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Liposome-mediated conformation transition of DNA detected by molecular probe: methyl green

Weimin Huang, Zheling Zhang, Xiaojun Han, Jilin Tang, Jianguo Wang, Shaojun Dong*, Erkang Wang*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

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Abstract

Recent studies have focused on the structural features of DNA–lipid assemblies. In this paper, we take methyl green (MG) as a probe molecule to detect the conformational change of DNA molecule induced by dimethyldioctadecylammonium bromide (DDAB) liposomes before the condensation process of DNA begins. DDAB-induced DNA topology changes were investigated by cyclic voltammetry (CV), circular dichroism (CD) and UV–VIS spectrometry. We find that upon binding to DNA, positively charged liposomes induce a conformational transition of DNA molecules from the native B-form to the C motif. Conformational transition in DNA results in the binding modes of MG to DNA, changing and being isolated from DNA to the solution. More stable complexes are formed between DNA and DDAB. That is also proved by the melting study of DNA.

Keywords: Voltammetry; Spectroscopy; MG; DDAB; Probe molecule; DNA

1. Introduction

The interaction between cationic lipids and various DNA is well documented. Interaction between DNA and lipids has become an active area of research recently due to the fundamental importance and various applications of such interaction. The ability of DNA to influence the structure of membranes and to initiate polymorphic phase transitions in bilayers might have important biological implications [1–4]. Cationic lipids can easily form stable complexes with DNA owing to the attraction forces between the opposite net charges of the lipid and DNA. In addition, DNA–lipid complexes are used as a non-viral system for gene delivery to target cells [5]. Nucleic acids are electroactive species producing reduction and oxidation signals in various polarographic and voltammetric techniques. Electrochemical methods have been successfully applied in the analysis of nucleic acids and their components. The oxidation of both guanine G and adenine A at carbon electrodes is expected to follow a two-step mechanism involving the total loss of four electrons and four protons [6,7]. The DNA-trapped compounds can either be detected directly if they are electroactive molecules or via changes in electrochemical DNA signal [8–12]. Recently, Wang et al. [13] and Tomschik et al. [14] showed that the sensitivity of DNA and RNA analyses could be greatly increased if the constant current chronopotentiometry and voltammetry with sophisticated baseline correction are used. These techniques are now applied to the development of DNA hybridization sensors and ultratrace measurements of nucleic acids [6,13–18]. Our group had studied the interaction between DNA and cationic surfactant cetyltrimethylammonium bromide (CTAB) [19]. We found that the complex MG–DNA–CTAB was formed. CTAB had the characteristic of surfactant behavior that can form micelle-like structure in solution. However, DDAB can form SUVs (liposome), which is the structure of bilayer lipid membrane. As it is known, a variety of bilayer structures formed by synthetic lipids have been used to mimic membrane properties, and they furnish unique opportunity to investigate the relationship between structure and function. The vesicle system is a self-closed bilayer aggregate and constitutes one of the most explored models of membranes. Compared to micelles, liposomes have a different intrinsic curvature and related bilayer structure exhibits their unique character to interact with DNA. Methyl green (MG) is a good redox and

* Corresponding authors. Fax: +86-431-5689711.
E-mail address: ekwang@ns.ciac.jl.cn (E. Wang).

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optical probe for the study of the interaction of DNA and liposome. On one hand, it can give fine amperometric response on the GCE, on the other hand it also has the intrinsic character whose spectrum signal range do not overlap with DNA's in absorption and CD spectrum. The most important is that it can interact with DNA by outside binding mode that is sensitive to the conformation of DNA. That is to say its control and release by DNA pertain to the conformational change in DNA.

In this paper, the small molecule MG was used as a probe to study small unilamellar vesicles (SUVs) formed by a simple artificial cationic lipid, dimethyldioctadecylammonium bromide (DDAB), to interact with calf thymus DNA (CT DNA) based on voltammetric and spectroscopic (UV–VIS and CD) methods. The results show DDAB liposomes induce a conformational transition of the DNA molecules from the native B-form to the C motif. The compact structure of C motif induced by DDAB liposomes makes MG isolated from DNA and a new complex between DNA and DDAB was formed. The complex formation also resulted in the increase of T_m on DNA, which indicates a more stable complex between DNA and DDAB. The process in which MG molecule was separated from DNA by DDAB lipid vesicles and the new complex formed between DNA and DDAB has also been discussed.

