Polycondensation of *N*-(*O*,*O*-di-*n*-hexadecyl)phophorylalanine at the air/water interface

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Dedicated to Professor Chengye Yuan on his 80th anniversary

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Abstract

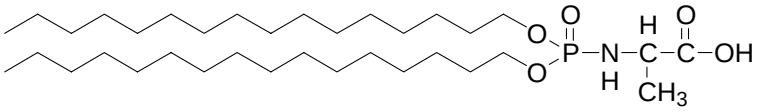
The polycondensation of N-(O,O-di-*n*-hexadecyl)phophorylalanine **1** at the air/water interface has been studied. It is found that it can form stable insoluble monolayer (Langmuir film) at the air/water interface. Among the materials collected from the air/water interface, dipeptide derivative was detected by electrospray ionization mass spectrometry (ESI-MS), and the structure was further identified by tandem mass spectrometry (MS/MS), which suggested that there formed the peptide without any coupling reagents. The transmission FT-IR spectra of the subsequent Langmuir-Blogget film (LB film) confirmed the polycondensation of **1** at the air/water interface.

Keywords: Polycondensation, Langmuir monolayer, phosphorylalanine, peptide, MS, IR

Introduction

Currently, there is great interest in liposomes, monolayers, bilayer membranes, and Langmuir-Blodgett multilayers as biomembrane models and furthermore because of their manifold potential applicability.[1] Interfaces in such systems, which permit a particular arrangement of molecules, are considered to play an important role in the reactions in biological systems. It has been shown in our laboratory that N-phosphorylamino acids and peptides are chemically active species,[2] which might be related to the phosphorylation or dephosphorylation of proteins. It is very interesting that the peptide formation of N-phosphorylamino acids proceeds in aqueous or organic solution without any coupling reagents,[3] which has led to the proposal of a model for the co-evolution of proteins and nucleic acids at the prebiotic stage.[4] In the present paper, we

synthesized the amphiphilic *N*-(*O*,*O*-di-*n*-hexadecyl)phophorylalanine **1** and investigated the peptide formation of **1** at the air/water interface as regard to the significance of self-assembling processes in the formation of primitive biopolymers at the early stages of evolution and the biosynthesis of biopolymer in ordered systems such as bilayer lipid membranes occurring in present life.[5] Actually, the polycondensation of amino acid based amphiphiles in ordered systems such as Langmuir monolayers, LB films and liposomes has been widely reported since the first publication by Katchalsy et al. [6]



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Scheme 1

Results and Discussion

The surface pressure per molecular area isotherm of **1** at 293K shown in Figure 1 indicates that the phosphorus amphiphile with amino acid moiety as head group can form rather stable insoluble monolayer (Langmuir film) at the air/water interface. The linear region of a condensed film of the amphiphile is extrapolated to the zero surface pressure, and the mean cross-sectional area per molecule of each *N*-phosphorylalanine is obtained to be 0.46nm^2. Both the hydrogen bond network throughout the highly polar head groups and the hydrophobic interaction between the long hydrocarbon chains can attribute to the stability of the Langmuir film.

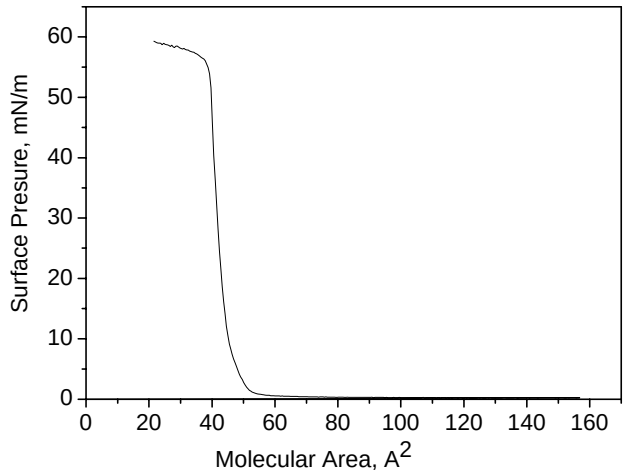
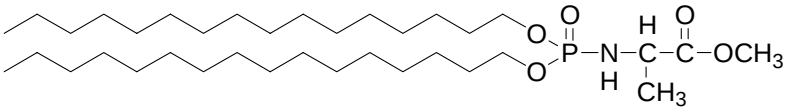


Figure 1. Surface pressure per molecular area isotherm of **1** at 293K.

