Study on the Aggregation of Fluorinated Surfactant FC-134 on the Surface of DNA

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Abstract

The aggregation of perfluoroalkylsulfonyl quaternary ammonium iodide (FC134) on DNA was studied by spectral method. The results showed that it is a good way to determine DNA with FC134 by RLS method. This method is reliable, precise and simple. FT-IR spectra and UV-spectra were used to study the mechanisms of the interaction. The results indicated that the conformation of the DNA has changed during the interaction because the microenvironment of DNA changed.

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Keywords; Fluorinated surfactant; DNA; Resonance light scattering; Conformation.

1. Introduction

Interactions between DNA and surfactants have been studied extensively in recent years [1, 2]. It is very important in biotechnological and biomedical applications, particularly for the possibility of using such system for in vivo gene delivery and gene transfer [3, 4]. The importance of the study of aggregate formation between DNA and cationic surfactants become evident recently due to growing interest in the use of cationic lipids [5, 6], and particularly quaternary ammonium surfactants [7, 8] for the construction of liposomal genetic delivery systems.

DNA can form tight complex with surfactants through hydrophobic or electrostatic binding [9-11]. Among three different kinds of surfactants, i.e., cationic, anionic and nonionic surfactants, the interaction of cationic surfactant with DNA attracts the most attention [12]. Although, there is a great deal of work on the study on the interactions between DNA and hydrogenated surfactants [9-12], an important lack can be observed on characterization the interactions between DNA and fluorinated surfactants. In fluorinated
surfactants, all or most hydrogens in the hydrophobic tail are replaced by fluorine atoms. The substitution of the larger and highly electronegative fluorine atoms for the smaller hydrogen atoms increases the hydrophobicity of the surfactant and lowers CMC. The aim of this work is to give experimental results and further evidences of the interaction of fluorinated surfactant with DNA through RLS technique. Perfluoroalkylsulfonyl quaternary ammonium iodide (FC134) is chosen as fluorinated surfactant for it is a quaternary ammonium cationic surfactant.

On the other hand, the resonance light-scattering technique has been developed as a sensitive instrumental analysis method in the application of determination of DNA \cite{13,14} in the recent years. It was found in the RLS spectrum that when DNA was added, the intensity of RLS enhanced, and there was a linear relationship between the enhanced intensity and the concentration of nucleic acid. The extent to which a particle absorbs and scatters light depends on its size, shape, and index of refraction relative to the surrounding medium, and scattering. Scattering in each sphere is proportional to the square of the volume. \cite{15,16} It was concluded that the RLS technique was based on the aggregate of probe molecules on the surface of nucleic acid, which resulted in enhanced RLS intensity.

Therefore, the aim of this work is to give original experimental results of the interaction of DNA with fluorinated surfactant perfluoroalkylsulfonyl quaternary ammonium iodides (FC134) by RLS technique. It might also be helpful for the development of the application of fluorinated surfactants for biomedical purpose.

2. Experimental

2.1. Apparatus

The resonance light-scattering spectrum and the intensity of resonance light scattering were measured with a Shimadzu RF-540 spectrofluorometer (Kyoto, Japan). A WH-2 vortex mixer (Huxi Instrumental Co., Shanghai, China) was used to blend the solution. Conductance was measured by using a DDS-11A conductivity meter (Shanghai Rex Instrumental Co., China).

2.2. Reagents

All reagents were of analytical-reagent grade, made in China. The working solution of Fluoro Surfactant FC134 was 1.1×10^{-3} mol·L^{-1}. The stock solution of DNA was prepared by dissolving commercially purchased DNA (Sino-American biotechnology company, China,) in doubly distilled water at 0~4°C. The working solution of the DNA was 20mg·L^{-1}. Doubly distilled water was used throughout. Doubly distilled water was used throughout.

