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# Chiral Recognition of L-Amino Acids on Liposomes Prepared with L-Phospholipid

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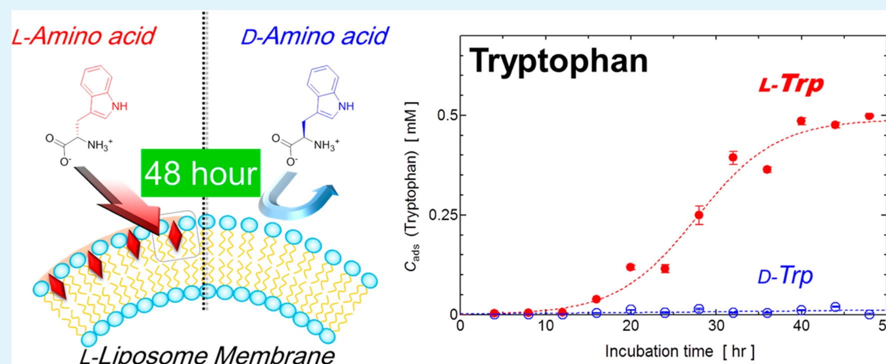
Supporting Information




**ABSTRACT:** In this study, we demonstrated that liposomes composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) can recognize several L-amino acids, but not their D-enantiomers, by analyzing their adsorptive behavior and using circular dichroism spectroscopy. Changes in liposomal membrane properties, determined based on fluorescent probe analysis and differential scanning calorimetry, were induced by L-amino acid binding. UV resonance Raman spectroscopy analysis suggested that the chiral recognition was mediated by electrostatic, hydrophobic, and hydrogen bond interactions, where the recognition site could therefore be constructed on the DPPC membrane. Our findings clearly indicate the potential function of liposomes in asymmetric recognition.

**KEYWORDS:** *liposomes, chiral recognition, amino acids, adsorption, electrostatic interaction, hydrogen bond, lock-and-key model*

Stereochemical structures of biomolecules play important roles in biological systems. In these biomolecules, homochirality is one of the essential properties. In general, the breaking of symmetry is observed in several molecules, for example, L-amino acids, L-phospholipids, and D-carbohydrates.[1] In contrast, in vitro chemical reactions with prochiral substrates usually produce *racemic* compounds. Although asymmetric catalysts have been developed to produce enantio-excess products,[2,3] the origin of such homochirality continues to attract much attention.[4]

The recognition and separation of L- and D-amino acids are essential processes from the viewpoints of scientific understanding and practical application. To obtain enantioselective products, chiral and racemic approaches have been developed, which are mainly based on the asymmetric synthesis of the pure enantiomers and on separating mixtures of the two enantiomers, respectively.[5] For instance, the membrane matrixes composed by cyclodextrin derivatives have been developed,[6] in which a specific "lock-and-key" interaction could be designed at the molecular surface region. In comparison to "rigid" recognition interfaces, there are some reports that supramolecular complex or flexible materials can be used to recognize enantiomers at their "self-assembled" surfaces.[7−10] By using a molecular imprinting membrane, Langmuir-type isotherms have been obtained in adsorption kinetics, indicating the enantioselective interaction of the target molecules at the recognition site in the membranes,[11] although in general Langmuir isotherms can be obtained for a homogeneous surface in the adsorption process. It is therefore expected that the design of recognition sites on the self-assembled membrane would enable the development of a platform for chiral separation.

It has been reported that self-assembled structures can act as an interface for enantioselective chemical processes.[12] The micelle−phenylalanine interaction has been applied to cascading ultrafiltration to achieve enantiomer separation.[13] Self-assembled monolayers at the air−water interface have also revealed selective partitioning of enantiomers of the amino acids Val, Leu, and Phe, depending on the surface pressure of the monolayer,[14] suggesting the possibility that self-assembled lipid membranes, for example, liposomal membranes, can be utilized for the chiral separation processes. It has been reported

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that liposomal membranes can accelerate the enantioselective amino acid polymerization from racemic *N*-carboxyanhydride amino acids.[15] These liposomal membranes contributed to the elongation of oligopeptides by providing a suitable platform (membrane surface) for the polymerization reaction, while a relatively insignificant difference in the chirality of tryptophan (Trp) oligomers was found in the presence of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) liposomes. The enantiospecific interaction of amino acid dimers (Trp-Trp) has been observed in phospholipid bilayers.[16,17] At the phospholipid liposomal or micellar surface, $^{1}$H NMR analysis showed that different conformation of Trp-Trp enantiomers were induced, in which the distance and dihedral angle of Trp-Trp differed between the enantiomers.[16] In our previous studies, liposomes were developed as a functional platform for recognition of biomolecules: proteins and enzymes,[18] amyloid beta,[19] single-stranded RNA,[20] and L-Trp.[21] These recognition events were significantly affected by the liposomal membrane properties such as fluidity and polarity. The phospholipid used to make the liposomes consisted of L-enantiomers; thus, the liposomal membranes contained chiral specificity. Therefore, it is expected that not only chemical properties of amphiphilic molecules but also the physicochemical properties of self-assembled membranes are possible clues to develop a novel, flexible recognition site (lock) on the membranes. On the basis of the characterization of physicochemical properties of membranes, an ideal membrane surface for molecular recognition can be designed.

