Biochemical and Biophysical Research Communications **263,** 41–46 (1999)
Article ID bbrc.1999.1266, available online at http://www.idealibrary.com on IDEAL®

DNA–Surfactant Interactions: Coupled Cooperativity in Ligand Binding Leads to Duplex Stabilization

Mrunalini V. Pattarkine and Krishna N. Ganesh[1]
Organic Chemistry Synthesis Division, National Chemical Laboratory, Pune 411008, India

Received July 3, 1999

The cooperative nature of interaction of cationic surfactants with short oligonucleotides leading to eventual stabilization of DNA duplexes is demonstrated. At submicellar concentrations and DNA:surfactant charge ratios of 0.2 to 0.8, the association of single chain (CTAB) and double chain (DOTAP) surfactants to oligonucleotides is initiated by electrostatic interaction of cationic ligands with polyanionic DNA that aligns the surfactant molecules on the DNA template. This is followed by binding of new surfactant ligands to the initial complex, driven cooperatively by the hydrophobic forces, leading to *in situ* formation of surfactant-bound and bare duplexes as separate species. These exhibit independent melting behaviour characterised by double transition in thermal UV profiles, with a higher T_m for surfactant–DNA complexes. Understanding the cooperative binding of the cationic surfactants to the DNA described here may have implications for rational design of DNA binding drugs and DNA delivery systems.

The understanding of the physical nature of DNA-cationic surfactant interaction has primary importance due to its significance in biomedical applications, particularly as efficient gene delivery vectors (1). DNA-surfactant complexes are generally insoluble in water and precipitation of DNAs by cationic surfactants is useful in DNA extraction and isolation protocols (2). Cationic polymers (3) and cationic lipids (4) condense DNA, leading to large multimolecular and polydisperse aggregates that are efficient in transfecting cells in culture but do not diffuse within a tissue. In comparison, cationic detergents also condense DNA into discrete particles (5), each consisting of a single nucleic acid molecule (6), which is released fast into cells with accompanying decondensation. This interesting feature of DNA-surfactant interaction has prompted biophysical characterization of complexes of large DNA molecules with various surfactants (6–9). However, parallel studies on interaction of short oligonucleotides either with lipids or detergents are lacking. The recent applications of short oligonucleotides and their analogues as antisense/antigene therapeutic agents (10) also need efficient delivery vectors necessitating a study of behaviour of oligonucleotide-surfactant complexes. In this connection, this paper reports on identification of discrete duplex–surfactant complexes that coexist with relatively free duplexes when DNA is treated with *submicellar* concentrations of detergents. Under these conditions, the co-operativity in surfactant binding to DNA is coupled to melting transition of DNA, leading to significant stabilization of DNA duplexes by cationic surfactants.

MATERIALS AND METHODS

All the chemicals used were of the highest purity available. Cetyltrimethyl ammonium bromide (CATB), tetramethyl ammonium bromide (TMAB) and cetyl alcohol was from Sigma and used as received. 1,2-Dioleoyloxytrimethyl ammonium propane (DOTAP) was sourced from Avanti Polar Lipids, Canada. The complementary oligonucleotides (ODN) GGAAAAAACTTCGTGC, 1 and GCACGAAGTTTTTTCC, 2, were synthesized by β-cyanoethyl phosphoramidite chemistry on a Pharmacia GA plus DNA synthesizer and purified by FPLC and rechecked by RP HPLC.

UV melting experiments on ODN duplex 1:2 (1 μM each strand) were performed in 10 mM NaCl using Perkin Elmer Lambda 15 UV/VIS Spectrophotometer fitted with a Julabo water circulator with programmed heating accessory. Appropriate complementary ODN pairs were taken together in 10 mM NaCl at a strand concentration of 1 μM each, heated in a water bath to 80°C for 3 minutes, slowly cooled to room temperature and then stored overnight at 4°C. The surfactants (CTAB or DOTAP) at desired concentrations were then added to the annealed duplexes and the samples were allowed to equilibrate for 30 min before starting the melting. The samples were heated at a rate of 0.5°C per minute and the thermal denaturation of the duplex was followed by monitoring changes in absorbance at 260 nm as a function of temperature.

