A DNA–Lipid Complex Soluble in Organic Solvents

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A DNA–lipid complex is readily prepared by mixing aqueous solutions of anionic DNA and cationic dialkyl amphiphiles, which thus forms a double helical structure and exhibits intercalation of dyes in chloroform solution.

Complementary base pairing and intercalation of dye molecules make DNA molecules of interest from a molecular recognition perspective. DNAs are polyanionic and so soluble only in aqueous media. If they could be solubilized in organic media and retain their stranded structures, they would become a useful tool in the study of double-helical structures and hydrogen bonds between base pairs in hydrophobic media. Previously, we have prepared lipid-coated enzymes, whose surface is covered with dialkyl amphiphile monolayers, that are soluble and catalyse reactions effectively in organic media.[1–4]

In this paper, we report a DNA–lipid complex prepared by

mixing DNA polyanions with cationic dialkyl amphiphiles. The DNA–lipid complexes obtained are soluble in most organic solvents, but not in aqueous media. Double-stranded structures and intercalation behaviour of the DNA–lipid complex were studied by using circular dichroism (CD) and UV spectra in chloroform.

The preparation of DNA–lipid complexes is simple: a charge equiv. of an aqueous solution (25 ml, pH 8.0, HEPES buffer) of DNA sodium salts (25 mg, from salmon testes, Sigma) and an aqueous dispersion (25 ml) of cationic dialkyl amphiphiles (**1**, **2** or **3**) were mixed at room temperature. The fibrous precipitate was centrifuged, freeze-dried, and obtained in a fair yield. The DNA–lipid complexes obtained were soluble in most hydrophobic organic solvents, such as benzene, chloroform and diethyl either, but not in aqueous media. Elemental analyses (P, N, C) confirmed that cationic amphiphiles attach to phosphate anions of DNA in a ratio of 1:1. A fibrous powder of the DNA–lipid complex shows typical IR absorption spectra at 3200 cm^{-1}, which indicates hydrogen bonds between complementary nucleic bases in double-helical structures.[5] When cationic single-chain amphiphiles such as hexadecyltrimethylammonium bromide (CTAB) were used instead of dialkyl amphiphiles **1–3**, the DNA–lipid complex obtained was not soluble in neither organic nor aqueous media.

When anionic dipalmitoylphosphatidic acid (DPPA), and zwitterionic dipalmitoylphosphatidylchyoline (DPPC) were used as lipid molecules, the DNA–lipid complex was not obtained as a precipitate upon mixing with aqueous DNA solutions. These results suggest that the cationic head groups of amphiphiles interact electrostatically with the phosphate anions of DNA and that the lipophilic double alkyl tails of the amphiphiles solubilize the complex in hydrophobic organic media, as shown in Fig. 1. Since no significant differences were observed in the spectrum of DNA–lipid complexes prepared from amphiphiles **1–3**, the amphiphile **1**, having a long spacer chain, was used as a counter amphiphile for DNA in the following experiments.

Fig. 2 shows the CD spectrum of the DNA–lipid **1** complex solubilized in chloroform containing water (11–110 mmol dm^{-3}). The CD absorption near 280 nm, which indicates a type of double-stranded structure of DNA, was very dependent on the chloroform water content: θ values increased linearly with increasing water content from 11 to 66 mmol dm^{-3} and then decreased with the increase to 110 mmol dm^{-3}. The intensity of CD spectra of native DNA in ethanolic solution or dry DNA cast films from aqueous solutions in air is known to change depending on the ethanol concentration in water or water moisture in the air, respectively, owing to slight changes of base-pair stacking forms (A, B or C form) in the stranded structure.[6,7] The CD spectrum of the DNA–lipid complex in chloroform dried with molecular sieves (11 mmol dm^{-3} water) was consistent with that of the C-form of native DNA as a well-dried cast film from aqueous solution or in an ethanolic solution. The CD spectra at the water-containing chloroform (45–110 mmol dm^{-3}) are similar to those of the A- or B-form of native DNA in aqueous solutions.[7] When the chloroform solution was dried using molecular sieves, the CD spectra at high water content reversibly changed to that observed at low water content. Thus, the DNA–lipid complexes form the regular double-helical structure in chloroform containing 45–110 mmol dm^{-3} water and their microenvironment for base-pair stacking is reversibly affected by the water content.

