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Neutron reflectivity studies on the DNA adsorption on lipid monolayers at the air–liquid interface

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Abstract

In situ neutron reflectivity was used to study the DC-Chol and TC-Chol monolayers at the air–liquid interface in the presence and absence of DNA in the subphase. It was found that the DC-Chol is more effective in adsorbing the DNA than the TC-Chol. It was also found that a compact DNA layer formed beneath the DC-Chol monolayer with a DNA gap spacing around 20 Å and a less compact DNA layer adsorbed to the TC-Chol monolayer with a DNA spacing around 60 Å, as estimated from the determined neutron scattering length density. From the determined neutron scattering length density profiles, the adsorbed DNA somewhat penetrates into the head group region of the charged lipids.
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Keywords: DC-cholesterol; TC-cholesterol; Langmuir–Blodgett film; Neutron reflectivity; DNA adsorption

1. Introduction

Recently, many studies have been made on the cationic lipid–DNA complex due to the noviral gene therapy [1]. These studies focused on the structure and interaction of cationic lipid–DNA complex in solution. However, there were only limited numbers of studies on the lipid–DNA interaction at the air–liquid interface. Some studies used the Langmuir–Blodgett technique to investigate the cationic lipid–DNA interaction at the air–liquid interface [2–8]. As revealed by atomic force microscopy studies, the DNA adsorbed to a supported cationic bilayer could form aligned two-dimensional structure with regular spacing [2]. By X-ray diffraction, Okahata et al. [6] found that the DNA molecules adsorbed to the cationic $2C_{18}$-glu-N+ amphiphile monolayer form aligned structure parallel to the dipping direction with spacing of 41 Å. The in situ X-ray reflectivity studies by Kago et al. [7] on the dimethyldioctadecylammonium lipid monolayer–DNA interaction showed that the adsorbed DNA could be modeled as two layers with a first layer of about 25 Å containing more DNA and a second layer of about 11 Å containing less DNA. A wavy conformation structure of the adsorbed DNA was suggested according to the finding of the two-layer model. The in situ grazing-incident-diffraction studies on the TODAB–DNA interaction at the air–liquid interface showed that the DNA adsorbed under cationic lipid monolayer at air–water interface has ordered structure with a spacing decreased from about 40 to 32 Å as the pressure increases from 10 to 50 mN/m [3].

There is very little or no studies so far on the structure and the interaction of DC-Cholesterol (DC-Chol) with DNA at the air–liquid interface, while DC-Chol is widely used in forming lipid/DNA complex for gene therapy-related studies. The purpose of this paper is to investigate the interaction and structure of DC-Chol monolayer in the presence and absence of DNA at air–water interface. The lipid–DNA interaction is different at the air–liquid interface from the interaction in bulk solutions where DNA interacts with lipid bilayer in both sides.

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In situ neutron reflectivity was used to study the DC-Chol and TC-Chol monolayers in the presence and absence of DNA in the subphase. The reflectivity measurements could provide information about the depth density profile, the thickness and roughness of the thin film [9]. Detailed results and discussions will be presented.

2. Experimental

Linear DNA-type XIV from herring testes sodium salt (Na content 6.2%, H_2O content 4.5%) was purchased from Sigma and used without further purification. Its molecular weight is between 400 and 1000 base pairs with a center of distribution at 700 bps [10]. DC-Chol (3-β-[*N*-(*N*′,*N*′-dimethylethane)carbamoyl]cholesterol) with molecular weight of 537.3 and TC-Chol (3-β-[*N*-(*N*′,*N*′,*N*′-trimethyl-ethane)carbamoyl]-cholesterol) with molecular weight of 551.3 were purchased from Sigma and used without further purification. D_2O was purchased from Sigma. The DNA solution was prepared to the concentration of 1 μM in D_2O and treated with sonication for 30 min for complete dissolving.

In situ neutron reflectivity measurements were carried out at the time-of-flight reflectometer ARISA [11], KENS, KEK, Japan. A Langmuir trough was used in the in situ reflectivity measurements. The temperature of the trough for all measurements is controlled at 20 ± 0.1 °C by external water circulation. The surface pressure was kept at 21 mN/m for the TC-Chol monolayer, 18 mN/m for the TC-Chol/DNA monolayer, and 25 mN/m for the DC-Chol/DNA monolayer.

