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Langmuir films of an amide extracted from *Piperaceae* and its interaction with phospholipids

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Abstract

In this work, we investigate Langmuir monolayers from an amide extracted from dried roots of *Ottonia propinqua*, a native Brazilian plant believed to exhibit anesthetic and hallucinogen activities. In addition to producing monolayers from the amide itself, we probe the molecular-level action of the amide on phospholipids employed as simple membrane models. The surface pressure–molecular area (π–A) isotherms for the amide were little affected by a number of subphase conditions. Almost no changes were observed upon varying the compression speed, spreading volume onto the surface, ions in the subphase, ionic strength and the solution solvent. However, stronger effects occurred when the subphase temperature and pH were altered, as the isotherms were shifted to larger areas with increasing temperatures and decreasing pHs. These results are discussed in terms of the molecular packing adopted by the amide at the air–water interface. In the mixed films with arachidic acid, the area per molecule varied linearly with the concentration of amide, probably due to phase separation. On the other hand, in the mixed films with dipalmitoyl phosphatidyl choline (DPPC), small amounts of the amide were sufficient to change the π–A isotherms significantly. This points to a strong molecular-level interaction, probably between the phosphate group in the zwitterion of DPPC and the nitrogen from the amidic group.

Keywords: Isotherms; Anesthetic; Membrane

1. Introduction

A plant of genus *Ottonia*, which belongs to the family of *Piperaceae* and contains 23 species, 21 of which native of Brazil, is used as anesthetic and hallucinogen by the local people and find applications in treating toothache and sore throats [1–3]. The main component of these plants is an alkaloid derivative from amides [1–4]. The anesthetic effect and toxicity of an ethanolic extract from the root of the *Ottonia propinqua* specie have been investigated by Kute Rates et al. [5], which is the same specie studied by our group in this work and in a previous report [6]. The extraction of the amide and its characterization in

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powder form using Raman scattering and infrared absorption spectroscopies were described in Ref. [6]. A molecular structure based on the vibrational spectroscopy was proposed for the amidic compound extracted, which was consistent with the theoretical spectra obtained from ab initio calculations using Gaussian 98. In the present work, Langmuir films of the amide itself are characterized by surface pressure–molecular area (π–A) isotherms under different experimental conditions, in addition to the fabrication of mixed Langmuir films containing amide either with a fatty acid or a phospholipid. The main goal is to explore possible interactions between the amide and monolayer-forming materials, which may be relevant for its interaction with biological membranes [7–13].

2. Experimental details

A solution of the amide, whose extraction is reported in detail in Ref. [6], was prepared using dichloromethane. Langmuir films were produced using either a Lauda or a KSV 5000 Langmuir trough, with ultrapure water supplied by a Millipore system (18.2 MΩ cm) to prepare the subphase. Systematic changes in experimental conditions were carried out for the fabrication of neat amide Langmuir films, as follows. The compression speeds were varied between 5 and 40 mm min^{-1}; spreading volume onto the water surface varied from 110 to 880 μL; addition of ions Na^+, K^+, Ca^{+2} and Cd^{+2} into the subphase; ionic strength varied between 0.0003 and 0.0473 mol L^{-1}; the solvent in which the amide was dissolved was varied, covering a range of dielectric constant between 4 and 21; subphase temperature varied from 15 to 44 °C and pH varied between 1.8 and 5.7. Unless otherwise stated, the standard parameters used for fabrication of Langmuir films were 10 mm min^{-1} for compression speed, 15 °C for subphase temperature, 110 μL as spreading volume (10^{-4} mol L^{-1}) and ultrapure water as subphase (pH = 5.7). Mixed Langmuir films containing either amide with a fatty acid (arachidic acid, AA) or amide with a phospholipid (dipalmitoyl phosphatidyl choline, DPPC) were produced by co-spreading the solutions of amide/AA or amide/DPPC (the mixing ratios are given in molar), which were prepared in the same flask using dichloromethane as solvent. The subphase was ultrapure water kept at 20 °C and the compression speed was 10 mm min^{-1}.

3. Results and discussion

Before investigating the interaction between the amide and lipid molecules in Langmuir monolayers, a study was carried out for the amide itself. Changes in compression speed, spreading volume, ions in the subphase, ionic strength and solution solvent, as indicated in the previous section, have not affected the π–A isotherms strongly. For instance, Fig. 1 presents the π–A isotherms for amide monolayers for various barrier speeds: 5, 10, 20 and 40 mm min^{-1}. There is only a slight shift of the isotherm towards smaller areas with decreasing speeds, which indicates that the Langmuir film is compressed practically under thermodynamic equilibrium [14,15]. Considering the dimensions of the amide molecular structure shown in Fig. 1, the extrapolated area A_{ext} (obtained by extrapolating the isotherm in the condensed phase to $\pi = 0$) and the absence of hysteresis in a compression–decompression cycle (figure not shown), one could speculate that the amide might form a truly monolayer at the air–water interface.

The amide monolayers do depend on the subphase temperature, as illustrated in Fig. 2. The π–A isotherms are shifted to larger areas with increasing temperatures. The important role played by tempera-

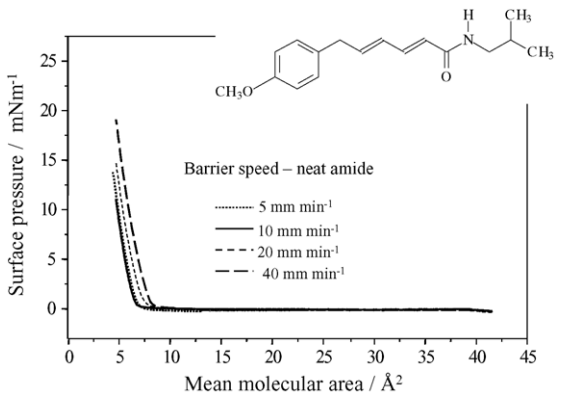

Fig. 1. π–A isotherms of the neat amide for different compression speeds and subphase temperature kept at 15 °C with pH 5.7. Inset: amide molecular structure.