2. Experimental and methods

2.1. Reagents

Calf thymus DNA (sodium salts, CT DNA) was obtained from Sigma (USA), dimethyldioctadecylammonium bromide (DDAB) was obtained from Aldrich (USA); all were used without further purification. Stock solution of DNA was prepared by dissolving commercial nucleic acid in buffer and stored at 4 °C for more than 24 h with occasional gentle shaking to get 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 from Shanghai No. 1 Chemical (Shanghai, China), and used after recrystallization from ethanol. Chloroform was analytical grade. Buffer solutions used in all experiments were prepared using analytical grade reagents: $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, NaCl and doubly purified water from Milli-Q system.

2.2. Sample preparation

For the preparation of DDAB vesicles, a dry film of 50 mg lipid was produced under rotary evaporation from a stock solution in chloroform and then organic solvent was removed by purified nitrogen. The lipid film was hydrated to the desired concentration with 10 mM phosphate buffer, pH 6.66. The resulting multilamellar liposome suspension was sonicated for several hours (in average about 2 h) in a bath sonicator, until a clear suspension of SUVs was obtained [20]. The vesicle sizes of a few representative samples were determined by dynamic light scattering measurement on a DynaPro-MS/X dynamic light scattering instrument. The DDAB lipid vesicles had diameters ranging from 40 to 70 nm. The molar ratios of lipid to DNA used for the experiments were lower than 5, which resulted in positively charged complexes avoiding a precipitation [21].

2.3. Electrochemical methods

CV was carried out with a CHI 630A electrochemistry workstation (CHI, USA). All electrochemical experiments employed a three-electrode cell (5 ml, single electrolyte compartment) with a glassy carbon electrode (GCE) working electrode, a platinum wire auxiliary electrode and an Ag|AgCl (sat. KCl solution) reference electrode. The potential of Ag|AgCl (sat. KCl solution) was taken as 0.198 V versus SHE. The buffers were purged with purified nitrogen (N_2) for 20 min prior to a series of experiments. A nitrogen environment was kept over solutions in the cell for exclusion of oxygen.

The electrochemical titration was conducted by keeping the concentration of MG or/and MG–CT DNA constant, and varying the DDAB concentration.

2.4. Spectra methods

UV–VIS absorption spectra were measured on a Carry 500 Scan UV–VIS–NIR Spectrophotometer (Varian, USA) using a 1.0-cm path length cell.

Melting temperatures (T_m) for free DNA, DNA–MG and DNA–liposome complex were measured by following the changes in absorption at 260 nm (A_{260}) as a function of temperature in a Carry 500 Scan UV–VIS–NIR Spectrophotometer (Varian). T_m was calculated by the software in the Carry WinUV software package (Varian).

Circular Dichroism (CD) for the difference in absorbance of left and right circularity polarized light was measured using a 62A 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). Four scans were averaged per spectrum.

The spectrum titration was conducted in the same way as the electrochemical one shown in Section 2.3. The spectrum obtained was subtracted from the background arising from the lipid vesicles alone.

3. Results

3.1. Cyclic voltammetry

The cyclic voltammograms (CV) of MG at the potential range of 0 to −0.9 V are shown in Fig. 1, curve a, dot line. Free MG yielded one irreversible electrochemical redox

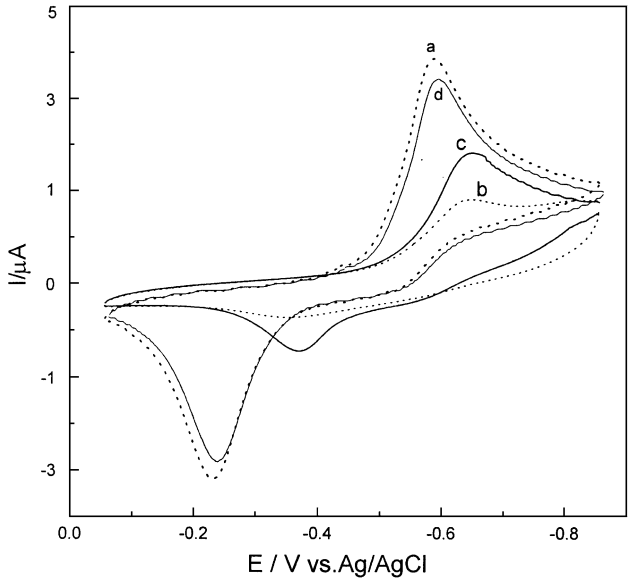

Fig. 1. Cyclic voltammograms of MG (1.1×10^{-5} M) in 10 mM phosphate buffer at pH 6.66 with 0.1 mol l^{-1} KCl at 25 °C. In the presence of (a) 0, (b) 6.4×10^{-6} M CT DNA, (c) (b) + 12.6×10^{-6} M DDAB, and (d) (c) + 17.4×10^{-6} M DDAB. The scan rate is 50 mV s^{-1}. After the interacting substances were introduced, the solution was allowed to stand for 30 min each time before measurements were taken in order to make sure that the measurements were done under equilibrium conditions. The molar concentration of DNA is related to the monomer content and it is the same as below.

