第 16 卷 第 1 期 物 理 化 学 学 报 Vol. 16, No. 1
2000 年 1 月 ACTA PHYSICO-CHIMICA SINICA January, 2000

Aggregates in Rhodamine-labeled Phospholipid Films Probed by Spectroscopy and Atomic Force Microscopy

Liu Tingting Yu Anchi Luo Guobin Zhao Xinsheng Ying Liming

(State Key Laboratory of Molecular Dynamic and Stable Structures, and Institute of Physical Chemistry, Peking University, Beijing 100871)

Huang Yanyi Huang Chunhui

(State Key Laboratory of Rare Earth Materials Chemistry and Applications, Peking University, Beijing 100871)

Abstract Molecular aggregates of rhodamine labeled phospholipid T1391 on LB films at different surface pressures have been investigated by optical spectroscopy and atomic force microscopy (AFM). Different types of molecular aggregates which show peak shifting and band broadening in the spectra were confirmed by the morphological AFM image. The rhodamine chromophores in the aggregates were suggested to orient at an angle of about 45 degree on the mica surface based on exciton theory and surface pressure-area isotherm.

Keywords: Aggregate, Exciton, AFM, Phospholipid, LB film, Spectroscopy

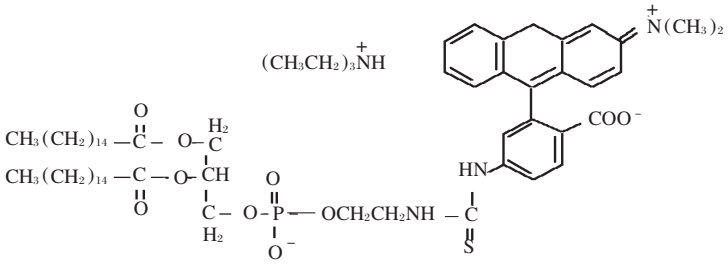
Extensive experimental[1-3] and theoretical[4, 5] investigations on aggregated molecular systems have been performed because of their similarities with biological structures as well as their intriguing optical and electronic properties. Advanced techniques such as second harmonic generation (SHG), scanning near-field optical microscopy (SNOM) and atomic force microscopy (AFM) have been used to probe the orientational anisotropy of organized molecular aggregates[6], to reveal the exciton dynamics of molecular J aggregates at about 100 nm resolution[7] and to view the organization of amphiphiles at interfaces with high spatial resolution, respectively. LB technique is an easy way to produce highly organized molecular assemblies. However, LB films are not as perfect as they seem. In addition to defects, the immicibility of components and the inhomogeneous distribution of functionalized groups cause the formation of aggregates and crystallites. Fluorescent dyes incorporated into phospholipid membrane provides a detectable information on the photophysical properties of the dyes in restricted geometries and the interaction between chromophores and the microenvironment, which may lead to better understanding of the correlation between photophysical and morphological characteristics of such systems. In an attempt to tackle the molecular self-assembly in fluorescent phospho-

Received 1999-03-29, revised 1999-06-23. Correspondent: Zhao Xinsheng. * The Project Supported by the National Natural Science Foundation of China and the Climbing Project

lipid membranes, we use rhodamine labeled DHPE LB monolayer as a model system to probe its microscopic and macroscopic properties by optical spectroscopy and atomic force microscopy.

1 Experimental

N-(6-tetramethylrhodaminethiocarbanoyl)-1, 2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (TRITC DHPE, T1391) was purchased from Molecular Probes Inc. (Eugene, Oregon USA) and its molecular structure is shown as



The cyclohexane and toluene (9∶1 volume ratio) solution of T1391 (0.1mg · mL^{-1}) was dropped on pure water subphase (pH = 5.6, 20℃) on a British NIMA Langmuir-Blodgett 622 trough. After evaporation of solvent, the monolayer was compressed at a speed of 40 cm^2 · min^{-1}, then the monolayer was transferred onto a freshly cleaved mica (for AFM) and pretreated quartz substrate in Z-type mode at a constant surface pressure of 20mN · m^{-1} and 40mN · m^{-1}. Transfer ratio is 1.0. Absorption spectra of T1391 LB monolayers and 8×10^{-7}mol · L^{-1} methanol solution were taken on Shimadzu UV-3100 UV-Vis-Nik Recording Spectrophotometer. AFM measurements were performed with a nanoscope IIIa controller (digital instrument, Santa Barbara, CA) equipped with a bioscope G scanner (90μm). LB monolayers in air-solid interface were imaged in tapping mode using silicon cantilevers with resonance frequency of 260 – 340kHz.

