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Voltammetric and spectroscopic studies on methyl green and cationic lipid bound to calf thymus DNA

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Abstract

DNA interaction with cationic lipids promises to be a versatile and effective synthetic transfection agent. This paper presents the study on binding of a simple artifical cationic lipid, cetyltrimethylammonium bromide (CTAB), to calf thymus DNA (CT DNA) prior to the condensation process, taking methyl green (MG) as a probe. The results show that the CTAB binds to DNA through electrostatic interaction forming a hydrophobic complex, thus changing the micro-environment of duplex of DNA, so the binding state of MG and DNA is changed, and a complex CTAB-CT DNA-MG is formed. This fact suggests a new way to mediate the conformation of molecular assemblies of DNA and lipids.

Keywords: Voltammetry; Spectroscopy; Methyl green; Cationic lipid; Calf thymus DNA

1. Introduction

Considerable interest in the DNA–lipid complexes has been generated by the observation that certain aggregates between DNA and cationic lipids are efficient vehicles for delivery of foreign DNA or RNA into a wide variety of eukaryotic cells. While the mechanism of transfection with these agents is not well understood, the lipids complexes are thought to facilitate the transfer of DNA through cell membrane [1–7]. Thus, there are several fundamental interactions important in stabilizig cationic lipid–DNA complexes, in which electrostatic interaction between the cationic head group and the negatively charged phosphate sites is thought to be the primary interaction, but the cooperative nature of the binding seems to be driven by hydrophobic association of the non-

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polar tails of the lipid [1,8]. However, the physical characteristics of the liposome/DNA complexes are poorly understood.

Because lipids have ill-defined voltammetric and spectroscopic characters, the study of the interaction of lipids with DNA is difficult using voltammetry and spectroscopy. Almost all of these works were carried out with microscopic methods, such as fluorescence microscopy, AFM and EM. [1–8]. Some small molecules show good voltammetric and spectroscopic characteristics, and interact with nucleic acid at the molecular level by specific modes [9–11]. The results of these various binding studies have been used in designing new and promising anti-cancer agents for clinical use, and in the study of the structure of nucleic acid, and also protein–nucleic acid recognition [9–11]. In this paper the small outside-binding (interactions which involve direct interactions of the bound molecule with the edges of base-pairs in either of the major or minor grooves of nucleic acids) molecule MG was used as a probe to study a simple artifical cationic lipid [1], cetyltrimethyammonium bromide (CTAB) interaction with calf thymus DNA (CT DNA) based on voltammetric and spectroscopic (UV-vis and CD) methods. The results show that the CTAB is electrostatically attached to the DNA phosphate sites and covered on duplex surface, which changes the binding state of MG and DNA before the CTAB interacting with DNA to form a hydrophobic complex. This fact suggested a new convenient and rapid way to mediate the conformation of molecular assemblies of DNA and lipids, and to design new and promising anticancer agents for clinical use.

2. Experimental and methods

Calf thymus DNA (sodium salts, CT DNA) and cetyltrimethyammonium bromide (CTAB) were obtained form Sigma Chemical Co. (USA), and used without further purification. Stock solution of DNA was prepared by dissolving commercial nucleic acid in buffer. DNA solution was stored at 4°C more than 24 h, with gentle shaking occasionally to achieve homogeneity, and used within 2 days. The concentrations of nucleic acid were calculated according to the absorbance at 260 nm by using $\varepsilon_{DNA} = 6600\ M^{-1}\ cm^{-1}$. MG was obtained form Shanghai No.1 Chemical Co. (Shanghai, China), and used after recrystallization from ethanol. Various kinds of buffer solutions with different ionic strengths used in all experiments were prepared using analytical grade reagents: CH_3COOH, NaOH, NaCl and doubly purified water from Milli-Q system. The pH of each solution was measured before being used.

