Colloid Polym Sci (2006) 285:239–243
DOI 10.1007/s00396-006-1547-y

SHORT COMMUNICATION

Fluorescence study on the domain formation of *N*-dodecanoyl-L-tryptophan within a liposome membrane

Kazuma Yasuhara · Toshinori Shimanouchi · Hiroshi Umakoshi · Ryoichi Kuboi

Received: 6 December 2005 / Accepted: 1 May 2006 / Published online: 25 August 2006

Abstract The aggregation behavior of *N*-dodecanoyl-L-tryptophan within a liposome membrane was investigated by fluorescence spectroscopy. Liposomes with a mean diameter of 100 nm, formed from either 1-2-dilauroyl-*sn*-glycero-3-phosphocholine, 1-2-myristoyl-*sn*-glycero-3-phosphocholine, or 1-2-palmitoyl-*sn*-glycero-3-phosphocholine, were used. Fluorescence measurements indicate that the sodium salt of *N*-dodecanoyl-L-tryptophan forms domains with the liposome membranes below the main phase transition temperature, T_m. Above T_m, the molecules are homogeneously dispersed in the liquid crystalline state of the membranes.

Keywords Liposome · Amino acid-type surfactant · Tryptophan fluorescence · Domain formation · Phase transition

Introduction

The domain formation of a number of different amphiphiles within a lipid bilayer is being studied with considerable interest [1–7]. It is well known that such domains play a key role in biological systems [1], and their structures are highly ordered. However, from the standpoint of physical chemistry, the mechanism for domain formation has not been clarified adequately. To gain insight into this mechanism, we used liposomes as model biomembranes. In recent years, several techniques, including NMR [2] and ESR [3], have been used to study the domain formation behavior within a liposome membrane [4–7]. Previously, we attributed the domain formation of various *N*-acyl amino acids to the leakage behavior of a fluorescent dye [8]. In this paper, we present the results obtained by using *N*-dodecanoyl-L-tryptophan, which is a fluorescent molecule and therefore allows for the application of fluorescence spectroscopy.

As observed in protein molecules, the fluorescence behavior of tryptophan residues sensitively reflects the local environment of the indole ring [9]. Based on this spectroscopic property, the fluorescence of sodium *N*-dodecanoyl-tryptophanate (SDTrp) in a liposome dispersion was measured and analyzed with respect to the formation of domains.

K. Yasuhara · T. Shimanouchi · H. Umakoshi · R. Kuboi (✉)
Department of Chemical Science and Engineering,
Graduate School of Engineering Science, Osaka University,
1-3 Machikaneyama-cho, Toyonaka,
Osaka 560-8531, Japan
e-mail: kuboi@cheng.es.osaka-u.ac.jp

Experimental

Materials

L-Tryptophan (H-L-Trp-OH) and dodecanoyl chloride were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used without further purification. *N*-dodecanoyl-L-tryptophan was synthesized by the reaction of H-L-Trp-OH with dodecanoyl chloride [10]. The crude product was washed by *n*-hexane several times to remove impurities. The purity of the product was confirmed by ^{1}HNMR spectroscopy. The sodium salt (SDTrp) was prepared from *N*-dodecanoyl-L-tryptophan by adding an equivalent quantity of sodium hydroxide. 1-2-Dilauroyl-*sn*-glycero-3-phosphocholine(DLPC),1-2-myristoyl-*sn*-glycero-3-phosphocholine (DMPC), and 1-2-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Avanti Polar Lipids (AL,

USA). All used chemicals except dodecanoyl chloride were of analytical grade.

Liposome preparation

Liposome suspensions were prepared by hydrating dry, thin lipid films (20 mg) with 2 ml of distilled water, followed by five freeze–thaw cycles (−80 and 50 °C). Finally, the liposome suspensions were extruded 11 times using a 100-nm polycarbonate membrane filter and a LiposoFast miniextruder from Avestin at 50 °C [11]. The lipid concentrations (13 mM) were determined by using phospholipase D and choline oxidase [12]. For the measurement, a special assay kit from Wako Pure Chemical Industries was used.

Surface tension measurement

To detect the aggregate formation of Dodec-Trp molecules, the surface tension of SDTrp aqueous solutions ranging in concentrations from 0 to 10 mM was measured. The measurements were performed at room temperature by using the surface tension meter CBVP-A3 (Kyowa Interface Science) and the Wilhelmy's hanging plate method.

Fluorescence measurement

For all the fluorescence measurements of liposome systems, the mixtures of 500 μl of each liposome suspension (13 mM) and 500 μl of 0.5 mM SDTrp solution were prepared. The final concentrations of the lipid and SDTrp were 6.5 and 0.25 mM, respectively. These solutions were kept at 4 °C overnight to complete the adsorption of SDTrp onto the vesicles. Fluorescence measurements were performed using the FP-6500 spectrofluorometer (Jasco). The excitation wavelength was 300 nm. The excitation and emission bandpasses were set at 3 nm. To measure the temperature dependency of the fluorescence intensities, the temperature of the measurement cell was controlled in a water bath, and the fluorescence intensity at maximal emission was automatically recorded as a function of temperature. The fluorescence measurements were performed in a range of 5 to 65 °C.