2 Results and discussion

2.1 π-A isotherm and surface morphology of the LB film

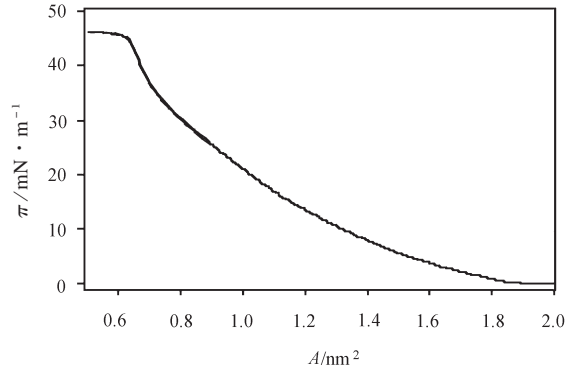


Fig. 1 Surface pressure-area isotherm of T1391 monolayer

A: Area per molecule; π: Surface pressure

The surface pressure-area (π-A) isotherm for T1391 molecule is shown in Fig. 1. The average molecular area at 20mN · m^{-1} and 40mN · m^{-1} is about 1.02 nm^2 and 0.67 nm^2, respectively. Comparing with the average molecular area (0.42 nm^2) of DHPE LB monolayer at 40 mN · m^{-1}[8], T1391 has larger molecular area because the big rhodamine 6G polar head takes up more space. It is possible that the double hydrophobic chains pack loosely on the surface due to the large head group. In this system, the long hydrocarbon chains attached to the phenyl ring may re-

strict its rotation[9], so the angle of the phenyl ring relative to the xanthene plane can not be changed at different surface pressures. AFM can give direct insight into the order of the molecular assemblies and was used to characterize the surface microscopic morphology of the LB films. Fig. 2 show the tapping mode AFM images of T1391 LB films on mica. Both LB monolayers at surface pressures of 20 mN · m^{-1} and 40 mN · m^{-1} exhibit phase separation, but in the latter case the phase separation is more obvious. The height difference between two types of domains in monolayers at low surface pressure were 0.5 ± 0.1nm (Average of 50 measurements). Three kinds of domains were observed in monolayers at higher surface pressure. The height differences between the two higher domains and the lowest domain are 0.8 ± 0.2 nm (Average of 50 measurements) and 1.4 ± 0.3 nm (Average of 50 measurements), respectively. According to the theory of Kuhn *et al*[4] and Kasha *et al.* [5], standing dipoles are in accordance with H aggregates and laying dipoles are in accordance with J aggregates. The domains with varied heights in AFM images of T1391 LB films on mica may be an indication of the formation of different kinds of aggregates. This AFM investigation further indicates that real LB monolayers[10] of amphiphiles are not as homogeneous and perfect as they are often considered.

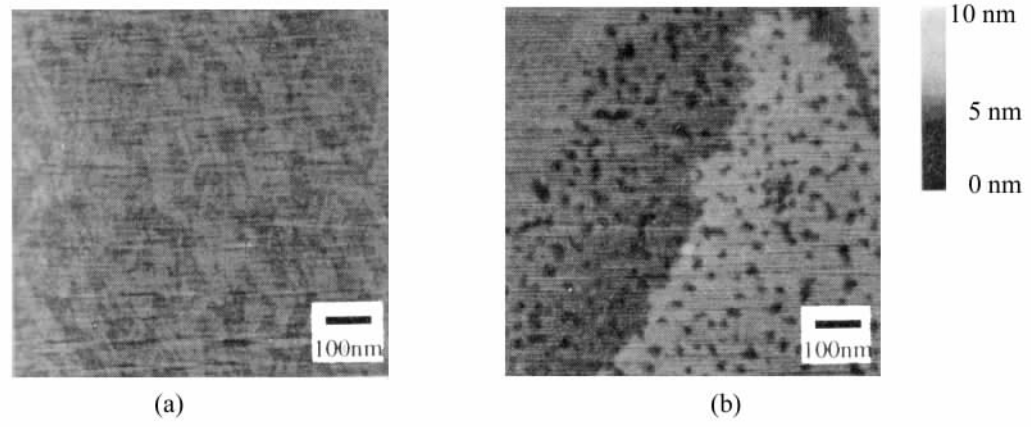


Fig. 2 AFM images of T1391 LB films at 20 mN · m^{-1} (a) and 40 mN · m^{-1} (b)