In this study, the chiral separation of L- and D-amino acids was performed using liposomes composed of L-DPPC. After 48 h of incubation of amino acids with L-DPPC liposomes, L-Trp and L-His (His = histidine) were effectively bound, but not D-Trp and D-His. The adsorption of L-Trp and L-His was shown to follow Langmuir-type adsorption kinetics. In addition, the L-amino acid-selective binding was also observed in racemic solution. To determine the effect of amino acid binding on the properties of liposomal membranes, the fluorescence spectra of membrane-binding 8-anilino-1-naphthalenesulfonic acid (ANS), differential scanning calorimetry (DSC), and dielectric spectroscopy analysis were performed. Finally, the interaction mechanisms of DPPC liposomes and L-Trp and L-His were estimated by UV resonance Raman spectroscopy, which suggested that multiple interactions between liposomes and L-amino acid molecules resulted in enantioselective amino acid binding.

## EXPERIMENTAL SECTION

**Materials.** 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC; carbon number/unsaturated bond = 16:0), was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). L-Trp, D-Trp, and other amino acids were purchased from Peptide Institute (Suita, Osaka, Japan). All amino acid reagents were over 98% purity of enantiomers. The fluorescent probe ANS was purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were purchased from Wako Pure Chemical Industry Ltd. (Osaka, Japan) and were used without further purification.

**Liposome Preparation.** A chloroform solution of phospholipids was dried in a round-bottom flask by rotary evaporator under a vacuum. The lipid films obtained were dissolved in chloroform, and the solvent was removed. These operations were repeated twice. The lipid thin film was kept under a high vacuum for at least 3 h, and then it was hydrated with ultrapure water at above the transition temperature ($T_m$). The vesicle suspension was frozen at −80 °C and was thawed at 60 °C to enhance the transformation of small vesicles into large multilamellar vesicles (MLVs). This freeze−thaw cycle was performed five times. MLVs were used to prepare the large unilamellar vesicles (LUVs) by extruding the MLV suspension 11 times through two layers of polycarbonate membranes with mean pore diameters of 100 nm using an extruding device (LiposoFast; Avestin Inc., Ottawa, Canada).

**Adsorption of Amino Acids to Liposomal Membrane.** The liposome suspensions (lipid: 4.5 mM) were mixed with L- or D-Trp and other amino acids with different equivalent ratios, and they were incubated at 25 °C for 48 h, to reach equilibrium of adsorption. After incubation, liposomes and adsorbed amino acids were separated by ultrafiltration membrane with the molecular cut of 50 000 Da (USY-5; Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The concentration of filtered amino acid ($C_{flt}$) was measured by the absorbance by UV spectrometer (UV-1800; Shimadzu, Kyoto, Japan) or fluorescence of fluorescamine (Ex: 390 nm, Em: 475 nm)[22] by spectrofluorometer (FP-8500; JASCO, Tokyo, Japan). The concentration of adsorbed amino acids ($C_{ads}$) and adsorbed amount of amino acids per lipid volume ($q$) were calculated by following equations.

$$C_{ads} = C_{ini} - C_{flt} \tag{1}$$

$$q = C_{ads}/C_{lip} \tag{2}$$

where $C_{ini}$ and $C_{flt}$ represent the concentration of amino acids in initial solution and supernatant, and $C_{lip}$ represents the concentration of liposomes. The adsorption isotherms were evaluated by the plot of $q$ versus $C_{flt}$ in 48 h of incubation (equilibrium concentration) at the same lipid concentration (4.5 mM). Especially, Langmuir isotherms were described by following equations.

$$q = q_{max}KC_{flt}/(1 + KC_{flt}) \tag{3}$$

where $q_{max}$ and $K$ represent the maximum of $q$ and a binding constant. The correlation for Langmuir isotherms and $q_{max}$ and $K$ values were estimated by the plot of $C_{flt}$ versus $C_{flt}/q_{max}$. From the $C_{ads}$ of L-amino acids and D-amino acids, separation parameter ($S_{L/D}$) was calculated by following equations.

$$S_{L/D} = C_{ads(lform)}/C_{ads(dform)} \tag{4}$$

where $C_{ads\ (L\ form)}$ and $C_{ads\ (L\ form)}$ represent the $C_{ads}$ values of L-amino acids and that of D-amino acids, respectively.

**Circular Dichroism Spectroscopy Analysis of Racemic Amino Acids.** To analyze the concentration ratio of L- and D-amino acids, circular dichroism (CD) spectra were measured by JASCO J-820 SFU spectropolarimeter (JASCO, Tokyo, Japan). The CD spectrum from 300 to 200 nm was measured with a quartz cell (0.1 cm path length) at a scan speed of 100 nm per minute and a width of 2 nm. Three scans excluding water background signals were obtained at 25 °C, and the data were calculated as molar ellipticities. In the case of racemic solutions, the initial concentrations of total amino acids were 1.0 mM. The racemic mixtures of L- and D-Trp or L- and D-His were incubated with DPPC liposome (4.5 mM) for 48 h at 25 °C, and the liposomes and adsorbed amino acids were removed by filtration shown in above.

