinity, as evidenced from the absorption titration. Strong hypochromism, spectral broadening and red-shifts in the absorption spectra were observed. Half-reciprocal plot constructed from this experiment gave binding constant of $5 \pm 0.5 \times 10^4$ M^{-1} in base molarity. We employed this anthryl probe-DNA complex for studying the effects of addition of various surfactant to DNA. Surfactants of different charge types and chain lengths were used in this study and the effects of surfactant addition to such probe-DNA complex were compared with that of small organic cations or salts. Addition of either salts or cationic surfactants led to structural changes in DNA and under these conditions, the probe from the DNA-bound complex appeared to get released. However, the cationic surfactants could induce such release of the probe from the probe-DNA complex at a much lower concentration than that of the small organic cations or salts. In contrast the anionic surfactants failed to promote any destabilization of such probe-DNA complexes. The effects of additives on the probe-DNA complexes were also examined by using a different technique (fluorescence spectroscopy) using a different probe ethidium bromide. The association complexes formed between the cationic surfactants and the plasmid DNA pTZ19R, were further examined under agarose gel electrophoresis and could not be visualized by ethidium bromide staining presumably due to cationic surfactant-induced condensation of DNA. Most of the DNA from such association complexes can be recovered by extraction of surfactants with phenol-chloroform. Inclusion of surfactants and other additives into the DNA generally enhanced the DNA melting temperatures by a few °C and at high [surfactant], the corresponding melting profiles got broadened.

Keywords: Surfactant; Cationic lipid; DNA; Intercalator probe; Agarose gel electrophoresis; DNA melting temperature

1. Introduction

Complexes formed between nucleic acids and cationic liposomes appear to be among the best candidates for the delivery (transfection) of DNA and RNA into a large variety of eukaryotic cells [1–3]. But all the factors that control the interactions between DNA and lipids and the nature of DNA-lipid complexes remain unclear. One of the critical factors in successful DNA delivery is the lipid composition of the cationic liposome [4]. The cationic lipids in these mixtures are amphipathic and can vary widely in their molecular structures. However, not all cationic lipids are capable of effecting transfection [5]. The presence of cationic surfactant in liposomes provides

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the vesicle with a positively charged surface which facilitates strong interactions between vesicles and plasmids. Presumably the resulting association complex carry a net positive charge which promote their approach to the cell surface through fusion and endocytosis [6]. However, while a lipid recipe may work with a particular cell line, the same may not be efficient for another type of eukaryotic system. Then a change or replacement of the surfactant with another amphiphile having different molecular structural features may bring back high transfection efficiency [7]. The elucidation of the optimal transfection recipe for any given cell line therefore appears to require a prior knowledge of surfactant, DNA/lipid ratio and the choice of the lipid composition. This in turn necessitates clear understanding of the nature of interaction between various surfactants/lipids and DNA.

Many lipids and surfactants used for this purpose by themselves also show interesting properties. For example, the double-chain cationic surfactant such as dimethyldialkylammonium bromide show interesting immunoadjuvant properties [8]. Cetylpyridinium chloride is another single-chain cationic surfactant with well-known antibacterial properties [9]. Several surfactants also find utility in the purification of DNA through precipitation [10,11]. Moreover, cetyltrimethylammonium bromide has been used to control the rate of DNA renaturation [12]. Recently, cationic surfactant induced coil to globular transition of DNA has been demonstrated by using fluorescence microscopy [13]. Okahata and co-workers have shown that DNA can be solubilized in organic solvents upon 1:1 complex formation with cationic surfactant molecules [14–16]. They also demonstrated that such DNA-cationic lipid complexes retain stranded structure and could therefore intercalate appropriate dye molecules in organic solvents such as chloroform. Independent evidence of formation of hydrophobic complexes between the cationic lipids and DNA has also been reported [17].

Due to our mutual interest in the chemistry and biology of DNA [18–20] and also in the molecular design of DNA-interactant lipids and surfactants [21–25], we thought that it would be useful to systematically explore the nature of interactions that prevail between DNA and lipids before undertaking an elaborate programme on the design of materials for DNA transfection. With this objective in mind, we developed a new probe, 9-anthrylmethyl trimethylammonium chloride, **1**, which was found to bind DNA with high affinity, as evidenced from strong hypochromism, spectral broadening and red-shifts in the absorption spectral titration. We utilized the resulting probe-DNA complex for studying the effects of addition of different surfactant of various charge types by recording the changes in the UV-visible absorption spectral characteristics. The effects of surfactant addition to such probe-DNA complex were also compared with that of the organic cations or salts. Addition of either salts or surfactants led to structural changes in DNA which induced dissociation of the probe from the DNA-bound probe complex. The effects of surfactant addition on DNA were further examined by using a structurally unrelated DNA intercalator, ethidium bromide as a probe using fluorescence spectroscopy which supported our findings from the UV-Vis absorption studies. To further understand the nature of the DNA surfactant association, the complexes formed between the plasmid DNA pTZ19R and cationic surfactants were also examined in terms of their mobilities and abilities to stain ethidium bromide under agarose gel electrophoresis. Based on these systematic studies, we have shown herein that there exists a clear difference between surfactant and ionic solutes (that cannot aggregate in water) in terms of their abilities to perturb DNA organization. These studies reveal how the hydrophobic effects and the specific surface charges play an important role in bringing about profound changes in DNA structures.

2. Materials and methods

2.1. Materials

2.1.1. Synthesis

*9-Trimethylaminomethylanthracene chloride, (probe **1**).* 9-chloro methyl anthracene was prepared by adaptation of a literature procedure [26]. The probe molecule, 9-(anthrylmethyl)trimethyl ammonium chloride was synthesized by passing dry trimethylamine gas through an acetone solution of 9-chloromethyl anthracene. Upon standing at room temperature, this resulted in the formation of an

orange precipitate which was recrystallized from 5% ethanol in ethyl acetate several times. Yield of the reaction is more than 95%, indicating virtually quantitative conversion. Satisfactory spectral and analytical data were obtained for this compound. Relevant spectral data: ^{1}H-NMR [90 MHz, D_2O]: δ 3.0 (9H, s), 4.95 (2H, s), 7.5–8.0 (6H, m), 8.0–8.25 (3H, m).

*Cetyldimethyl glycinate, (**8**, CDMG).* 500 mg (3.5 mmol) of dimethyl glycine ethyl ester (Sigma) was refluxed with 1.4 g (4.6 mmol) of cetyl bromide (Aldrich) in dry ethanol for 3 days. The solvent was removed after the end of this period and the solid material thus obtained was recrystallized from ethyl acetate. Yield 70%. ^{1}H-NMR: [90 MHz, $CDCl_3$]: δ 0.9 (3H, t), 1–1.5 (31H, br m), 3.5–3.9 (8H, s + br t), 4.1–4.6 (2H, q), 4.95 (2H, s).

The above quaternary ester 1.1 g (2.5 mmol) was refluxed for 12 h in 5 ml of aqueous 2N HBr. Then, the solvent was removed under vacuum and the solid obtained was recrystallized from ethyl acetate-methanol (9:1). Yield 80% (white crystalline solid). ^{1}H-NMR: [90 MHz, $CDCl_3$ + DMSO(D_6)]: δ 0.9 (3H, t), 1–1.6 (28H, br m), 3.25 (6H, s), 3.5 (2H, t); 4.36 (2H, s).

