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Modulation of membrane properties by DNA in liposomes: A spectroscopic study

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ABSTRACT

Liposome mediated DNA transport possesses a number of preventing diseases in clinical trials, thus, the study of interaction between DNA and liposomes has become a hot research direction. In this paper, the adsorption behavior of DNA onto two representative lipids had been studied by the fluorescence spectrum measurement, Ultraviolet absorption spectrum and Langmuir-Blodgett technology. The results of fluorescence spectrum measurement indicated that the fluorescence liposomes were quenched statically by DNA at all three temperatures. Thermodynamic analysis displayed that the intermolecular forces between DNA and liposomes were van der Waals forces and Hydrogen bonding. The experimental results of Ultraviolet absorption spectrum and Langmuir-Blodgett technology further verified these mechanisms. This work provides useful theoretical basis for the development of novel DNA delivery materials.

1. Introduction

As a biological macromolecule with ordered structure, DNA was used frequently in gene therapy with high biocompatibility [1–3], these years. However, the poor stability of DNA limited its transfer efficiency and application. Based on this, researchers have focused on the development of steady DNA transfer system [4,5]. Among all common DNA transport systems, the liposome-mediated gene transfer is the most widely used approach due to its good targetability and some other fine features [6–8]. To develop more stable liposome-mediated transport systems, it is important to study its interaction with membranes.

With so many studies of interaction between DNA and membranes by Langmuir-Blodgett technology [9–12], however, only a few researches have touched upon its optical properties, especially fluorescence properties. In recent years, great progress has been made in the study of the fluorescence properties of biomacromolecules, such as proteins [13], drugs [14], nanoparticles [15] and the like.

Lipids are the major constituents of cell membranes and the outer membrane of eukaryotic cells are rich in phosphocholine (PC) lipids [16]. In our previous study, the adsorption behavior of DNA on DPPC and POPC mixed lipid monolayers was investigated [17], which had demonstrated that DNA trended to adsorb on the polar heads of lipids by van der Waals force. Based on this, to study the interaction mechanism between DNA and model cell membranes by fluorescent technique, we selected DPPC and DMPC to prepare liposomes in this paper. In summary, this research provides useful information for the development of novel DNA liposome vectors.

2. Materials and methods

2.1. Chemicals

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) with high purity (>99%) were purchased from Sigma-Aldrich, and dissolved in chloroform/methanol (3:1, v/v) to reach a concentration of about 0.2 mg/mL. Calf thymus DNA (ctDNA) was purchased from Sigma-Solarbio Chemical Company and dissolved in MES buffer to the concentration of 1 mg/mL. All other chemical reagents were analytically pure. The water used was ultrapure water (resistivity = 18MΩcm).

2.2. Preparation and dynamic light scattering (DLS) of DMPC/DPPC liposomes

Liposomes were prepared using the ultrasonic film dispersion method, which made some minor improvements on the basis of other former researches [18].The mixture of DMPC/DPPC (1:1, mol:mol) and 1% Rhodamine B were heated and evaporated by a rotary evaporator at 60 °C, forming to lipid films. Then, 30 mL ultrapure water was added to the above system in order for the liposomes to be fully hydrated. After that, large unilamellar vesicles (LUVs) were obtained. Finally, LUVs were smashed to smaller liposomes by using cell disruptor.

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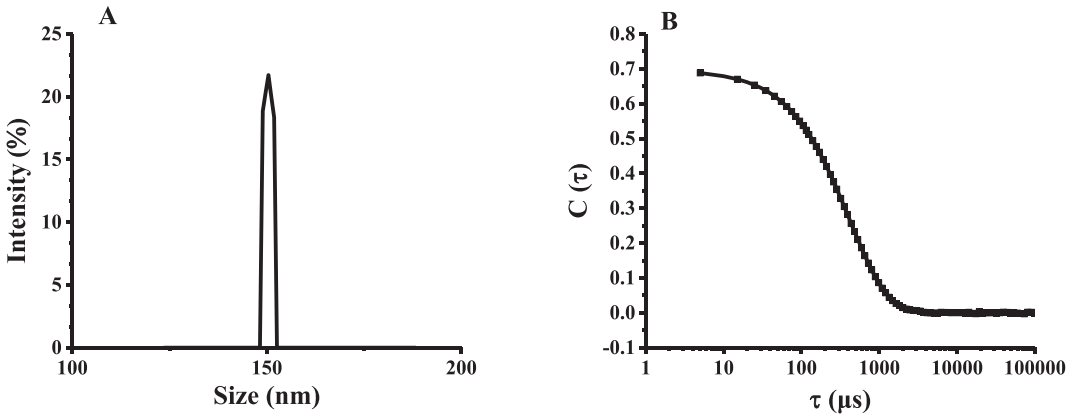

Fig. 1. A: DLS spectra of the liposome dispersed in PBS buffer (pH 7.4) with intensity-based distributions. B: Autocorrelative function of liposome diffusion.