2.2 Molecular aggregates and exciton model

The absorption characteristics of rhodamine dyes have been studied extensively in a variety of media and have been well documented. The phenomenon of rhodamine aggregation has been shown to occur at relatively high concentration in solutions[11], in LB films[12], in phospholipid vesicles[13] and on fused silica surfaces[6]. Before we go to the analysis of the optical absorption properties of LB films of T1391, we must consider the high concentration environment of the surface and the possibility of formation of the dimers and higher aggregates. Exciton model[5] summarized briefly below is a theoretical method to describe the excited state dipole-dipole interaction between molecules in van der Waal′s aggregates which can well interpret the absorption, fluorescence and dynamics of molecular aggregates. The dipole-dipole interaction, viewed as slight perturbation to the individual molecular states, causes these identical energy states to split into an exciton band . The exciton band

energy diagram for different types of molecular dimer is shown in Fig. 3. In J aggregate, the bottom exciton state is allowed for electric-dipole transition while the others are forbidden, so red shifted absorption band can be formed. In H aggregate, only the top exciton state is allowed, so H aggregate is characterized by blue shifted absorption band. The forbidden lowest exciton level usually makes energy transfer to the lowest excited triplet states more probable than emission. The shift of the aggregate′s absorption band compared to the monomer′s peak absorption is defined as equation (1)[5],

$$\Delta\nu = \frac{2}{hc}\frac{N-1}{N}\frac{\mu^2}{\gamma^3}(1-3\cos^2\theta) \quad (1)$$

where $\Delta\nu$ is the shift of the exciton band, N is the number of monomers in an aggregate, μ is the dipole moment, θ is the angle between the dipole and the vector joining the centers of the dipoles, r is the length of the vector.

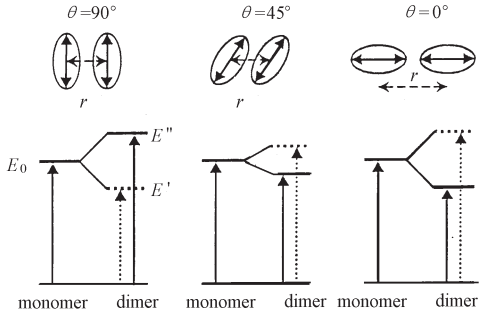


Fig. 3 Exciton band energy diagram for three types of molecular dimers

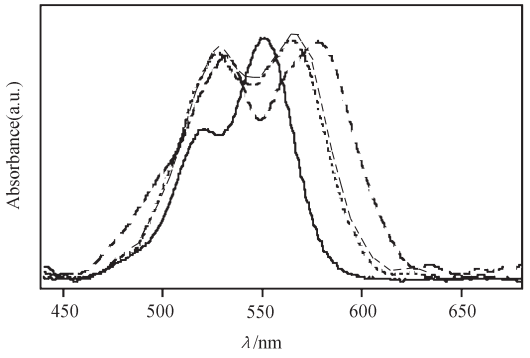


Fig . 4 Absorption spectra of T1391 in methanol solution (solid line) and LB films at 20 mN · m^{-1} (dotted line), 40 mN · m^{-1} (dashed line)

2.3 Absorption spectra

Fig. 4 shows the absorption spectra of T1391 in LB films at two different surface pressures. Solution spectrum at very low concentration is also illustrated for comparison. Apparently, the absorption spectrum of T1391 in methanol shows an intense band at 550 nm and a shoulder at 522 nm. Due to the structural similarity between T1391 and rhodamines, the intense band can be attributed to the 0-0 absorption band corresponding to the S_0-S_1 transition that is directed parallel to the long axis of the chromophore[10]. The weak shoulder may be attributed to the H dimers of T1391, which is consistent with the observation reported for rhodamines. It has been reported that rhodamine dyes have a tremendous propensity to aggregate even at very low concentration (10^{-6}mol · L^{-1})[10].

However, it should be noted that the vibrational band of the absorption may be buried with the dimer absorption. The spectra of monolayers splits into two nearly equal bands. The blue shifted bands (528.2 nm on 20 mN · m^{-1} and 531.0 nm on 40 mN · m^{-1}) and the red shifted bands (565.6 nm on 20 mN · m^{-1} and 577.5 nm on 40 mN · m^{-1}) can be assigned to H aggregates and J aggregates, respectively. Various kinds of aggregate states can coexist in the LB film, as observed by Menzel and coworkers[14]. As expected, the shifts of J aggregate bands increase with compressing of the film. However, small change was observed in the blue bands corresponding to H aggregates. It

has been reported recently that trans-stilbene fatty acid derivatives form H aggregates at the air-water interface prior to compression even in very dilute solution[15]. Our experimental results may be interpreted in a similar way. When spreading on air-water interface, T1391 molecules assemble into H aggregates spontaneously, while J aggregates grow during the compression of the molecules. Therefore, the distances between the molecules and the orientations of the dipoles in J aggregates are strongly surface pressure dependent. On the contrary, because the H aggregates have already formed at air-water interface, compression would have little effect on them.