The double chain surfactant, dihexadecyldimethylammonium bromide (**3**, DHDAB) was prepared by adaptation of a literature procedure [27]. The other surfactants, e.g., cetyltrimethylammonium chloride (**2**, CTAC) cetylpyridinium chloride (**5**, CPC), sodium dodecyl sulphate (**7**, SDS), and salts, e.g., NaCl and tetramethylammonium chloride (**6**, TMAC), and the probe ethidium bromide were purchased from Sigma or Aldrich Chemical Company. Dioctyldimethylammonium bromide (**4**, DODAB) was obtained from Lonza, Inc. USA. Solvents and other reagents for synthetic work and buffer preparation were of highest quality available commercially. All buffers and solutions were prepared with double-distilled water or deionized water (millipore).

2.2. Vesicle preparation

The vesicles of DHDAB were generated by probe sonication method [28]. Typically, 0.01 mmol of DHDAB were dissolved in 0.1 ml of chloroform in a vial and the solvent was removed under high vacuum. To the resulting film of the lipid in the vial, ca. 10 ml of deionized water was added. The dispersions were then heated in a hot water bath at $\sim$ 50°C for 5 min and then briefly sonicated with an immersion probe sonicator at 25 W (model XL-2020, Heat systems ultrasonic processor) for 2–3 min. The size of the DHDAB vesicles ranged from 800–1000 Å based on transmission electron microscopy (Jeol 200-CX, staining with 0.5% uranyl acetate).

2.3. DNA preparation

Calf-thymus DNA (CT DNA) was obtained from Sigma Chemical Company (St. Louis, MO) was purified by the literature method [29] involving phenol-chloroform extraction followed by ethanol precipitation. CT DNA thus purified was dissolved in 10 mM Tris-HCl, 1 mM EDTA (pH 7.4) buffer and was used for T_m measurements and spectroscopic binding analysis. The DNA concentrations were determined by using an extinction coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm and expressed in terms of base molarity [30].

The plasmid DNA pTZ19R used herein were grown in *E. coli* cells and isolated in our laboratory as described earlier [18].

2.4. Absorption titrations

Absorption titrations were carried out by keeping the concentration of the probe constant, while adding concentrated solution of the CT DNA in progressively increasing amounts into both the cuvettes till the saturation in hypochromism was observed. The intrinsic binding constant for the probe with CT DNA was determined by the half-reciprocal plot method using the literature procedure [31]. The intrinsic binding constant (K) for a given complex with CT DNA was obtained from a plot of $D/\Delta\epsilon_{ap}$ vs. D according to equation $D/\Delta\epsilon_{ap} = D/\Delta\epsilon + 1/(\Delta\epsilon \times K)$.....1, where D is the concentration of DNA in base molarity, $\Delta\epsilon_{ap} = |\epsilon_a - \epsilon_f|$ and $\Delta\epsilon = |\epsilon_b - \epsilon_f|$, where, ϵ_b and ϵ_f are respective extinction coefficient of the complex in the presence and absence of DNA. The apparent extinction coefficient, ϵ_a, was obtained by calculating $A_{obsd}/$[complex]. The data were fitted to the equation, with a slope equal to $1/\Delta\epsilon$ and an y-intercept equal to $1/(\Delta\epsilon \times K)$. The intrinsic binding constant (K) was determined from the ratio of the slope to *y*-intercept.

2.5. Effects of surfactant / salt on the UV-Vis absorption spectra of anthryl probe-DNA complex

The effects of addition of various concentrations of salt, or organic cations or surfactants on the UV-Vis absorption spectra of intercalatively bound anthryl probe-DNA complex were studied in the following manner. Progressively increasing amounts of solutions of different additives were added separately into solutions containing probe-DNA complex (Tris-HCl buffer, pH 7.4). After each addition the solutions were mixed carefully followed by recording the corresponding spectrum. The changes in the absorption peaks due to DNA-bound **1** were plotted against the concentration of each additive to determine the effects of different additives.

2.6. Effects of surfactant / salt on the fluorescence properties of ethidium bromide-DNA complex

The effects of addition of various concentrations of salt, organic cations or surfactants on the fluorescence emission spectra of intercalatively bound ethidium bromide-DNA complex were examined. Known concentrations of different additives were separately added in small increments into solutions containing probe-DNA complex (5 mM Tris-HCl buffer, pH 7.4). After each addition, the mixtures were carefully stirred followed by recording the corresponding fluorescence emission spectrum (550–700 nm) upon excitation at 266 nm. The changes in the fluorescence emission peak ($\sim$590 nm) due to DNA complexed ethidium bromide were plotted against the concentration of each additive. The concentration of additives required to obtain 50% quenching of the maximal fluorescence was compared to find out the relative efficiencies of different additives.

2.7. Agarose gel electrophoresis

The additive-DNA association complexes using various concentrations of different surfactants or salts were made by mixing the same amount of plasmid, pTZ19R (1 μg/reaction) at pH 7.4 Tris-HCl (20 mM) followed by incubation at 37°C for 5 min in 20 μl total volume. An aliquot of 5 μl of this solution was directly loaded into 1% agarose (Pharmacia) gel without further purification. The residual 15 μl was diluted to 100 μl to which an equal volume of phenol was added, mixed, centrifuged at 5 K for 10 min and the top layer was collected in a new Eppendorf tube. Then, 100 μl of $CHCl_3$ was added to this and thoroughly mixed. After phase separation, the top layer was collected. The DNA from this supernatant was precipitated by the addition of 0.3 M NaOAc and 300 μl ethanol and the resulting mixture was cooled at -20°C for 30 min and finally centrifuged at 13K for 40 min at 4°C. The supernatant thus produced was discarded and the DNA pellet was taken and washed with 70% aqueous ethanol and dried. The treated DNA samples were then loaded into agarose gel with loading dye (bromophenol blue and xylene cyanol FF) parallel to the DNA samples (that were not purified after the addition of various surfactants) for comparison. All the DNA samples as well as the dye from the respective lanes were electrophoresed under a constant electric field of 80 V for 90 min and then stained with ethidium bromide (0.5 μg/ml) for 2 h. Bands of DNA were detected and photographed (Canon SLR camera with an orange filter) under UV light (Photodyne Transilluminator, 312 nm) in a darkroom.

2.8. Melting temperature measurements

Melting temperatures (T_m) for pure DNA or additive-DNA complexes were measured by following the changes in absorption at 260 nm (A_{260}) as a function of temperature in a Shimadzu Model UV-2100 UV-Vis spectrophotometer. Temperature of the cuvette chamber was maintained using a Julabo Model F-10 water circulating bath and the temperatures inside the cuvette were measured before and after recording each spectrum. All the experiments were carried out with 38 μM (base molarity) CT DNA at pH 7.4, 5 mM Tris-HCl buffer. The absorption intensities at 260 nm were plotted against individual temperatures and the mid-points of inflection region in the temperature-A_{260} curves were taken as the corresponding T_m values [32].