The DLS was performed by Nano Brook Omni (Brookhaven, USA). For size measurements, DMPC/DPPC liposomes were diluted with PBS buffer.

2.3. Fluorescence spectrum measurements

Fluorescence spectrum was measured by a Photon Technology International (PTI) QM/TM-40 spectrofluorimeter equipped with a 75 W Xenon lamp and a thermostat bath (HS-4 (B), Chengdu Instrument Factory, China). The excitation and emission wavelengths were 540 nm and 550–650 nm. The concentrations of DNA used in this measurement were 8 μM, 16 μM, 24 μM, 32 μM, 48 μM and 64 μM. And three selected temperatures were 296 K, 303 K, and 313 K, respectively.

2.4. Ultraviolet absorption spectrum

The UV1901PC spectrophotometer (Shanghai AuCy Technology Instrument Co., LTD.) was used to measure the UV–vis absorption spectra. The 1 cm quartz cuvette was used for all spectral detections. DNA dissolves in water at a solution of 1 mg/ml. All experiments were repeated three times to ensure the reliability of the results.

2.5. Langmuir monolayer measurements

The surface pressure-area (π-A) isotherms and compression-expansion cycles curves were recorded by using KSV instrument (Helsinki, Finland). In an experimental operation, for π-A isotherms, certain

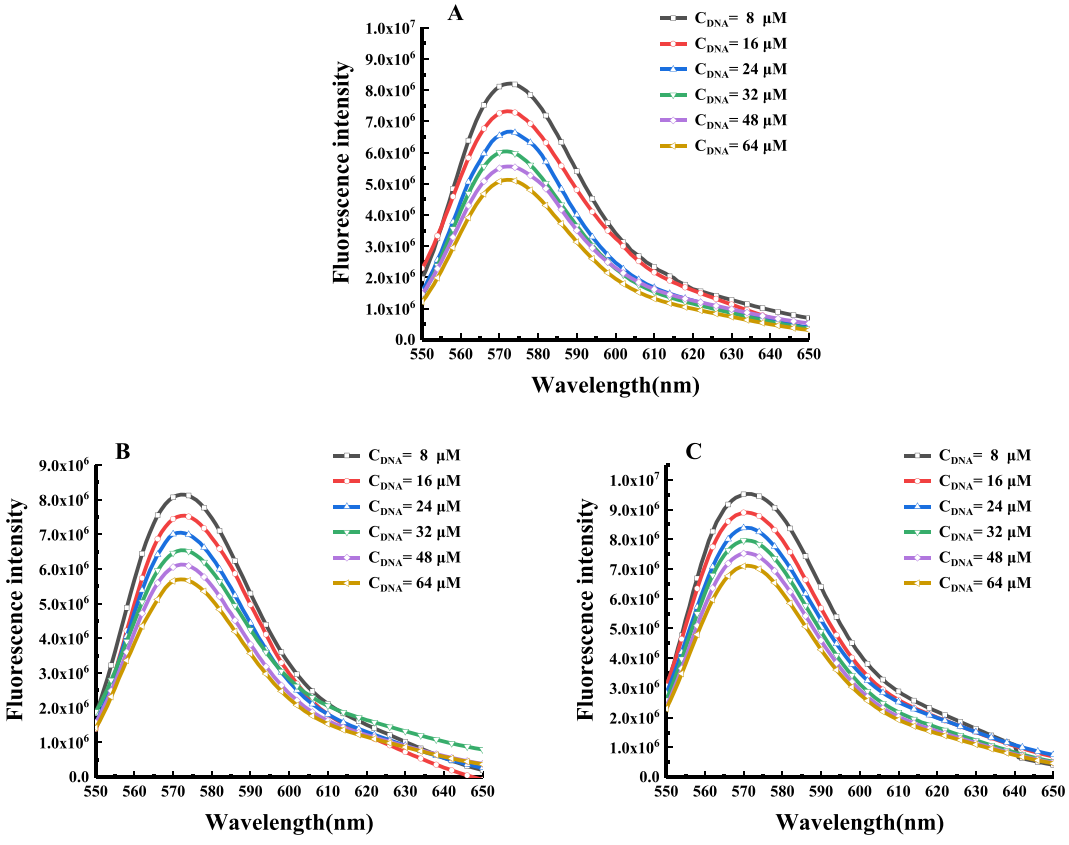

Fig. 2. Fluorescence spectra of DMPC/DPPC liposome labeled by Rhodamine B in various concentrations of DNA in aqueous solution (pH = 7.4) at 296 K (A), 303 K (B) and 313 K (C).