All fluorescence measurements were done on a Perkin Elmer model LS 50-B spectrofluorimeter at 25°C, with slit widths of 3 nm for excitation and 15 nm for the emission monochromators. Light scattering measurements were done by setting the excitation and emission monochromators of the fluorimeter at 550 nm. The surfactants (CTAB and DOTAP) were titrated into a solution of the duplex 1:2 (1 μM) and the measured intensity at 550 nm was plotted as a

[1] To whom correspondence should be addressed. Fax: 91 20 589 3153. E-mail: kng@ems.ncl.res.in.

0006-291X/99 $30.00

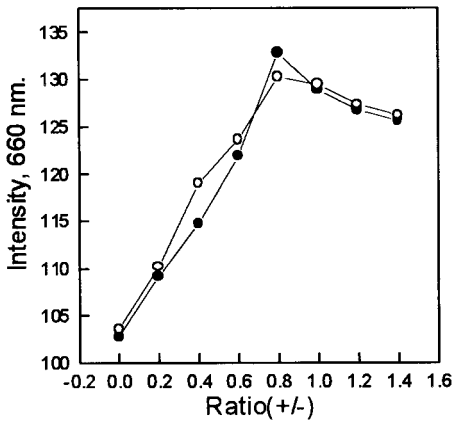

FIG. 1. Light scattering intensity vs mole ratio of CTAB (○) and DOTAP (●) to ODN-phosphate (30 μM).

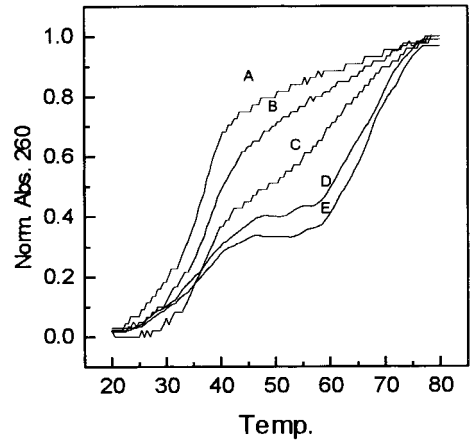

FIG. 2. UV–Temperature plot for ODN duplex 1:2 in presence of increasing concentrations of CTAB (A) 0 μM, (B) 6 μM, (C) 12 μM, (D) 18 μM and (E) 22 μM.

function of the mole ratio of cationic ligand to the ODN phosphate for CTAB and DOTAP (Fig. 1). The ODN samples were prepared in 10 mM NaCl unless stated otherwise. Ethidium bromide (excitation 480 nm, emission 595 nm) displacement assays were carried out by using duplex 1:2 (1 μM) and ethidium bromide (1.26 μM) in 10 mM NaCl, into which was added CTAB solution (1 μM/1 μL). Experiments using the dye Hoechst 33258 (Sigma), were done with 0.03 μM solution of the dye in 10 mM NaCl and recording the excitation (360 nm) and emission spectra (510 nm) for the dye in the absence and presence of CTAB (22.5 μM), in presence of the duplex 1:2 1 μM alone (ODN: dye, 1:100) and with both, duplex and 22.5 μM CTAB.

All CD studies were done on Jasco J715 Spectropolarimeter. The CD spectra of the free duplex 1:2 (1 μM) in 10 mM NaCl and with 22 μM CTAB were recorded at different temperatures (20°C–80°C).

RESULTS AND DISCUSSION

The interaction of cationic lipids with DNA is known to induce condensation and subsequent precipitation of the condensate (11–13). In order to optimise the surfactant concentration at which its DNA complex can still be kept soluble in aqueous solutions, light scattering measurements were made on ODN solutions at different mole ratios of CTAB to oligonucleotide. Figure 1 shows the light scattering data and as seen, an increase in CTAB/ODN ratio gradually leads to an enhanced scattering due to the increased size of the resulting complex. At a ratio of CTAB to phosphate of about 0.75–0.8, the solution turned cloudy with a drop in scattering due to the on-set of precipitation of complex. This is consistent with earlier data seen in case of *E. coli* DNA, indicating that the precipitation behaviour is dependent on charge ratio rather than the length/size of DNA (8). For the ODN duplex used in the present study, the CTAB concentration at a charge ratio of 0.8 corresponds to 22 μM, which is much below the critical micelle concentration of CTAB (92 μM). Hence the binding of CTAB to ODN under these conditions occurs predominantly in monomeric form and not in micellar form. All further studies were therefore done at CTAB concentrations below 22 μM to avoid both precipitation of the nucleic acid and complexities in interpretation of observed data.