3. Results and discussion

3.1. Choice of probes

Recently Kumar et al. introduced a probe for studying DNA binding phenomena [33]. The presence

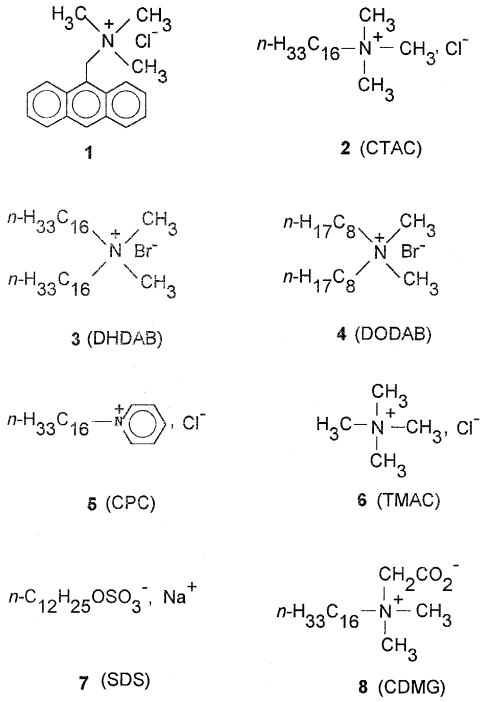

Fig. 1. Structures and abbreviations of the probe and of different surfactants and additives used in study.

of anthryl moiety in this probe gives sensitive spectrophotometric and fluorimetric handles to examine the nature of its interactions with DNA. The well-resolved vibronic transition of the probe in the 300–400 nm region offers an indication of the local microenvironment that the probe experiences upon binding with DNA. Variation in the intensities of these transitions can be used to determine the nature and the strength of the stacking interactions between the probe and DNA under different conditions. Generally, the probes that stack with DNA are planar and planarity is an important criterion for effective intercalation into the helix [34]. Consequently, the pronounced hydrophobic character of the planar anthryl moieties promotes interaction of the probe into a less polar interior of the DNA double-stranded helix. However, the presence of the ammonium group (NH_3^+) in the probe used in the reported method [33] allows an additional mode of interaction with DNA through hydrogen bonding. In order to confine the DNA-probe interaction specifically to *electrostatic* and *intercalative* modes, we deliberately modified the probe by incorporation of a trimethylammonium group (NMe_3^+) in the place of an ammonium group (NH_3^+) (see structure **1**, Fig. 1). The NMe_3^+ group in **1** bears a positive charge irrespective of the solution pH and thus offers greater utility for DNA binding studies, particularly when studies need to be done at different pH values.

To verify our results further involving surfactant-DNA interactions (see below), we also used another DNA intercalator ethidium bromide (a fluorogenic probe) to understand the nature of the surfactant-DNA interaction.

*3.2. Absorption titration of the probe **1** with CT DNA*

As expected, probe **1** was found to interact very strongly with CT DNA. Fig. 2 shows the UV-Vis absorption titration of the probe with CT DNA. Upon addition of increasing amounts of CT DNA into a solution containing probe of fixed concentration, spectral broadening, a progressively greater extent of hypochromism in the absorption intensities, and red-shifts were observed, eventually reaching a saturation in the observed phenomena. The peaks at 355, 372

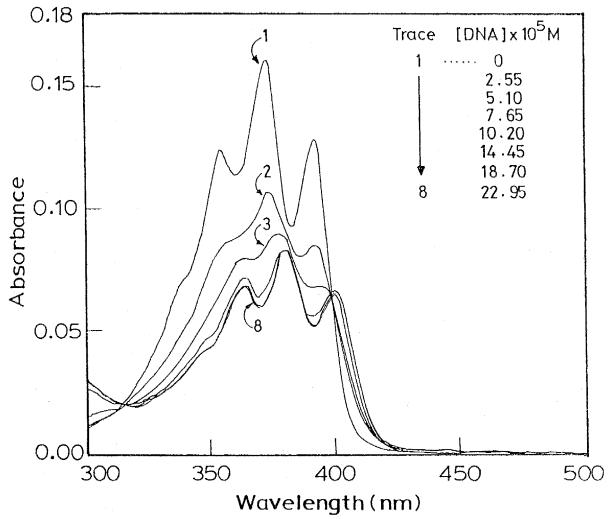

Fig. 2. Absorption titration of the probe **1** with CT DNA. UV-visible (UV-Vis) absorption titration was carried out by adding increasing amounts of CT DNA, maintaining the concentration of the probe **1** constant. Trace 1: UV-Vis absorption spectra due to the probe alone (2.43×10^{-5} M). Traces 2–8 are absorption spectra due to the progressive addition of CT DNA into the solution containing the probe. Respective concentrations of CT DNA for different spectra are also given.

and 392 nm represent the absorption due to the free probe **1** in solution, while the corresponding peaks at 364, 382 and 401 nm represent the red-shifted intercalation complex between the probe **1** and CT DNA. Isosbestic points are clearly observed in Fig. 2 near 312 and 400 nm for binding of **1** with CT DNA. These results suggest that there exists a chemical equilibrium between the bound and the free probe with no spectroscopically detectable intermediate states.

The saturation in the hypochromism is shown in Fig. 3A. A maximum extent of $\sim$ 62% hypochromism was observed. The pronounced hypochromism suggests a strong interaction between the electronic states of the probe **1** with DNA. The variation in the absorbance of **1** at 372 nm with continually increasing amounts of CT DNA addition was used to construct the half-reciprocal plot as given by the equation (see Section 2). The absorption titration data fitted nicely and the plot of $D/\Delta\epsilon_{ap}$ vs. D, resulted in a straight line (Fig. 3B). The binding constant was estimated as $5 \pm 0.5 \times 10^4$ M^{-1} from the ratio of the slope to the *y*-intercept. As indicated by the previous study [35], the pronounced hypochromism as well as $\sim$ 10 nm red-shift are indicative of strong intercalation of the probe **1** with CT DNA. The observed, large extent of hypochromism points toward a close positioning of the probe to the DNA bases, presumably as a consequence of intercalation of the anthryl unit into the helix and due to an appreciable overlap of the π-π^* states of the chromophore with electronic states of the CT DNA.

3.3. Effect of surfactant addition on DNA-bound probe 1 using UV-Vis spectroscopy

In order to decipher the nature of surfactant-DNA interactions, we have examined the effect of addition of various surfactants on the absorption spectra of DNA-bound **1**. At the same time, to provide a comparative perspective, we have also studied the effect of addition of salts and small organic cations (such as tetramethylammonium ion) into the CT DNA-bound **1**. Table 1 summarizes relevant parameters of this study involving the effect of surfactant addition on DNA-bound probe.

Fig. 4A shows the changes in the absorption spectra upon addition of increasing amounts of NaCl into the CT DNA-bound **1**. Trace 1 in Fig. 4A represents the spectrum due to the free probe **1**, in the absence of any DNA. Trace 2 shows fully DNA-bound probe **1** (peak maximum at $\sim$ 382 nm). Addition of progressively increasing amounts of NaCl to fully DNA-bound probe **1** causes a blue shift ($\sim$ 10 nm)

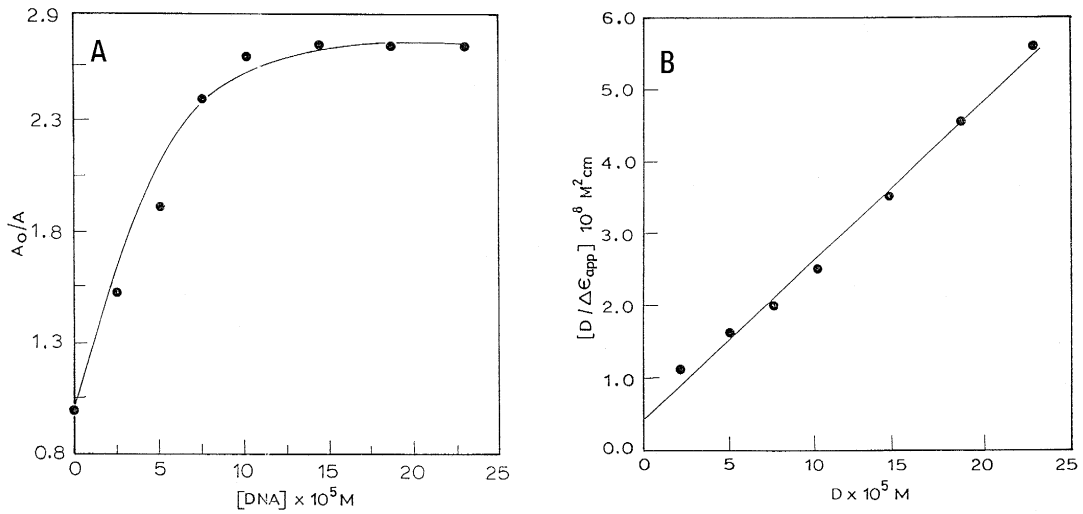

Fig. 3. A: Saturation plot of absorption titration of the probe **1** with CT DNA. It was obtained by plotting A_0/A vs [DNA] where A_0 denotes absorption intensity of the free probe **1** at 372 nm and A is the observed absorption intensity for the probe **1** in the presence of varying concentration of CT DNA. Concentrations of DNA was expressed in base molarity. B: Half-reciprocal plot of the absorption titration. It was obtained by plotting $D/\Delta\epsilon_{ap}$ vs. D according to the equation, as described in Section 2.