2.3. Method

Appropriate working DNA and FC134 solution were added to a 25mL volumetric flask. The mixture was diluted to 10mL with doubly distilled water and vortexed. Five minutes later, all the absorption and RLS measurements were obtained against the blank treated in the same way without DNA. The RLS spectrum was obtained by scanning simultaneously the excitation and emission monochromator of the RF-540 spectrofluorometer through the wavelength range 300~600 nm with Δλ=0 nm. The RLS intensity was measured at the maximum wavelength 370 nm.
3. Results and discussion

3.1. Spectral characteristics

Figure 1 was obtained according to the standard procedure. Fig.1 showed that FC134 had weak RLS peaks at 370 and 470 nm. When ctDNA was added, enhanced RLS peaks could be observed at 370 and 470 nm, which mainly resulted from the long range assembly of FC134 on the molecular surface of nucleic acid. The extent to which a particle absorbs and scatters light depends on its size, shape, and index of refraction relative to the surrounding medium, and scattering. Scattering in each sphere is proportional to the square of the volume. Therefore, the amount of scattering is directly proportional to the volume of each sphere. Thus, the larger the aggregation, the greater the scattering. FC134 is a positive ion in solution, according to literature, as a positively charged molecule, it has a condensing effect on nucleic acids. When the molar ratio of the FC134 to nucleic acid is rather high at a lower ionic strength, the FC134 molecule assembles and aggregates on the molecular surface of nucleic acid. This leads to long range assembly, which likely induces the formation of suprahelical structures of nucleic acids. When incident light shines on the suprahelical structures of nucleic acids, resonance occurs. Therefore, since the aggregation of FC134 on the molecular surfaces of nucleic acids produces large particles in size, strong enhanced resonance light scattering can be observed.

The experiment showed that the enhanced intensity of RLS took on an excellent linear relationship when the concentration of DNA was lower than 1.0 mg·L⁻¹ and concentration of FC134 was in the range of 2.2×10⁻⁶~1.1×10⁻⁵ mol·L⁻¹. Experiments showed when the concentration of FC134 was 6.6×10⁻⁶ mol·L⁻¹, the linear range and the correlation coefficient of the similar linear regression equation were both the best, as shown in Table 1. Therefore, it could be used for the determination of DNA with FC134 using RLS technique.

3.2. Aggregation of FC134 on DNA

Fig. 2 shows the plots of the conductivity against surfactant concentration. The inflection observed in curves at a certain concentration of FC134 is considered to be the CMC of the micelles. It is obvious that the CMC of FC134 is 1.27×10⁻⁴ mol/L. All experiment were conducted under the CMC of FC134, in order that FC134 in the solution was monomer.
Fig. 3 was the effect of molar ratio on the RLS intensity of FC134-DNA system. It showed that when the ratio of FC134/DNA in the range of 0.05-0.3, the RLS intensity of FC134-DNA system enhanced linearly. When the ratio is higher than 0.3, the RLS intensity began to decrease, and when the ratio continue, the RLS intensity did not change any more and keep invariance to increase in the range of 0.5-1.0. It could be concluded that FC134 and DNA formed a complex with a near proportion of 1:1. The RLS intensity first increased and then decreased, finally invariance. The phenomena can be explained that the FC134 ions absorbed electrostatically phosphate groups of opposite charge on the surface of DNA driving by hydrophobic interaction, inducing FC134 aggregation, which lead to the enhancement of RLS intensity. However, FC134 is fluorinated surfactant. Fluorinated surfactant has larger and highly electronegative fluorine atoms which enhance hydrophobic nature and the rigidity of the C-F bond is able to stiffen the perfluorooalkanoate chain, consequently it has strong hydrophobic interaction. Therefore, the conformation of DNA changed for the strong hydrophobic interaction of FC134 at high surfactant concentration during FC134 aggregation, which resulted in the decreased RLS intensity. When FC134 aggregated until saturation, the RLS intensity did not change any more, which could be considered to form a stable macromolecule complex.

Table 1 Effect of concentration of FC134 on the linear relationship

<table>
<thead>
<tr>
<th>concentration of FC134 (mol·L⁻¹)</th>
<th>Linear range of DNA (mg·L⁻¹)</th>
<th>linear regression equation</th>
<th>correlation coefficient</th>
<th>Detection limit (µg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2×10⁻⁶</td>
<td>0.4~0.8</td>
<td>I=6.75C+20.133</td>
<td>0.9563</td>
<td>22.2</td>
</tr>
<tr>
<td>4.4×10⁻⁶</td>
<td>0.016~0.8</td>
<td>I=19.63C+24.017</td>
<td>0.9756</td>
<td>7.6</td>
</tr>
<tr>
<td>6.6×10⁻⁶</td>
<td>0.068~1.0</td>
<td>I=41.83C+20.819</td>
<td>0.9993</td>
<td>3.5</td>
</tr>
<tr>
<td>8.8×10⁻⁶</td>
<td>0.076~1.0</td>
<td>I=40.16C+22.457</td>
<td>0.9983</td>
<td>3.8</td>
</tr>
<tr>
<td>1.1×10⁻⁵</td>
<td>0.08~1.0</td>
<td>I=37.85C+25.016</td>
<td>0.9984</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Fig. 4 showed the effect of the ratio of FC134/DNA on the A₂₆₀/A₂₉₅ of FC134-DNA system. It could be seen that the ratio of A₂₆₀/A₂₉₅ decreased sharply and keep constant with the increase of FC134 concentration, which indicated a specific influence of FC134 on DNA secondary structure. It was the proof of the conformation of DNA transformation from B type to Z type form.

