

## Investigation of Spontaneous Condensation of an Amino Acid Based Amphiphile on Cast Film by *in situ* Transmission FT-IR Spectroscopy

Hai Yan WANG, Yan Mei LI\*, Yi XIAO, Yu Fen ZHAO

The Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084

**Abstract:** The spontaneous condensation of the amphiphilic N-(*O,O*-dihexadecyl)phosphoryl-alanine on the cast film was observed by *in situ* investigation of transmission infrared spectroscopy. The particular orientation and ordered packing of the monomers within the multilayers of the cast film is concluded to attribute to the spontaneous condensation between the monomers.

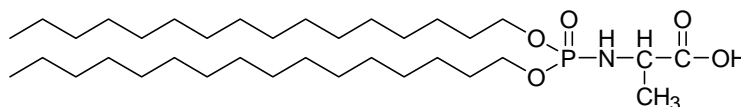
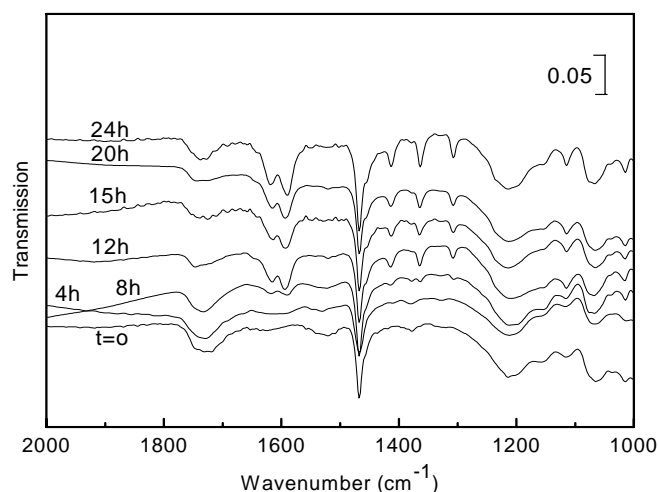
**Keywords:** Amphiphile, cast film, condensation, FT-IR, N-(*O, O*-dihexadecyl)phosphoryl alanine.

The reactions within highly ordered supramolecular systems such as Langmuir-Blodgett films (LB films) and liposomes *etc.* are of great interest<sup>1,2</sup> for their outstanding rapidity and structural specificity from the arrangement of the functional groups in the molecules. Since the condensation of amphiphilic amino acid derivatives in LB films was first reported in the year of 1948 by Katchalsy *et al.*<sup>3</sup>, this research field has drawn much attention as regard to possible approaches to the new materials compatible between artificial materials and natural biomaterials due to the amino acid moiety<sup>4-7</sup>. It has been previously reported that amphiphilic N-phosphorylamino acids could self-assembly into highly ordered supramolecular structures and the specific arrangement and orientation of the molecules in the highly ordered structures could permit the reaction between the monomers<sup>8,9</sup>. The cast film of the amphiphiles could provide particular orientation of the molecules on the solid surface and could serve as molecular templates for chemical reaction in highly ordered systems<sup>10,11</sup>. We present here the condensation of a synthetic amphiphilic amino acid derivative N-(*O,O*-dihexadecyl) phosphorylalanine (**Figure 1**) on the cast film monitored by *in situ* transmission infrared spectroscopy.

The starting material was synthesized and purified as described previously<sup>12</sup>. The cast film was prepared by spreading a drop of dilute solution in chloroform on the surface of thoroughly cleaned CaF<sub>2</sub> substrate. Immediately after the volatilization of the solvent the cast film was monitored by transmission FT-IR at different intervals. The

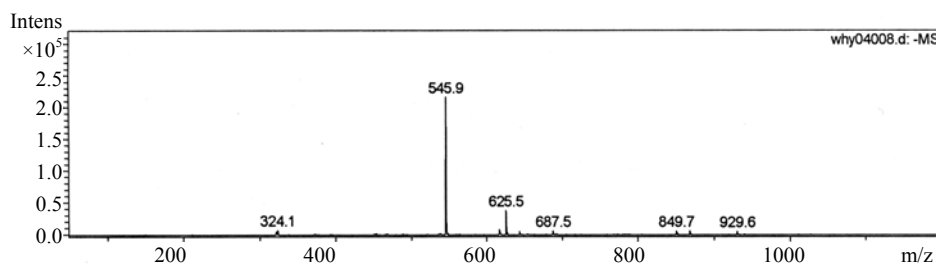
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\* E-mail: liym@mail.tsinghua.edu.cn

**Figure 1** The molecular structure of N-(*O,O*-dihexadecyl) phosphorylalanine**Figure 2** The time-dependent transmission FT-IR spectra of N-(*O,O*-dihexadecyl) phosphorylalanine cast film

time-dependant transmission FT-IR spectra of the cast film in the spectral range 1000-2000  $\text{cm}^{-1}$  is shown in **Figure 2**. The spectra of the cast film display the appearance and increase in intensity of the broad band in the spectral range 1540-1660  $\text{cm}^{-1}$  (overlapping of the amide I and amide II band), with the expense of the decrease of the C=O absorption at 1736  $\text{cm}^{-1}$ , which indicates the formation of amide bond between the alanine head groups in N-(*O,O*-dihexadecyl) phosphorylalanine<sup>4,5,8,9</sup>. A series of increasing peaks distributed at 1417  $\text{cm}^{-1}$ , 1365  $\text{cm}^{-1}$  and 1311  $\text{cm}^{-1}$  respectively could be assigned to the side chain methyl group in the resultant peptide derivative, which could also be found in the FT-IR spectrum of the cast film prepared from chemically synthesized N-(*O,O*-dihexadecyl)phosphorylalanine. The strong peak at 1468  $\text{cm}^{-1}$  attributed to the  $\delta$  ( $\text{CH}_2$ ) bending mode indicates the chain packing of hexagonal subcell structure in the multi-layered cast film, and the small shoulder observed at approx. 1456  $\text{cm}^{-1}$  is due to the  $\delta$  ( $\text{CH}_3$ ) bending mode. The  $\nu$  ( $\text{CH}_2$ ) frequencies at 2918 and 2850  $\text{cm}^{-1}$  of cast film (not shown), diagnostic of the all *trans* zigzag conformation of hydrocarbon chains, implies that the highly ordered multi-layered structures on cast film<sup>13,14</sup>. To confirm the condensation within the cast film, the material on the aged cast film was collected and analyzed by ESI-MS. The peak of  $m/z$  687 attributed to N-(*O,O*-dihexadecyl)phosphorylalanine was distinctly discernable in the negative mode

Figure 3 ESI-MS spectra of the material collected from the aged cast film



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 *N*-(*O,O*-dihexadecyl)phosphorylalanine chemically synthesized, which confirms the presence of dipeptide derivative. We also observed that the FT-IR spectra of solid powder and solutions in chloroform remained unchanged at different time intervals under ambient conditions for a long time (over days), which suggests that the amphiphilic phosphorylalanine is very stable in bulk phase without any condensation.

From these results, it could be concluded that it is the particular orientation and ordered packing of the monomers within the multi-layered structures on the cast film that allows the occurrence of spontaneous condensation.

In summary, a novel amphiphilic amino acid derivative was found to condense spontaneously on the thin film cast on the  $\text{CaF}_2$  substrate due to the molecular arrangement and orientation in the layered structure of the cast film. In our opinion, the research may consummate the theory of *N*-phosphorylamino acid as the co-evolution model of life origin<sup>15</sup> as regard to the formation of biopolymer in ordered systems similar as the natural process occurring in cell membranes<sup>16</sup>.

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