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含缩醛正离子类脂分子与蛋白质的相互作用*

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摘 要： 合成系列含缩醛的双链正离子类脂分子，并用荧光光谱研究其与牛血清蛋白（BSA）的相互作用. 通过荧光的变化，解释蛋白质构象的变化. 在低类脂浓度时，少量类脂分子束缚在牛血清蛋白周围，荧光有很大幅度的淬灭，蛋白质本身肽链被解开，与此同时最大发射波长从（344 ± 1） nm 蓝移到（331 ± 1） nm. 由于疏水相互作用，更多类脂分子不断地聚集在蛋白质周围，牛血清蛋白中的两个色氨酸残基被完全地包裹在类脂分子形成的双分子膜中，荧光强度不断增加直到恒定不变.

关键词： 正离子类脂；缩醛；牛血清蛋白；荧光光谱
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A Fluorescence Study on the Interactions of Cationic Lipids with Bovine Serum Albumin*

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Abstract Interactions of a series of dialkyl cationic lipids linking with bovine serum albumin (BSA) through acetal (linker) have been studied by the fluorescence spectroscopy. At low concentrations of cationic lipids, the fluorescence intensity of BSA decreased with binding of cationic lipid, and the maximum of emission wavelength shifted from (344 ± 1) nm to (331 ± 1) nm. It indicates that the BSA goes to uncoiled flexible conformation from its native structure. When the concentrations of lipids increased, the fluorescence intensity increased rapidly and then maintained unchanged. It reveals that two tryptophan residues of BSA are all enwrapped in the bilayer membrane, owing to the hydrophobic interactions between lipids and BSA.

Keywords Cationic lipid, Acetal, Bovine serum albumin, Fluorescence spectroscopy

1 Introduction

Lipids are widely used in the chemistry and biochemistry, such as catalysis, separation[1], supramolecular assembly, pharmacology[2] and gene delivery[3]. For these applications, it is very important to study the interaction of lipids with their targets. The fluorescence spectroscopy is a powerful tool to detect the binding of lipids to protein, DNA and other biomolecules[4-7]. Applications of lipids in molecular recognition of peptides[8], 2D protein recrystallization[9], protein targeting[10] and biological sensing[11] have been reported.

The interaction of lipid with water-soluble protein has been studied in recent years[12-14]. Some models for the structure of monoalkyl lipids-protein complexes

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have been proposed[12]. The interactions of bovine serum albumin (BSA) and human serum albumin (HSA) with the anionic or cationic monoalkyl lipid led to fluorescence quenching, and the cationic lipid could recover partly quenching by inducing the enhancement of fluorescence for BSA[14]. For BSA as a water-soluble globular protein, its primary structure was known for a long time and its tertiary structure was determined by X-ray crystallography a few years ago[15,16]. There are two tryptophan residues, one is exposed and the other is buried in the interior of BSA in the native conformation. The fluorescence intensity and the maximum emission wavelength would be changed when environments of two tryptophan residues changed in the protein-lipids system.

In the previous work[17], considering in the acidic environment inside of cells (pH = 2 ~ 5), the ketals should be easy to break up resulting in little toxicity, while in the neutral environment outside of cells (pH = 7), we designed and synthesized a series of cationic lipids with ketal as a linker bond as gene vectors. The gene delivery in vitro experiments showed that they are efficient in transfecting a few kinds of cell lines. The acetal is stable in the neutral or basic environment, and unstable in acid. The cationic lipid containing the acetal may be a novel carrier as the gene vector in the gene delivery. In this paper, we wish to report the interaction of BSA with cationic lipids bearing two alkyl chains as the hydrophobic tail and acetal as the linker by the fluorescence spectroscopy.