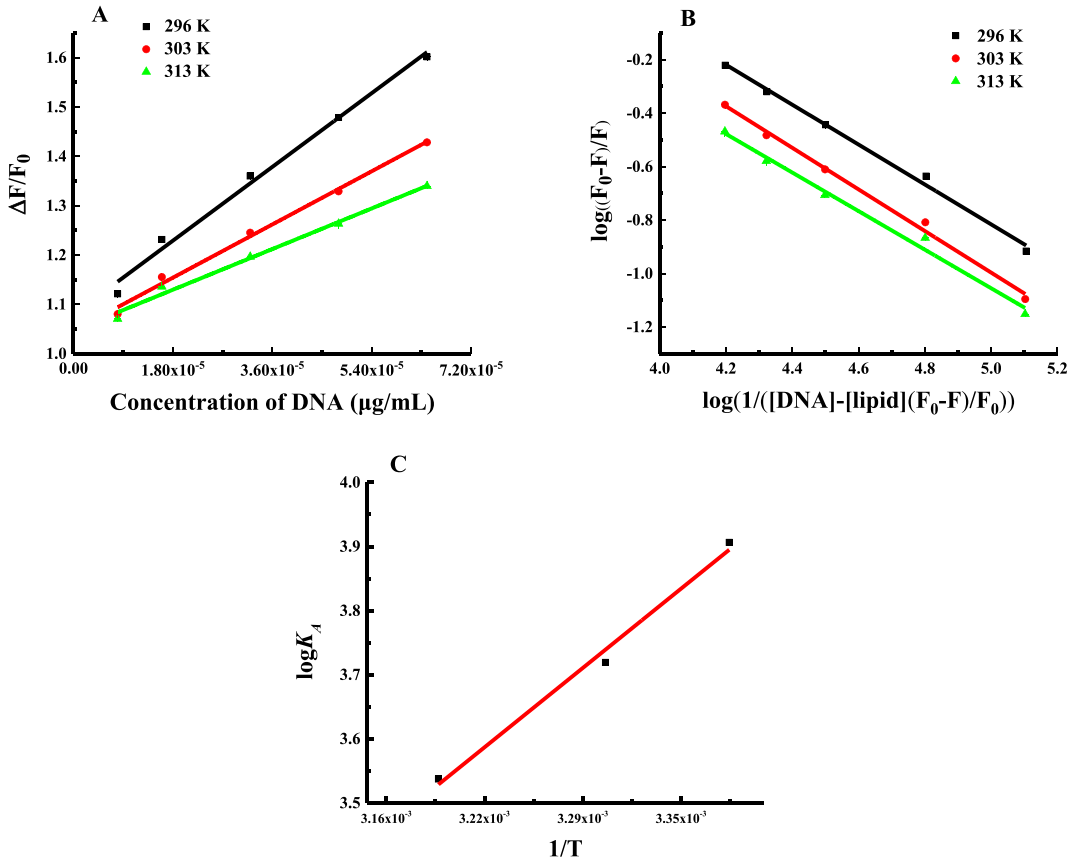

Fig. 3. A: The linear Stern-Volmer plot of $\Delta F/F_0$ against the concentration of DNA at different temperatures (296 K, 303 K, 313 K). B: The plot of log $((F_0 - F)/F)$ vs log $(1/([DNA] - [lipid](F_0 - F)/F_0)$. C: Van't Hoff plot of DNA-liposome.

volumes of DMPC and DPPC were dropped onto the air-water surface and DNA was added into the subphase with an appropriate Hamilton microsyringe. Then, a 15 min was given for the evaporation of the solvent. After that, we compressed the films with symmetrical barriers at a certain speed of 1 mm/min. For compression-expansion cycles curves, we compressed the films to 30 mN/m. And then, barriers were released to the surface pressure was 0 mN/m. The process is repeated twice. Every curve was measured at least three times to ensure their repeatability. All measurements were carried out at the temperature of 20 ± 1 °C.