**Hydrophobicity Analysis of the Membrane Surface by ANS.** The local hydrophobicity of the liposomal membrane was characterized by using an environmentally sensitive probe; ANS.[23] ANS dissolved in ethanol were added to the liposome suspension or the preincubated mixture of liposome and amino acids. The final concentrations of lipid, ANS, and amino acids were 100 $\mu$M, 1 $\mu$M, and 10 $\mu$M, respectively. The fluorescence spectra of ANS were measured by using a fluorescent spectrometer after incubation for 30 min. ANS was excited at 350 nm, and the emission spectra were measured from 375 to 530 nm.

**Dielectric Dispersion Analysis (DDA) for the Bound-Water in Surface Regions of Liposomal Membranes.** The dielectric permittivity ($\varepsilon'$) and dielectric loss ($\varepsilon''$) were measured at the frequency range from 1.0 and 6.0 GHz, by using a network analyzer (Keysight Technologies, PNA-X N5245A, 10 MHz to 50 GHz). In this region, the relaxation properties of the bound water of liposomal

membrane surfaces can be observed together with bulk water signals.[24,25] The measurements were performed at 25 °C, and the concentrations of lipid or amino acids of liposome suspension were 100 and 15 mM, respectively.

**Differential Scanning Calorimetry Analysis of Liposomal Membranes.** A differential scanning calorimeter (DSC-60; Shimadzu, Kyoto, Japan) was used for calorimetric measurements of liposomes. Liposome suspensions (100 mM) with or without amino acids (15 mM) were incubated for 48 h at 25 °C before DSC measurements. The 20 $\mu$L sample solution was sealed in an alumina hermetic pan. Thermograms were obtained with a heating and cooling rate of 2 °C/min between 25 and 50 °C. There were no significant differences between the thermograms in heating and in cooling processes for one sample. At least three cycles of heating/cooling were repeated in each experiment, and the accumulated data were used for the calculation of the enthalpy variation ($\Delta H$) and transition temperature ($T_m$) values.

**Raman Spectroscopy Analysis of Trp and His.** UV resonance Raman spectra of Trp and His were measured by a confocal Raman microscope (LabRAM HR-800; HORIBA, Ltd., Kyoto, Japan) at a excitation wavelength of 266 nm, with laser power of 50 mW. The data accumulation was 30 s, and the scan was repeated three times for each sample.

**Statistical Analysis.** Results are expressed as mean ± standard deviation. All experiments were performed at least three times. The distribution of data was analyzed, and statistical differences were evaluated by use of Student's *t* test. A *P*-value of <0.05 was considered significant.

## RESULTS AND DISCUSSION

**Adsorption Behavior of Amino Acids by Liposome Membrane.** To investigate the adsorption behavior of amino acids on liposome membranes, the adsorption of the L- or D-amino acids on the liposome membrane was evaluated, where Trp and His were selected as target molecules.

Figure 1 shows the time course for adsorption of the amino acids on DPPC liposomes. The adsorbed amounts of L-Trp and L-His gradually increased after 16 h of incubation, and reached a plateau after 48 h, at which point almost all of the amino acids were adsorbed on the DPPC liposomes. It was also found that the adsorption kinetics of the L-amino acids were sigmoidal with a lag time (no adsorption from 0 to 16 h). However, negligible amounts of D-Trp and D-His were adsorbed on the DPPC liposomes even at 48 h. Sarangi et al. reported that L-Trp was adsorbed by DPPC supported lipid bilayers (SLB) via interaction with the DPPC head groups,[26] although the authors did not refer to the D-Trp enantiomer. The asymmetric selectivity of Trp was partly shown in our previous report. L-Trp selectively partitioned to the liposomal membrane, while we did not observe selective partitioning of L-Trp in a liquid–liquid two-phase system in the presence of phospholipids (no self-assembly).[21] These results suggest that the self-assembled lipid membrane structure plays an important role in the asymmetric recognition of amino acids.

The adsorptive behaviors of L-amino acids on liposomal membranes can be regarded as those of guest molecules onto host materials (e.g., molecular imprinted membrane).[7] To estimate the adsorption type of Trp and His, the adsorbed amounts ($q$) versus the equilibrium concentration of amino acids ($C$) was plotted at different molar ratios of amino acids and DPPC (Figure 2). The linear correlation between equilibrium and adsorbed concentrations, as revealed by the Langmuir plot (Figure S1), suggests that the adsorption isotherms of L-Trp and L-His with DPPC liposomes are of the Langmuir type. It has been reported that the adsorption of L-arginine on 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine

