Interaction between nitric oxide and lipid-like DDPA LB film investigated with SHG and AFM

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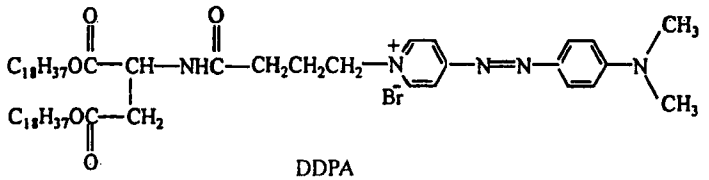
Interactions between Nitric oxide (NO) and DDPA Langmuir-Blodgett (LB) film are investigated with second harmonic generation (SHG) and atomic force microscopy (AFM). It has been found that the adsorption of NO molecules on DDPA LB film only changes the value of the second-order susceptibility of the DDPA molecule on film but not its orientation.

Keywords NO molecule, adsorption, LB film, second harmonic generation (SHG), atomic force microscopy (AFM)

Introdution

Nitric oxide (NO) is an important bio-regulatory molecule. It has been implicated in many physiological processes including vascular smooth muscle relaxation,[1-4] neuronal communication,[5] immune regulation,[6-8] olfactory memories formation,[9,10] *etc*. Biological roles of NO molecule have been studied extensively by many groups.[1-13] However, the details on the interaction of NO with bio-membrane or protein have not yet been understood thoroughly. Second harmonic generation (SHG) is a surface-specific and *in-situ* technique to study the reactions and the properties of molecules on the surface.[14,15] Atomic force microscopy (AFM), a surface probe technique as well, has been widely used to study the surface morphology of lipid monolayers and bilayers.[16-19] Here, a lipid-like molecule, dioctadecyl-*N*-[4-(*p*-*N*,*N*-dimethylaminophenylazo pyridiniumbutyryl)-*L*-aspartate bromide (DDPA), which has large second-order susceptibility,[20,21] was selected to simulate the phospholipid molecule.

The interactions between NO and the DDPA Langmuir-Blodgett (LB) film are investigated with SHG and AFM. We report a novel phenomenon that the adsorption of NO only changes the value of the second-order susceptibility of the DDPA molecule on film but not its orientation.



DDPA

Experimental

The experimental setups of SHG measurements have been described elsewhere previously.[22,23] Monolayer *Z*-type mode DDPA LB films were deposited on the hydrophilically pretreated quartz substrates[24,25] at a con-

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stant surface pressure (30 $mN \cdot m^{-1}$) by the vertical dipping method on a British NIMA Langmuir-Blodgett 622 trough. The subphase is pure water (pH = 5.6), and the surface-pressure *via* area (π-A) curve of the DDPA monolayer has been shown elsewhere.[20,21] The AFM measurements of the DDPA LB films were performed in a tapping mode with a Nanoscope IIIa (Digital Instruments) equipped with a bioscope G scanner (90 μm). The 2.0% NO/N_2 (mol/mol) mixture used in this experiment was purchased from the National Research Center for Certified Reference Materials of China.

Results and discussions

Fig. 1 shows the decay curve of the SHG electric intensity with *p-p* combination after the injection of NO/N_2 mixture into the gas cell where the DDPA LB film was located. The open circles are the experimental data, and point B is the time origin of injection. The pressure of the gas cell was maintained at $(8.7 \pm 0.4) \times 10^4$ Pa, which was measured by a Baratron (MKS). The inset shows the stabilization of the signal from the DDPA LB film when no

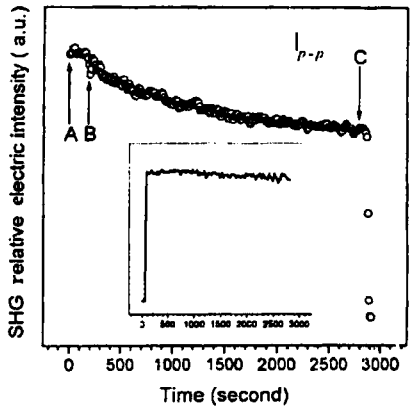

Fig. 1 Decay curve of the SHG electric intensity with the *p-p* combination after the injection of the NO/N_2 into the gas cell where the DDPA LB film was located. The inset shows the stabilization of the signal from the DDPA LB film under laser irradiation.