3. Results

3.1. Characterization of liposomes

Dynamic light scattering measurement was applied to gauge the size of DMPC/DPPC liposomes, whose results were showed in Fig. 1. The diameter characterized by intensity of liposomes was about 170 nm, while the intensity-based size distribution was centered at about 5 nm (Fig. 1A). Fig. 1B was the autocorrelative function of liposome diffusion, which showed the DMPC/DPPC liposomes had an awesome dispersion in PBS buffer (pH 7.4). The above results showed that DMPC/DPPC liposomes have moderate particle size and good dispensability in solvents, which is suitable for the study of this experiment.

3.2. DNA adsorbed by liposomes

The fluorescence spectra of liposomes labeled by Rhodamine B in various concentration of DNA at 296 K, 303 K, and 313 K were collected and showed in Fig. 2. It could be found that DNA reduced the fluorescence intensity of liposomes at each temperature and higher the DNA concentration, the more the fluorescence intensity decreased. It was known that the ability of different molecular interactions to reduce fluorescence intensity is called fluorescence quenching [19]. Thus, the interaction between DNA and liposomes made fluorescence quenching of liposomes, which proved DNA had a strong interaction with fluorescence liposomes.

For different lipids, the phase transition temperature is an important parameter to measure their physiological characteristics. Lipids are more likely to form membranes of liposomes while the ambient temperature exceeds the phase transition temperature of lipids. Based on this, the three temperatures of 296, 303 and 313 K were selected for fluorescence spectrum experiment, since the phase transition temperatures of DMPC and DPPC were 296 and 313 K, respectively. In Fig. 2, the effect of DNA on fluorescence characteristics of fluorescent liposomes at different temperatures was measured. The results showed that the fluorescence intensity of fluorescence liposomes was quenched by DNA at all three temperatures. In addition to this, quenching phenomenon was more obvious with the increase of DNA concentration, which basically presented a linear quenching relationship. As for the specific quenching mechanism, further data processing was required for analysis.

Table 1
Binding parameters and thermodynamic parameters of DNA-liposomes system.

T	K_{SV}	K_A	ΔH	ΔS	ΔG
(K)	($\times 10^3$ L/mol)	($\times 10^3$ L/mol)	($\times 10^4$ K·J/mol)	(J/mol·K)	($\times 10^4$ K·J/mol)
296	8.306	8.062			−2.208
303	5.991	5.239	−3.8199	−54.47	−2.169
313	4.577	3.447			−2.115

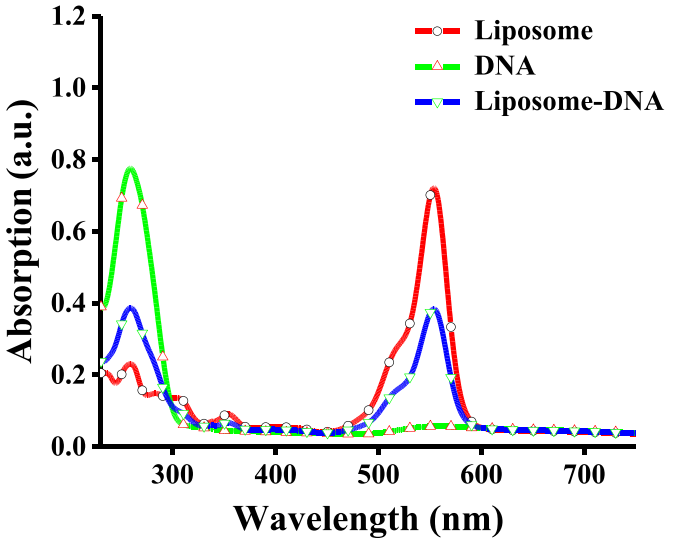

Fig. 4. The Ultraviolet absorption spectrum of liposome and DNA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fluorescence quenching can be divided into dynamic quenching and static quenching according to different quenching mechanisms [19]. Dynamic quenching refers to fluorescence quenching of excited fluorescent molecule by energy transfer or physical collision with quencher. While, complexes are formed by weak binding between ground state fluorescent molecules and quenching agents, and the phenomenon that the fluorescence is completely quenched by the complex was called static quenching. The type of fluorescence quenching could be distinguished by classical Stern-Volmer equation (Eq. (1)) [20], and the mechanism of fluorescence quenching could be inferred. The fluorescence quenching data at different temperatures (296 K, 303 K and 313 K) were statistically analyzed by linear regression analysis.

