Available online at www.sciencedirect.com
ScienceDirect
Physica B 385–386 (2006) 841–844
PHYSICA B
www.elsevier.com/locate/physb

Neutron and X-ray reflectivity studies on DNA adsorption on mixed DPPC/DC-Cholesterol monolayers

Jui-Ching Wu[a], Tsang-Lang Lin[a,*], U-Ser Jeng[b], Hsin-Yi Lee[b], Thomas Gutberlet[c]

[a]*Department of Engineering and System Science, National Tsing Hua University, Hsinchu 300, Taiwan*
[b]*National Synchrotron Radiation Research Center, Hsinchu 300, Taiwan*
[c]*Paul Scherrer Institut, CH-5232 Villigen-PSI, Switzerland*

Abstract

We have studied DNA adsorption on mixed DPPC/DC-Chol monolayers. Solid supported mixed monolayers on silicon wafers were prepared using Langmuir–Blodgett (LB) dipping technique. Neutron and X-ray reflectivity measurements were used to characterize these LB monofilms. For LB monofilms with DNA adsorption, the reflectivity data of the DPPC/DNA film are very close to that from the DPPC film, which indicates only minor DNA adsorption on the pure DPPC monolayer. Increasing the percentage of DC-Chol, film thickness increases. The DC-Chol/DNA film is thicker than the pure DC-Chol film (film thickness 18 Å) by about 9 Å due to the presence of adsorbed DNA. A model is presented to explain the structure of the lipid/DNA film.
© 2006 Elsevier B.V. All rights reserved.

PACS: 87.14.Gg

Keywords: DNA adsorption; DPPC; DC-cholesterol; Mixed monolayer; Langmuir–Blodgett film; Reflectivity

1. Introduction

In recent years, DNA interaction with lipids has been widely studied due to the potential application in gene therapy [1–5]. It was found that using cationic lipids, such as DODAB [1], DOTAP [2] and DC-cholesterol (3β-[*N*-(dimethylaminoethane)-carbamoyl]-cholesterol) [5], result in higher transfer efficiency. The synthetic charge DC-Chol (DC-cholesterol) has been widely used in gene therapy studies. So far, there have only been a few studies on the interaction of DNA with lipid monolayer at the air–liquid interface. The interaction of DNA with lipid monolayer at the air–liquid interface differs from the interaction with lipid bilayers (in bulk solution). In the former case, DNA interacts only on one side of the DNA, not on both sides as is the case in bilayers. The purpose of this study is to understand the interaction of the mixed DPPC/DC-Chol monolayer with DNA and the structure of such mixed complex LB monofilms.

X-ray and neutron reflectivity measurements were employed to study the mixed DPPC/DC-Chol LB monolayer supported on silicon wafer with and without DNA adsorption. The reflectivity measurements provide information about the depth density profile, thickness and roughness of the thin monofilm. Detailed results will be presented and discussed.

2. Experimental

Linear DNA-type XIV from herring testes sodium salt (Na content 6.2%, H_2O content 4.5%) was purchased from Sigma. Its molecular weight is between 400 and 1000 base pairs with a center of distribution at 700 bps [6]. DC-Chol was purchased from Sigma. Deuterated DPPC (dDPPC) was purchased form Cambridge Isotope Laboratories, Inc. The DNA solution was prepared at a concentration of 1 μM in de-ionized water and sonicated for 30 min. Surface pressure–area isotherms were measured with a Langmuir-Blodgett trough (type 601B, NIMA). The temperature of the trough for all experiments was controlled at 20 ± 0.1 °C by external water circulation. LB films for reflectivity

*Corresponding author. Tel.: +886 3 5742671; fax: +886 3 5728445.
E-mail address: tllin@mx.nthu.edu.tw (T.-L. Lin).

0921-4526/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.physb.2006.05.121

measurements were transferred onto silicon wafers at constant pressure.