process, with the anodic peak potential (E_{pa}) at −0.23 V, the cathodic peak potential (E_{pc}) at −0.59 V and the peak separation ΔE_p was 0.36 V. In the presence of CT DNA, the peak current of MG decreased and the peak potential shifted negatively (shown in Fig. 1, curve b, dot line). The current decrease was due to very small concentration of the free MG itself when binding to DNA compared with that of the free MG in the absence of DNA. When DDAB lipid vesicles were added to the solution, a positive shift of peak potential and a continuing increase of peak current were found which, finally, was almost the same with free MG alone (shown in Fig. 1, curves c and d, solid line). From the results, we can see that in the presence of DDAB, the equilibrium concentration of free MG in the solution increased. There was a competitive binding to DNA between DDAB and MG. The outside binding of MG to DNA was even more weakened in the presence of DDAB. So the DDAB can break the complex between DNA and MG. The interaction between DNA and DDAB made the MG isolated from DNA and a new complex between DNA and DDAB was formed. Actually, we have found that the CV of MG did not show obvious changes in the presence of DDAB SUVs (data not shown). It indicated that adsorption of DDAB did not affect the result from Fig. 1. The reason could be the hydrophobic surface of GCE repulsed the liposome, which was hydrophilic in outside layer. The applied potentials for CV measurement were done below 0 V. In these applied potentials, the surface of GCE was negatively charged and it also repulsed the negatively charged DNA molecules. This lessened the chance of adsorption of DNA to GCE.

3.2. Absorption spectra studies

Fig. 2 shows the visible spectra of MG in the absence and presence of CT DNA. MG interacting with CT DNA led to substantial red shifts of the maximum absorption wavelength (λ_{max}) from 632 to 641 nm with the largest hypochromicity of 23% (Fig. 2, curves a and b). Two isosbestic points (data not shown) were observed indicating the interaction of MG and CT DNA forming a complex without any intermediate. Hypochromism was suggested due to strong interaction between the electronic state of the chromophore and that of DNA base pairs [20–23].

When DDAB lipid vesicles were added into the solution (Fig. 2, curves c and d), with increasing concentration of DDAB, a blue shift of λ_{max} from 641 to 632 nm and the largest hyperchromic effect was observed, and the spectrum recovered to be almost the same with free MG. The absorbance increase can be interpreted as a release of the MG from the double helix into the buffer in the presence of DDAB and MG recovered to the original state (free MG) in the solution. It may imply that a change in DNA conformation has occurred. Conformation change in DNA caused a drop of outside binding MG molecules from DNA to the solution.

3.3. CD spectral studies

It is considered that MG does not display CD spectrum in the absence of nucleic acids, but the CD spectrum can be

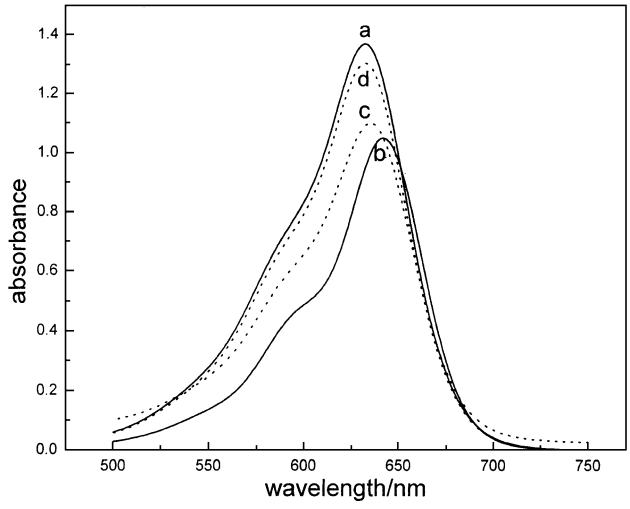

Fig. 2. UV–VIS absorption spectral change of MG on the addition of CT DNA and DDAB, 0.11 mM MG. The spectra recorded in 10 mM phosphate buffer solution (pH 6.66) in the presence of (a) 0, (b) 6.4×10^{-5} M CT DNA, (c) (b) + 12.6×10^{-5} M DDAB, and (d) (c) + 17.4×10^{-5}M DDAB. After the interacting substances were introduced, the solution was allowed to stand for 30 min each time before measurements were taken in order to make sure that the measurements were done under equilibrium conditions.