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \tag{1}$$

where F_0 and F are the fluorescence intensities of liposomes in the absence and presence of DNA, respectively; K_{SV} is the Stern-Volmer quenching constant.

Fig. 3A presented the dots and linear regression plot of $\Delta F/F_0$ against [DNA] (R^2 >0.98 at least). The results showed that the K_{SV} of liposomes and DNA were negatively correlated with temperature rise. This phenomenon indicated that the quenching mode of DNA and liposomes was static quenching, which demonstrated that there had a weak interaction between them [21]. In our previous study [17], the adsorption behavior of DNA on DPPC and POPC mixed lipid monolayers was investigated, which had demonstrated that DNA trended to adsorb on the polar heads of lipids by van der Waals force and hydrogen bond. Based on this theoretical basis, the mechanism of quenching had also been verified. For further investigated the binding properties of DNA and liposomes, the binding constant K_A was calculated by Eq. (2) and showed in Fig. 3B. The slope of the curve in Fig. 3B was the combination constant K_A at different three temperatures, which was 8.062×10^3 L/mol, 5.239×10^3 L/mol, 3.447×10^3 L/mol, respectively. The binding constant decreases as the temperature increased, which demonstrated that the fluorescence quenching phenomenon of DNA on liposomes was static quenching from the binding level.

$$\log\frac{F_0 - F}{F} = n\log K_A - n\log\left(\frac{1}{[Q] - \frac{(F_0 - F)[P]}{F_0}}\right) \tag{2}$$

where K_A is the binding constant and n is the number of binding sites; [P] denotes the concentration of DNA [22].

The main driving forces involved in the interaction between small molecules and biological macromolecules are hydrophobic, hydrogen bonding, electrostatic attraction and van der Waals forces [23,24]. According to previous reports [22], the type of interaction force could be analyzed through the calculation of thermodynamic parameters used Eqs. (3) and (4).

$$\log K_A = -\frac{\Delta H}{2.303RT} + \frac{\Delta S}{2.303R} \tag{3}$$

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

where ΔH, ΔS and ΔG are the change of enthalpy, entropy and Gibbs free energy, R denotes the gas constant and T is the experimental temperature.

ΔH and ΔS were estimated from the slope of linear and the intercept of the Y axis after the linear regression of log K_A vs 1/T showed in Fig. 3C. The values of ΔH and ΔS were −3.8199 × 104 K·J/mol and −54.47 J/mol·K, respectively, from Table 1, which meant the force between DNA and liposomes were van der Waals forces and Hydrogen bonding according to Table S1.

The UV–visible absorption spectrum is often used to characterize Chemical bonding between different biomacromolecule. Fig. 4 showed the UV–vis spectrum of liposomes, DNA and liposomes-DNA. It could be found that the maximum absorption peaks of liposomes and DNA were about 550 nm, 260 nm, respectively. These results were consistent with previous reports [25]. From the blue line in Fig. 4, the maximum

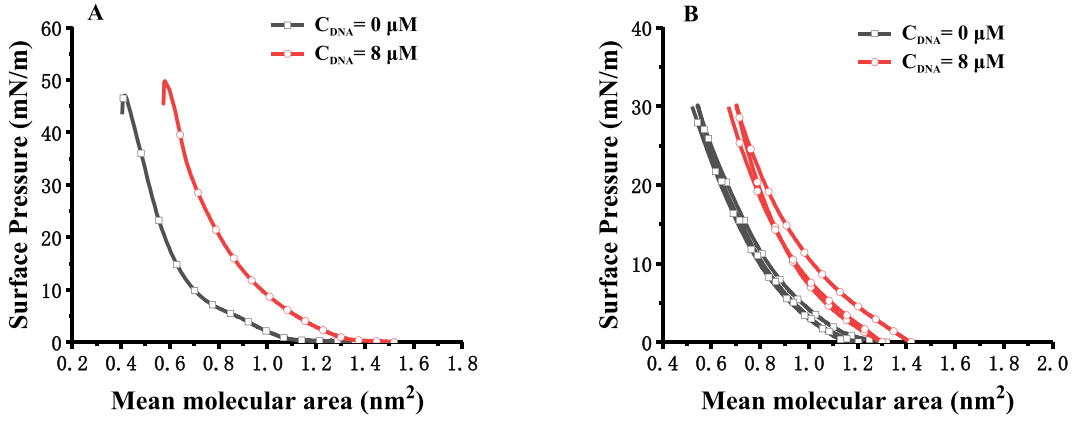

Fig. 5. A: Surface pressure-area isotherms for DMPC and DMPS (1:1, mol/mol) monolayers with DNA. B: Compression-expansion cycles of DMPC and DMPS containing DNA at pH 7.4.