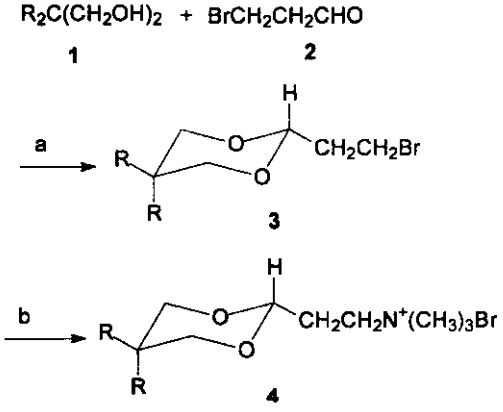
2 Experimental

2.1 Instruments

^{1}H NMR spectra were recorded with Bruker DMX-500 spectrometers with $CDCl_3$ as the solvent and TMS as the internal standard. IR spectra were recorded with Bruker Vector 220 infrared spectrometer. The elemental analysis was performed on the Perkin-Elmer 240C analytical instrument. The fluorescence spectra were measured with 970-CRT spectrofluorimeter.

2.2 Reagents

The bovine serum albumin (BSA) was purchased from Sigma and used as received. The bovine serum albumin solution (5.0 μmol/L) and phosphate buffer (pH = 7.0, 0.02 mol/L) were prepared in the triply distilled water. All the other reagents were analytical grade and the triply distilled water was used. Compound 3 was prepared by a literature procedure[18]. A solution of compound 3 (5 nmol) and 25 mL 30% trimethylamine-methanol in autoclave was heated at 65℃ for 24 h. After the methanol was removed, the residue was separated by silica-gel column with petroleum ether/ethyl acetate to give compound 4 (Scheme 1). The structures of 4a ~ 4c were characterized by IR, ^{1}H NMR and elemental analysis.



4a : R = C_6H_{13}, 4b : R = $C_{10}H_{21}$, 4c : R = $C_{14}H_{29}$

Scheme 1 Synthesis of 4. Reagents and conditions: a. p-TsOH, CH_2Cl_2, room temperature, 24 h, b. $N(CH_3)_3$, MeOH, 65 ℃, 24 h.

4a : ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, J = 7Hz, 6H, CH_3 ×2), 0.97 (m, 2H, CH_2CH_3), 1.10 ~ 1.30 (m, 16H, $(CH_2)_4$ ×2), 1.54 (m, 2H, CH_2), 2.08 (m, 2H, CH_2CH_2N), 3.38 (d, J = 11.5Hz, 2H, H_a), 3.49 (s, 9H, $N(CH_3)_3$), 3.64 (m, 2H, CH_2CH_2N), 3.75 (d, J = 11.5Hz, 2H, H_e), 4.68 (t, J = 4Hz, 1H, OCHO); IR (KBr) ν: 2956、2850、1493、1468、1419、1392、1108、721 cm^{-1}; Elemental analysis: calculated for $C_{21}H_{44}NO_2Br$: C 59.72%, H 10.43%, N 3.32%; found C 59.64%, H 10.27%, N 3.29%.

4b : ^{1}H NMR ($CDCl_3$, 500MHz) 0.88 (t, J = 7Hz, 6H, CH_3 ×2), 0.96 (m, 2H, CH_2CH_3), 1.10 ~ 1.30 (m, 32H, $(CH_2)_8$ ×2), 1.53 (m, 2H, CH_2), 2.08 (m, 2H, CH_2CH_2N), 3.38 (d, J = 11.5Hz, 2H, H_a), 3.50 (s, 9H, $N(CH_3)_3$), 3.65 (m, 2H, CH_2CH_2N),

3.75(d,J=11.5Hz,2H,H_e), 4.64(t,J=4Hz,1H, OCHO); IR (KBr) ν: 2958、2850、1492、1468、1419、1392、1110、721 cm^{-1}; Elemental analysis: Calculated for $C_{29}H_{60}NO_2Br$: C 65.17%, H 11.24%, N 2.62%; found C 65.25%, H 11.08%, N 2.57%.