Fig. 3 shows the UV spectral changes observed when acriflavine responds to the addition of an excess of native DNA in the aqueous buffer solution and the DNA–lipid **1** complex in chloroform solution containing 110 mmol dm^{-3} of water. In aqueous solution, intercalation of acriflavine into DNA base pairs causes both hypochromism (*ca.* 50%) and a 15 nm red shift of the absorption maximum. These observations are explained by stacking interactions of intercalated dye with base pairs in DNA and electrostatic interactions with phosphate anions of DNA, respectively.[8] In chloroform solution, the absorption maximum of the acriflavine itself was

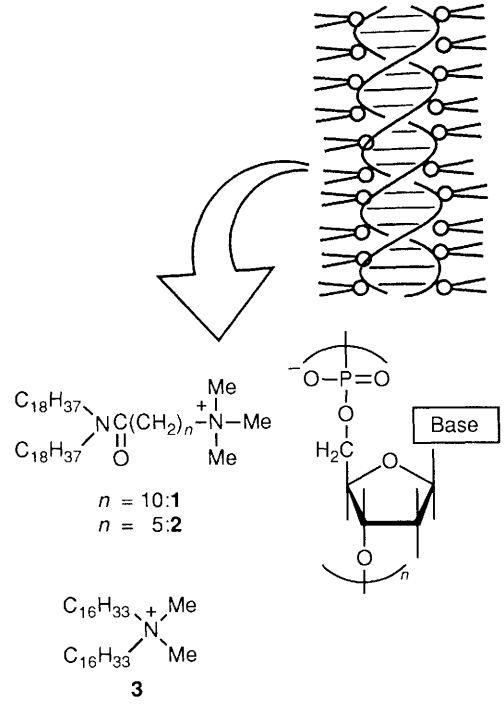

Fig. 1 A schematic illustration of a DNA–lipid complex from DNA polyanions and cationic dialkyl amphiphiles

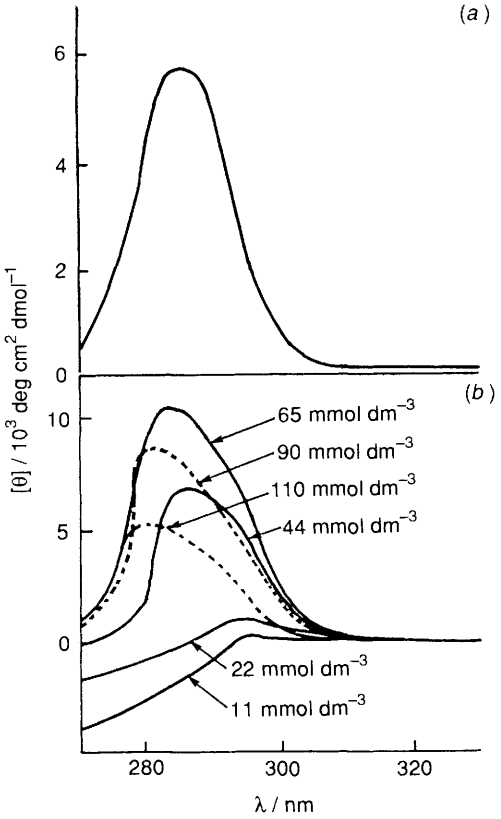

Fig. 2 Circular dichroism (CD) spectra of (*a*) native DNAs (1×10^{-3} unit mol dm^{-3}) in aqueous solution (pH 8.0, HEPES buffer) and (*b*) a DNA–lipid **1** complex (1×10^{-3} unit mol dm^{-3}) in chloroform depending on water content (11–110 mmol dm^{-3}) at 25 °C. The numbers in (*b*) indicate water content in chloroform solution determined by the Karl–Fisher method.