Table 1 Calculated distances (nm) between chromophores at different surface pressures and orientation angles*

Orientation angle	Surface pressure π/mN · m^{-1}	
θ/(°)	20	40
0	1.57	1.29
30	1.34	1.10
45	0.99	0.81
60	0.66	0.69
90	1.05	1.10

* N was assumed to be infinitely great [16]. The transition-dipole moment of T1391 molecule was calculated from the absorption spectra of it's monomer in methanol.

According to equation (1) and absorption spectra, distances between chromophores at different surface pressures and orientation angles were calculated and the results are shown in Table 1.

The calculated distances between chromophores when orientational angle of the dipole is around 45 degree are consistent with the average molecular area at different surface pressure which is 1.02 nm^2 at 20 mN · m^{-1} and 0.67 nm^2 at 40 mN · m^{-1} respectively and also confirm that the chromophores in J aggregates are further approaching with the increase of the surface pressure.

3 Conclusion

The T1391 molecules in LB films exist predominantly as different kinds of aggregates. Both H and J type molecular aggregates have been observed based on the absorption spectra. AFM images further prove the inhomogeneity of T1391 monolayer. Theoretical calculation based on the exciton model can well explain the experimental results. To explore the exact physical picture of the mechanism of aggregates formation, one needs to know the distribution of orientational angle of the rhodamine head group. The accuracy of our calculation based on exciton model is largely dependent on it. The system under current investigation is apparently inhomogeneous, SHG may not be a good method to obtain the orientational angle on the surface. However, total internal reflection fluorescence (TIRF) coupled with SHG would be a good method to solve this problem and preliminary experiments at air-water surface are under going.

References

1 Argyrakis P, Basko D M, Drobizhev M A, *et al. Chen. Phys. Lett.*, **1997, 268**: 372

2 Khairutdinov R F, Serpone N. *J. Phy. Chem. B*, **1997, 101**: 2602

3 Kemnitz K, Tamai N, Yamazaki I, *et al. J. Phys. Chem.*, **1986, 90**: 5094
4 Czikklely V, Forsterling H D, Kuhn H. *Chem. Phys. Lett.*, **1970, 6**(3): 207
5 Mcrae E G, Kasha M. *J. Chem. Phys.*, **1958, 28**: 721
6 Kikteva T, Star Dmitry, Zhao Z F. *J. Phys. Chem.*, **1999, 103**: 1124
7 Higgins. D A, Reid P J, Barbara P F. *J. Phys. Chem.*, **1996, 100**: 1174
8 Solletti J M, Botreau M, Sommer F, *et al. J. Vac. Sci. Technol. B*, **1996, 14**(2): 1492
9 Vuorimaa E, Ikonen M, Lemmetyinen H. *Thin Solid Film*, **1992, 214**: 243
10 Dutta A K, Lavoie H, Ohta K, *et al. Langmuir*, **1997, 13**: 801
11 Burghardt T P, Lyke J E, Ajtai K. *Biophys. Chem.*, **1996, 59**: 119
12 Vuorimma E, Ikonen M, Lemmetyinen H. *Chem. Phys.*, **1994, 188**: 289
13 Deumie M, El Baraka M J. *Photochem. Photobiolol. A*: *Chem.*, **1995, 89**: 239
14 Menzel H, Weichert B, Schmidt A, *et al. Langmuir*, **1994, 10**: 1926
15 Whitten D, Chen L H, Geiger H C, *et al. J. Phys. Chem.*, **1998, 102**: 10098
16 Gavish M, Popvitz-Biro R, Lahaav M, *et al. Science*, **1990, 250**: 973

罗丹明标记的磷脂分子聚集态的光谱与 AFM 研究*

柳汀汀　于安池　罗国斌　赵新生　应立明
(北京大学分子动态与稳态结构国家重点实验室和物理化学研究所,北京　100871)
黄岩谊　　黄春辉
(北京大学稀土材料化学与应用国家重点实验室,北京　100871)

摘要　在不同表面压的 LB 膜上,应用光谱学和原子力显微镜学研究了由罗丹明标记的膦脂分子形成的多种聚集态. 这些分子聚集态使光谱的峰值位移,谱带加宽. AFM 的形貌图进一步说明了膦脂单分子膜的不均一性. 根据激子理论,以 45° 取向角存在的罗丹明染料聚集体更符合 π-A 曲线.

关键词:　聚集态,　激子,　AFM,　膦脂,　LB 膜,　光谱学
学科代码:　B030503,　B030105

1999-03-29 收到初稿,1999-06-23 收到修改稿. 联系人:赵新生.　　*国家自然科学基金和攀登计划资助项目