The monolayer was compressed at a constant rate until the surface pressure reached 35mN/m. Then the monolayer area was kept constant and the surface pressure was recorded versus time to obtain the surface pressure relaxation curve of the monolayer. As shown in Figure 2, the surface pressure of the monolayer decreased gradually and remained almost unchanged after 2 hours. It is demonstrated that three factors may lead to the decrease of the surface pressure in the constant monolayer area: i) the dissolution of some of the film forming molecules into the subphase under the floating film, ii) the rearrangement of the film forming molecules at the interface or even the phase separation in the Langmuir film and iii) the chemical reaction of the film forming molecules at the air/water interface.[7] It is noted that the surface pressure decreased relatively rapidly at the very beginning but almost kept unchanged after a few hours. It implies that the dissolution of the amphiphile does not seem to be a dominating factor, because the surface pressure should decrease in a more proportional way in that case. Therefore, it is reasonable to exclude the impact of the dissolution of the molecules. Since the compression of the film is just a process of quasi equilibrium, the rearrangement and even the phase separation are inevitable.



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Scheme 2

To clarify the complicated situation we compare the surface pressure relaxation curves of the Langmuir film of **1** with its non-polymerizable derivative Methyl *N*-(*O*,*O*-di-*n*-hexadecyl)phophorylalaninate **2**. In the case of **2**, the decrease of the surface pressure also occurred to a mild extent. Dissolution of the molecules in the case of **2** could be neglected because the solubility of **2** in water is less than that of **1** due to its less polarity of the head group. Thereby the decrease in surface pressure in the case of **2** was attributed mainly to the rearrangement of the molecules. Thus it can be concluded prudently that the difference of decrease of the surface pressure between **1** and **2** arises from the condensation of the molecules at the air/water interface in the case of **1**.

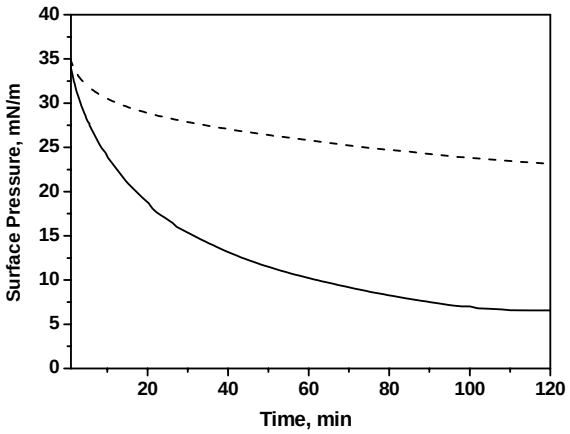
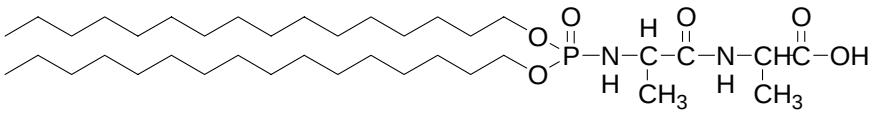


Figure 2. Surface pressure relaxation curves at 293K. solid line: **1**; dashed line: **2**.