Voltammetric experiments were carried out with Model 630 electrochemical Analyzer (CH Instrument, USA) and a home-made electrochemical cell. The cell design was similar to that previously reported [12]. The working electrode was a glassy carbon disk (0.75 cm^2) and the reference electrode was a saturated calomel electrode (SCE), all potentials were reported vs. the SCE. A platinum disk was used as the counter electrode. The working electrode was polished to a mirror finish with 1 μm, 0.3 μm and 0.05 μm α-Al_2O_3, respectively, and washed in an ultrasonic bath with pure water to remove any adhesive particles.

UV-vis absorption spectrum was measured on a Vis-723 spectrophotometer (Shanghai No. 3 instrument factory, Shanghai, China) using a 1.0-cm path length cell. Circular Dichroism (CD), the difference in absorbance of left and right circularity polarized light, was measured using a 62-A DS CD spectrometer (AVIV, USA) with a 1.0-cm path length rectangular quartz cell at 25°C controlled by a thermoelectric cell holder (AVIV).

The CD absorption titrations were conducted by keeping the concentration of CT DNA and/or MG-CT DNA constant, and varying the CTAB concentration.

3. Results and discussion

3.1. Circular dichroism (CD) of nucleic acid

The CD spectrum in the UV range sensitive to the conformational change of the helix, would bring about different results on the binding reaction [13]. The UV circular dichroic spectrum of

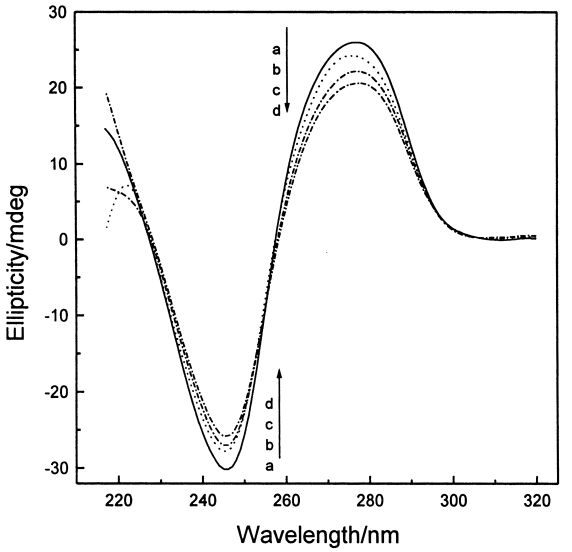

Fig. 1. CD spectral change of CT DNA (1.01×10^{-4} M) on the addition of CTAB in 0.05-M HAC-AC$^-$ buffer solution (pH: 7.04). CTAB concentration: (a) 0-M, (b) 1.1×10^{-5}-M, (c) 5.5×10^{-5}-M, (d) 1.1×10^{-4}-M,

CT DNA exhibits a positive band at 278 nm (UV: λ_{max} 260 nm) due to the base stacking and a negative band at 245 nm due to the helicity of B-DNA as shown in Fig. 1. Upon the incremental addition of the CTAB to DNA, the intensity ($\Delta\varepsilon$) of both the positive and negative bands decreases; however, the overall contour of the spectrum is maintained. This suggests that the DNA binding with CTAB induces certain conformational changes, and these changes correlate with the helix unwinding [13]. Changes in the intensity of the CD peak at 278 nm have been associated with alteration of hydration of the helix in the vicinity of phosphate or ionic concentrations of the ribose ring. It would be reasonable to suggest that exchanging a cationic lipid, such as CTAB, with a sodium ion would lead to a change in hydration near the phosphate group of the DNA helix, particularly since the alkyl chain of the lipid is quite hydrophobic [1,13–15]. The inability of CTAB to unwind supercoiled DNA indicates that electrostatic interaction exists between DNA and CTAB, and the positively charged trimethylammonium group of CTAB interacts with the backbone phosphate groups of DNA [1]. The DNA molecules exist in a form that is coated by fatty acid chains, the composition of which is determined by the CTAB used [1,3,8,13,14].