and apparent hyperchromism (~ 74%). In the inset of Fig. 4A is shown the saturation plot of the hyperchromism observed due to NaCl addition (A_a/A_b vs. [NaCl]), where A_b is the absorption intensity at the λ_{max} of completely DNA-bound probe **1** and A_a is the observed absorption intensity at the altered λ_{max} due to probe **1** upon inclusion of an increasing amount of additive (NaCl). A minimum of ~ 120 mM of NaCl was required to obtain a saturation in the apparent hyperchromism. Fig. 4B,C shows the effect on the absorption spectra of CT DNA-bound probe **1** due to addition of increasing amounts of NH_4Cl and NMe_4Cl. Note that with both of these systems (salt or organic cation) hyperchromism was seen as was the

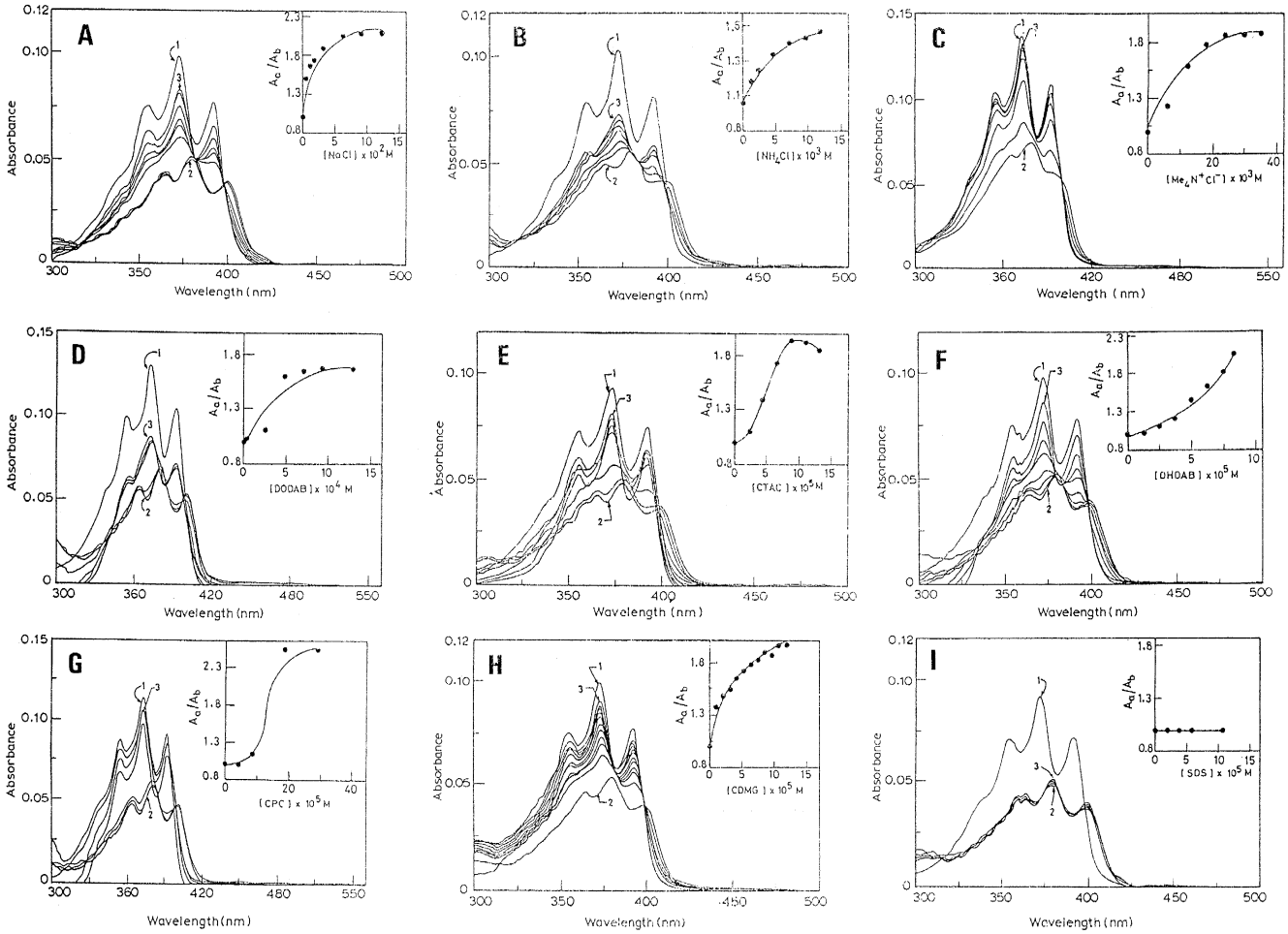

Fig. 4. The effect of addition of different additives on DNA-bound probe **1**. This experiment was carried out by adding increasing amount of additives into the probe **1**-DNA complex (CT DNA, 8.5×10^{-5} M, base molarity) following the absorption spectra after each addition. In all figures (A–I), trace 1 represents the absorption spectra due to probe **1** alone, trace 2 is absorption spectra of fully CT DNA-bound probe **1** (in the absence of any additives) and trace 3 represents the final spectra (after the attainment of saturation in hyperchromism) obtained upon addition of saturating concentration of the additive into the probe-DNA complex. The addition of progressively increasing amount of additives into the probe-DNA complex resulted in the spectra from 2 towards 3. Effects of additives: (A) NaCl; saturating concentration of the NaCl ~ 120 mM. (B) NH_4Cl; [NH_4Cl] at saturation ~ 12 mM. (C) NMe_4Cl; [NMe_4Cl] at saturation ~ 24 mM. (D) DODAB; [DODAB] at saturation ~ 0.9 mM. (E) CTAC; [CTAC] at saturation ~ 0.08 mM. (F) DHDAB); [DHDAB] at saturation ~ 0.08 mM. (G) CPC; [CPC] at saturation ~ 0.18 mM (H) CDMG; [CDMG] at saturation ~ 0.09 mM. (I) SDS; No change in the spectrum was observed. In all the figures, the respective insets were obtained by plotting A_a/A_b vs [additive], where A_b stands for the absorption intensity at the λ_{max} of completely DNA-bound probe **1** and A_a denotes the observed absorption intensity at the altered λ_{max} due to the chromophore **1** in the presence of increasing amounts of the respective additive.

