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DNA STRONGLY BINDS TO MICELLES AND VESICLES CONTAINING LIPOPOLYAMINES OR LIPOINTERCALANTS.

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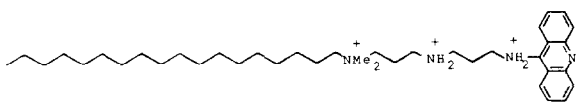
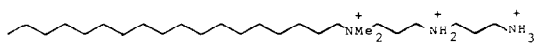
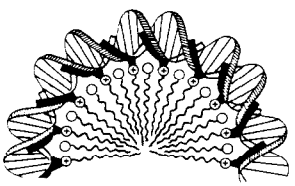
Abstract. *Gel electrophoresis and U.V. spectroscopy show that DNA binds to the surfaces of both small unilamellar vesicles and micelles coated with tris-ammonium or acridinyl-trisammonium head groups.*

The design of small nucleic acid-binding molecules that either interfere passively with biochemical processes, or result in strand scission or chemical modification of the nucleic acid itself has become a very stimulating field of research in chemistry. In comparison, much less effort has been put into the conception of synthetic macromolecular systems[1] with multiple nucleic acid binding sites, and such studies have been mainly restricted to polymers containing basic and aromatic aminoacids[2] or pendant nucleic bases[3]. However such systems could have the unique property of a very strong cooperative affinity for the genetic material, and of eventually adapting the shape of the natural molecule to that of the synthetic one, mimicking for instance the packed forms of cellular DNA. In chromatin, DNA is organized as globular (60x110 Å) particles consisting of a protein core surrounded by 1.75 nucleic acid turns. Remarkably, the size of the nucleosome core particle falls in the range of certain lipid aggregates sizes; DNA could wrap around those as well, provided they contain attracting head groups. These guidelines led to the design of *lipopolyamines* (1) and *lipointercalants* (2) (i.e. amphiphilic molecules possessing a long hydrocarbon tail for anchoring into the hydrophobic core particle and a headgroup capable of interacting with nucleic acids).

(1) 

(2) 



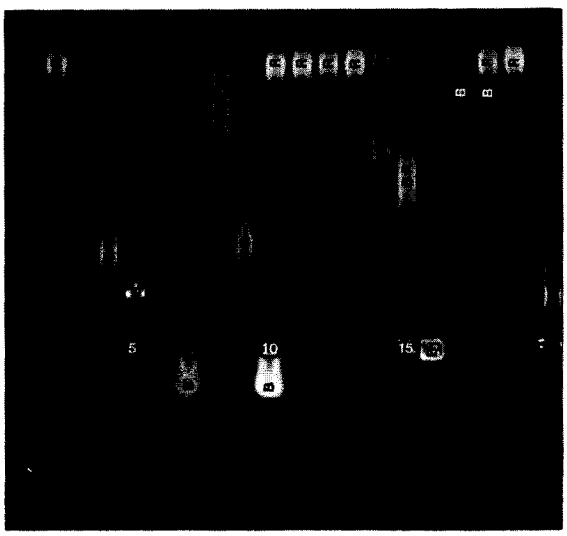
The present communication reports some preliminary results on the interaction of nucleic acids with micelles and small unilamellar bilayer vesicles; incorporation of lipopolyamine (1) or lipointercalant (2) leads to globular supramolecular assemblies of adjustable size and multiple binding sites. DNA is shown to bind very strongly to these particles, giving discrete and soluble molecular complexes.
The following compounds were synthesized:

CH_3-$(CH_2)_{17}$-NMe_2^+-$(CH_2)_3$-NH_2^+-$(CH_2)_3$-NH_2^+-R (1):R=H (2):R=9-acridinyl

CH_3-$(CH_2)_{15}$-NH_2^+-R (3):R=H (4):R=9-acridinyl

HO-$(CH_2)_3NH_2^+$-(9-acridinyl) (5).

Lipopolyamine (1) was obtained[4] (65%) from n-octadecylbromide in three straightforward steps involving successive reaction with dimethylamine, 1,3-dibromo- and 1,3-diamino- propane. Reaction with 9-chloroacridine afforded (2) (50% yield). Similarly, acridines (4) (80%) and (5) (70%) were obtained from stearylamine (3) and propanolamine, respectively. Calf thymus DNA (Sigma Co.) was sonicated[5] and purified further by 2% agarose gel electrophoresis to an average length of 500-600 base pairs. Small unilamellar vesicles of c.a. 100 Å radius were prepared by high speed ultracentrifugation[6] of a sonicated egg-yolk lecithin (EYL, Sigma Co.) or dipalmitoylphosphatidic acid (PA, Fluka Co.) suspension in 40 mM Tris-acetate pH 8 buffer. Neutral detergent micelles of c.a. 20 Å core radius were formed with eicosaethyleneglycol monohexadecylether[7] ($C_{16}E_{20}$, Brij 58, Fluka Co.).