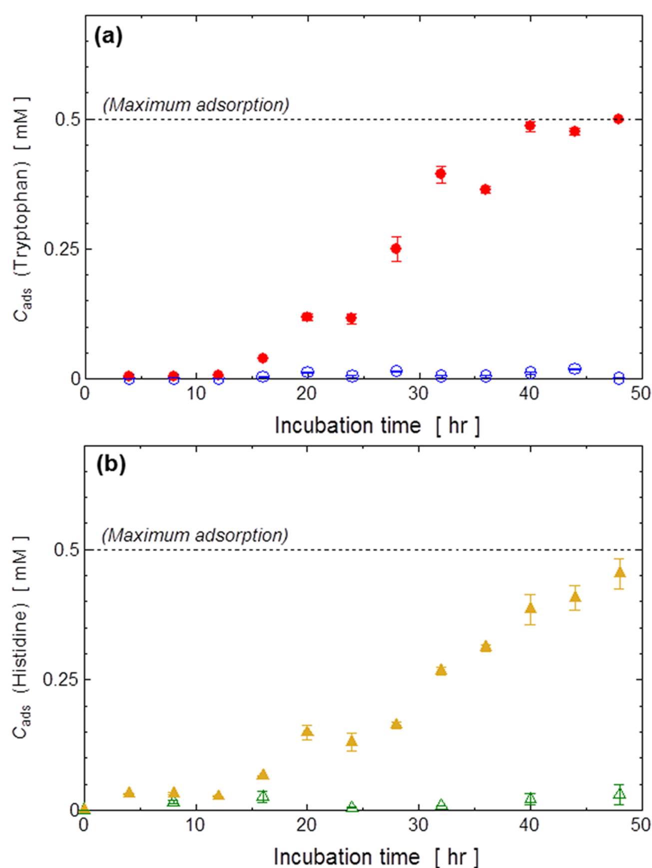


**Figure 1.** Time course of adsorbed concentration ($C_{ads}$) of L or D forms of amino acids. (a) $C_{ads}$ of L-Trp (●) and D-Trp (○). (b) $C_{ads}$ of L-His (▲) and D-His (△). In all samples, the initial concentrations of amino acid and DPPC were 0.5 and 4.5 mM, and the incubation temperature was 25 °C.

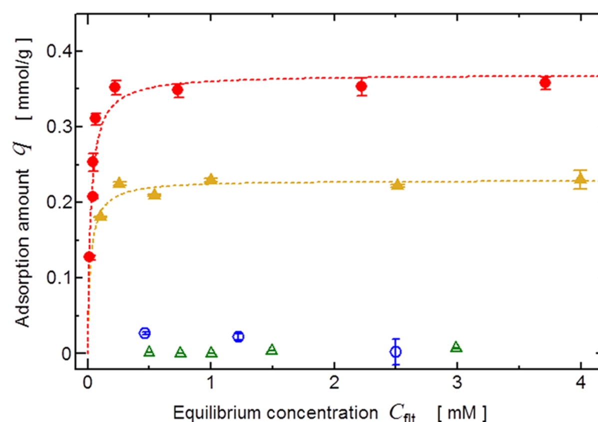


**Figure 2.** Evaluation of adsorption isotherms of L-Trp (●), L-His (▲), D-Trp (○), and D-His (△) on DPPC liposomes (4.5 mM). The fitting curve of Langmuir isotherms eq 3 are shown by dotted line (Langmuir plots of L-Trp and L-His were shown in Figure S1. All samples were incubated for 48 h at 25 °C, and the adsorption amounts are calculated based on the eqs 1 and 2.

(DMPC) or 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) liposomes was also correlated with Langmuir isotherms.[24] In the case of D-amino acids, reliable correlations were not obtained for $C$ versus $q$ due to their negligible adsorption on DPPC. According to the hypothesis, a monolayer adsorbed to the surface adsorption site is known



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as Langmuir model. Our results therefore suggest that the self-assembled membrane structure formed a "uniform adsorption site" for the binding of L-Trp and L-His molecules at the membrane surface of DPPC liposomes due to the rearrangement of phospholipid molecules, to decrease binding free energy in a "local minimum." The Langmuir isotherms indicate binding constants for L-Trp and L-His of 39.6 and 42.3 $mM^{-1}$, respectively. These values suggest a slightly stronger binding of L-Trp compared with binding of L-His to DPPC liposomes. The ratio of DPPC per adsorbed L-Trp and L-His was ~3.9 and 8.8, respectively, indicating that the chiral recognition sites were composed of multiple DPPC molecules. It was therefore investigated whether the self-assembled membrane structure of the DPPC liposomes showed a higher chiral recognition for L-Trp and L-His.

The above results suggest that liposomes can be applied to chiral separation of racemic solutions. Thus, the adsorptive behaviors of Trp and His in their racemic solutions were determined using CD spectroscopy. We found that the peak intensities at 222 nm (Trp) and 213 nm (His) corresponded to the ratio of L- and D-amino acids at the same total concentration of amino acids (1.0 mM; Figure S2a,b). Racemic mixtures of Trp or His were incubated with DPPC liposomes for 48 h, and the samples were filtered based on the procedure described in the Materials and Method section. As shown in Figure 3a,b, the