DNA: 2.2×10⁻⁶ mol·L⁻¹

Fig. 4 The effect of the ratio of FC134/DNA on the A₂₆₀/A₂₉₅

DNA: 5.7×10⁻⁵ mol·L⁻¹

Fig. 4 showed the effect of the ratio of FC134/DNA on the A₂₆₀/A₂₉₅ of FC134-DNA system. It could be seen that the ratio of A₂₆₀/A₂₉₅ decreased sharply and keep constant with the increase of FC134 concentration, which indicated a specific influence of FC134 on DNA secondary structure. It was the proof of the conformation of DNA transformation from B type to Z type form.
Fig. 5 and Fig.6 were UV spectra of FC134–DNA system. Fig.5 showed that the light absorption of a DNA system increased obviously at a wavelength of 225 nm when DNA was added. This may be explained by the fact that FC134 aggregated on the surface of the nucleic acid, which is in accordance with the enhanced RLS intensity (Fig.1). According to literature \cite{18}, enhanced absorption exposed the electrostatic interaction. As FC134 is a cationic surfactant, phosphate group of DNA could attract electrostatically FC134. It could be concluded that electrostatic interaction is one of the driving forces of FC134 aggregating on the surface of DNA. Fig.6 showed the effect of FC134 concentration on the absorption of DNA. It could be seen that with the increase of the concentration of FC134, the absorbance of DNA at 260 nm increased. As FC134 has an absorption at 225 nm, the peak shape of DNA at 260 nm was affected, but it could be still observed that the absorption increased. Besides, the absorption also increased when wavelength is beyond 320 nm. The increase in absorbance at $\lambda > 320$ nm with the increase of the concentration of the FC134 resulted from the formation of compact light scattering particles \cite{19}.

3.3. UV Spectra of FC134-DNA system

![Figure 5: Absorption spectra of FC134–DNA system.](image)

![Figure 6: The UV spectra of FC134-DNA complex.](image)

FC134: $1.1 \times 10^{-5}\text{mol}\cdot\text{L}^{-1}$; DNA(1-2):0, 0.4$\text{mg}\cdot\text{L}^{-1}$. DNA:0.8$\text{mg}\cdot\text{L}^{-1}$; FC134(1-3):0, $1.1 \times 10^{-5}$, $1.5 \times 10^{-5}\text{mol}\cdot\text{L}^{-1}$;

4. Conclusion

It is of great importance to study the binding of surfactant with DNA in Gene therapy, not only for the treatment of diseases with genetic defects, but also in the development of strategies such as cancer, cardiovascular diseases, and rheumatoid arthritis. Especially, fluorinated surfactants often occur together with proteins in the formulations of the chemical, biosciences, cosmetic and medical industries, so these applications of fluorinated surfactants demand the further understanding on their interactions with biomacromolecules. It has never been reported the interaction between fluorinated surfactants and DNA, therefore, the interaction between FC134 and DNA is studied in this paper. The determination of macro amount DNA with FC134 by resonance light scattering method is proposed. This method is sensitive and convenient. FC134 is a new kind probe for the determination of DNA. Besides, UV-spectra was used to study the effect of the concentration of FC134 on the conformation transition of DNA, and IR spectra proved the FC134-DNA complex had been formed and the interaction had altered the conformation of DNA. The experiments results showed that FC134 aggregated on the surface of DNA through hydrophobic interaction and electrostatic interaction to form a hydrophobic complex, thus changing the
conformation of DNA from B type to Z type. Therefore, FC134 could be used as a non-viral vector which will be studied in further work.

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References