4c: ^{1}H NMR ($CDCl_3$,500MHz) 0.88(t,J=7Hz, 6H,$CH_3\times2$), 0.96(m,2H,CH_2CH_3), 1.10~1.30 (m,48H,$(CH_2)_{12}\times2$), 1.54(m,2H,CH_2), 2.08 (m,2H,CH_2CH_2N), 3.37(d,J=11.5Hz,2H,H_a), 3.47(s,9H,$N(CH_3)_3$), 3.64(m,2H,CH_2CH_2N), 3.75(d,J=11.5Hz,2H,H_e), 4.68(t,J=4Hz,1H, OCHO); IR (KBr) ν: 2958、2850、1493、1465、1418、1392、1108、721 cm^{-1}; Elemental analysis: Calculated for $C_{37}H_{76}NO_2Br$: C 68.73%, H 11.76%, N 2.10%; found C 68.60%, H 11.65%, N 2.19%.

2.3 Fluorescence Measurements

The cationic lipid was dissolved in chloroform. After removing chloroform under a stream of nitrogen, the lipid was dried in vacuum for 3 h, then sonicated for 30 min after adding 5 mL of the triply distilled water. 1 mL phosphate buffer and 1 mL BSA (5 μmol/L) were added when it was cooled to room temperature. The mixture was diluted to 10 mL with the triply distilled water and maintained at 25℃ for 4 h. Its fluorescence intensity was measured in a 1 cm quartz cell with the excitation wavelength of 295 nm and emission wavelength from 300 nm to 450 nm. Both the excitation and emission band-pass widths were 10 nm. The wavelength scanning speed was set at 150 nm/min.

3 Results and discussion

The interactions between BSA and cationic lipids (4a, 4b, 4c) were studied in aqueous solution at room temperature by the steady-state fluorescence spectroscopy. The results are shown in Fig. 1. From the figures it can be seen that at low concentration of lipid a strong fluorescence quenching occurred. The fluorescence intensity decreased to 85%, 75% and 70% of the initial intensity for 4a, 4b and 4c, respectively. With 4b and 4c, when the concentration of lipids was more than 10^{-5} mol/L, the fluorescence intensity increased rapidly and even greater than the initial one, then the flu-