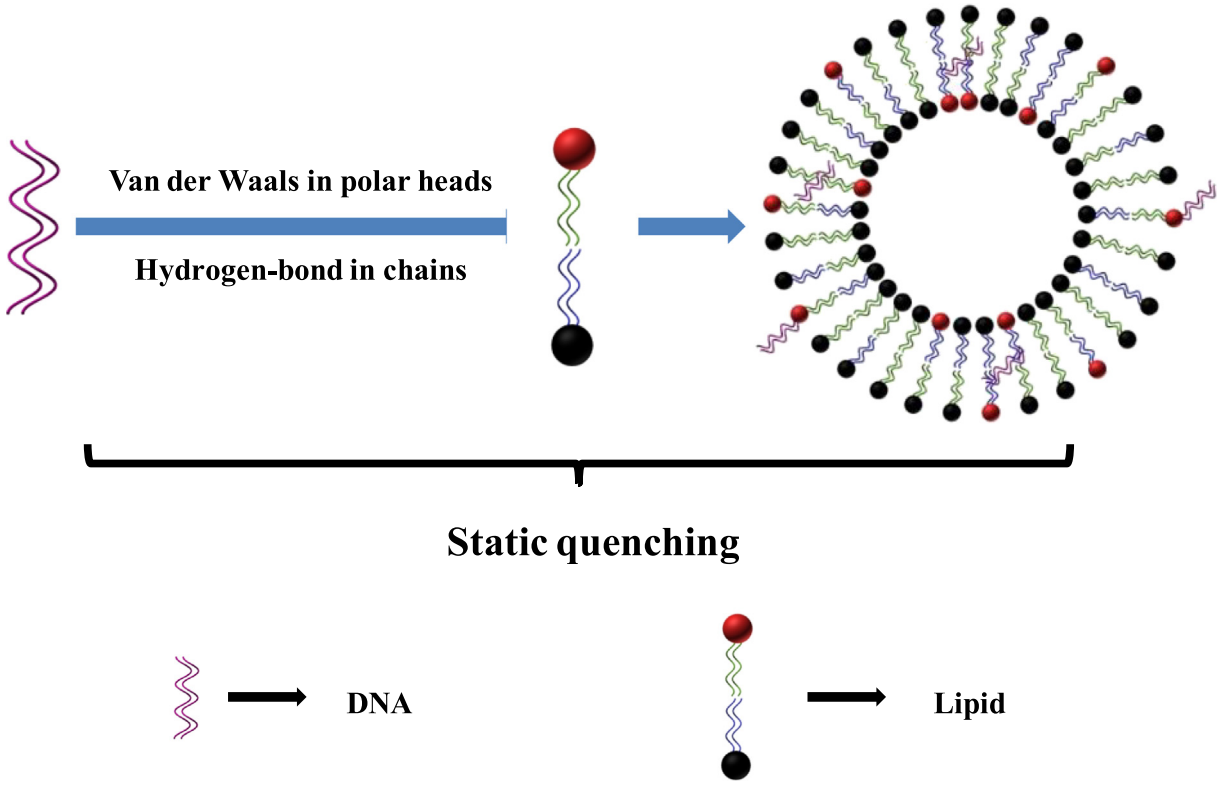

Fig. 6. The schematic of interaction between DNA and DMPC/DMPC liposomes.

absorption peak in 260 nm disappeared nearly, which indicated the prosperous combination of liposomes and DNA.

3.3. Langmuir monolayer measurements

The adsorption behavior of DNA on the bilayer model had been studied by the above fluorescence spectrum experiments. Moreover, Langmuir films which self-assembly by lipids can serve as a model for single cell membrane. To further confirm the adsorption behavior of DNA on DMPC/DPPC in another aspect, LB measurements had been done and were shown in Fig. 5. The isotherms of pure DMPC/DPPC without DNA started to rise at the mean molecular area of 1.08 nm^2. In comparison to the pure lipid monolayer, the isotherms lifted up to larger mean molecular area and the collapse pressure had a slight increase in presence of DNA, which demonstrated that DNA could be attached to mixed lipid monolayer by hydrogen bonds with the tail chain and van der Waals forces with polar heads of lipids. The results in Fig. 5B showed that DNA was inserted into the lipid monolayers making it significantly less steady than the pure lipids. These results were consistent with previous fluorescence spectrum experiments.