NO-film interaction was introduced. The SHG signal can not be recovered when the NO/N_2 mixture was pumped out from the gas cell, and then it remains the same after long time (1 h) pumping, showing that the NO molecules can be strongly adsorbed on the DDPA LB film. From Fig. 1, it is found that the SHG electric intensity decreases with the increasing of the interaction time between NO and the DDPA LB film, which shows some typical characteristrics of adsorption. The adsorption process can be symbolically described by

$$\mathrm{DDPA} + \mathrm{NO(gas)} \xrightarrow{k} \mathrm{DDPA \cdot NO}$$

The rate equations can be written as

$$\frac{d[\mathrm{DDPA}]}{dt} = -k[\mathrm{DDPA}][\mathrm{NO}] \quad (1)$$

$$\frac{d[\mathrm{NO}]}{dt} = 0 \quad (2)$$

$$\frac{d[\mathrm{DDPA \cdot NO}]}{dt} = k[\mathrm{DDPA}][\mathrm{NO}] \quad (3)$$

where [DDPA], [NO] and [DDPA·NO] stand for the concentrations of DDPA, NO and DDPA·NO, respectively and k is the rate constant. The initial conditions are

$$[\mathrm{DDPA}] = [\mathrm{DDPA}]_0, [\mathrm{NO}] = [\mathrm{NO}]_0, [\mathrm{DDPA \cdot NO}] = 0$$

From Eqs. (1), (2) and (3) and the initial conditions, the concentrations of DDPA and DDPA·NO can be derived as

$$[\mathrm{DDPA}] = [\mathrm{DDPA}]_0 \exp(-k[\mathrm{NO}]_0 t) \quad (4)$$

$$[\mathrm{DDPA \cdot NO}] = [\mathrm{DDPA}]_0 (1 - \exp(-k[\mathrm{NO}]_0 t)) \quad (5)$$

Then the total SHG electric intensity can be given as

$$I = I_a[\mathrm{DDPA}] + I_b[\mathrm{DDPA \cdot NO}] = [\mathrm{DDPA}]_0 (I_b + (I_a - I_b)\exp(-k[\mathrm{NO}]_0 t)) \quad (6)$$

where I_a and I_b are the SHG electric intensities from unit concentration of [DDPA] and [DDPA·NO], respectively. The solid line shown in Fig. 1 is the simulation result based on Eq. (6). The simulated parameters are $I_a : I_b = 1.5$, $k = 1.3\ s^{-1} \cdot mol^{-1} \cdot L^3$ which is within the

range of other studies in biological systems.[26,27] The above analysis indicates that the decay of the SHG electric intensity of the DDPA LB film is due to the adsorption of NO. The detected molecular averaged orientation angles at points A and C are $38.3 \pm 0.4°$ and $38.5 \pm 0.4°$ respectively, showing that the adsorption of NO does not destroy the structure of the DDPA LB film. The adsorption of NO on the DDPA LB film only changes the value but not the orientation of the second-order susceptibility of the DDPA molecules on film. The SHG electric intensity with $s-p$ combination of the DDPA LB film displays the similar decay after NO/N_2 mixture injection.

The tapping mode AFM images of the DDPA LB film on quartz substrates are given in Fig. 2. The quartz substrate has been examined by AFM, and the surface roughness is found to be less than 0.5 nm. Fig. 2 (A) and (B) are the typical images of the DDPA LB film before and after NO/N_2 mixture injection. In both images, the brighter area corresponds to the DDPA film and the darker area corresponds to the defects. The thickness and coverage of the DDPA LB film are nearly equal. No evidence of the LB film destroyed (film detachment or aggregation) is found by AFM investigation after NO/N_2 mixture injection. In present resolution, the DDPA LB film retains its basic membrane structure after NO/N_2 mixture exposure. This is consistent with the SHG measurement that the adsorption of NO on the DDPA LB film only changes the value not the orientation of the second-order susceptibility of DDPA molecules on film.

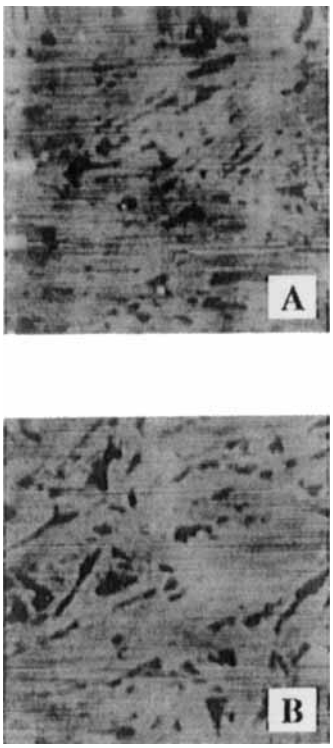

Fig. 2 Tapping mode AFM images of the DDPA LB film on quartz substrates. 1 × 1 μm scan range, 10 nm grayscale. (A) before NO/N_2 injection; (B) after NO/N_2 injection.

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