3. Results and discussions

Fig. 1 shows the in situ neutron reflectivity data of cationic cholesterol monofilms at the air–liquid interface in the absence and presence of DNA in the subphase. The

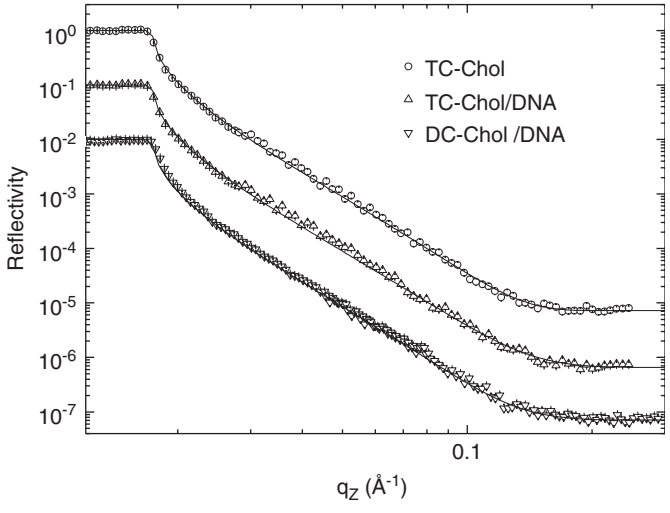

Fig. 1. In situ neutron reflectivity of TC-Chol and DC-Chol monolayers at the air–liquid interface with and without DNA in the subphase.

Table 1
The data fitting results of neutron reflectivity

	Layer	Thickness (Å)	NSLD ($\times 10^{-6}$ Å^{-2})	σ (Å)
TC-Chol	1	12	2.0	2
	2	6	3.0	2
	D_2O	—	6.0	2
TC-Chol/DNA	1	10	1.5	2
	2	4	2.5	2
	3	12	5.5	3
	D_2O	—	6.0	10
DC-Chol/DNA	1	10	1.5	2
	2	4	2.5	2
	3	12	5.0	2
	D_2O	—	6.0	8

Parratt32 (developed by Hahn-Meitner Institute) fitting program is used to analyze the reflectivity data. The fitted curves are also shown as solid lines. The fitting results are listed in Table 1. The reflectivity curves for TC-Chol, TC-Chol/DNA and DC-Chol/DNA all look similar. However, there is still difference in the reflectivity curves.

For the pure TC-Chol monofilm, a two-layer model is used to fit the reflectivity data. The first layer containing the hydrocarbon tail has a thickness of 12 Å and a neutron scattering length density (NSLD) of 2×10^{-6} Å^{-2}. The second layer containing the hydrophilic head group of the charged lipid has a thickness of 6 Å and a NSLD of 3×10^{-6} Å^{-2}. The head group layer may also contain some D_2O. This would cause the NSLD of the head group layer to become higher than the NSLD of the head groups alone.

In the presence of DNA in the subphase, the reflectivity data can be fitted with a three-layer model. Both the TC-Chol/DNA and DC-Chol/DNA cases have similar structure. The layer thickness is determined to be 10, 4 and 12 Å, respectively, for the layers 1, 2 and 3. Layer 1 should contain the hydrocarbon tails of the charged lipids. Layer 2 should contain the hydrophilic head group, D_2O and part of the adsorbed DNA, while layer 3 should contain the adsorbed DNA and D_2O. This can be judged from the NSLD determined from the fitting. The NSLD of the first layer is found to be 1.5×10^{-6} Å^{-2} and the NSLD of the second layer is 2.5×10^{-6} Å^{-2} for both cases. However, the NSLD for the third layer is higher for TC-Chol/DNA case, 5.5×10^{-6} Å^{-2} and it is 5.0×10^{-6} Å^{-2} for the DC-Chol/DNA case. The NSLD of DNA along is around 4×10^{-6} Å^{-2}. This means if there is more DNA adsorption (a compact DNA layer) underneath the lipid monolayer, the NSLD of this layer will deviate from the NSLD of D_2O toward the NSLD of DNA. Thus, it is obvious that the DC-Chol is more effective in adsorbing the DNA than the TC-Chol. It would need about 50% of the volume in this layer to be occupied by the DNA to cause the NSLD to drop from the pure D_2O (6×10^{-6} Å^{-2}) value to 5.0×10^{-6} Å^{-2} for the DC-Chol/DNA case. Considering that the diameter of the DNA is about 20 Å, the spacing between the DNA would be around 20 Å or even smaller if