To study the effect of the cationic ligands on the stability of the ODN duplex in the ODN-surfactant complex, the thermal melting of the duplex 1:2 was studied in presence of varying concentrations of different cationic ligands. In the presence of increasing concentrations of CTAB (0–22 μM), the duplex 1:2 showed a melting profile as in Fig. 2. It is seen that the T_m gradually enhanced with increase in CTAB concentration. A sigmoidal curve characteristic of the duplex melting with the transition around 40°C were seen upto a CTAB concentration of 12 μM. Beyond this, the sigmoidal profile slightly broadened out and at 18 μM CTAB, it decomposed into two transitions (Fig. 2) suggesting the formation of a second species that is thermally more stable than the initially observed one. The higher melting transition moved further up when CTAB concentration was increased to 22 μM, while no significant changes were seen with the T_m of lower transition (Table 1).

The interaction of CTAB with ODN can either be electrostatic one occurring between the cationic headgroup of the detergent and the anionic backbone phos-

TABLE 1

UV-T_m of Duplex in the Presence of Various Amounts of CTAB[a]

[CTAB]	[DNA]/[CTAB]	T_{m1}°C	T_{m2}°C
0	0	41.0	—
6	0.2	43.0	—
12	0.4	42.8	54
22.5	0.6	42.0	65

[a] All experiments done in 10 mM NaCl. Concentrations of DNA and CTAB are expressed in μM. T_m's are accurate to ±0.5°C.

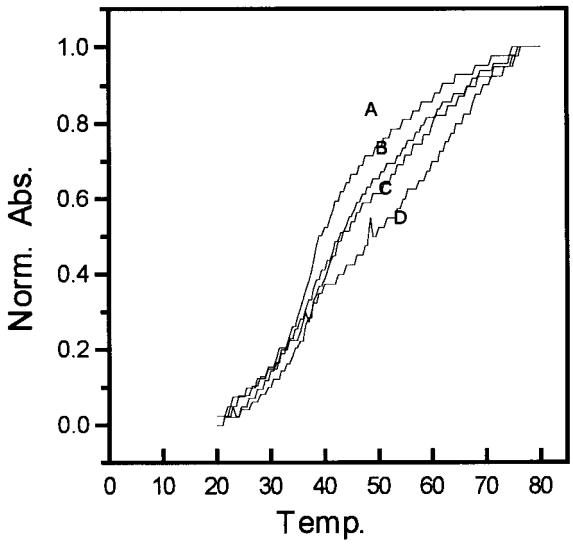

FIG. 3. UV–temperature plot for the duplex 1:2 in presence of increasing concentrations of DOTAP, (A) 0 μM (B) 6 μM, (C) 12 μM, (D) 18 μM.

phates or hydrophobic one where the chains of the detergent interact with the bases of oligonucleotide to result in the observed splitting in melting profile. To find out if this pattern resulted from such charge-charge interactions or hydrophobic effect, the melting studies were carried out using tetramethylammonium bromide [$(CH_3)_4N^+Br^-$] which lacks the hydrocarbon chain, but has a similar cationic headgroup. In the same concentration range as that of CTAB, this did not have any effect on the T_m of the duplex 1:2. To delineate the contribution of the alkyl chain, the melting of duplex 1:2 was done using cetyl alcohol as a ligand. Even in this case, no splitting of the melting transition was seen, unlike the case with CTAB. Thus, either the cationic headgroups or the hydrophobic tails, on their own, are unable to induce splitting in the melting profile. Only when the ODNs interact with the molecules that have the charged headgroup and the hydrophobic tail covalently linked amphiphiles, double transitions were observed in the melting profile. To further examine the possible role of the hydrophobic chain in complexation with ODN, the melting was carried out using DOTAP, a double chain cationic detergent. This experiment qualitatively showed a stabilization of the duplex with increasing concentrations (0–22 μM) and appearance of a second melting around 60°C, similar to CTAB (Fig. 3). However, in this case the resolution of the transitions was not as clear as that seen in the presence of CTAB. It is possible that the detergent molecules might bind to single stranded DNA resulting from the duplex melting and the observed high temperature transition corresponds to such a melting of CTAB-single strand ODN complex. To test such an event, the UV-temperature plot of the ss ODN 1-CTAB (22 μM) complex was examined and this showed a lack of a sigmoidal behavior.