3.2. Cyclic voltammograms of MG-CT DNA-CTAB

The cyclic voltammograms (CV) of MG are shown in Fig. 2, the free MG shows one irreversible redox peak with anodic peak potential (E_{pa}) at -0.18 V and cathodic peak potential (E_{pc}) at -0.53 V, and peak separation (ΔE_p) of 350 mV (shown in Fig. 2a). In the presence of CT DNA the equilibrium concentration of MG decreases, resulting in a decrease of peak current (shown in Fig. 2b). In the presence of 5.47×10^{-3} M CT DNA, the CV curve shows $E_{pa} = -0.31$ V, $E_{pc} = -0.59$ V and $\Delta E_p = 280$ mV. The decrease of ΔE_p indicates that presence of DNA effects the kinetics of electron transfer. The current decrease is due to the diffusion coefficient of the MG becoming much smaller when bound to DNA than that of the free complex. In the presence of CTAB, the CV curve (shown in Fig. 2c) is very similar to that of Fig. 2b, both ΔE_p and peak

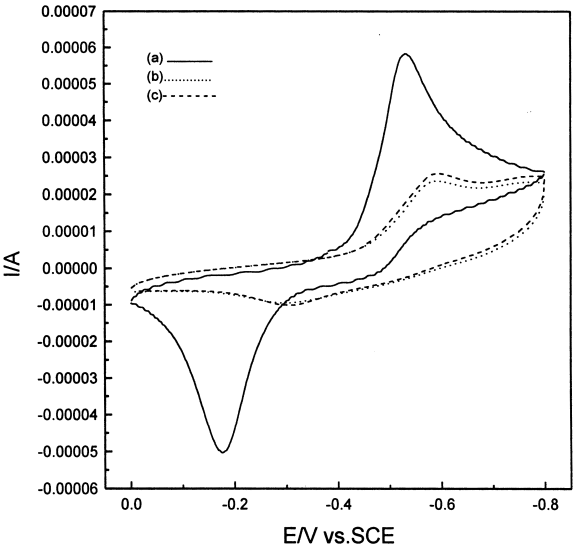

Fig. 2. Cyclic voltammograms of (a) MG (5.47×10^{-4} M), (b) MG-CT DNA (1:10) and (c) MG-CT DNA-CTAB (1:10:10) in 0.1-M HAC-AC$^-$ buffer solution (pH: 7.04).

current decrease, indicating that the MG is not replaced by the CTAB, no free MG is produced while the CTAB interacts with DNA. A MG-DNA-CTAB is formed following the CTAB interaction with CT DNA.

3.3. Absorption spectra of nucleic acid–dye complexes

The absorption spectra of MG interaction with CT DNA are shown in Fig. 3. The free MG has a maximum absorption peak at 615 nm (Fig. 3a), while the MG interacts with CT DNA producing isosbestic points at 635 nm and 531 nm, a red shift of the maximum absorption peak from 615 nm to 628 nm (Fig. 3b), and a large hypochromicity 31% at $0 \le R \le 10$, where the R is the ratio of the concentration of CT DNA to that of MG. The spectral changes observed (hypochromicity, red-shift and isobestic points, the data are not shown) are consistent with the outside-binding the chromophore with the stack DNA base pairs [11,16]. Therefore, MG is considered as an outside-binding of DNA. The absorption spectrum of MG interaction with CT DNA-CTAB is shown in Fig. 3c, there is certain change in the spectrum of MG-CT DNA, the absorbance increases about 10% and blue-shifts 4 nm from 628 nm to 624 nm, indicating that the MG interaction with DNA is weakened while the CTAB interacts with CT DNA.