X-ray reflectivity measurements were carried out at the in-house X-ray laboratory at the National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan. Neutron reflectivity measurements were carried out at SINQ, PSI, Switzerland, using the neutron reflectometer AMOR. The parameters of the surface oxide layer used in the data fitting for all cases are thickness 5 Å, SLD (scattering length density) 14.3×10^{-6} Å^{-2} for X-ray and 3.4×10^{-6} Å^{-2} for neutron. The Parratt32 (Hahn-Meitner Institute) fitting program is used to analyze the reflectivity data.

3. Results and discussions

Both neutron and X-ray reflectivity fitting results are listed in Tables 1 and 2. The total film thickness can be more accurately determined from the X-ray reflectivity data due to the much wider q-range covered by X-ray reflectivity measurements. The neutron reflectivity data were limited in q-range; however, the neutron reflectivity could distinguish the deuterated hydrocarbon layer and the hydrophilic/DNA layer better than X-rays due to the difference in their neutron scattering length densities. Much more reliable results can be obtained by such contrast variations using both the neutron and X-ray reflectivity measurements simultaneously.

The X-ray reflectivity data of dDPPC and DC-Chol with and without DNA on silicon wafer are shown in Fig. 1. The fitting curves are shown as solid lines. There is almost no difference in the reflective curves between the dDPPC film and the dDPPC/DNA film. This indicates that only very minor amounts of DNA adsorb to the dDPPC monolayer film. This can be attributed to the weak interaction between the zwitter-ionic dDPPC head group and the negatively charged DNA. For the DC-Chol/DNA film, it is easy to see from the X-ray reflectivity data that the film thickness is larger than the film thickness of DC-Chol monolayer. The neutron reflectivity data of mixed dDPPC/DC-Chol with and without DNA are shown in Figs. 2 and 3. The fitting curves are shown as solid lines. Both the X-ray data and the neutron data can be fitted with the same structural models but with different scattering

Table 1
Reflectivity data fitting results of mixed dDPPC/DC-Chol films without DNA

dDPPC/DC-Chol	Layer*	Thickness (Å)	X-ray SLD ($\times 10^{-6}$ Å^{-2})	Neutron SLD ($\times 10^{-6}$ Å^{-2})	σ (Å)
100:0	1	18	9.1	7.6	2.7
	2	6	13.0	1.6	2.0
80:20	1	18	9.9	7.2	2.0
	2	5	13.7	1.6	3.5
50:50	1	17.2	10.4	6.8	3.0
	2	4	13.3	1.6	3.0
20:80	1	17	9.1	5.0	3.0
	2	3	13.0	1.0	3.0
0:100		18	11.6	5.0	3.6

*Layer 1 is lipid tail. Layer 2 is lipid head.

Table 2
Reflectivity data fitting results of mixed dDPPC/DC-Chol films with DNA

dDPPC/DC-Chol	Layer*	Thickness (Å)	X-ray SLD ($\times 10^{-6}$ Å^{-2})	Neutron SLD ($\times 10^{-6}$ Å^{-2})	σ (Å)
100:0/DNA	1	18	9.7	7.6	2.0
	2	6	13.3	1.6	3.5
80:20/DNA	1	18	8.2	6.4	2.0
	2	5	12.8	3.8	3.0
50:50/DNA	1	16	9.4	6.0	3.8
	2	7.2	11.3	3.8	2.0
20:80/DNA	1	16	8.2	4.6	3.0
	2	9	12.0	3.8	3.0
0:100/DNA	1	16	8.0	4.5	4.6
	2	11	9.2	3.8	2.0

*Layer 1 is the top layer (mostly the lipid tails). Layer 2 is the second layer (lipid head/DNA).