induced in the visible region based on MG interaction with nucleic acids. The induced CD spectra of MG provide significant information about microenvironment surrounding the DNA molecule. The nucleic acids had no absorption bands in visible region, only the induced CD spectrum of MG was observed.

The induced CD of the MG was observed as shown in Fig. 3A. MG did not display CD spectrum in the absence of nucleic acids (shown in Fig. 3A, curve a, solid line). In the presence of DNA (shown in Fig. 3A, curve b, solid line), a positive band at 658 nm and a negative band at 628 nm were observed in the induced CD spectrum corresponding to the characteristic spectrum of the groove binding with stacking. The result shows that the MG was bound on the outside of the helix and stacking [22–25]. When DDAB was added to the solution (shown in Fig. 3A, curves c and d, dot line), the induced CD spectrum of MG disappeared gradually and no obvious CD signal was found at last with the increasing concentration of DDAB. It indicates that there is no obvious interaction between MG and DNA. MG was driven out of grooves of DNA to solution because of the strong interaction between DDAB and DNA. The same result was obtained from the absorbance spectrum shown above.

The CD spectrum in the UV range can be used to monitor the conformational transition of DNA [22–24]. When MG–DNA was titrated with DDAB, the positive Cotton effect of B-form DNA at 270 nm and the negative Cotton effect at 245 nm were all shifted to long wavelength (shown in Fig. 3B). At the same time, the $\Delta\varepsilon$ value was decreased gradually. The spectrum showed a positive Cotton effect at 285 nm and a negative Cotton effect at 251 nm, which are characteristics of C-form DNA in aqueous solution [26,27]. B-form DNA is most common which shows a stereochemical double helical structure with Π-electron-rich base-pair stacking. Prive et al. [28] reported that water molecules were important to form B-structures of DNA in which water molecules interacted with oxygen of ribose and phosphate and minor or major groove of DNA strands. In low humidity, B-form DNA helix can change to C-form reversibly [29]. B to C secondary conformational change makes DNA collapse into a tightly packed phase. The compact spatial structure gave MG a spatial hindrance and squeezing effect in grooves of DNA. There was no enough space for MG outside binding in grooves of DNA, and electrostatic repulsion between MG and DDAB also existed; these made MG isolated from DNA. Furthermore, DNA and DDAB can form a kind of sandwich-like complex [30]. The complex structure gave no chance for MG to approach the DNA molecule anymore. The result necessarily proves that binding state changes of MG can imply conformation change of DNA induced by DDAB lipid vesicles.