Based on all these results, we proposed the schematic diagram of interaction between different concentrations of DNA and DMPC/DPPC liposomes labeled by Rhodamine B, which was shown in Fig. 6. From this schematic diagram, the intermolecular forces between DNA and liposomes could be seen clearly. It still has abundant biological significance although this is only a model study. In the study of biomolecular interaction, atomic force microscopy (AFM) was widely used to observe the changes of surface morphology [26–30]. We will use AFM to further observe the interaction between fluorescent liposomes and DNA in the future, and use its fluorescence characteristics to study targeted liposomes carrying DNA in cells, mice and other organisms.

4. Conclusion

In this paper, the adsorption behavior of DNA onto two representative lipids had been studied by the fluorescence spectrum measurement, Ultraviolet absorption spectrum and Langmuir-Blodgett technology. The binding of DNA to mixed lipids was mainly the adsorption behavior on DMPC/DPPC liposomes. The more electronegative atoms of DNA, such as oxygen, nitrogen and the like, formed hydrogen bonds with a large number of hydrogen atoms in the lipid tail chain. Besides, the DNA itself, as a polar molecule, bound to the polar head of lipids through van der Waals forces. These two forces enabled DNA to adsorb to lipid molecules and generate a static quenching of fluorescent labeled liposomes.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2019.117459.

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References

[1] T. Kurosaki, N. Higuchi, S. Kawakami, Y. Higuchi, T. Nakamura, T. Kitahara, M. Hashida, H. Sasaki, Self-assemble gene delivery system for molecular targeting using nucleic acid aptamer, Gene 491 (2012) 205–209.
[2] A.O. Elzoghby, W.M. Samy, N.A. Elgindy, Protein-based nanocarriers as promising drug and gene delivery systems, J. Control. Release 161 (2012) 38–49.
[3] N. Allon, A. Saxena, C. Chambers, P. Bhupendra, A new liposome-based gene delivery system targeting lung epithelial cells using endothelin antagonist, J. Control. Release 160 (2012) 217–224.
[4] D. Mirska, K. Schirmer, S.S. Funari, A. Langner, B. Dobner, G. Brezesinski, Biophysical and biochemical properties of a binary lipid mixture for DNA transfection, Colloids Surf. B 40 (2005) 51–59.