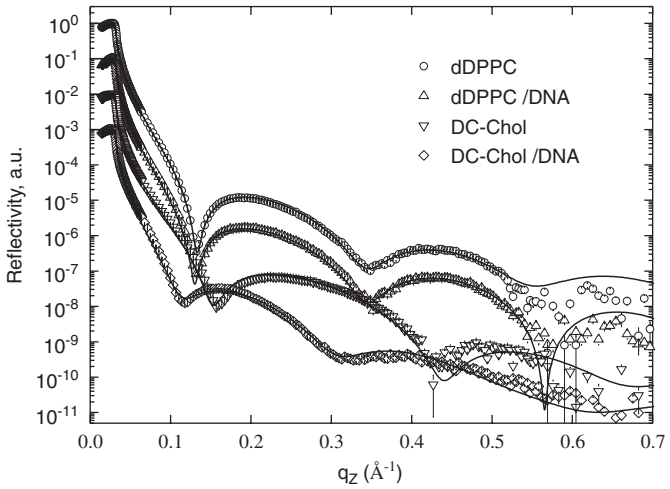

Fig. 1. X-ray reflectivity of dDPPC and DC-Chol films with and without DNA adsorption on silicon wafer.

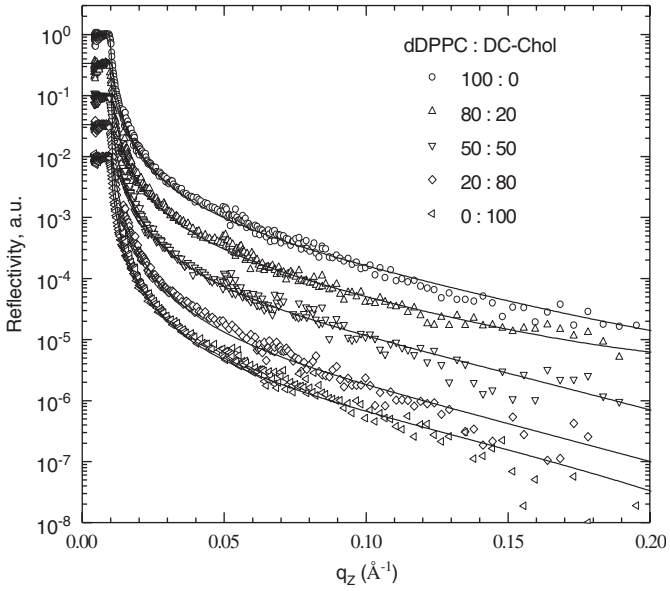

Fig. 2. Neutron reflectivity of mixed dDPPC/DC-Chol films on silicon wafer.

length densities. Due to the adsorption of DNA, the total film thickness is larger than the pure monolayer film thickness. The increment in film thickness is 0, 0, 2, 5 and 9 Å, respectively for the 0%, 20%, 50%, 80% and 100% DC-Chol mixed monolayer. It seems that the percentage of DC-Chol needs to be much higher than 50% to be more effective to adsorb the DNA. Even for the 80% DC-Chol, the effectiveness of DNA adsorption can still not be compared with the100% DC-Chol monolayer.

For neutron reflectivity, it is easy to distinguish dDPPC and DC-Chol due to the deuterated tails of dDPPC with higher SLD. Besides, DNA also has a high neutron SLD of about 4×10^{-6} Å^{-2}. Hence, it is easy to distinguish the lipid head group region and DNA by neutrons. As shown in the fitted results, the neutron SLD of the hydrocarbon region of the mixed lipids decreases with increasing DC-Chol

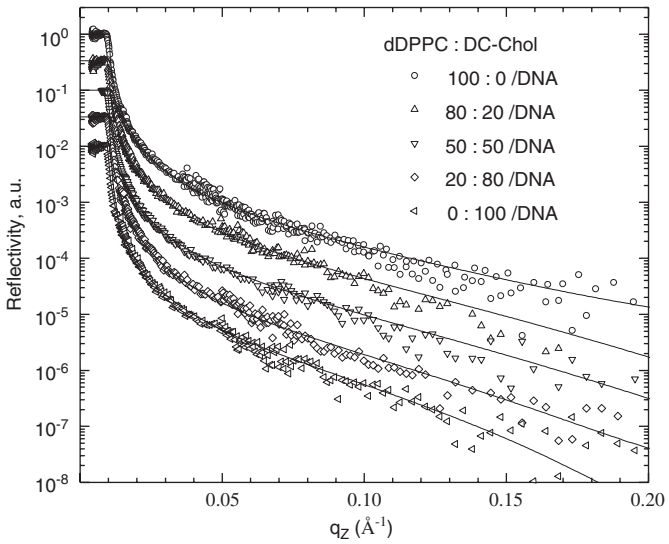

Fig. 3. Neutron reflectivity of mixed dDPPC/DC-Chol/DNA films on silicon wafer.