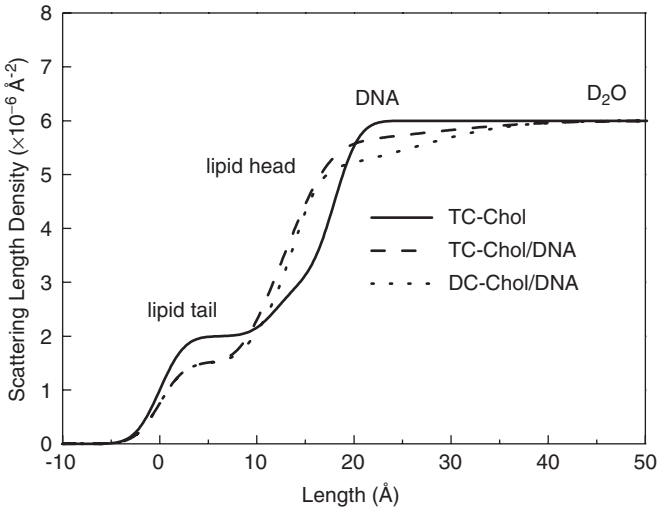

Fig. 2. The NSLD profiles determined from the in situ neutron reflectivity data.

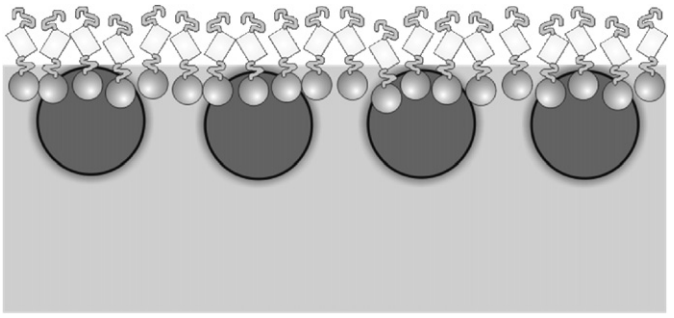

Fig. 3. A probable structure of DC-Chol/DNA at air–water interface.

we further consider the water molecules contained within the DNA diameter (in the groves of the DNA). Similarly, the adsorbed DNA would occupy about 25% of the space in this layer for the TC-Chol case. The spacing between the adsorbed DNA would be at least 60 Å for the TC-Chol case.

The roughness of the D_2O layer becomes much larger in the presence of the DNA in the subphase. The roughness of the D_2O layer is 10 Å for the TC-Chol/DNA case, and is 8 Å for the DC-Chol/DNA case, which are larger than the 2 Å for the TC-Chol case. The NSLD profiles determined from the in situ neutron reflectivity data are shown in Fig. 2. It is easier to see the actual structural from the density profiles than the meaning of the layer thickness and roughness values. From the density profiles, one can see that the presence of DNA would cause the NSLD in the 10–20 Å region (corresponding to the head group region) to increase. This indicates the presence of DNA in this region and the DNA penetrates into the head group region. The NSLD in the 20–30 Å region is lowered due to the presence of DNA adsorption. As discussed previously, this originally pure D_2O region is now partly occupied by DNA. According to the above discussions, a probable structure of DC-Chol/DNA at air–water interface is shown in Fig. 3.

4. Conclusions

In situ neutron reflectivity was used to study the DC-Chol and TC-Chol monolayers at the air–liquid interface in the presence and absence of DNA in the subphase. It was found that the DC-Chol is more effective in adsorbing the DNA than the TC-Chol. It was also found that a compact DNA layer formed beneath the DC-Chol monolayer with a DNA gap spacing around 20 Å and a less compact DNA layer adsorbed to the TC-Chol monolayer with a DNA spacing around 60 Å, as estimated from the determined NSLD. From the determined NSLD profiles, the adsorbed DNA somewhat penetrates into the head group region of the charged lipids.

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