[5] K. Chen, L. Guo, J. Zhang, Q. Chen, K. Wang, C. Li, W. Li, M. Qiao, X. Zhao, H. Hu, D. Chen, A gene delivery system containing nuclear localization signal: increased nucleus import and transfection efficiency with the assistance of Ran GAP1, Acta Biomater. 48 (2017) 215–226.
[6] S. Castano, B. Delord, A. Février, J. Lehn, P. Lehn, B. Desbat, Asymmetric lipid bilayer formation stabilized by DNA at the air/water interface, Biochimie 91 (2009) 765–773.
[7] C. Janich, A. Hädicke, U. Bakowsky, G. Brezesinski, C. Wölk, Interaction of DNA with cationic lipid mixtures–investigation at Langmuir lipid monolayers, Langmuir 33 (2017) 10172–10183.
[8] Y.U. Bae, J.W. Huh, B.K. Kim, H.Y. Park, Y.B. Seu, K.O. Doh, Enhancement of liposome mediated gene transfer by adding cholesterol and cholesterol modulating drugs, Biochim. Biophys. Acta 1858 (2016) 3017–3023.
[9] T. Hianik, P. Vitovic, D. Humenik, S.Y. Andreev, T.S. Oretskaya, E.A.H. Hall, P. Vadgama, Hybridization of DNA at the surface of phospholipid monolayers. Effect of orientation of oligonucleotide chains, Bioelectrochemistry 59 (2003) 35–40.
[10] B. Juskowiak, A. Swiatkowska, Study of the G-quadruplex–dipalmitoylphosphatidylcholine interactions at the air/water interface, Colloids Surf. A Physicochem. Eng. Asp. 417 (2013) 250–255.
[11] Z. Zhang, C. Hao, H. Qu, R. Sun, Studied on the dynamic adsorption process of Lycium barbarum polysaccharide in the POPC/DPPC monolayers, Colloids Surf. B Biointerfaces 178 (2019) 38–43.
[12] C. Hao, J. Li, W. Mu, L. Zhu, J. Yang, H. Liu, Bin Li, Adsorption behavior of magnetite nanoparticles into the DPPC model membranes, Appl. Surf. Sci. 362 (2016) 121–125.
[13] S. Kundu, H. Matsuoka, H. Seto, Zwitterionic lipid (DPPC)–protein (BSA) complexes at the air–water interface, Colloids Surf. B: Biointerfaces 93 (2012) 215–218.
[14] A. Casadó, G.M. Chiara, S.L. Sagristá, F. Castelli, M. Pujol, M.A. Alsina, M. Mora, Langmuir monolayers and Differential Scanning Calorimetry for the study of the interactions between camptothecin drugs and biomembrane models, Biochim. Biophys. Acta 1858 (2016) 422–433.
[15] E. Guzmán, E. Santini, M. Ferrari, L. Liggieri, F. Ravera, Interfacial properties of mixed DPPC–hydrophobic fumed silica nanoparticle layers, J. Phys. Chem. C 119 (2015) 21024–21034.
[16] G. Neunerta, J. Makowiecki, E. Piosik, R. Hertmanowski, K. Polewski, T. Martynski, Miscibility of dl-α-tocopherol β-glucoside in DPPC monolayer at air/water and air/solid interfaces, Mater. Sci. Eng. C 67 (2016) 362–368.
[17] H.J. Qu, C.C. Hao, Z.Y. Zhang, Z.W. Xu, R.G. Sun, Adsorption behavior of DNA on phosphatidylcholine at the air-water interface, Mater. Sci. Eng. C 99 (2019) 505–510.
[18] Yibo Liu, Juewen Liu, Adsorption of nanoceria by phosphocholine liposomes, Langmuir 32 (2016) 13276–13283.
[19] M.I. Halawa, W. Gao, M. Saqib, S.A. Kitte, F.X. Wu, G.B. Xu, Sensitive detection of alkaline phosphatase by switching on gold nanoclusters fluorescence quenched by pyridoxal phosphate, Biosens. Bioelectron. 95 (2018) 8–14.
[20] T. Li, Z. Cheng, L. Cao, X.H. Jiang, Comparison of interactions between three food colorants and BSA, Food Chem. 194 (2016) 740–748.
[21] M. Mansouri, M. Pirouzi, M.R. Saberi, et al., Investigation on the interaction between cyclophosphamide and lysozyme in the presence of three different kind of cyclodextrins: determination of the binding mechanism by spectroscopic and molecular modeling techniques, Molecules 18 (2013) 798–813.
[22] A. Papadopoulou, R.J. Green, R.A. Frazier, Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study, J. Agric. Food Chem. 53 (2005) 158–163.
[23] Y.J. Hu, Y. Liu, L.X. Zhang, R.M. Zhao, S.S. Qu, Studies of interaction between colchicine and bovine serum albumin by fluorescence quenching method, J. Mol. Struct. 750 (2005) 174–178.
[24] Y.Q. Wu, H.G. Zhang, Y.Q. Wang, Conformational and functional changes of bovine serum albumin induced by TiO_2 nanoparticles binding, J. Mol. Liq. 243 (2017) 358–368.
[25] J.I. Paredes, S. Villarrodi, A. Martínezalonso, J.M. Tascón, Graphene oxide dispersions in organic solvents, Langmuir 24 (2008) 10560–10564.
[26] J. Zhong, C.H. Yang, W.F. Zheng, L.X. Huang, Y.K. Hong, Lijun Wang, Y.L. Sha, Effects of lipid composition and phase on the membrane interaction of the prion peptide 106–126 amide, Biophys. J. 96 (2009) 4610–4621.
[27] J. Zhong, From simple to complex: investigating the effects of lipid composition and phase on the membrane interactions of biomolecules using in situ atomic force microscopy, Integr. Biol-UK (6) (2011) 632–644.
[28] J. Zhong, D. He, Recent Progress in the Application of Atomic Force Microscopy for Supported Lipid Bilayers, vol. 18, 2012 4148–4155.
[29] C.P. Shi, Y.N. He, M.Z. Ding, Y.F. Wang, J. Zhong, Nanoimaging of food proteins by atomic force microscopy. Part I: components, imaging modes, observation ways, and research types, Trends Food Sci. Technol. 87 (2019) 3–13.
[30] C.P. Shi, Y.N. He, M.Z. Ding, Y.F. Wang, J. Zhong, Nanoimaging of food proteins by atomic force microscopy. Part II: application for food proteins from different sources, Trends Food Sci. Technol. 87 (2019) 14–25.