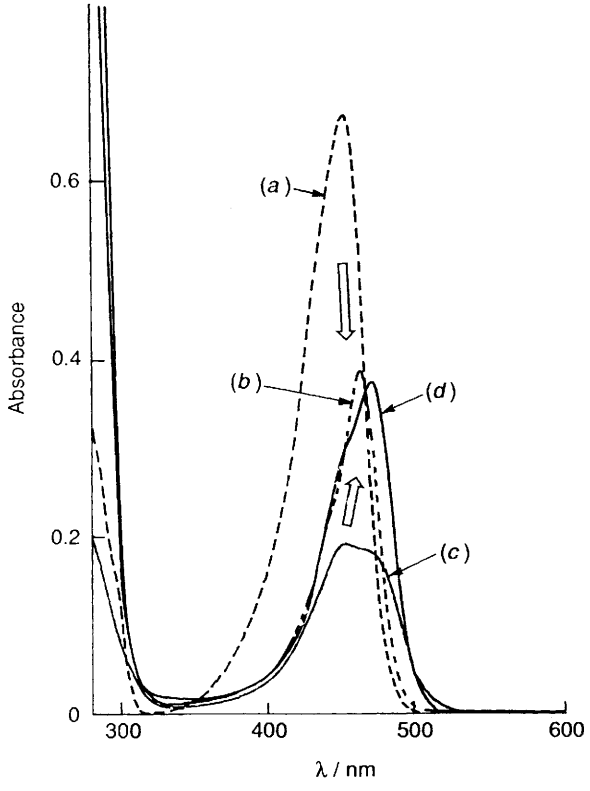

Fig. 3 UV–VIS spectra of acriflavine (1×10^{-5} mol dm^{-3}) at 25 °C. (*a*) In aqueous media (pH 8.0, HEPES buffer), (*b*) in aqueous media in the presence of 1×10^{-3} mol of native DNA, (*c*) in chloroform solution (45 mmol dm^{-3} of water), and (*d*) in chloroform in the presence of 1×10^{-3} mol dm^{-3} of the DNA–lipid **1** complex.

a little broad and very reduced in hydrophobic media compared with that in aqueous solutions. The presence of an excess of the DNA–lipid complex in chloroform increased the absorption of acriflavine by *ca.* 200% and showed the red shift of 15 nm of the absorption maximum. The explanation of this is that the environment of the dye molecule becomes more hydrophilic and more electrostatic by intercalation into DNA base pairs in chloroform solution. Thus, the dye molecules intercalated between base pairs in DNA showed similar absorption spectra independent of media.

The fluorescence spectrum of acriflavine is known to greatly increase in the presence of an excess of DNA in aqueous solution owing to intercalation.[9] The fluorescence spectrum of acriflavine also increased in response to the addition of the DNA–lipid complex in chloroform. This result also suggests intercalation behaviour in chloroform.

The UV absorption changes increased with increasing concentration of DNA in solution. From reciprocal plots, an apparent binding constant of acriflavine per binding site (*K*/*n*) was determined to be 2.1×10^4 dm^3 mol^{-1} for the DNA–lipid complex in chloroform, where *n* indicates the number of nucleic acids per binding site. This value is lower than the *K*/*n* = 1.3×10^5 dm^3 mol^{-1} for acriflavine in native DNA in aqueous solution.[10] The binding constant of intercalation is reported to decrease with decreasing relative permitivity of the aqueous solutions of DNA.[11]

The binding constant (*K*/*n*) of acriflavine to the DNA–lipid complex was also decreased by lower water content in the chloroform solution. This is consistent with the effect of water content on CD spectra shown in Fig. 1. Thus, at lower water content, the DNA–lipid complex takes the C-form and dye molecules barely intercalate between base pairs. However, at higher water content, the DNA–lipid complex shows A- or B-forms and dye easily intercalates between base pairs in the stranded structure.

In summary, DNA can be solubilized in organic media as the 1:1 complex with cationic lipid molecules, and the DNA–lipid complex forms a stranded structure in which dye molecules can intercalate even in organic media. The micro-environment near base pairs in the DNA–lipid complex and intercalation behaviours were greatly affected by the water content in organic media. The DNA–lipid complex could become a new tool in the study of DNA structures in organic solvents. In addition, it can form a self-standing film, insoluble in aqueous solution by casting from the organic solution, or a Langmuir–Blodgett film by spreading the organic solution on a water subphase.

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