Table 1
Effect of surfactant addition on DNA bound probe, **1**

Additive [a]	Hyperchromism (%) [b]	[Additive]10^5 M [c]
NaCl	74	12000
NH_4Cl	44	1200
NMe_4Cl	89	2400
DODAB	45	90
CTAC	73	8 [d]
DHDAB	78	8 [e]
CPC	84	18
CDMG	83	9
SDS	- [f]	- [f]

[a] See Fig. 1 for structures and notations of the additives.
[b] Percent of apparent hyperchromism at saturating concentration of the additive.
[c] The saturating concentration at which maximum hyperchromism was observed.
[d] At a still higher concentration of CTAC precipitation was observed.
[e] At still higher concentration solution became turbid.
[f] No hyperchromism was observed even up to addition of 1 mM SDS.

case with NaCl. But in order to achieve the same extent of hyperchromism as was seen with NaCl, nearly one-tenth concentration of either NH_4Cl or NMe_4Cl with respect to [NaCl] was required.

Fig. 4D shows the changes in the absorption spectra upon addition of dioctyldimethyl ammonium bromide, DODAB, into the DNA-bound **1**. The same extent of hyperchromism as was seen with NaCl could now be achieved even with two orders of magnitude *less* concentration relative to [NaCl]. This could be due to the introduction of two *n*-octyl chains in the additive, DODAB. Further lengthening of the chain length of the additive structure indeed increased the propensity of inducing destabilization of DNA-**1** complex (Fig. 4E,F). Interestingly, single-chain cationic surfactant, CTAC (obtained by introducing one *n*-hexadecyl chain instead of a methyl group in TMAC), effected an equal extent of destabilization of the probe-DNA complex at almost two orders of magnitude less concentration relative to that of TMAC or almost three orders of magnitude less concentration relative to that of NaCl (Fig, 4E). Interestingly, however, when an additive containing two *n*-hexadecyl chains (DHDAB) was employed, the efficiency of promoting hyperchromism appeared to have gone up only modestly over its single-chain counterpart, CTAC (Fig. 4F). However, the precise nature of interactions between the vesicle-forming DHDAB with CT DNA could be different from that between micelle-forming, single-chain, CTAC and CT DNA (see below). Fig. 4G shows the effect of addition of CPC in which the polar head group included a flat, pyridinium moiety. When employed upon probe-DNA complex, the nature of the saturation in the observed hyperchromism appears to be non-monotonous in this instance, although it is almost equally efficient with respect to CTAC in effecting the destabilization of DNA-**1** complex. We also employed one single-chain *zwitterionic*, CDMG and one *anionic* SDS surfactant for the same studies as described above (Fig. 4H,I, respectively). It is interesting that while the *zwitterionic* surfactant, CDMG, appears to be equally efficient with respect to the *cationic* CTAC in affecting DNA-**1** complex stability (Fig. 4H), the *anionic* SDS does not appear to possess any capacity to induce destabilization of the probe-DNA complex (Fig. 4I). Net *anionic* phosphatidate lipids have earlier been shown to be inert toward intercalated acridine-DNA complexes [36]. We believe that although CDMG itself is a zwitterionic surfactant, the betaine type COO^- unit on its head group could get protonated in the close vicinity of the polyanionic phosphodiester moieties of DNA. This would render the head group charge of CDMG net cationic, particularly during its interactions with DNA. This could explain why its interaction pattern simulates that of CTAC.

The hyperchromisms observed at different concentrations of various additives allow us to assess the relative abilities of different additives to induce the 'destabilization' of probe-DNA complex (Table 1). While salts or organic ions such as NaCl or $NMe_4^+Cl^-$ and *cationic* surfactants promote destabilization of the probe-DNA complex, the *anionic* surfactants do not affect the stability of such complex. This could be due to repulsive interactions between anionic surfactant aggregate and DNA-**1** complex polyanion. To bring about the comparable effects on the dissociation of **1** from the complex in which the probe **1** is DNA-bound, salts like NaCl need as high as 100 mM, while NH_4Cl and NMe_4Cl require only 10 mM as much concentration. This could be due to the intrinsic differences in the cation hydration and the affinities toward polyanionic DNA phosphate back-

bone. Replacement of the two methyl groups of NMe_4Cl by the two *n*-octyl group led to greater propensity to destabilization of the probe-DNA complex. Thus, the same extent of destabilization could be achieved at 1 mM of DODAB as against 10 mM NMe_4Cl. Increasing the chain length to *n*-hexadecyl (both in case of CTAC and DHDAB) leads to an increase in approximately another order of magnitude in the efficiency of inducing destabilization of the probe-DNA complex. The enhanced efficiencies of inducing destabilization observed with cationic long hydrophobic chain containing surfactants in contrast to their small organic cation counterparts or salts could be due to cumulative effects of electrostatic binding and strong hydrophobic association between the hydrocarbon chains and hydrophobic interior of DNA polymer.

3.4. Effect of surfactant addition on DNA-ethidium bromide complex using fluorescence spectroscopy

The strong intercalative ability of ethidium bromide is well-known in literature. The effects of addition of surfactants/salts on the ethidium bromide-DNA complex were also examined by adding progressively increasing amounts of surfactants or salts to solutions containing CT DNA-bound ethidium bromide in 5 mM Tris-HCl (pH 7.4) and by following the changes in the fluorescence emission spectra of the resulting mixture after each addition of the additives. To verify our results from the previously described UV-Vis experiments about surfactant DNA interactions, we chose five additives, e.g., NaCl, NMe_4Cl, CTAC, DHDAB and SDS, for fluorescence studies.

Fig. 5A shows the changes in the fluorescence emission spectra upon the addition of increasing amounts of NaCl into the CT DNA-bound ethidium bromide. Trace 1 represents the spectrum due to ethidium bromide alone (8.98×10^{-6} M) in 5 mM Tris-HCl (pH 7.4). Addition of excess DNA (5.3×10^{-5} M) into this solution led to enhancement in the fluorescence emission intensity of the resulting mixture (trace 2). The progressive addition of aqueous NaCl solution into this DNA-ethidium bromide complex led to gradual fluorescence quenching, finally reaching a saturation. Trace 3 in Fig. 1A represents the maximally quenched fluorescence spectrum obtained due to the addition of NaCl into DNA-bound probe solution.

Then we separately examined the effects of the addition of progressive amounts of a solution of a small organic cation TMAC into solutions containing probe-DNA complexes. A similar kind of quenching in the fluorescence spectral features was observed as described in the cases of NaCl or CTAC (not shown).

Fig. 5B shows the changes in the fluorescence emission spectra upon addition of increasing amounts of a solution of a single chain cationic surfactant, CTAC, into the solution of ethidium bromide-DNA complex. Traces 1 and 2 in Fig. 5B represent the spectrum due to the free ethidium bromide (probe) and DNA-bound probe and trace 3 represents the fluorescence spectrum obtained upon addition of maximum amount of CTAC into DNA-bound probe solution to achieve a saturation in the observed fluorescence quenching. Although the fluorescence quenching phenomena were seen in both instances involving the addition of NaCl or CTAC into the solution containing ethidium bromide-DNA complex, the remarkable differences lay in the concentration of NaCl or CTAC required to induce an equal extent of fluorescence quenching (see below).

Fig. 5C shows the changes in the fluorescence emission spectra upon addition of increasing amounts of a vesicular dispersion of DHDAB, into the solution of ethidium bromide-DNA complex. Spectral traces 1 and 2 in Fig. 5C are due to the free probe and DNA-bound probe respectively. Trace 3 represents the fluorescence spectrum obtained upon addition of maximum amount of DHDAB into a solution containing DNA-bound probe to achieve a saturation in the observed fluorescence quenching. The fluorescence quenching phenomena observed with DHDAB are similar to that seen in both instances involving the addition of NaCl or CTAC into the solution containing ethidium bromide-DNA complex. But notably almost one-fifth of the concentration of DHDAB compared to that of CTAC was required to induce an equal extent of fluorescence quenching.