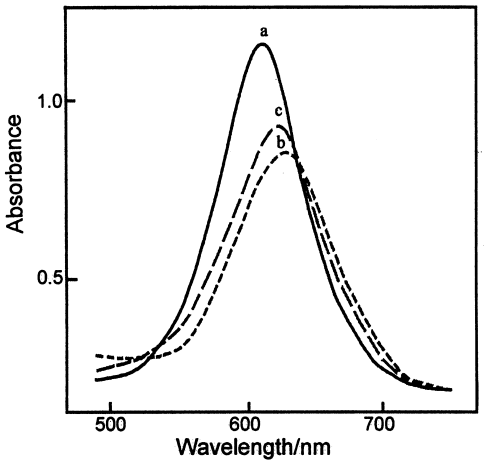

Fig. 3. Absorption spectral change of MG (1.05×10^{-5} M) on the addition of CT DNA in 0.05-M HAC-AC$^-$ buffer solution (pH: 7.04). (a) MG, (b) MG-CT DNA (1:10), (c) MG-CT DNA-CTAB (1:10:10).

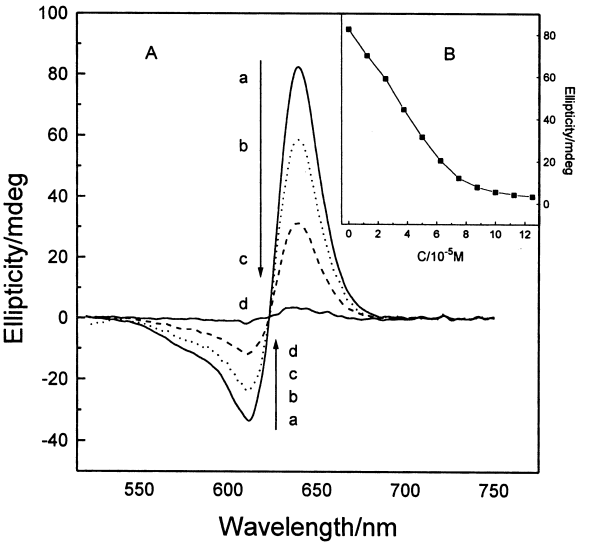

Fig. 4. (A) Induced CD spectra of MG-CT DNA (MG 1.25×10^{-5} M MG: CT DNA = 1:10) on the addition of CTAB in 0.05-M HAC-AC$^-$ buffer solution (pH: 7.04). CTAB concentration: (a) 0-M, (b) 2.5×10^{-5}-M, (c) 5.0×10^{-5}-M, (d) 1.25×10^{-4}-M. (B) The relationship between concentration of CTAB and Ellipticity at 639 nm.

3.4. Induced circular dichroism of MG-CT DNA-CTAB

It is considered that MG does not display CD spectrum in the absence of nucleic acids, but the CD spectrum can be induced in the visible region based on MG interaction with nucleic acids. The induced CD spectra of MG provide significant information about micro-environment surrounding the DNA molecule. The nucleic acids have no absorption bands in the visible region, only the induced CD spectrum of MG is observed, and the representative spectrum is shown in Fig. 4, curve a. The induced CD spectrum of MG displays a positive band at 639 nm and a negative band at 612 nm, indicating that MG is outside-binding to CT DNA through A–T base pairs with light stacking [11,16–19]. The absorption of the induced CD

is dependent on the ratio of CT DNA to MG, when the ratio increasing the absorbance increases. The absorption is affected by increasing the concentration of CTAB in solution, when the solution is titrated by CTAB, the induced CD spectra of MG decrease (shown in Fig. 4 curve b–d), indicating the environment change of CT DNA due to CTAB interaction with CT DNA.