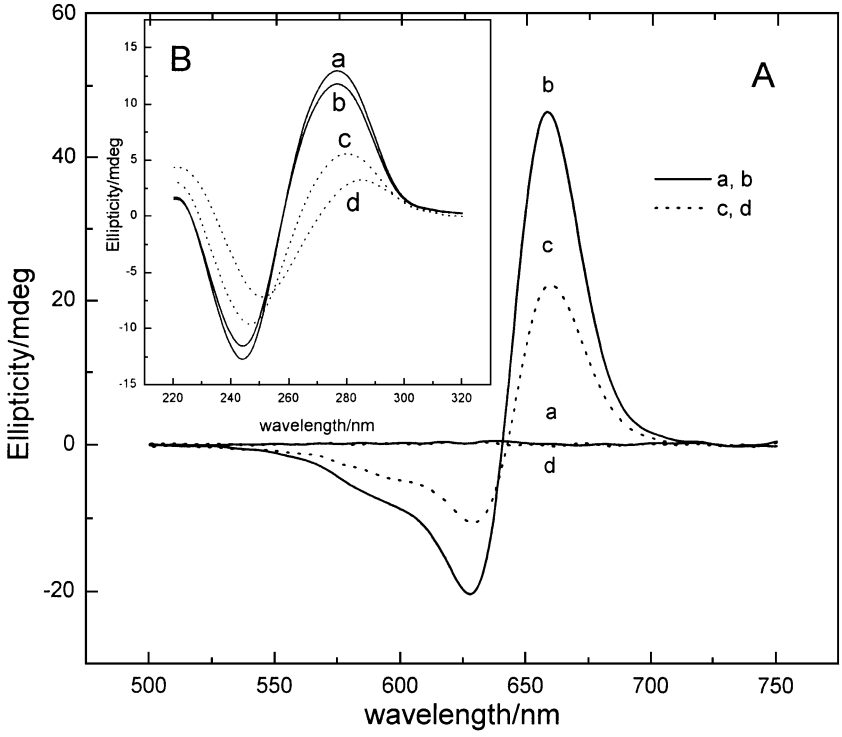

Fig. 3. (A) CD spectra of MG (0.11 mM) on the addition of CT DNA and DDAB in 10 mM phosphate buffer solution (pH 6.66) in the presence of (a) 0, (b) 6.4×10^{-5} M CT DNA, (c) (b) + 12.6×10^{-5} M DDAB, and (d) (c) + 17.4×10^{-5} M DDAB. (B) CD spectra of DNA (6.4×10^{-5} M) on the addition of MG and DDAB in 10 mM phosphate buffer solution (pH 6.66) in the presence of (a) 0, (b) 1.1×10^{-4} M MG, (c) (b) + 12.6×10^{-5} M DDAB, and (d) (c) + 17.4×10^{-5} M DDAB. After the interacting substances were introduced, the solution was allowed to stand for 30 min each time before measurements were taken in order to make sure that the measurements were done under equilibrium conditions.

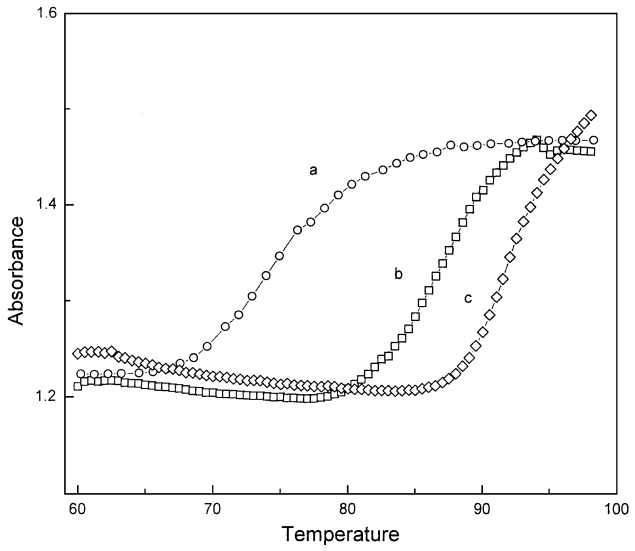

Fig. 4. UV melting of the thermal denaturation of CT DNA in 10 mM phosphate buffer solution (pH 6.66) in the presence of (a) 0, (b) 1.1×10^{-4} M MG, (c) 3.0×10^{-4} M DDAB. Melting temperatures (T_m) for free DNA, DNA–MG and DNA–liposome complex were measured by following the changes in absorption at 260 nm (A_{260}) as a function of temperature.

3.4. UV-melting study

T_m increase was actually used to prove that there is strong binding of a ligand to the helical form of DNA [31]. Fig. 4 shows the melting curves for free DNA, DNA–DDAB and DNA–MG. CT DNA in aqueous solution has a melting temperature of 75 °C (curve a). This means that with the increase of temperature the double-helical structure of DNA is disturbed, and DNA melts, or undergoes a helix-coil transition of its secondary structure; the thermal denaturation curve for CT DNA was 88 °C when mixed with MG (curve b), and the thermal denaturation curve for CT DNA was 92 °C when mixed with DDAB (curve c). From the result, we can see that when mixed with DDAB, DNA displayed an increase of A_{260} at about 4 °C than that when mixed with MG. It indicates that a stronger interaction between DNA and DDAB began than it did between DNA and MG. The stronger interaction between DNA and DDAB resulted in a more stable complex between DNA and DDAB.