Fig. 5D shows the effects of addition of increasing amounts of an anionic surfactant SDS into the probe-DNA complex solution. Notably, after addition of several aliquots of SDS solution into the probe-DNA complex, the fluorescence spectra of the DNA-bound probe did not alter significantly. This is in marked

contrast to what was observed in the experiments involving addition of either CTAC, NaCl or DHDAB.

The changes in the fluorescence emission intensity (at 590 nm) due to DNA-bound ethidium bromide were plotted against the concentration of each additive (shown as insets in Fig. 5). This plot gave relative estimates about the efficiencies of different additives in inducing destabilization of the ethidium bromide-DNA complex. Amount of additives required to effect the same extent (50%) of fluorescence quenching, varied remarkably as the nature of additives differed in their hydrophobic and electrostatic character. Under comparable conditions, to effect 50% of quenching of the fluorescence due to intercalated ethidium bromide, as high as ~ 0.4 M of NaCl was required. In contrast, to bring about an identical effect in the fluorescence spectra (50% quenching), the corresponding concentrations required for TMAC, CTAC and DHDAB were $\sim 1.7 \times 10^{-1}$ M, 7.5×10^{-5} M and 1.7×10^{-5} M respectively. Thus, the efficiencies of single-chain or double-chain cationic surfactants in bringing about same extent of fluorescence quenching are almost four to five orders of magnitude greater respectively over their small, non-hydrophobic, organic cationic counterpart tetramethylammonium chloride or salt, e.g., NaCl. Note that DHDAB is approximately five-fold more efficient than CTAC in perturbing DNA ethid-

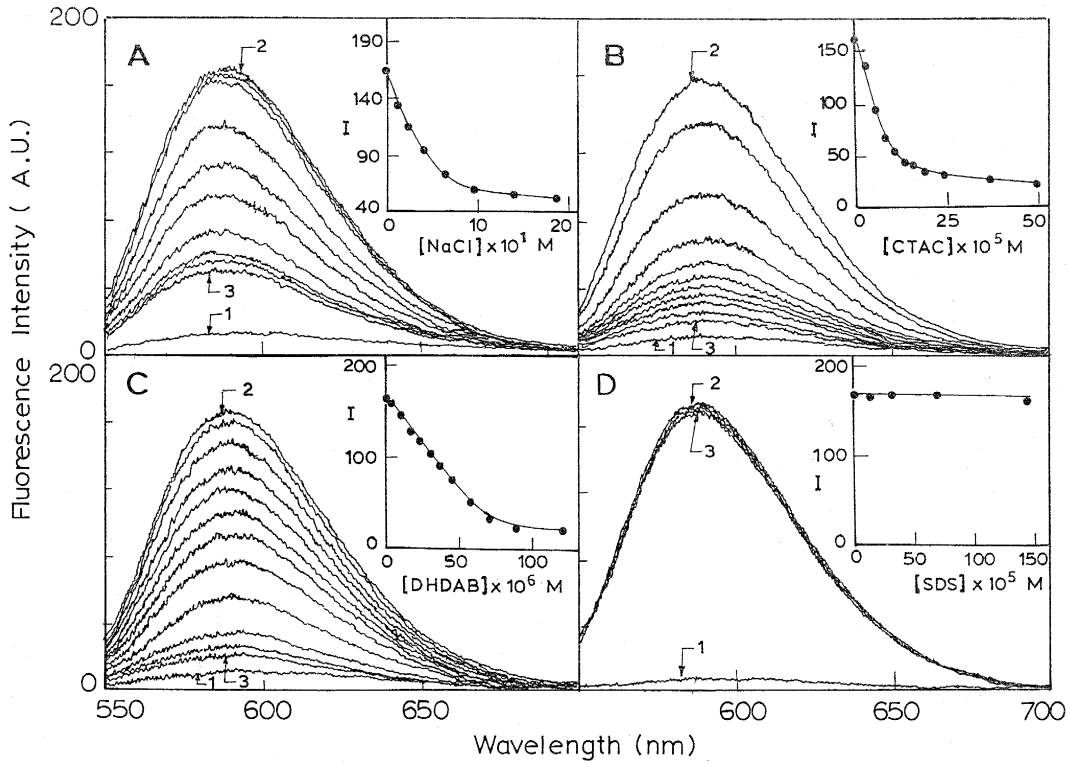

Fig. 5. Effect of addition of different additives on DNA-bound ethidium bromide (probe). This experiment was carried out by adding increasing amounts of additives into the probe-DNA complex (CT DNA, 8.5×10^{-5} M, base molarity), followed by recording the fluorescence emission spectra after each addition (excitation at 266 nm and emission in the range 550–700 nm). Panels A, B, C and D in this figure show the effect of additions of NaCl, CTAC, DHDAB and SDS respectively into the intercalated ethidium bromide-DNA complex. In all the figures (A, B, C and D), trace 1 represents the fluorescence emission spectra due to 8.98×10^{-6} M ethidium bromide in 5 mM Tris-HCl buffer (pH 7.4) at ambient temperature. Trace 2 represents the absorption spectra of fully CT DNA-bound ethidium bromide ([DNA] $= 5.3 \times 10^{-5}$ M) and trace 3 represents the final spectra (after the attainment of saturation in fluorescence quenching) obtained upon addition of a saturating concentration of the additive into the probe-DNA complex. The addition of progressively increasing amount of additives into the probe-DNA complex resulted the spectra from 2 towards 3. The corresponding insets were obtained by plotting the fluorescence emission intensity (I) against additive concentrations.

ium bromide complex. This is a notable difference between the anthryl probe and the ethidium bromide probe.

The apparent quenching in the fluorescence emission intensity upon the addition of increasing amounts of salts or cationic surfactants could be due to a gradual release of the free probe (ethidium bromide) out of the probe-DNA complex. This must be due to salt or cationic surfactant-induced perturbation of DNA organization leading to dissociation the probe from the probe-DNA complex. Although the efficiency in effecting the destabilization of the probe-DNA complex varies widely, all additives, salts, organic cation or cationic surfactant could influence the probe-DNA complex stability. Notably, however, the addition of SDS could not affect the stability of DNA-bound ethidium bromide complex to any significant extent even at a high concentration. This could be due to lack of interaction between anionic surfactant aggregates and the polyanionic DNA.

While the results obtained with absorption titration experiments involving probe **1**-DNA complexes are qualitatively similar to that obtained in the fluorescence titration employing ethidium bromide-DNA complex, a closer look at the data reveals some differences in relative efficiencies in destabilising probe-DNA complexes in two cases. This probably originates from the differences in interaction of two probes with DNA. Note that both the probe **1** and ethidium bromide are cationic. The anthryl probe contains a positive charge away from the polyaromatic skeleton. In contrast ethidium bromide is a heterocyclic fluorophore with a localized positive charge. These features, in addition to their differences in overall structure, bring about considerable differences in their binding constants with a given sample of DNA. Note that under comparable conditions, the probe ethidium bromide has almost one order of magnitude higher binding constant with calf thymus DNA [37] over that of the anthryl probe.