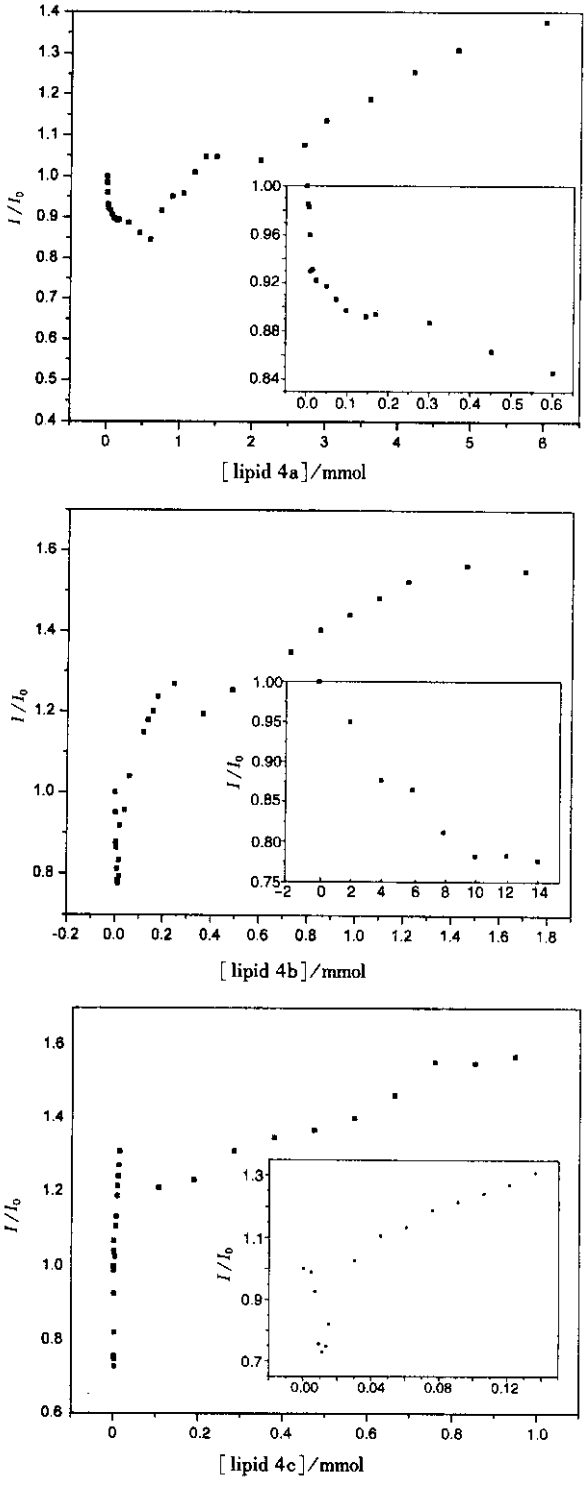


Fig. 1 The relative fluorescence intensity I/I_0 of BSA in the presence of different concentrations of lipid (4a,4b,4c) at the room temperature in buffer solution

I_0 is the fluorescence intensity of BSA in the absence of (4a,4b,4c), λ_{max} = 344 nm, I is the fluorescence intensity in the presence of (4a,4b,4c) with the different concentration.

orescence intensity (I/I_0 = 1.55) reached its maximum value (Fig. 1). But for cationic lipid 4a, the fluorescence intensity decreased at low concentration (Fig. 2), while at higher concentration the fluorescence intensity increased directly (Fig. 3). The fluorescence spectra of BSA in aqueous solution showed a maximum emission wavelength at 344 nm. Upon addition of cationic lipid, the maximum emission is blue-shifted from (344 ± 1) nm to (331 ± 1) nm. This observation indicates clearly that the polarity of the environment around tryptophan residues in BSA is much lower than that of the pure aqueous solution (Fig. 2).

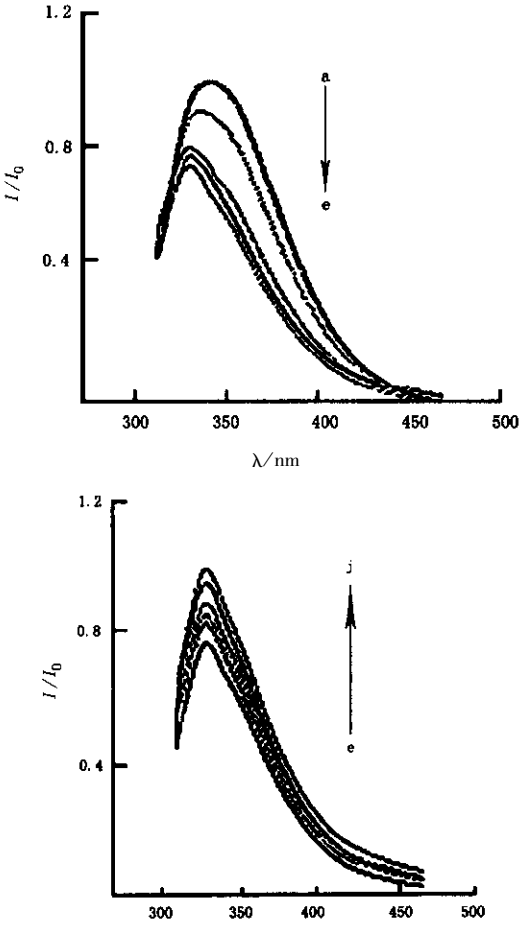
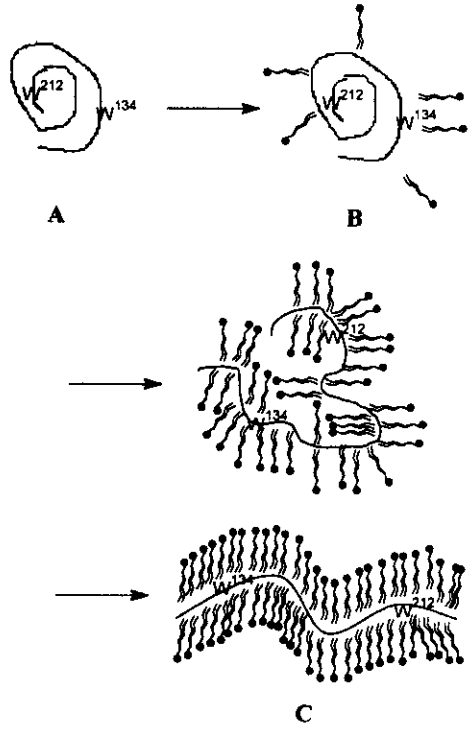