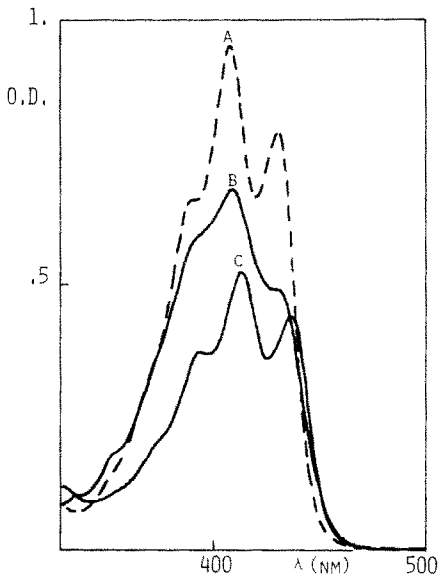


Figure 1 (left). Agarose gel electrophoresis of sonicated calf thymus DNA mixed with micelles or vesicles containing (1)-(5); concentration ranges 0.02 μM for DNA, 10-20 μM and 0.2-1μ M for the small and large particles respectively, loaded with an average of 5 and 150 molecules of (1)-(4) (50 μl samples in 40 mM Trisacetate pH8 buffer). The acridine chromophore shows a blue (B) fluorescence and the ethidium-revealed nucleic acid is red (R). Lanes 2 and 19, DNA alone. Lanes 3-6, DNA plus [(2)/C16E20] micelles, base pair (bp)/acridine (a) c.a. 5,3, 1,0.5. Lane 7, [(2)/C16E20] alone. Lanes 8-9, same as for 3-4 with (1) instead of (2). Lanes 10-13, DNA plus [(4)/C16E20], [(3)/C16E20], (5) and spermidine, respectively; (5) moved out of the plate. Lanes 14-15, DNA plus [(2)/EYL], bp/a c.a. 5 and 2. Lanes 16-17, [(2)/EYL] and [(2)/PA] vesicles alone. Lane 18, DNA plus [(2)/PA], bp/a c.a. 2.

Figure 2 (right). Optical density (1 cm path) changes for a 10^{-4}M solution of (2). (a) in 8 MeOH: 2 Trisacetate aqueous buffer; (b) in 10^{-3}M C16E20 micellar solution (λ_{max} = 408 nm); (c) as (b) plus DNA, bp/a = 2, (λ_{max} = 414 nm).

Of the possible techniques for detecting the macromolecular association between DNA and lipid aggregates, agarose gel electrophoresis was found to be the most straightforward one[8]. Complex formation could be detected visually, since the individual species had

different colors and mobilities; indeed under UV irradiation, ethidium bromide-revealed DNA appears as a red spot (R; Fig. 1) whereas acridine-containing aggregates show up as blue-green (B)[9]. Figure 1 summarizes the most striking features of the interaction between calf thymus DNA and compounds (**1-5**). DNA (R, lane 2) and the cationic micelles containing (2) (B, lane 7) move as expected in opposite directions when driven by the electric field. Evidence that mixtures of $C_{16}E_{20}$ (c.m.c. of 2.10^{-6}M, aggregation number of 120)[7] and (2) form an homogeneous population of micelles comes from the observation of a defined, slow-moving spot (as compared to (5), lane 12) whose mobility increases with the (2)/$C_{16}E_{20}$ ratio (not shown). Solutions of DNA plus [(2)/$C_{16}E_{20}$] give a new blue and red fluorescent band (BR, lanes 3-6) which implies strong association. The streaking and migration of this band decrease with the DNA/[(2)/$C_{16}E_{20}$] ratio, and free [(2)/$C_{16}E_{20}$] micelles appear when all sites on the nucleic acid are occupied. In contrast to other complex DNA-polyfunctional macromolecular systems, the species remain soluble even at saturation. Thus, although both DNA and [(2)/$C_{16}E_{20}$] have multiple binding sites, such behaviour strongly suggests the formation of discrete supermolecular entities and rules out reticulation.