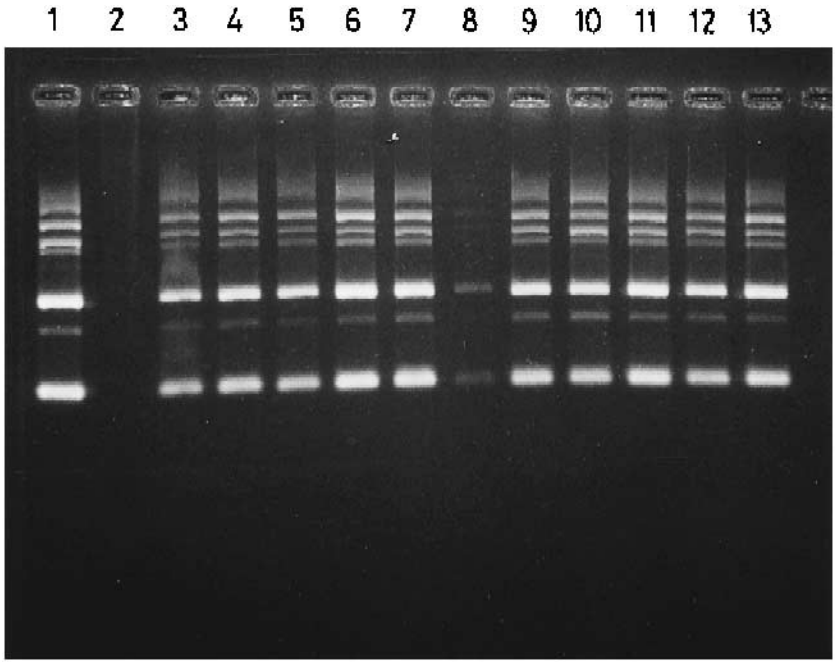

Fig. 6. Effect of cationic surfactants on the electrophoretic mobility of the plasmid DNA pTZ19R in agarose (1%) gel. Lane 1: DNA alone, lanes 2, 3 and 4: plasmid DNA samples were treated with 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M of CTAC respectively. lanes 5, 6 and 7 contain plasmid DNA that was initially treated with 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M of CTAC respectively and then subjected to phenol-chloroform extraction followed by ethanol precipitations prior to loading in agarose gel for electrophoresis. Lanes 8, 9 and 10 contain plasmid DNA that were treated with 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M of DHDAB respectively. Lanes 11, 12 and 13 contained plasmid DNA that were initially treated with 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M of DHDAB respectively and then subjected to phenol-chloroform extraction followed by ethanol precipitations prior to loading in agarose gel.

3.5. Agarose gel assay

Agarose gel electrophoresis allows one to compare the relative location of the DNA fragments under a constant electric field and gel electrophoresis is routinely used to separate linear double-stranded DNA pieces of different sizes. Each band could be detected visually by ethidium bromide staining and under UV exposure. Since ethidium bromide is probably the well-studied intercalator of DNA, [38] we thought that the interaction between plasmid DNA and different additives could additionally be probed by using agarose gel electrophoresis of the complexes formed between different additives and DNA.

Fig. 6 summarizes the salient features of the interactions between the DNA and different surfactant additives. Lane 1 shows plasmid DNA pTZ19R alone, while lanes 2, 3 and 4 show DNA in the presence of 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M CTAC respectively. Lanes 5, 6 and 7 contain DNA samples which were prepared in the following manner. DNA was first treated with 1×10^{-3} M, 1×10^{-4} M, and 1×10^{-5} M CTAC respectively and then the different CTAC-DNA mixtures were subjected to phenol-chloroform extraction followed by ethanol precipitation prior to loading into agarose gel. This afforded an opportunity to examine whether the surfactant from the surfactant-DNA complexes could be removed by this organic solvent extraction treatment. Lanes 8, 9 and 10 show DNA complexed in the presence of 1×10^{-3} M, 1×10^{-4} M and 1×10^{-5} M DHDAB. Lanes 11, 12 and 13 contained DNA samples that were first complexed with 1×10^{-3} M, 1×10^{-4} M, and 1×10^{-5} M DHDAB respectively and then each of the mixtures was subjected to phenol-chloroform extraction and ethanol precipitation prior to loading into agarose gel.

The above experiments reveal that at high concentration (1×10^{-3} M or more) of the cationic surfactant (CTAC or DHDAB), the DNA-surfactant com-

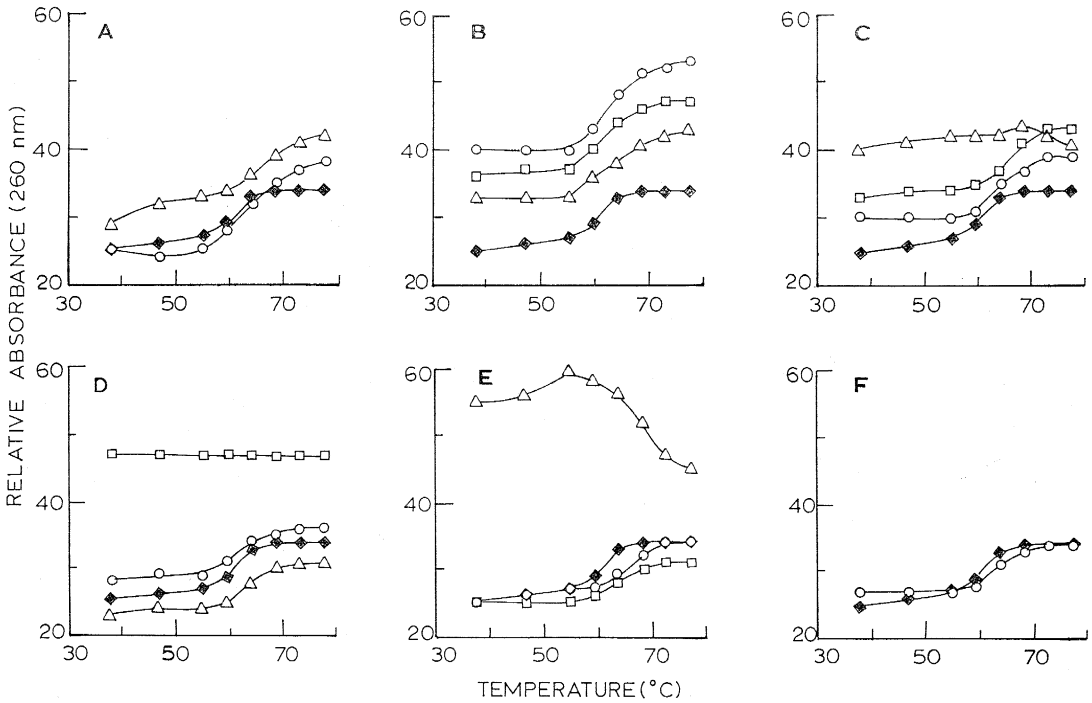

Fig. 7. Melting profiles of CT DNA in the presence and absence of different additives at varying concentrations. In all the figures (A–F) the curve with (♦) represents the melting profile of free CT DNA (3.8×10^{-5} M) in the absence of any additive. (A) Melting profiles of CT DNA in the presence of 1×10^{-3} M (△) and 1×10^{-4} M (○) of NMe_4Cl. (B) Effect of DODAB: 1×10^{-4} M (○), 1×10^{-5} M (□) and 1×10^{-6} M (△). (C) Effect of CTAC: 1×10^{-4} M (△), 1×10^{-5} M (□) and 1×10^{-6} M (○). (D) Effect of DHDAB: 1×10^{-4} M (□), 1×10^{-5} M (△) and 1×10^{-6} M (○). (E) Effect of CPC: 1×10^{-4} M (△), 1×10^{-5} M (□) and 1×10^{-6} M (○). (F) Effect of SDS: 1×10^{-4} M (○).

plexed bands in agarose gel could not be visualized under UV exposure even after 2 h staining with ethidium bromide (lanes 2 and 8 respectively for CTAC and DHDAB). At a lower concentration (1×10^{-4} M) of either CTAC or DHDAB, faint staining of the DNA bands could be observed. However, at a still lower concentration (1×10^{-5} M) of the surfactants, ethidium bromide staining after the gel-electrophoresis is considerably improved. The invisibility or faint visibility of the DNA bands in the presence of a larger concentration of cationic surfactants in the agarose gel even after long ethidium bromide staining suggest that plasmid DNA-surfactant complexes lost the ability of intercalation towards the intercalator ethidium bromide. This could be due to several reasons. One possibility is the formation of compaction between DNA and cationic surfactant, presumably through the charge neutralization and concomitant alteration in the native DNA structure in water. Although the DNA conformation under this situation is changed to a compact structure, this form of DNA can still retain the double-stranded organization [13]. In fact this was also found to be the case in organic solvents as shown by Okahata et al. [14]. It has been also shown that the addition of cationic liposomes to DNA lead to condensation of DNA structures [39]. This leaves insufficient space available for ethidium bromide to intercalate and stain. Such condensed DNA makes itself inaccessible to small intercalators such as ethidium bromide. Furthermore, in the condensed state of DNA-liposome complex, *DNAase I* cannot degrade DNA. Condensation is a phenomena that has been thoroughly examined by Bloomfield [40].