Circular dichroism studies. In order to find out whether surfactant binding induces any structural/conformational changes in the ODN, temperature-dependent circular dichroism (CD) spectra of the duplex in the absence and presence of CTAB were studied. In the absence of CTAB, the CD profile of duplex is typical of the B-form, with a positive peak at 273 nm, a negative peak at 245 nm and a crossover near the UV absorption maximum (14). The addition of CTAB led to a slight decrease of the ellipticity in the 273-nm peak accompanied by a red shift to 278 nm and an increase in the intensity of negative peak at 250 nm (Fig. 4). However, significant differences were seen in their CD patterns upon increasing temperature. The free duplex showed a gradual decrease in positive intensity band at 273 nm and an abrupt decrease in the

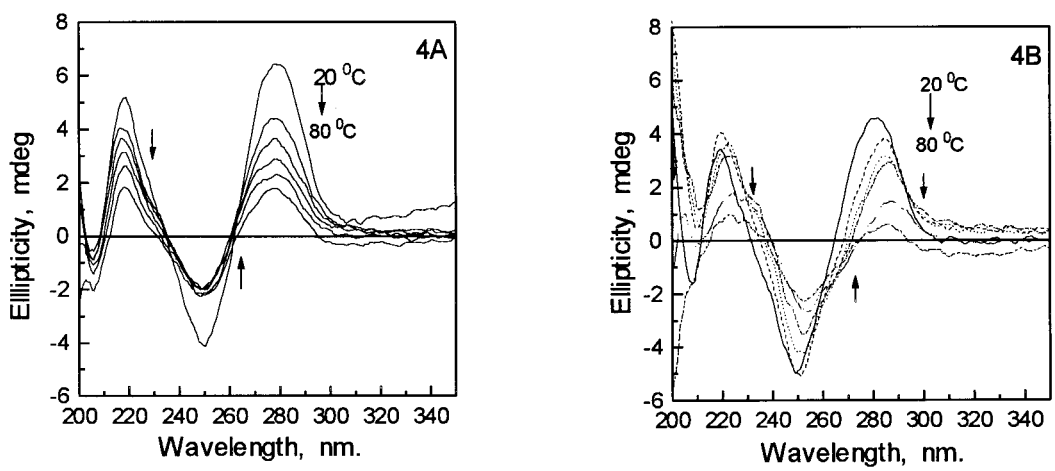

FIG. 4. Temperature-dependent CD spectra of (A) free duplex 1:2 and (B) duplex-surfactant complex at temperatures 20, 30, 40, 50, 60, 70 and 80°C. The CD spectral changes under the experimental conditions are indicated by arrows.

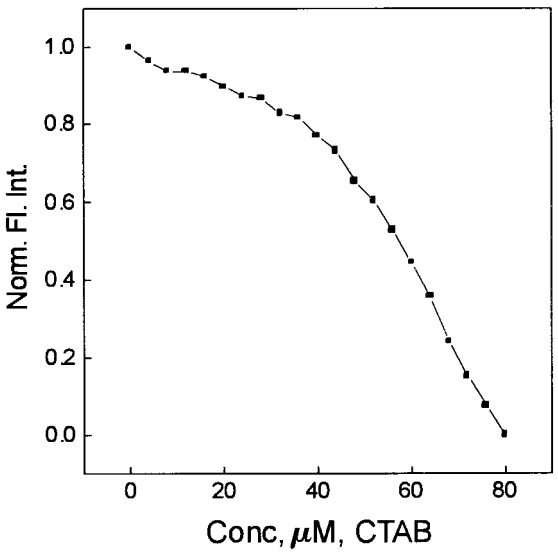

FIG. 5. Fluorescence intensity changes upon ethidium bromide displacement from duplex 1:2 by incremental addition of CTAB.

negative band without any shifts in their peak positions (Fig. 4A). In contrast to this, the CD spectra of duplex-surfactant complex (Fig. 4B), exhibited a gradual decrease in intensity accompanied by shift in peak positions of both positive (220 and 278 nm) and negative (250 nm) bands and 5–7 nm shift in cross over point.