Spectroscopic evidence for intercalation of the acridine headgroups of [(2)/$C_{16}E_{20}$] into DNA is shown in Figure 2. At an average[10] load of ca. 10 molecules of (2) per micelle, the visible spectrum of the chromophore (b) is broad and shows a lower absorbance than in methanol solution (a). (These differences tend to disappear when the (2)/$C_{16}E_{20}$ ratio is decreased, suggesting some headgroup stacking[11] when the local concentration of (2) is high). Addition of DNA results in a maximum 45% hypochromic effect and a 6 nm bathochromic shift for a base pair over acridine ratio of 2 (curve (c)). Such behaviour is typical of acridine intercalation[11].

Model compound (**5**) shows spectra identical to those of [(2)/$C_{16}E_{20}$] (Fig. 2, curves (a) and (c) for the free and complexed states, respectively); nevertheless, being only a monointercalator, the complex does not withstand the electrophoresis conditions, where the equilibrium remaining free species have opposite mobilities (Fig. 1, lane 12). In [(**4**)/$C_{16}E_{20}$] micelles, the acridine moieties are buried in the micellar surface, preventing them from entering the grooves and intercalating. Indeed, the broad visible spectrum of [(**4**)/$C_{16}E_{20}$] is unaffected by DNA, and the two species move separately in the electric field (lane 10).

Polyintercalation is not the only phenomenon which holds (2) micelles and DNA together. Indeed, particles coated with the trisammonium head group of (**1**) also bind very strongly DNA (lanes 8-9), although [(**3**)/$C_{16}E_{20}$] (lane 11) and spermidine (lane 13) do not, at least in the electrophoresis conditions. Polyamines are well-known to stabilize duplex polynucleotides[12], as detected by the melting temperature (Tm) increase of the double to single strand transition. With this technique, which relies on recording the 260 nm O.D. increase with temperature, spermidine shifts the Tm of polyrA.polyrU from 45°C to 70°C (Trisacetate). Interestingly, [(**1**)/$C_{16}E_{20}$] induces biphasic behaviour for this duplex, the magnitude of the original 45°C transition decreasing and being replaced by another one above 95°C; this shift is complete for bp/(1) ~ 2 which reinforces the conclusions inferred from the gel electrophoresis experiments.

Results from experiments with small unilamellar EYL vesicles coated with (1)-(4) lead to roughly the same conclusions as for the neutral $C_{16}E_{20}$ micelles, the lecithin core being zwitterionic and thus having no net charge (see for instance lanes 14-16)[13]. Yet the DNA-vesicle species (lane 15) shows a surprisingly fast moving and streakless band as compared to the smaller DNA-micellar one (lanes 4-5), a feature which may be a consequence of the relative dimensions of the interacting partners. Indeed, the 500 base pair-long DNA (~ 1500 Å) takes up (and possibly wraps around) many 20 Å core radius micelles with few (5-10) binding sites before its own length and binding sites are exhausted (a biochemically comparable situation would be that of histone H_1-depleted chromatin). On the contrary, the EYL vesicles (100 Å radius, 600 Å circumference, 100-200 binding sites) are large enough to allow the entropically favoured 1/1 cooperative association to occur[14], and the resulting two DNA turns-coated liposomes display a mobility (Fig. 1, lanes 14-15) comparable to phosphatidic acid vesicles (lane 17). Although the DNA wrapping needs to be confirmed by another technique, it can already be stated that micellar and vesicular assemblies containing nucleic acid binding headgroups have a promising future as packing matrixes for DNA, or as structural probes if further coated with nucleic acid damaging molecules.

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8 It appears that gel electrophoresis of micelles or liposomes is not described in the litterature; the use of this technique follows the observation that viral particles move on the gel (A.J. Blacker and J.M. Lehn).

9 The acridine fluorescence is almost quenched in the micellar environment as compared to homogeneous organic solution; furthermore (2) may compete with ethidium for binding to DNA and thus fluorescence intensities underestimate concentrations.

10 Interparticle exchange of (1)-(4) is fast, so that not only the distance between binding sites but also their number can adapt for optimal interaction with DNA.

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13 Experiments performed with cationic dioctadecyldimethylammonium vesicles resulted in precipitation, and anionic phosphatidic acid vesicles containing (2) showed no interaction with DNA (lanes 17-18).

14 Further support comes from the presence of excess free DNA in equilibrium with the associated form (lane 14), and from a spectroscopically detected slow equilibration after mixing (turbidity decrease) as compared to the micellar solution.

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