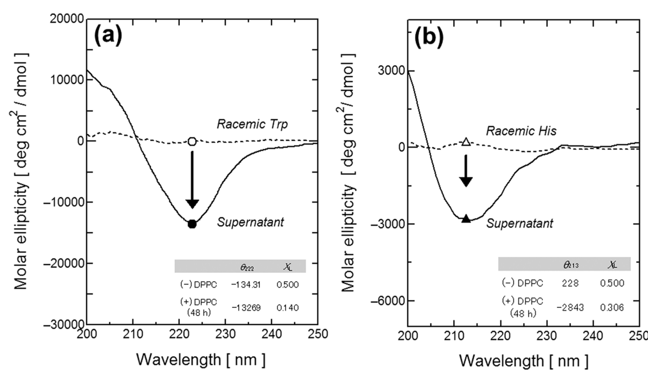


**Figure 3.** CD spectroscopic analysis of racemic amino acid solutions before (−) and after (+) incubation with DPPC liposomes. (a) CD spectra of initial racemic (dotted line) and supernatant (solid line) solutions of Trp after adsorption. (inset) The molar ratio of L-Trp ($X_L$) calculated by the molar ellipticities at 222 nm ($\theta_{222}$; calibration curve is shown in Figure S2). (b) CD spectra of initial racemic (dotted line) and supernatant (solid line) solutions of His after adsorption. (inset) The molar ratio of L-His ($X_L$) calculated by the molar ellipticities at 213 nm ($\theta_{213}$; calibration curve is shown in Figure S2). In all samples, the initial concentrations of total amino acid and DPPC were 1.0 and 4.5 mM, and the incubation time and temperature were 48 h and 25 °C, respectively.

deracemization of both Trp and His solutions was observed in the supernatants, which indicates the removal of L-amino acids from solution by binding to DPPC liposomes.[27] The molar fractions of L-amino acids ($X_L$) for L-Trp and L-His were 0.140 and 0.306, respectively. In previous reports, a monolayer of cholesterol-armed cyclen $Na^+$ and a cellulose acetate polymer imprinted with enantiomer glutamic acids produced enantiomeric ratios of 0.36 and 0.13, respectively.[14,28] Although it is difficult to directly compare our data with these values, our results show a higher chiral selectivity for L-Trp and L-His. The above results indicate the preferential adsorption of L-Trp and L-His on DPPC liposomes in racemic solutions; however, the adsorbed amounts were not as large as with the enantiomerically pure (L-form) solutions (Figure 1). Such preferential adsorption could also be observed by using liposome-immobilized hydrogels. We hypothesize that the racemic amino acid solutions resulted in inhibition of the adsorption of L-forms. Although D-form Trp or His showed little adsorptions on DPPC liposomes, there still exists a possibility that D-forms weakly interact with DPPC membranes, resulting in an occupancy of the amino acid binding site of L-forms. In the case of the enatiomerically pure samples, the interactive moieties (carboxyl group, amine group, side chain) in L-form amino acid molecules could fully interact with DPPC liposome, while those in D-forms did partially, and then, resulted in the enantioselective adsorption. According to the specific interaction of amino acids, the physicochemical membrane properties can be affected. To achieve better separation using liposomes, it is critical to investigate the dynamic properties of liposomal membranes during chiral recognition.

**Characterization of Physicochemical Properties of Membranes during Amino Acid Recognition.** The physicochemical properties of liposomal membranes (e.g., micropolarity)[29] are possible clues to enable regulation of the interactions between guest molecules and liposomes. Therefore, we further studied the chiral recognition of the L- and D-amino acids using membrane surface hydrophobicity and DSC analyses.

The properties of liposomal membranes during amino acid adsorption were characterized by using the fluorescence probe 8-anilino-1-naphthalenesulfonic acid (ANS). ANS is known as a polar environment-sensitive probe,[30] which has been extensively applied to the analysis of membrane hydrophobicity. Figure 4 shows the time course of the fluorescence intensity

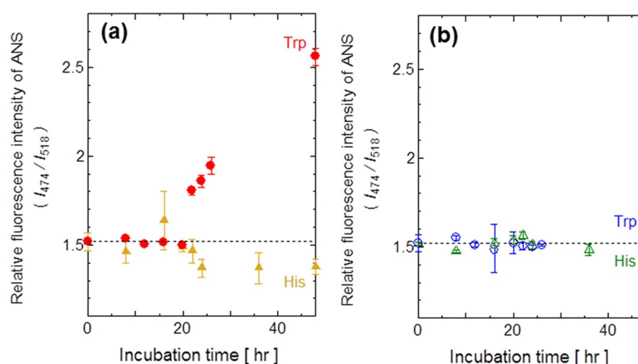


**Figure 4.** Time course of the relative fluorescence intensity of ANS with amino acids. The ratio of fluorescence intensities at 474 and 518 nm ($I_{474}/I_{518}$) is measured in the presence of L-Trp (●), L-His (▲), D-Trp (○), and D-His (△). The $I_{474}/I_{518}$ values are evaluated against the dielectric constant of several conditions of solvent. In all samples, the concentrations of amino acid and DPPC were 0.1 and 1.0 mM, and the incubation temperature was 25 °C before measurements.

ratios ($I_{474}/I_{518}$) of ANS in the DPPC liposome, where $I_{474}/I_{518}$ indicates the hydrophobicity degree at the liposomal membrane surface (Figure S3). In the presence of L-Trp, the $I_{474}/I_{518}$ values increased after 20 h of incubation, while the opposite tendencies were observed in the presence of L-His (Figure 4a). These results imply that the adsorption of amino acids could induce changes in the hydrophobicity of the liposomal membrane surface. The adsorption of L-Trp resulted in the exclusion of water molecules from the DPPC liposomes,