Notably, no significant difference in the mobilities of different forms of plasmid DNA was observed in the presence of varying concentrations of cationic surfactants under the conditions of gel electrophoresis. After mixing the DNA with cationic surfactant aggregates in water, we attempted to recover the plasmid DNA by using a phenol-chloroform extraction procedure [41]. Although, at high concentrations of the cationic surfactant, 'tight' DNA-surfactant complexes are formed, as evidenced by the lack of ethidium bromide staining on agarose gel, and also from absorption titration results (discussed above), the DNA from these complexes could be recovered when subjected to the phenol-chloroform extraction followed by ethanol precipitation of the aqueous layer, as evidenced by agarose gel (lane 5 and 11 for CTAC and DHDAB respectively). This suggests that after the removal of the cationic lipid or surfactants from the surfactant-DNA complex by organic solvent treatment, the plasmid DNA can regain its original conformation so that ethidium bromide intercalating abilities of the *recovered* DNA could be retained.

The effects on electrophoretic mobilities or stainabilities of DNA in the presence of other surfactants with anionic (SDS) and zwitterionic (CDMG) headgroups were also examined (not shown). Under comparable conditions as used with cationic lipids, these surfactants affected neither the DNA staining capacities with ethidium bromide nor the band mobilities of the resulting surfactant-DNA mixtures.

3.6. Effects on DNA melting

We also examined the effects of inclusion of different surfactants, organic ion and salts on the melt-

Table 2
Thermal denaturation of calf thymus DNA in the presence of added surfactants

Additive [a]	10^6[additive],M [b]	T_m [c]	dT [d]
None	-	60	13
NMe_4Cl	1000	68	17
	100	64	18
DODAB	100	64	22
	10	63	20
	1	62.5	15
CTAC	100	[e]	[f]
	10	63	18
	1	64	18
DHDAB	100	[e]	[f]
	10	64	18
	1	64	18
CPC	100	[e]	[f]
	10	64	18
	1	64	18
SDS	100	62	13

[a] See Fig. 1 for structures and notations of the additives.
[b] The concentration of DNA in base molarities (3.8×10^{-5} M) was kept constant for all the experiments.
[c] See text for conditions. The T_m values are accurate within ± 1°C.
[d] Widths of thermal denaturation; these values are accurate within ± 1°C.
[e] Normal DNA melting transition profile is either abolished or distorted.
[f] Not determined.

ing properties of CT DNA. The thermal denaturation studies on CT DNA were conducted at varying [additive]/[DNA] ratios in the presence of 5 mM Tris-HCl (pH 7.4) buffer (Fig. 7). The relevant data are summarized in Table 2.

As is evident from Table 2, the DNA melting behavior in the presence of cationic compounds are roughly the same. Modest rises in T_m values with concomitant broadening of the melting profile as a function of surfactant concentration were seen. In contrast to the results observed with cationic surfactants, the addition of *anionic*, single-chain surfactant, SDS (1×10^{-4} M), did not significantly influence the melting behavior of the native DNA.

4. Conclusions

Metal ions in the salts have been shown to influence DNA organization in several possible ways. These include salt induced B to A transition [42], B to C to Z conformational alteration [43], helix to coil transition [44], helical destabilization, melting [45], and DNA aggregation and condensation into compact structures [46]. Such structural alterations also occur as a function of salt concentration [47]. However, little is known as to how inclusion of surfactants or organic cations of different charges and hydrophobicities affect the properties of DNA. Particularly, how the intercalative capacities and mobilities under the conditions of gel electrophoresis are affected upon complexation with aggregates of detergents or lipids remain unclear.

The present investigation clearly brings out several points of similarities and differences between salt and surfactant effects on DNA and revealed how the hydrophobic effects and the specific surface charges play an important role in bringing about profound changes in DNA structures. We have introduced a new chromogenic probe that interacts strongly with DNA to form an intercalated complex. The stability of the probe-DNA complex is influenced by the addition of surfactants or salts leading to the dissociation of the probe from the complex. The effect is most pronounced when the surfactant is *cationic*. The efficiency of the cationic surfactants in destabilizing the probe-DNA complex is nearly three to four orders of magnitude greater over the corresponding capacity of NaCl. Importantly the surfactants CTAC or DHDAB were at least two orders of magnitude more effective in dissociating the probe from this complex relative to that of the related organic cation $NMe_4^+Cl^-$. In a parallel experiment we also used a well-known fluorogenic probe to demonstrate that cationic surfactant could indeed perturb probe-DNA complexes at a concentration orders of magnitude lower compared with their non-aggregating counterparts or simple salts.

What could be the plausible reason for such observations with cationic surfactants? Because of the presence of positive charges on different lipids and surfactants, electrostatic binding of these self-organizing molecules to the anionic DNA phosphates are facilitated. This results in the charge neutralization at the DNA backbone. This in turn reduces the inter-(that prevail between two different strands) and intra-strand (that occur within two regions of the same strand) electrostatic repulsions present in the native DNA phosphate backbone. As a result, under these circumstances, the DNA duplexes pack in more compact fashion, leaving insufficient space available for the accommodation of the incoming guest molecule (intercalator) or of the ones pre-existing within the double strands. Thus this results in the destabilization of probe-DNA complex. This conclusion is further supported by the lack of ethidium bromide staining of DNA bands in the presence of a high concentration of cationic surfactants in agarose gel electrophoresis experiments. The cationic surfactants also affect the DNA melting behavior as evidenced by modest increases in T_m for double to single strand transition.

If we compare the structures of the cationic surfactants with that of salt or small organic cations used herein, it becomes clear that, in addition to the common positive charge, surfactants or lipids contain one or more long hydrophobic chains. The presence of these hydrophobic chains gives them the additional properties of various modes of aggregation upon dispersal in water. These special abilities of the cationic surfactants over salts must be responsible for their abilities to achieve greater structural distortion of DNA at a concentration orders of magnitude lower. Although, at this level of knowledge, it is difficult to assign the exact mode of interaction, several possibilities exist. The lipophilic alkyl chains can interact

with the hydrophobic interior of the DNA. The propensity of the hydrophobic chains of the surfactants to interact with the hydrophobic interior of DNA is further necessitated from the spontaneous tendency of surfactant hydrocarbon chains to minimize water contacts. The complexation of surfactant with DNA might also lead to important changes in the 'structure' of water molecules around the DNA backbone. As a matter of fact, a similar concept was initially proposed by Herskovits and co-workers, and was expanded upon by Falk, Hartman and Lord [48,49]. The elucidation of all the factors that are responsible for such behavior may require additional studies. Nevertheless the findings described herein clearly show the differences between salt and surfactants on their modes of interactions with DNA and are important for understanding the nature of DNA-cationic lipid/surfactant complexes.

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