Electrospray ionization mass spectrometry (ESI-MS) is a powerful tool in structure analysis of amino acids and peptides.[8] Especially, the component in a mixture can be studied through the tandem mass spectrometry (MS/MS) without further separation. ESI-MS has been extensively used in the study of phosphorylamino acids and related peptides, and it has been previously reported that the signal response in ESI-MS can be enhanced largely with the introduction of a phosphoryl group at the N terminus of amino acids or peptides, especially in negative mode.[9] Therefore, it is possible to analyze the trace substances at the air/water interface by ESI-MS. To confirm the condensation at the air/water interface, the material at the water surface was analyzed by ESI-MS. The peak of m/z 687 attributed to dipeptide derivative **3** was distinctly discernable in the negative mode ESI-MS spectroscopy as shown in Figure 3, and its structure was further identified by multistage mass spectrometry. The peak of m/z 687 exhibits the same fragmentation pattern in MS/MS spectrometry as that of the dipeptide derivatives **3** chemically synthesized (Figure 4), which confirms the presence of dipeptide derivative. No higher peptides were discernable in MS due to the possible dissolution of the molecules into the subphase and the resulting trace amount of the samples at the air/water interface.



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Scheme 3

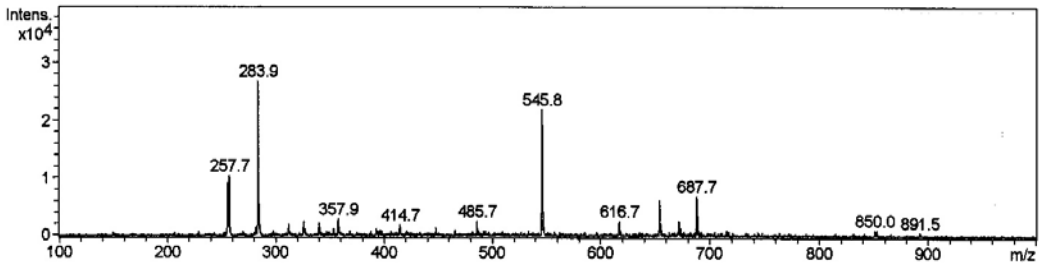


Figure 3. ESI-MS spectra of the material collected at the air/water interface.

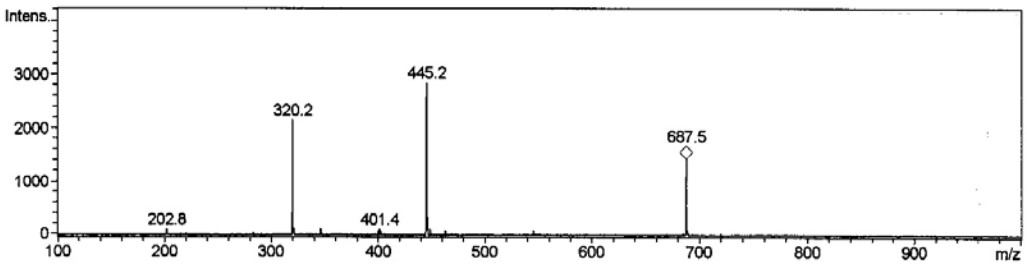


Figure 4. ESI-MS/MS spectra of **3**.

The polycondensation of **1** at the air/water interface was further confirmed by the transmission IR spectra of the subsequent LB film. The monolayer was deposited onto the CaF_2 substrates successfully by the well-described LB technique after the monolayer was allowed for sufficient reaction at the air/water interface. The transmission IR spectra of the 33-monolayer LB films shown in Figure 5 display a distinct broad band in the spectral range 1500-1660 cm^{-1} (overlapping of the amide and amide band), with the expense of the disappearance of the C=O absorption at 1736 cm^{-1}, as compared with their KBr-plate IR spectra for powder (not shown). The $\nu(CH_2)$ frequencies at 2918 and 2850 cm^{-1} of the 33-monolayer LB film (not shown) indicates the highly ordered *trans-zigzag* conformation of hydrocarbon chains, [10] which implies that the supramolecular structures were maintained during the polycondensation process for the monolayer to be deposited onto the CaF_2 substrates successfully. The IR spectrum of the LB film kept unchanged after exposure to air at ambient temperature for 48 h, which implies that the spontaneous polycondensation occurs at the air/water interface rather than after the deposition onto the CaF_2 substrates.