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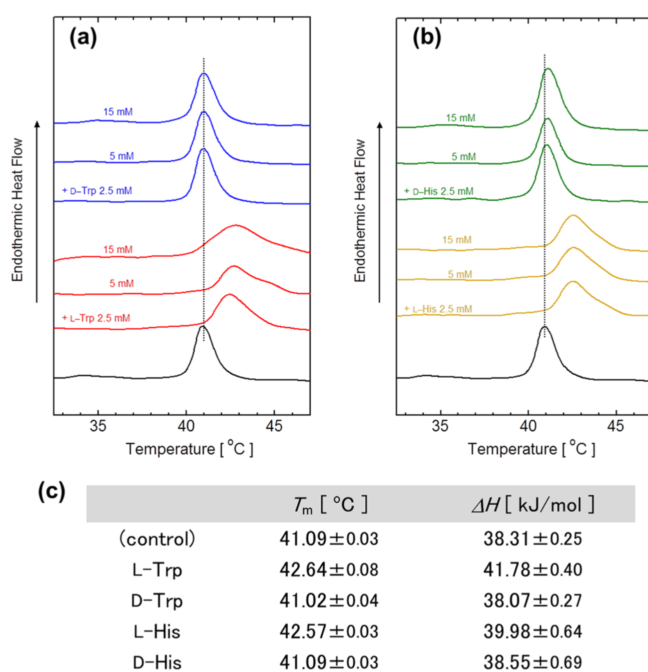
whereas L-His induced the hydration of the membrane surface, possibly because His is less hydrophilic than Trp.[31] Conversely, there were no significant changes in the amount of D-Trp and D-His, even after 48 h (Figure 4b).

Polarity changes in the hydrophobicity of the liposomal surface were also observed by adsorption of L-Trp, as determined by dielectric dispersion analysis (Figure S4). The suppression of this shoulder peak corresponds to diminishing the dielectric relaxation by insertion of cholesterols or proteins.[25] It was thus considered that the adsorption of L-Trp resulted in the exclusion of water molecules from the DPPC liposome, while L-His induced the hydration of the membrane surface. Collectively, these results suggest that the chiral recognition of amino acids induced changes in the membrane surface properties.

The phase transition of the DPPC liposomes was analyzed by DSC, and pretransition and main transition peaks were observed at 34.69 and at 41.09 °C, respectively. The main phase transition temperature ($T_m$) slightly increased by the addition of L-Trp or L-His, whereas no changes were induced by D-amino acids (Figure 5a,b). Such a variation of DPPC liposomes in $T_m$ is corresponding to the previous report about the incorporation of cationic lipid, owing to the electrostatic interaction between lipid molecules.[32] In the case of anionic liposomes, $T_m$ was increased by the addition of poly(L-lysine) or poly(L-arginine), owing to the stabilization of the gel phase via the restriction of electrostatic repulsion between neighboring phospholipid molecules.[33,34] Considering our results, the slight increases in the $T_m$ values indicate that the electrostatic interactions between L-amino acids and liposomal membranes induced the stabilization of the membrane packing. The adsorption of L-amino acids is also suggested by the suppression of the pretransitions to a ripple gel phase only in the presence of L-amino acids. The enthalpy ($\Delta H$) was also calculated for DPPC liposomes incubated with the amino acids (Figure 5c). The $\Delta H$ values slightly increased with the addition of L-amino acids, but no significant increase was observed in the presence of D-amino acids. Furthermore, the increase in $\Delta H$ values was proportional to the amino acid concentrations (Figure S5). These results suggest that such variations in $T_m$ and $\Delta H$ correspond to the adsorption of the L-enantiomers on DPPC liposomal membranes. It has been suggested that these endothermic changes could be related to the stabilization of acyl chain packing via interactions with L-amino acids.[35,36] Therefore, we investigated that whether the enantioselective adsorption of L-Trp and L-His on DPPC liposomes induced the membrane to become more ordered, in which electrostatic and van der Waals interactions are possible driving forces of their interaction.




| | $T_m$ [ °C ] | $\Delta H$ [ kJ/mol ] |
|---|---|---|
| (control) | 41.09±0.03 | 38.31±0.25 |
| L-Trp | 42.64±0.08 | 41.78±0.40 |
| D-Trp | 41.02±0.04 | 38.07±0.27 |
| L-His | 42.57±0.03 | 39.98±0.64 |
| D-His | 41.09±0.03 | 38.55±0.69 |

**Figure 5.** DSC analysis of DPPC liposomal membranes with or without amino acids. The thermograms of pure DPPC liposomes are shown as black lines. The main transition of DPPC liposomes (41.09 °C) is shown as dotted line. (a) DSC thermograms of DPPC liposomes mixed with L-Trp (red) or D-Trp (blue) at the different concentrations. (b) DSC thermograms of DPPC liposomes mixed with L-His (yellow) or D-His (green) at the different concentrations. (c) Table of phase transition temperatures ($T_m$) and enthalpies ($\Delta H$) determined by the main phase transition and liposome amounts (2.0 $\mu$mol). All samples were incubated by 25 °C in 48 h before measurements. The concentrations of amino acids and liposome were 15 and 100 mM, respectively.