In order to locate the possible binding site of CTAB on the duplex, fluorescence experiments were done, using an intercalator ethidium bromide (15) and a minor groove binding agent HOECHST (16) as probes. The titration of CTAB into duplex ethidium bromide complex led to a gradual decrease in ethidium bromide fluorescence intensity at 510 nm upto a CTAB concentration of 40 μM, beyond which a rapid fall resulted due to disassociation of the intercalated probe (Fig. 5). This behaviour is characteristically elicited by minor groove binding agents and hence competition experiments were done with HOECHST. The addition of CTAB to duplex-HOECHST complex did not result in displacement of the probe, but the fluorescence observables (excitation and emission maxima) of HOECHST changed significantly, reflecting an alteration in HOECHST environment.

Nature of surfactant-DNA interaction: phase separation of free and surfactant-bound DNA. UV melting studies of the duplex 1:2 indicated that the mode in which CTAB and DOTAP bind to the ODN duplex leads to at least two species with distinct melting transitions. The first melting is almost identical to the parent duplex, whereas the second melting is dependent on detergent concentration. The lack of sigmoidal behavior in single strand ODN-surfactant melting indicates that the high temperature transition in the melting of ODN duplex in presence of CTAB/DOTAP does not arise from a simple complexation of detergent to ss ODN liberated during duplex melting. The initial interaction is electrostatic stabilization of cationic surfactant-ODN complexes. The increase in T_m that can be attributed solely to the replacement of the Na^+ counterions by tetralkylammonium counterion is not likely to be more than 5°C and the observed ΔT_m of 20–25°C can be accounted for only by considering the involvement of additional stabilizing interactions. When surfactant initially binds to ODN, the non-polar hydrocarbon chains protrude out of ODN duplex into aqueous environment (Fig. 6B). As the surfactant concentration is increased, new surfactant molecules prefer to approach a duplex that already carries bound surfactants rather than a free duplex (Fig. 6C). This type of binding excludes unfavorable interaction of non-polar surfactant chains with water molecules in vicinity and surfactant interaction with the duplex is thus dictated by the lateral association of hydrophobic chains leading to a cooperative binding. Self-association of surfactants to form micelles is unlikely at concentrations used here that are much below the critical micellar concentrations, and hence the surfactant binding to ODN is in monomolecular form rather than as micelles. The cooperativity in surfactant-ODN binding is thus driven by hydrophobic association of non-polar chains initially templated on DNA by cation-anion electrostatic interaction. This process leads to a phase separation of surfactant-saturated-duplex from sparsely bound duplex in solution (Fig. 6D). Upon heating, the unbound duplex melts first followed by melting of the duplex–surfactant complex. The latter transition occurs at a higher temperature since ODN melting in the complexes requires disaggregation of non-polar chains templated on duplex. The observed broad, ill-defined transition in case of DOTAP complex as compared to well resolved transitions with CTAB is perhaps a consequence of the different nature of the aggregates from single chain (CTAB) and double chain (DOTAP) surfactants on duplex template. Similar biphasic transitions have been noticed in the binding of spermine-cholic acid conjugates as a result of DNA based nucleation of amphiphiles (17).

The CD studies indicated no major changes in conformation of DNA structure as CTAB binds to duplex. The slight decrease in 275-nm band accounts for hydration changes accompanying exchange of Na^+ counterion by the hydrophobic tetraalkylammonium counterion (18). The observations suggest a minor conformational adjustment in duplex as a consequence of CTAB binding, but within the overall B-geometry. It has been reported earlier that only bilayer forming double chain lipids induce larger conformational changes in DNA (19). The results of fluorescence experiments strongly suggest that the surfactant molecules bind ODN duplex from the minor groove side,

