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Thermodynamic analysis and fluorescence imaging of homochiral amino acid–amino acid interactions at the air/water interface

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ABSTRACT

Surface pressure-molecular area (Π-A) isotherms and fluorescence microscopy were used to investigate the interactions between *N*-stearoyl-glutamic acid (L- and D-) and L-arginine at the air/water interface. *N*-stearoyl-glutamic acids (C_{18}-Glu) with different chirality (L- and D-) were spread at the air–water interface onto subphases containing varied concentrations of L-arginine at pH 5. The apparent binding affinity of C_{18}-Glu to L-arginine was obtained by fitting the plots of the change in mean molecular area of C_{18}-Glu vs. L-arginine concentration. The thermodynamic properties of the binding events such as binding constant and Gibbs free energy were estimated from the binding curves. We found that *N*-stearoyl-L-glutamic acid (C_{18}-L-Glu) had a stronger binding affinity to L-arginine as compared to *N*-stearoyl-D-glutamic acid (C_{18}-D-Glu) at low to moderate surface pressures (below ~22 mN/m). The C_{18}-D-Glu had stronger binding to L-arginine at higher surface pressure. Domains with different shapes in C_{18}-L-Glu and C_{18}-D-Glu monolayers were observed under a fluorescence microscope in situ at the air/water interface. Herein, we present a mechanism for C_{18}-L-Glu and C_{18}-D-Glu interacting with L-arginine at the air/water interface.

1. Introduction

Chirality is one of the most distinctive biological signatures of nature and the chiral interactions in living systems are fundamental in chemical biology and pharmacology [1–6]. Ever since Louis Pasteur discovered the existence of two non-superimposable forms of tartrate crystals, the phenomenon of chirality has inspired scientists from various fields [3,7–17]. Building blocks of life, such as amino acids, sugars, proteins, and DNA, and many natural products like steroids, hormones, and pheromones are chiral. In nature, amino acids in proteins are exclusively in L form, while sugars in DNA and RNA are all in D form [18]. Homochirality is critical for molecular recognition and replication processes in living systems [19,20]. Enantiopure molecules such as enzymes help to direct the synthesis of further enantiopure molecules in organisms. The presence of single wrong-handed amino acid can disturb a stable α-helical structure in proteins. Chiral interactions are very important to living systems; however, understanding the natural mechanism of homochiral selectivity remains as a significant challenge [20–22].

Herein, Langmuir monolayers of amino acid surfactant at the air/water interface are employed as a model system to elucidate the underlying mechanism of chiral interactions in biological systems by quantifying the interactions between amino acids. In order to study the chiral recognition phenomena, protein–protein interactions, and protein–lipid interactions, monolayers at the air/water interface have been used as a model for biomembranes [5,23–36]. It is expected that understanding chiral interactions in two-dimensional monolayers is simpler than that in three-dimensional systems [31,37]. One notable advantage of the air/water interface is that both the molecular conformation and molecular separation of surface-active chiral molecules can be tuned by mechanical compression of the monolayer [31]. In our studies, molecules of glutamic acid with a long hydrocarbon chain (C_{18}-Glu) are spread at the air/water interface, while L-arginine is dissolved in the subphase as shown in Fig. 1. Surface pressure-molecular area (Π-A) isotherms and fluorescence microscopy are used to explore the interaction between L-L and D-L complexes. The apparent binding constants and Gibbs free energy for glutamic acid–arginine associations were obtained. At low to moderate surface pressures, homochiral interactions are preferred over heterochiral interactions that C_{18}-L-Glu shows stronger binding to L-arginine as compared to C_{18}-D-Glu. A model is proposed to explain the chiral selectivity we observed in this study. In nature, almost all of the homochiral interactions and recognition events happen in the absence of mechanical forces. Our results provide some insights into understanding the origin of homochiral selectivity in nature from thermodynamic perspectives. Moreover, the method

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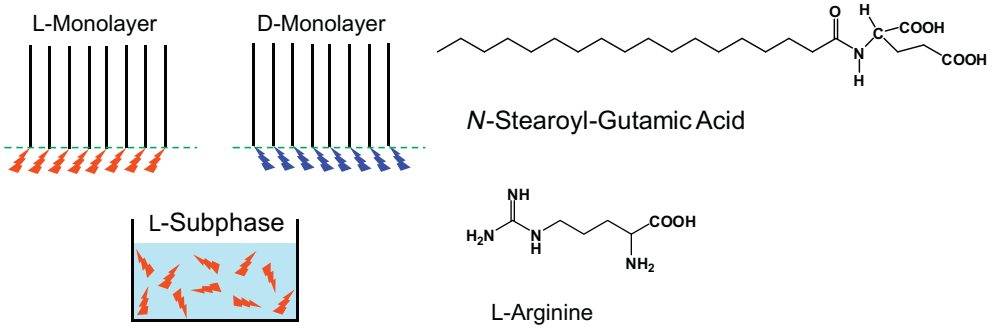

Fig. 1. Schematic illustration of amino acid–amino acid interactions at the air/water interface and the chemical structures of *N*-stearoyl-glutamic acid (C_{18}-Glu) and arginine.

developed in this study enable us to investigate the interactions between amino acid pairs that are known to be important in protein folding, enzyme specificity, receptor binding, cell signaling, and protein–protein interactions.