**Possible Mechanism of Chiral Recognition.** A possible model for the asymmetric recognition of amino acids by liposomal membranes was investigated based on UV resonance Raman spectroscopic analysis. UV resonance Raman signals of Trp and His can be distinguished based on their constituents: for Trp, the peaks at 1467, 1550, 1576, and 1616 $cm^{-1}$ were assigned either as $N_1$−H bending or as $C_2$−$C_3$ or $C_3$−$C_\beta$ stretching, respectively;[37−39] for His, the peaks at 1498, 1576, and 1632 $cm^{-1}$ were assigned as stretching mode of the imidazole ring, $C_1$−$C_2$ stretching mode, and $C_4$−$N_5$ stretching mode, respectively[36] (Figure S6). Figure 6a,b shows the UV resonance Raman spectra of L- and D-Trp in the presence of DPPC after 48 h of incubation. In the presence of DPPC liposomes, the peak intensities at 1467, 1550, and 1576 $cm^{-1}$ of L-Trp decreased, while no significant changes were observed in the case of D-Trp. A decrease in the peak intensities of L-His was also observed, but not in the case of D-His (Figure 6d,e) These results suggest that L-Trp and L-His interacted with the DPPC liposomes. In addition, the decrease in the peak intensities was proportional to the lipid concentration (Figure 6c,f). The Raman peak intensity of Trp is sensitive to environmental hydrophobicity.[40] It has been reported that the adsorption of cysteine ethyl ester on DPPC liposomal membranes resulted in a decrease in its Raman peak intensities, in which the interaction between the phospholipids and amino acid side chains could be induced by the replacement of water molecules, with a consequent weakening of the vibrational force constants.[41] Furthermore, nucleobases, which are hydrophobic moieties in RNA molecules, can interact with the liposomal membranes via hydrophobic and hydrogen bonding interactions.[20] The log $P$ values of the indole ring (Trp) and imidazole ring (His) are 2.1 and −0.1, respectively (cf. amino acid backbone (Gly): −3.2)), and the hydrogen bond donors and acceptors are accumulated at the hydrophobic−hydrophilic interface of membranes. Taken together, these data suggest that the side chains of L-amino acids interacted with the hydrophobic regions of the liposomal membranes, in which hydrogen bonds could be formed between L-amino acids and DPPC molecules. The influence of ionic strength and the surrounding pH on the adsorption of Trp was furthermore investigated, indicating the decrease of adsorption by effects of both pH and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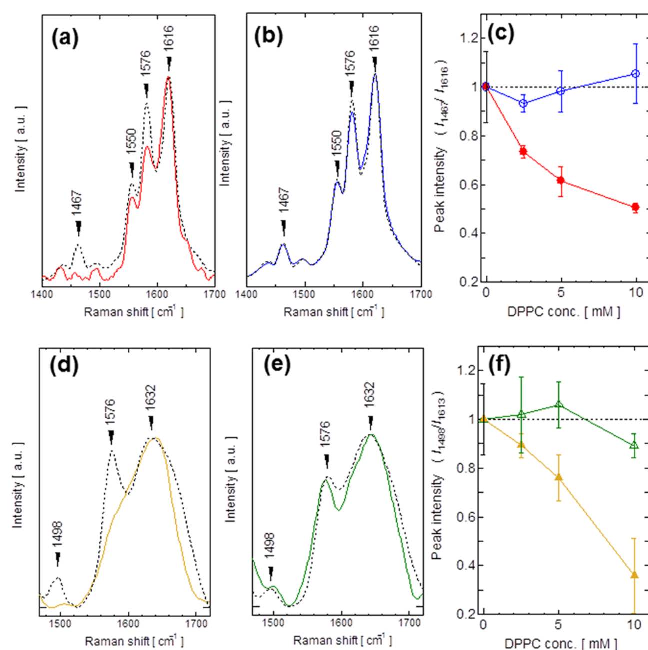


**Figure 6.** UV resonance Raman spectroscopic analysis of Trp and His with DPPC liposomes. Raman spectra of each amino acid are measured without (dotted line) or with DPPC liposomes (solid line): (a) L-Trp, (b) D-Trp, (d) L-His, and (e) D-His. (c) Relative Raman peak intensity of Trp (1467 $cm^{-1}$) with DPPC liposomes. The similar analysis for His (1498 $cm^{-1}$) was shown in (f). In all measurement, the concentrations of amino acids and DPPC were 15 and 100 mM. All samples were incubated at 25 °C for 48 h before measurements.

ionic strength. In our experiments, the aqueous bulk pH changes of the mixed solutions were within 0.4; thus, the effect of pH on their adsorptive behavior is negligible. This result suggests that electrostatic interactions could also contribute to the asymmetric recognition.