4. Discussion

DNA–cationic liposome assemblies become the subjects of intensive studies due to their unique properties and their high efficiency in acting as vehicles for DNA delivery into eucaryotic cell. Two interaction processes were shown to occur between positively charged liposome and DNA: a DNA-induced membrane fusion and a liposome-mediated DNA condensation [32]. The liposome-mediated B to C transition could be suggested on the basis of the high positively charged density and reduced relative humidity prevailing near the surface of the charged lipid bilayer, which was reported previously [33]. Some small molecules are good probing molecules to interact with nucleic acid at the molecular level by specific mode detected by voltammetric and spectroscopic methods [19]. Actually, MG molecule has outside-binding mode to DNA. This property was used here to probe the interaction of DDAB lipid vesicles with double-stranded DNA in this interaction that will change the DNA conformation. Moreover, the conformation change of DNA can influence the binding state of MG to DNA. In other words, we can get the information about conformational change of DNA by detecting the binding state changes of MG to DNA. Combining our cyclic voltammograms, UV–VIS and CD spectrum results, we propose the process shown in Fig. 5. MG can interact with DNA by outside-binding mode (shown in A), as is proved by CV (Fig. 1), UV–VIS (Fig. 2) and CD (Fig. 3). Anyway the interaction cannot change B conformation of DNA. However, when DDAB cationic lipid vesicles were added to the solution (shown in B of Fig. 5) at this kind of binding state of MG, we can get the increasing concentration of free MG in solution through the CV and UV–VIS results, accompanied by the increase of the concentration of DDAB. With the sandwich-like complex (shown complex A in Fig. 5) formed by DNA with DDAB, DNA changed its conformation from B to C gradually. Because of the conformation transition of DNA, the grooves

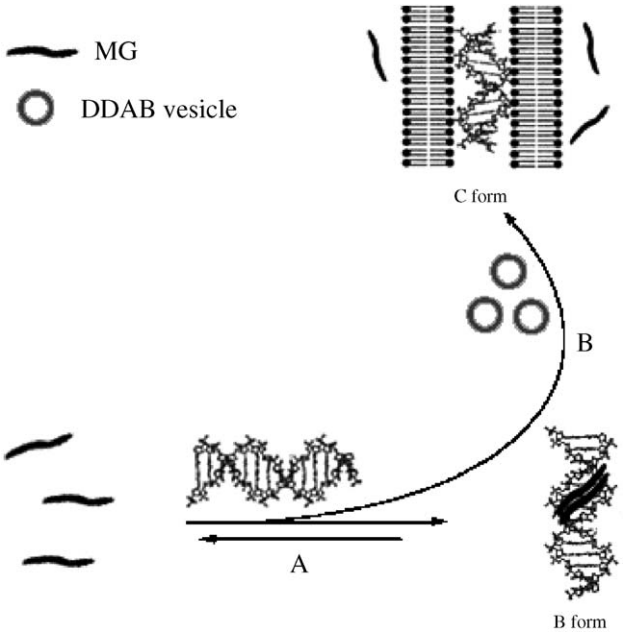

Fig. 5. The process of the competitive interactions between MG and DDAB with DNA. A represents the interaction between DNA and MG. MG interacts with DNA by outside-binding and DNA still keeping its B conformation. B represents the competitive interaction between DDAB and MG with DNA. DDAB breaks the binding of MG to DNA by forming a sandwich-like complex with DNA and induces DNA to change its conformation from B to C.

of DNA became crowded and it cannot accommodate the MG anymore; MG fell off from the DNA to the solution and recovered to the free MG while a more stable complex was formed between DNA and DDAB. This was proved by CD and melting results. At the same time, the lipid membrane outside of DNA prevented the MG from crossing the membrane and reaching the surface of DNA molecule. However, the limitation of the new protocol is that it is an indirect way to learn the conformation change of DNA induced by DDAB SUVs by detecting the change of signal of the probe on electrochemistry and spectroscopy. In order to know where the specific conformation change of DNA begins, we have to combine the CD spectrum of DNA in the UV region.

5. Conclusion

The probe molecule was used to detect positively charged liposome to induce a secondary conformational transition of the DNA molecules from the native B-form to the C-form by CV, UV–VIS and CD methods. The process was simplified by introducing probe molecule to the system. The changes of the binding mode of the probe molecule to DNA reflected the secondary conformational transition induced by DDAB cationic lipid vesicles. The redox and optical probe was sensitive to the conformation change of DNA. Its control and release by DNA reflect the conformation change of DNA. The characters give us a way in which we can detect the sensitive change of signal of the probe on electrochemistry and spectroscopy to know whether the conformation of DNA changed or not induced by DDAB SUVs indirectly. In the meantime, it makes up the deficiency of lipids that have the ill-defined voltammetric and spectroscopic characters and that make it easy to study the interaction of liposomes with DNA by voltammetry and spectroscopy.

Acknowledgements

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