2. Materials and methods

2.1. Materials

C_{18}-L-Glu and C_{18}-D-Glu were synthesized from stearoyl chloride and L- and D-glutamic acid in a mixture of water and acetone as described previously [12,38]. Texas Red ®1,2-Dihexadecanoyl-*sn*-Glycero-3-Phosphoethanolamine, Triethylammonium Salt (Texas Red® DHPE) was from Invitrogen (Grand Island, NY). Low-conductivity water was produced from a NANOpure Ultrapure Water System (Barnstead, Dubuque, IA) with minimum resistivity of 18 MΩ cm. Extra dry chloroform (99.90%, stabilized) was ordered from Fisher Scientific.

2.2. Surface pressure-molecular area isotherms

C_{18}-L-Glu and C_{18}-D-Glu were dissolved in ethanol and dispersed into chloroform to reach a concentration of 1×10^{-3} M ($V_{ethanol}$:$V_{chloroform}$ = 1:24). The monolayer was prepared by spreading the chloroform/ethanol solution of C_{18}-Glu over 10 mM phosphate buffer at pH 5 with varied L-arginine concentration at 22 ± 1 °C. After 30 min for solvent evaporation, the surface pressure versus mean molecular area isotherms (Π-A isotherms) were recorded by using a KSV Mini-trough (KSV Instrument Ltd., Finland) with an area of 273 cm^2 at a constant barrier speed of 7.5 cm^2/min. To ensure the interaction between the monolayer and L-arginine reach an equilibrium, different times ranging from 15 min to 2 h were waited before the monolayer was compressed, but no significant differences were observed in the isotherms.

2.3. Epifluorescence microscopy of monolayers

Texas-Red®DHPE was added to the chloroform/ethanol solution of C_{18}-Glu at 0.5 mol% in order to image the domain morphology of the surfactant monolayers at the air/water interface. It was assumed that the introduction of Texas-Red®DHPE into the monolayer does not affect the interactions between C18-L-Glu or C18-D-Glu and L-arginine. The surfactant solution was spread onto the air/water interface and compressed under the same conditions as described above and imaged with epifluorescence microscopy. The microscope used in this study was an upright 80-*i* fluorescence microscope from Nikon (Tokyo, Japan) equipped with a 10× objective and a Texas Red HYQ filter (excitation range: 532–587 nm and emission range: 608–683 nm). All the images were captured on a Roper Scientific CCD camera using NIS-Elements AR 3.2 software. Fluorescence images were acquired for the monolayers of C_{18}-L-Glu and C_{18}-D-Glu on the subphases with various L-arginine concentrations.

3. Results and discussion

Surface pressure-molecular area (Π-A) isotherms are used to evaluate the interactions between L-arginine and glutamic acid with different chiral configurations. Fig. 2 shows the Π-A isotherms of C_{18}-L-Glu and C_{18}-D-Glu monolayers on L-arginine subphase at varied concentrations. Π-A isotherms indicate the formation of stable monolayers at the air/water interface. The monolayers collapse at approximately 40 mN/m for at all of the L-arginine concentrations investigated. The Π-A isotherms shift to larger mean molecular area as L-arginine concentration increases for both C_{18}-L-Glu and C_{18}-D-Glu. The increase in mean molecular area of C_{18}-Glu is an indication of interactions between glutamic acid and L-arginine at the air/water interface [31]. The isoelectric point (pI) for glutamic acid and arginine are 3.08 and 10.76, respectively [39]. Therefore, at pH 5 C_{18}-Glu is negatively charged and arginine is positively charged resulting in primarily electrostatic interactions between them. The electrostatic interactions and stereochemistry determine the relative orientation between arginine and glutamic acid at the air/water interface. The isotherms for C_{18}-L-Glu and C_{18}-D-Glu in the absence of L-arginine in the subphase show a plateau at a surface pressure around 20 mN/m. The plateau region is due to the orientational change of the surfactant molecules in the monolayer [40]. With the increase of L-arginine concentration in the subphase, the plateau region shift to higher molecular area. For the C_{18}-L-Glu monolayer, the plateau disappears when the concentration of L-arginine in the subphase reaches 30 mM, while the plateau in the C_{18}-L-Glu monolayer disappears at a lower L-arginine concentration (1 mM). The difference in the shift of plateau region between C_{18}-L-Glu and C_{18}-D-Glu monolayers suggests that L-arginine interact with C_{18}-L-Glu and C_{18}-D-Glu differently.

The interaction between glutamic acid and L-arginine at the air/water interface can be modeled by ligand-receptor binding with an assumption that the change in mean molecular area of C_{18}-Glu is proportional to the amount of L-arginine binding to the monolayer [31]. L-arginine in solution associates with glutamic acid in the monolayer leading to the formation of a glutamic acid–arginine complex: $E + R \leftrightharpoons ER$, here E is the glutanic acid in the monolayer and R is the L-arginine in the subphase. The process is characterized by an association constant, K ($K = \frac{[ER]}{[E][R]}$). The reciprocal K ($1/K = K_d$) is the dissociation constant, which is equal to the concentration of L-arginine at which half of the binding is reached. The lower K_d, the tighter binding. We compared the change in mean molecular area on the L-arginine subphase at varied concentrations with that on buffer in the absence of L-arginine. This change is plotted against L-arginine concentration in Fig. 3 for both C_{18}-L-Glu and C_{18}-D-Glu