The adsorption of 10 different amino acids on DPPC liposomes was measured after 48 h of incubation (Figure 7). For almost all amino acids (except for Ser) the adsorption of the L-form was dominant, indicating that DPPC liposomes could be widely applied to recognize the chirality of amino acids. Higher L-selectivity was obtained especially with Trp and His ($S_{L/D}$ > 1000). In the case of Tyr and Pro, L-selectivity was also observed with $S_{L/D} \approx 100$, owing to the hydrogen donors or acceptors in their side chains, indicating that the formation of hydrogen bonds at the aromatic structure could play a crucial role in their high L-selectivity. In contrast, amino acids possessing hydrophobic (nonpolar) side chains (Leu and Val) showed lower L-selectivity ($S_{L/D}$ < 10). In addition, no L-selectivity was observed with Ser ($S_{L/D} \approx 1$). These results indicate that the hydrophobicity of the side chains might not be an important factor for inducing asymmetric recognition. Although Ser and Cys possess relatively smaller side chains, their adsorptive behaviors were opposite: higher adsorption amounts of both L- and D-Ser (without selectivity) and lower adsorption amounts for Cys. It is therefore hypothesized that the hydrogen bond is a key driving force for the binding of amino acids to liposomal membranes; their enantioselective binding can be attributed to stereospecific hydrogen bond formation at their side chains.

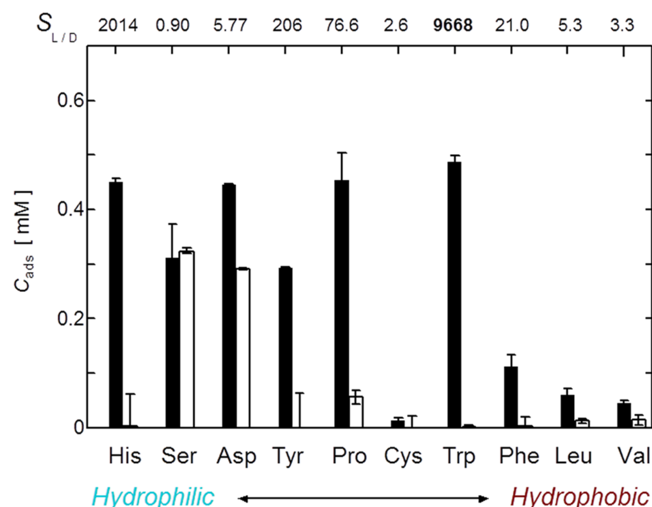


**Figure 7.** Adsorbed concentration ($C_{ads}$) of L or D forms of 10 amino acids on DPPC liposomes. The order of these amino acids corresponded to the hydrophobicity[31] from left to right. Separation parameter ($S_{L/D}$) of each amino acid is described above the corresponding bars. In all samples, the initial concentration of amino acids and DPPC were 0.5 and 4.5 mM, and the incubation temperature was 25 °C.

## CONCLUSION

Chiral recognition by L-DPPC liposomes was demonstrated by their L-amino acid-selective adsorptive behavior after 48 h of coincubation. Changes in surface hydrophobicity and thermodynamics properties of DPPC liposomes were observed during the adsorption of L-Trp and L-His. The obtained Langmuir isotherms indicate the adsorption of L-Trp and L-His at the membrane surface. These results suggest that L-selective adsorption could be caused by the formation of chiral recognition sites on the membrane: a dynamic rearrangement of self-assembled phospholipid molecules could be induced by the specific interactions as well as a lock-and-key model (Figure 8). In this model, even minor stereochemical recognition of L-

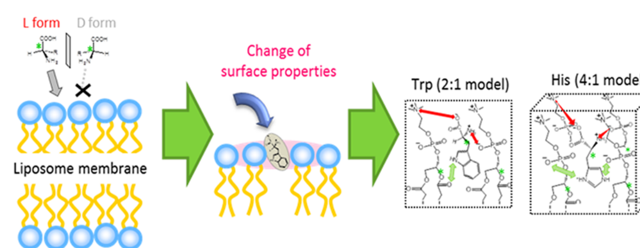


**Figure 8.** Conceptual illustration of the mechanism of chiral recognition on liposomal membrane.

amino acids induces changes in physicochemical properties of the liposomal membranes, resulting in specific adsorption. Chiral recognition by the L-phospholipid (e.g., DPPC) liposomes was also estimated for various types of amino acids. The important factor for chiral recognition is the formation of multiple interactions as a "three-point interaction."[42] In our case, L-phospholipids (DPPC) showed a better matching with L-amino acids (Trp, His) in the earlier step of adsorption, and then high selectivity was induced, which would be features of using a flexible interface.[43] Owing to multiple interactions between the side chains and phospholipids, a novel



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chiral recognition platform by utilizing a self-assembled, liposome-based system can be developed. It is also possible to design a high-performance membrane for chiral recognition by focusing on the physicochemical membrane properties of the recognition sites on membrane surfaces.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b07198.

> Langmuir plot about the adsorption of L-Trp and L-His; CD spectra and its calibration curve of the ratio of L/D form of Trp and His; fluorescence spectra and the ratio of fluorescence intensities of ANS in the mixture of water and 1,4-dioxane; spectra of dielectric loss of DPPC suspension with Trp or His; variation of the main transition enthalpy ($\Delta H$) in different concentration of amino acids; Raman peak assignments of Trp and His. (PDF)

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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