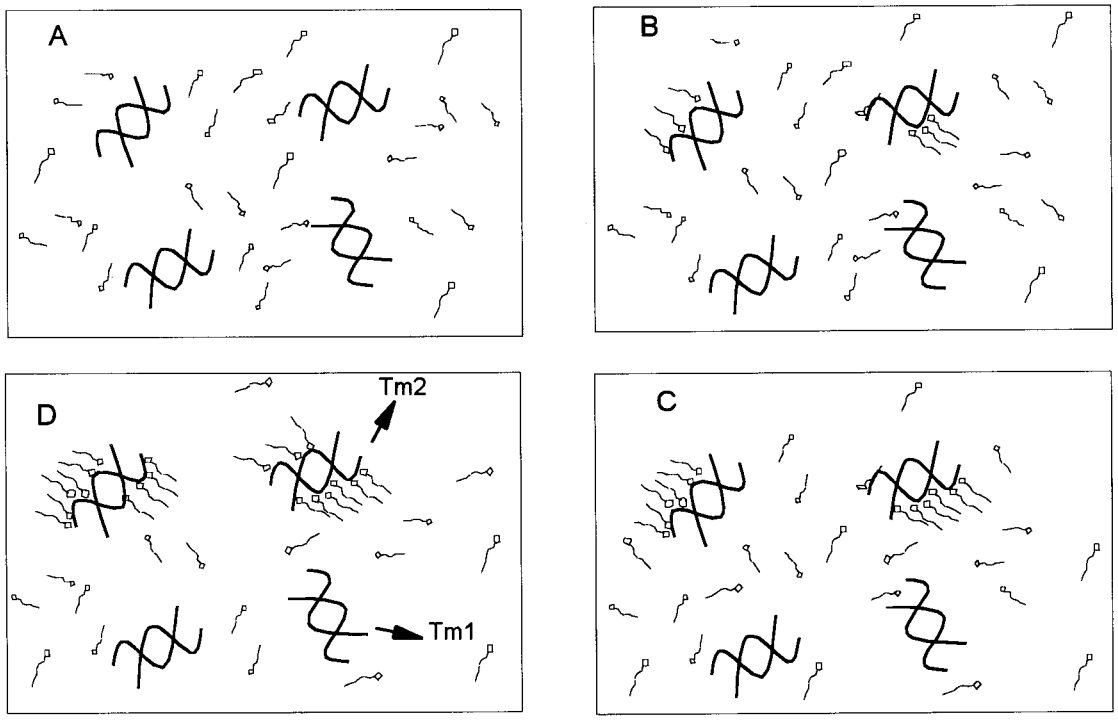

FIG. 6. Schematic representation of model for DNA–surfactant interactions. (A) DNA and surfactant at sub-micellar concentrations. (B) Initial binding of surfactant to some DNA molecules by electrostatic interactions. (C) Hydrophobic interactions among surfactant chains, co–operatively drive new surfactant molecules to bind DNA molecules carrying pre-bound surfactants. (D) Phase separation 'in solution' of surfactant-bound and free duplexes, leading to two melting transitions T_{m2} and T_{m1}.

since CTAB disintercalates ethidium bromide and change the environment around HOECHST bound in the minor groove. The lesser interstrand phosphate distance in the minor groove perhaps allows a better chain aggregation for co-operative binding of surfactant to duplex.

It was previously observed that the rate of renaturation for complementary DNA strands can be enhanced 10^4 fold by the addition of simple cationic detergents, which is certainly mediated by a combination of electrostatic and hydrophobic association (2, 20). The study of interaction between CTAB and large T4DNA by potentiometric titration suggested a bimodal distribution between the elongated coil and compact globule states (6). The transition between the two states is cooperative but not continuous and occurs in discrete steps for each DNA chain. The thermodynamic results on CTAB-T4 phage DNA complexes resolved cooperative binding from isolated site binding in terms of enthalpic and entropic contributions, strongly invoking hydrophobic interactions (8). These studies involved use of large DNA molecules, leading to conclusion that cationic surfactants bind to both helical and single strand forms and the observed biphasic melting is due to successive melting of helical and single strand complexes. Our results suggest that binding of the surfactant with single strand short oligonucleotides is not cooperative probably due to the random structure of oligonucleotides, but however in duplexes, the structure is highly ordered due to the base pairing. Thus coexistence of surfactant-bound and bare duplexes as separate species within solution and their sequential melting leads to a biphasic melting profile. This model simultaneously incorporates the bimodal distribution as well as cooperative ligand binding assisted-enhancement in duplex stability (enhanced renaturation rate) seen with large DNA molecules. The surfactants prefer to bind to ODN duplex from the minor groove side.