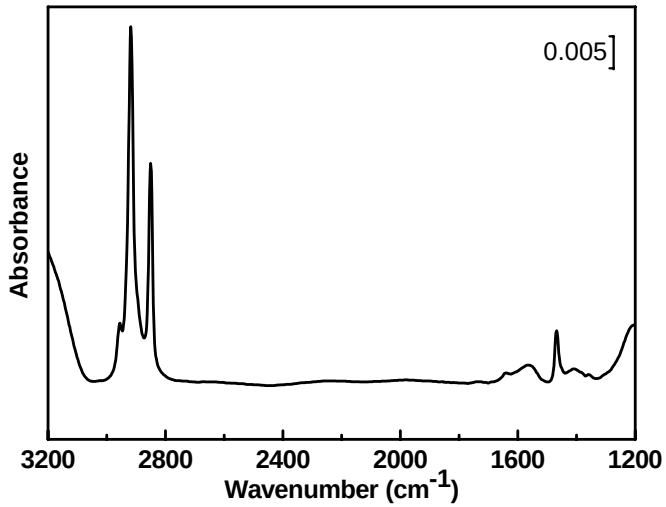


Figure 5. Transmission IR spectrum of 33-monolayer LB film of **1**.

Conclusions

In summary, polycondensation of amphiphilic *N*-(*O*,*O*-di-*n*-hexadecyl)phophorylalanine was demonstrated in Langmuir film at the air/water interface. Although the peptide formation of phosphorylamino acids was previously reported in aqueous or organic solution, the polycondensation in our case cannot be explained simply with the interpretation for the above systems. It is estimated that the particular orientation and packing of the monomer molecules at the air/water interface are favorable for the polycondensation of the amino acid based amphiphile in the highly ordered Langmuir film.

Experimental Section

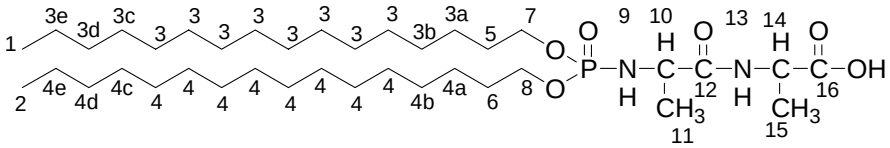
General Procedures. ESI mass spectra in negative mode were acquired on a Bruker ESQUIRE~LC^{TM} ESI ion trap spectrometer equipped with a gas nebulizer probe capable of analyzing ions up to *m/z* 6000. Samples were continuously pumped into the ESI chamber at a flow rate of 10 μl/min. The nebulizer gas was delivered at a flow pressure of 7 psi. The source temperature was maintained at 300°C. Data were acquired at 0.6 Th intervals from *m/z* 100 to 1000 with a scan rate of 13000 Th·S^{-1}. Transmission FT-IR spectra were recorded on Bruker IFS 66V spectrophotometer equipped with a DTGS detector. Typically, 2048 interferograms were collected to obtain a satisfactory signal-to-noise ratio at resolution of 4 cm^{-1}. The film spectra were obtained by subtracting the spectrum of CaF_2 blank plate from the corresponding sample spectra. NMR spectra were recorded on a Bruker AC 200 for ^{31}P and JEOL ECA 300 for ^{1}H and

JEOL ECA 600 for ^{13}C spectrometers using standard conditions. Chemical Shifts (δ, in ppm) are referred to internal Me_4Si for ^{1}H and ^{13}C and to external aqueous 85% H_3PO_4 solution for ^{31}P.