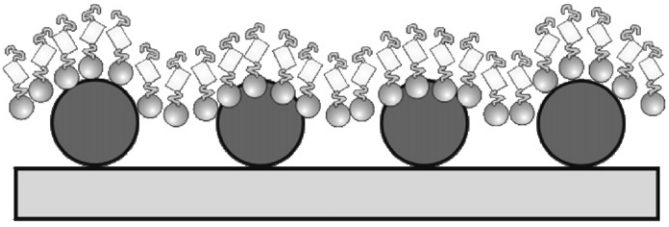

Fig. 4. Schematic model of the LB monofilm of DC-Chol/DNA on silicon wafer.

percentage. For the mixed lipid with DNA adsorption, the neutron SLD of the second layer containing the lipid head group and the DNA is significantly higher than the neutron SLD of the second layer (lipid head group) of mixed lipid monolayer without DNA adsorption. This also indicates the presence of DNA adsorbed.

For the DC-Chol/DNA LB film, the first layer thickness is 16 Å and the second layer thickness is 11 Å. However, the overall diameter of DNA is around 20 Å. As depicted in the model shown in Fig. 4, there should be some spacing between neighboring DNA due to charge repulsion between DNA molecules. Therefore, firstly, the DC-Chol monolayer could be modulated conformably with the DNA layer. Secondly, the DC-Chol head group could dip slightly into the DNA grooves. These factors cause the total film thickness of the DC-Chol/DNA LB film to be smaller than the sum of the film thickness of DC-Chol 18 Å and the DNA diameter 20 Å [7]. Part of the DNA should be counted in the first layer and most of the DNA is counted in the second layer.

4. Conclusions

LB monofilms of mixed dDPPC/DC-Chol monolayer with and without absorption of DNA on silicon wafer were

studied by both neutron and X-ray reflectivity. From neutron and X-ray reflectivity results, the mixed film thickness is found to decrease as the percentage of DC-Chol increases. With the adsorption of DNA, the film thickness is larger than films without DNA adsorption. It seems that a high percentage of DC-Chol is needed in the mixed monolayer to be more effective in adsorbing DNA.

Acknowledgements

We would like to thank the NSRRC for the X-ray reflectivity measurements. Part of this work was performed at the SINQ, Paul Scherrer Institut, Villigen Switzerland. This research is supported by the NSC, grant nos. NSC93-2113-M-007-018 and NSC94-2623-M-007-014, and the Ministry of Education, ROC, Grant no. 91-I0037-J4.

References

[1] D.D. Lasic, H. Strey, M.C.A. Stuart, R. Podgornik, P.M. Frederik, J. Am. Chem. Soc. 119 (1997) 832.
[2] J.O. Rädler, I. Koltover, T. Salditt, C.R. Safinya, Science 275 (1997) 810.
[3] C.S. O'Hern, T.C. Lubensky, Phys. Rev. Lett. 80 (1998) 4345.
[4] B.J. Battersby, R. Grimm, S. Huebner, G. Cevc, Biochim. Biophys. Acta. 1372 (1998) 379.
[5] C.M. Wu, H.L. Chen, T.L. Lin, W. Liou, J.S. Lin, Langmuir 20 (2004) 9432.
[6] R. Dias, S. Mel'nikov, B. Lindman, M.G. Miguel, Langmuir 16 (2000) 9577.
[7] K. Kago, H. Matsuoka, R. Yoshitome, H. Yamaoka, K. Ijiro, M. Shimomura, Langmuir 15 (1999) 5193.