It is suggested on the basis of the combined evidence that the formation of a simply intercalated species requires G–C base pairs, and that either a partially intercalated complex or an outside-bound complex (perhaps in a groove, such as minor groove in A–T sequences) is formed within the A–T regions of DNA [20,21]. The result of induced CD indicates that MG can fit into the helical curve of the minor groove with displacement of the water from the groove, given the correct twist of its linked aromatic ring that can fit snugly into the minor groove. Electrostatic interaction of cationic group of MG with the negative electrostatic potential in the minor groove plus close van der Waals contacts with the 'walls' of the minor groove provide additional favorable components to the free energy of binding [20,21]. Hydrogen bonds can be formed between A–T base pairs and the bound molecule, MG. The electron structure of MG is changed by these interactions, so the induced CD of MG is obtained. While DNA, a multivalent anion, is binding to cationic lipids which are arranged at the surface of DNA with their hydrophobic chains extending into the solution and their ionic head groups orientating toward the duplex, that heteromolecular complexes between DNA and lipid are achieved initially through electrostatic interaction. Because CT DNA is a rod-like polyanion, a model which seems reasonable is that the cationic group of CTAB binds phosphate sites along the DNA chain, while the CTAB hydrocarbon chains are hydrophobically associated with each other along the double helix again to exclude water near the hydrophobic chains [1,2,14,21] in the absence of DNA condensation or aggregation. Provided sufficient CTAB is bound, the resulting complex will exhibit hydrophobic characteristics. Hydrophobic interactions between the hydrocarbon chains of CTAB upon binding to the DNA-phosphate sites are likely to be responsible for the highly cooperative nature of the binding of this lipid to DNA [3]. The hydrophobic complex changes the micro-environment of the CT DNA leading to the binding state of MG-CT DNA being changed such that the induced CD decreases, and the difference between the induced CD of MG may be attributed to a DNA structure phase modulation which occurs precisely at the liposome-to-DNA ratio.

3.5. Effect of the ionic strength

In order to test if there is an electrostatic interaction between CTAB and nucleic acid, the strong electrolyte, NaCl is used. The spectra of MG-CT DNA-CTAB at different ionic strengths are shown in Fig. 5. The induced CD is increasing while the concentration of NaCl increases in solution which shows that the interaction CTAB with CT DNA is weak. As is pointed out earlier for the CTAB systems with nucleic acids, the equilibrium constant for binding is dependent on ionic strength [3,6,8]. The effect may be explained as a

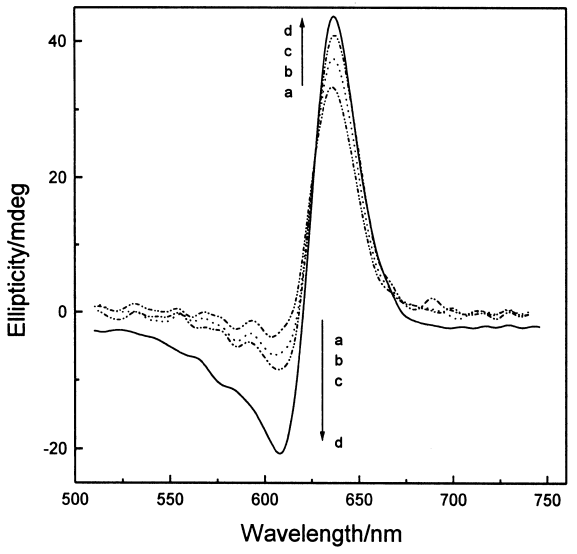

Fig. 5. Absorption change of the induced CD of MG-CT DNA-CTAB (1:10:2) on the addition of NaCl. NaCl concentration: (a) 0-M, (b) 0.05-M (c) 0.15-M, (d) 0.2-M.

competition between CTAB and NaCl with nucleic acids. The first addition of cations assemble near the anionic nucleic acid, which hinder the interaction of nucleic acids with other cations added afterwards. If other cations, such as Na^+ occupied the effective range of electrostatic interactions, the affinity of CTAB with nucleic acid would be greatly weakened. Thus, increasing the salt concentration leads to dissociation of CTAB-nucleic acid complexes. These observations are in agreement with previous work on CTAB and other cationic lipids binding to DNA [3,6,8].

4. Conclusions

In summary, the CTAB bound to DNA through electrostatic interaction forms a hydrophobic complex, then changes the microenvironment of duplex DNA, which decreases the induced CD of MG, but does not displace the outside-binding reagent MG. The complex MG-CT DNA-CTAB is formed.

Acknowledgements

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