LB Trough Measurements. An LB trough (KSV 2000 Minitrough, KSV Instruments LTD, Finland) equipped with a Wilhelmy balance was employed for monolayer spreading as well as the relative experiments. The water used for subphase was double-distilled and the pH value was 5.8. The temperature of the subphase was kept constant at 20. Monolayers were obtained by spreading by a microsyringe certain volume of chloroform solutions composed of **1** or **3** with the concentration of 0.5 mM onto pure water. After spreading, 15 min was allowed for solvent evaporation. To obtain the surface pressure per molecular area isotherms, the monolayers were compressed at the rate of 15 cm^2/min until the collapse points were countered. To obtain the surface pressure relaxation curves, the monolayers were compressed at the rate of 15 cm^2/min up to 35 mN/m and the trough barriers were fixed and the surface pressure versus time were recorded. To collect the material at the air/water interface for ESI-MS spectrometry, the monolayers were compressed at the rate of 15 cm^2/min up to 25 mN/m and the surface pressure was kept constant at 25 mN/m. The monolayers were held for 4 hours allowing the occurrence of the reaction at the air/water interface. Then the monolayers were compressed with the barriers, and the material, observed by visual inspection, was collected form the water surface, transferred to a glass vial, and dried under vacuum. The 33-monolayer LB films were fabricated as following. The monolayers were compressed at the rate of 15 cm^2/min up to 25mN/m and the surface pressure was kept constant at 25 mN/m. The monolayers were held at the surface pressure for at least 4 hours before being deposited onto the CaF_2 substrates. The LB films were deposited from pure water onto CaF_2 substrates by vertical method (dipping/lifting rate 4 mm/min) at fixed surface pressure 25 mN/m with a transfer ratio of nearly unit. After deposition of the first monolayer, the substrates were kept dry in air for 30 min so that the following monolayers were successfully transferred.

Syntheses

The syntheses of *N*-(*O*,*O*-di-*n*-hexadecyl)phophorylalanine (1) and Methyl *N*-(*O*,*O*-di-*n*-hexadecyl)phophorylalaninate (2) has been described in a previously paper.[11]



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***N*-(*O*,*O*-di-*n*-hexadecyl)phophorylalanyl alanine (3).** To a mixture of methyl alaninate hydrochloride (73 mg, 0.5 mmol) and *N*-methylmorpholine (54mg, 0.5 mmol) in anhydrous THF (2 ml), were added with stirring at ambient temperature 1 (340mg, 0.55mmol) and HOBt (71mg, 0.51mmol). To the resulting mixture was added with stirring in ice bath DCC (126mg,

0.52mmol) in THF (3ml). The mixture was stirred at ambient temperature overnight and then was filtered. The filtrate was concentrated *in vacuo* and pale yellow powders were obtained. The powders were added into saturated aqueous $NaHCO_3$ solution (40 ml) and the resulting mixture was extracted with $CHCl_3$ (3×15ml). The combined organic extracts were washed sequentially with saturated aqueous $NaHCO_3$ solution (3×15 ml) and 10% aqueous citric acid solution (3×15 ml) and saturated aqueous NaCl solution (3×10 ml), dried (Na_2SO_4) and filtered, and the filtrate was concentrated *in vacuo* and pale powders were obtained. The powders were dissolved in EtOH (25 ml). To the mixture was added dropwise with stirring at ambient temperature 1.1 M aqueous NaOH solution (1 ml). Then the mixture was stirred overnight. Then to the mixture was added H_2O (10 ml). To the mixture was added in stirring aqueous 3M HCl in ice bath to adjust the pH value to 2. The resulting mixture was extracted with $CHCl_3$ (3×15ml) shortly after the acidification. The combined organic extracts were washed with H_2O (3×15ml), dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo* and pale powders were obtained. The residue was purified via recrystallization with EtOAc/petroleum ether. Pure 3 (311mg, yield 82%, m.p. 84°C) was thereby obtained as a white solid. ^{31}P NMR ($CDCl_3$, δ): 8.52; ^{1}H NMR ($CDCl_3$, δ): 0.88 (t, J=6.2Hz, 6H, (2-CH_3)-1,2), 1.25 (s, 52H, (26-CH_2)-3,4), 1.40 (m, 6H, (2-CH_3)-11,15), 1.62 (m, 4H, (2-CH_2)-5,6), 1.93 (bd, 1H, NH-9), 3.72 (m, 1H, CH-10), 3.96 (m, 4H, (2-CH_2)-7,8), 4.56 (m, 1H, CH-14), 7.52 (d, 1H, NH-13); ^{13}C NMR ($CDCl_3$, δ): 14.14 (C-1,2), 18.32 (C-15), 20.91 (C-11), 22.72 (C-3e,4e), 25.54 (C-3a,4a), 29.40 (C-5,6,3b,3c), 31.95 (C-3d,4d), 48.25 (C-10), 51.17 (C-14), 67.09 (C-7,8), 173.03 (C-12) , 175.37 (C-16); ESI-MS: $[M+1]^+$ m/z 690, $[M+Na]^+$ m/z 712, $[M-H]^-$ m/z 688. Anal. Calcd. for $C_{38}H_{77}N_2O_6P$: C, 66.24; H, 11.26: N, 4.07. Found: C, 66.02; H, 11.23; N, 4.12%.