CONCLUSIONS

Cationic surfactants interact with anionic ODN by a combination of initial electrostatic interaction followed by a cooperative binding of surfactant ligands to the same duplex, driven by hydrophobic forces. The formation of a bound layer of CTAB along the ODN helix provides an efficient mechanism to minimise contact of hydrocarbon chain with water. At submicellar concentrations of surfactants, free/unbound duplex coexists with surfactant-saturated duplex and sequential melting leads to biphasic behavior in thermal transition. Understanding of the cooperative surfactant binding to ODN and stabilization of the ODN duplex by amphiphiles as described here may have implications for rational design of drugs, DNA delivery systems and new performance materials. Template oligomerization of DNA bound cations have recently been used to pro-

duce nanometric particles (21, 22) and DNA-lipid complex in organic media is shown to form an aligned cast film (23). The study of large DNA-cationic surfactant complex in presence of alcohols has enabled observation of isolated giant DNAs complexed with cationic surfactant by fluorescence microscopy (24). Since by choice of the right sequences, DNA can be induced to form a variety of structures such as hairpins, cruciforms, triplexes and tetraplexes, template can be tailored to align functionalised surfactant discretely to form novel complexes and further process the alignments to generate new materials.

ACKNOWLEDGMENTS

M.V.P. thanks the CSIR, New Delhi, for providing the research associateship. K.N.G. is an Honorary Senior Faculty of Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore.

REFERENCES

1. Miller, A. D. (1998) *Angew. Chem. Int. Ed. Eng.* **37,** 1768–1785.
2. Pontius, B. W., and Berg, P. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8237–8241.
3. Tang, M., and Szoka, F. C. (1997) *Gene Ther.* **4,** 823–832.
4. Labatmoleur, F., Steffan, A. M., Brisson, C., Perron, H., Feugeas, E., Furstenberger, P., Oberling, F., Brambilla, E., and Behr, J. P. (1996) *Gene Ther.* **3,** 1010–1017.
5. Behr, J. P. (1986) *Tetrahedron Lett.* **27,** 5861–5864.
6. Melnikov, S. M., and Sergeyev, V. G. (1995) *J. Am. Chem. Soc.* **117,** 9951–9956.
7. Sergeyev, V. G., Mikhailenko, S. V., Pyshkina, Yaminsky, I. V., and Yoshikawa, K. (1999) *J. Am. Chem. Soc.* **121,** 1780–1785.
8. Spink, C. H., and Chaires, J. B. (1997) *J. Am. Chem. Soc.* **119,** 10920–10928.
9. Melnikov, S. M., Sergeyev, V. G., and Yoshizava, K. (1995) *J. Am. Chem. Soc.* **117,** 2401–2408.
10. Bennett, C. F. (1998) *in* Applied Antisense Oligonucleotide Technology (Stein, C. A., and Craig, A. M., Eds.), pp. 129–146, Wiley–Liss, NY.
11. Bloomfield, V. A. (1996) *Curr. Opin. Struct. Biol.* **6,** 334–341.
12. Gershon, H., Ghirlando, R., Guttman, S. B., and Minsky, A. (1993) *Biochemistry* **32,** 7143–7151.
13. Wong, F. M. P., Reimer, D. L., and Bally, M. B. (1996) *Biochemistry* **35,** 5756–5763.
14. Roger, A., and Norden, B. (1997) *Circular Dichroism and Linear Dichroism*, Oxford Univ. Press, NY.
15. Cain, B. F., Baguley, B. C., and Denny, W. A. (1978) *J. Med. Chem.* **21,** 658–668.
16. Breslauer, K. J., and Jin, R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8939–8942.
17. Janout, V., Lainer, M., Deng, G., and Regen, S. L. (1997) *Bioconjugate Chem.* **8,** 891–895.
18. Hanlon, S., Brudno, S., Wu, T. T., and Wolf, B. (1975) *Biochemistry* **14,** 1648–1660.
19. Akao, T., Fukumoto, T., Ihara, H., and Ito, A. (1996) *FEBS Lett.* **391,** 213–216.
20. Sikorov, J.-L., and Church, G. M. (1991) *J. Mol. Biol.* **222,** 1085–1108.
21. Blessing, T., Remy, J.-S., and Behr, J.-P. (1998) *J. Am. Chem. Soc.* **120,** 8519–8520.
22. Blessing, T., Remy, J.-S., and Behr, J.-P. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 1427–1431.
23. Tanaka, K., and Okahata, Y. (1996) *J. Am. Chem. Soc.* **118,** 10697–10683.
24. Yoshikawa, K., Yoshikawa, Y., Koyama, Y., and Kanbe, T. (1997) *J. Am. Chem. Soc.* **119,** 6473–6477.