Fig. 2　The fluorescence spectra of BSA (0.5 μmol/L) in the absence of lipid 4a (a) and in the presence of lipid 4a with different concentration (μmol/L): b. 2.4, c. 4.8, d. 7.2, e. 9.6, f. 12, g. 14.4, h. 16.8, i. 19.2, j. 21.6.

It is well known there are two important fluorophores tryptophan residues W^{134} and W^{212} in BSA. In the native conformation of water-soluble protein BSA, a substantial fraction of hydrophobic side chains including the W^{212} moiety are typically buried in the interior of the protein. At low concentration of the cationic lipid, intermolecular interactions between cationic lipids and tryptophan residue (W^{134}) occurred with binding of lipids to BSA. In the changes near W^{134} residue, either a closer binding of lipid to W^{134} or changes in the vicinity of W^{134} can induce the fluorescence quenching. The maximum emission wavelength was blue-shift from (344 ± 1) nm to (331 ± 1) nm, indicating that there were some different species in the solution: native BSA, lipid/BSA complex and partially denatured BSA[14]. With binding of lipids to BSA, the environment of tryptophan residues changed and an increase of hydrophobicity in the vicinity of the tryptophan residues took place, blue shift occurred (Scheme 2).



Scheme 2　Schematic presentation of the interactions of lipids with BSA

When the concentration of cationic lipid increased, more binding of lipid occurred to BSA. The energy gained in this process[12] made protein uncoiling. In the uncoiling flexible conformation of BSA, part of hydrophobic moiety tryptophan residue (W^{212}) was exposed to bulk aqueous solution, BSA went from

its native coiled state A to uncoiled state B in Scheme 2, and the fluorescence intensity increased and even greater than the initial one. At higher concentration of cationic lipid, more and more lipid molecules bound to uncoiled BSA, two tryptophan residues were all enwrapped in vesicle C, and the fluorescence intensity of BSA did not change at maximum value ($I/I_0 = 1.55$). It was reported that in case of surfactant with the monoalkyl tail at low concentration binding of surfactant to W^{134} residue induces the fluorescence quenching, and the change around W^{212} residue is responsible for the quenching by anionic surfactant and a little enhancement by the cationic surfactant at high concentration[14]. For the cationic lipids (4a, 4b and 4c) synthesized in this work, the fluorescence increased rapidly with the concentration of lipid increasing. It is clear that the fluorescence enhancement of BSA is due to the interactions of tryptophan residues with the lipids. The lipids containing dialkyl tails could form the bilayer membrane. The typtophan residues were enwrapped partly in the bilayer membrane. With the increasing concentration of lipids, two tryptophan residues of BSA were all located in the bilayer membrane, the maximum fluorescence intensity was reached. Based on the observation and discussion above, we can conclude that the change in the conformation of protein BSA induced by the binding of lipids, in turn, which led to the change of fluorescence intensity.

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