Acknowledgements

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References

1. (a) Mueller A.; O'Brien D. F. C*hem. Rev.* **2002**, *102*, 727. (b) Lu Q.; Liu M. H. *Thin Solid Films*, **2003**, *425*, 248.
2. (a) Xue, C. B.; Yin, Y. W.; Zhao, Y. F. *Tetrahedron Lett.* **1988**, *29*, 1145. (b) Ma, X. B.; Zhao, Y. F. *J. Org. Chem.* **1989**, *54*, 4005. (c) Fu, H.; Li, Z. Z.; Zhao, Y. F.; Tu, G. Z. *J. Am. Chem. Soc.* **1999**, *121*, 291. (d) Chen, Z. Z.; Tan, B.; Li, Y. M.; Zhao Y. F. *J. Org. Chem.* **2003**, *68*, 4052.

3. (a) Deng, C. H.; Li, Y. M; Zhao, Y. F. *Phosphorus Sulfur Silicon Relat. Elem.* **2000**, *163*, 203. (b) Chen, Z. Z.; Tong, Y. F.; Chen, S. B.; Li, Y. M.; Chen, Y.; Zhao, Y. F.; Wang, J. F. *Chin. Sci. Bull.* **2002**, *47*, 1866.
4. (a) Zhao, Y. F.; Cao, P. S. *J. Biol. Phys.* **1994**, *20*, 283. (b) Zhou, W. H.; Ju, Y.; Zhao, Y. F.; Wang, Q. G.; Luo, G. A. *Origins. Life Evol. B.* **1996**, *26*, 547.
5. (a) Fukuda, K.; Shibasaki, Y.; Nakahara, H.; Liu, M. H. *Adv. Colloid. Interfac.* **2000**, *87*, 113. (b) Shibata, A.; Hashimura, Y.; Yamashita, S.; Ueno, S.; Yamashita, T. *Langmuir* **1991** *7*, 2261. (c) Zepik, H.; Shavit, E.; Tang, M.; Jensen, T. R.; Kjaer, K.; Bolbach, G.; Leiserowitz, L.; Weissbuch, I.; Lahav, M. *Science* **2002,** *295*, 1266. (d) Weissbuch, I.; Bolbach, G.; Zepik, H.; Shavit, E.; Tang, M.; Frey, J.; Jensen, T. R.; Kjaer, K.; Leiserowitz, L.; Lahav, M. *J. Am. Chem. Soc.* **2002**, *124*, 9093.
6. Baniel, A.; Frankel, M.; Friedrich, I.; Katchalsky, A. *J. Org. Chem.* **1948**, *13*, 791.
7. Vollhardt, D.; Emrich, G.; Gutberlet, T.; Fuhrhop, J. H. *Langmuir* **1996**, *12*, 5659.
8. Zhu, X. G.; Desiderio, D. M. *Mass. Spectrom. Rev.* **1996,** *15*, 213.
9. Chen, J.; Chen, Y.; Gong, P.; Jiang, Y.; Zhao, Y. F. *Phosphorus Sulfur Silicon Relat. Elem.* **2002**, *177*, 2087.
10. (a) Macphail, R. A.; Strauss, H. L.; Snyder, R. G.; Elliger, C. A. *J. Phys. Chem.* **1984**, *88*, 334. (b) Du, X. Z.; Liang, Y. Q. *J. Phys. Chem. B* **2000**, *104*, 10047.
11. Deng, C. H.; Li Y. M.; Xu, N.; Zhao, Y. F. *J. Chem. Research (S)* **1999**, 589.