Results and discussion

First, the fluorescence behavior of SDTrp in an aqueous solution was examined. Fluorescence measurements of SDTrp aqueous solutions were performed for several concentrations; corresponding spectra are shown in Fig. 1. Maximal emission was observed around 360 nm for an aqueous solution due to the fluorescence of the indole group of the SDTrp molecule. It was observed that the fluorescence intensity decreased upon increasing the concentration of SDTrp above 1 mM. Between 7 and 10 mM, the fluorescence intensity remained consistently low (see Fig. 2). These results suggested that fluorescence quenching occurs at the concentration that is sufficiently high enough for aggregate formation (cf. micelles). To clarify the aggregate formation of SDTrp, the surface tension of different concentrations SDTrp in aqueous solutions was measured. Figure 2 shows the concentration dependency of both the surface tension and the fluorescence intensity at 360 nm. It was observed that the surface tension decreased with increasing concentration up to 1 mM, which indicated the critical micellar concentration of SDTrp before leveling off. Therefore, SDTrp micelles were formed in the aqueous solution at concentrations above 1 mM. This concentration corresponds to the maximum of the fluorescence intensity vs concentration curve. From these results, we concluded that the quenching of the tryptophan fluorescence was a result of aggregate formation and that this behavior was due to a collision of each tryptophan head on the surface of the micelles.

Because the fluorescence intensity of SDTrp reflects the formation of aggregates, we applied the above phenomena for detecting the association of SDTrp within a liposome membrane. In the case of liposome systems, the maximal emission of SDTrp was observed at 350 nm by excitation at 300 nm. Therefore, the temperature dependencies of the fluorescence intensity at 350 nm for the several kinds of liposome and SDTrp mixtures were examined and are shown in Fig. 3. Although the fluorescence intensity in general, decreased gradually with rising temperature, a discontinuation of that trend was observed at approximately 42 °C for the DPPC liposome system and 24 °C for the DMPC liposome system (closed triangles). These temperatures correspond to the gel-to-liquid crystalline phase transition temperatures (T_m) for these phospholipids [13]. In the case of the DLPC liposome system, there was no discontinuation. This result is consistent with the fact that there is no phase transition of the DLPC liposome in the given temperature range (T_m=0 °C) [13]. Furthermore, for the DPPC system, the intensity is also slightly increased at approximately 30 °C (open triangle). This tiny peak occurs at a temperature which is known as "pretransition temperature" as observed by DSC [13]. Because the wavelength of the emission maximum of SDTrp did not change even after heating at 60 °C (data not shown), we propose that the location across the membrane of SDTrp molecules in the liposome is not changed by varying the temperature. In general, the wavelength of the emission maximum of tryptophan reflects the polarity of the surrounding environment [14, 15]. We concluded that the localization of the indole ring with respect to the position within the membrane is the same above and below T_m.

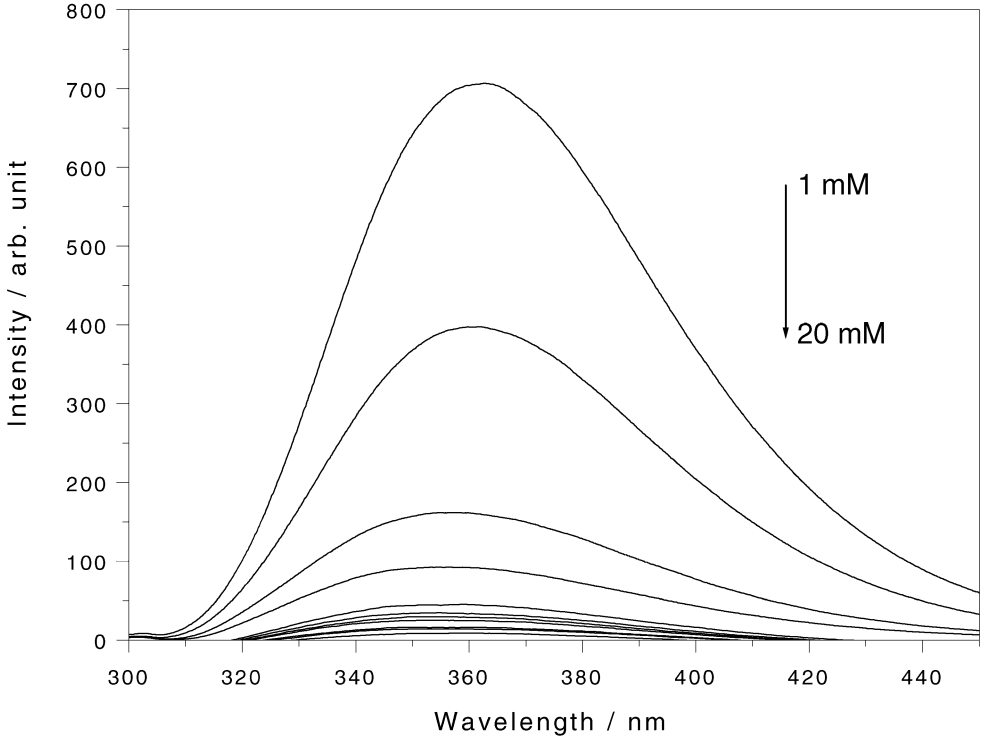

Fig. 1 Emission spectra of SDTrp aqueous solutions (pH=7) excited at 300 nm at room temperature. Concentrations of SDTrp were 1–20 mM at steps of 1 mM

It is assumed that aggregate formation of SDTrp in the liposome system leads to fluorescence quenching similar to that observed in the micellar solution. In other words, when some types of aggregates (domains) are formed within the liposome membrane, the tryptophan fluorescence is quenched. This concept permits us to explain the experimental results in Fig. 3. For the DPPC and DMPC systems, the fluorescence intensities drastically increased at T_m, reflecting a transition from aggregated SDTrp molecules to dispersed SDTrp molecules. We suppose that, below T_m, SDTrp molecules are condensed in a certain area within the liposome membrane, thereby quenching the fluorescence of

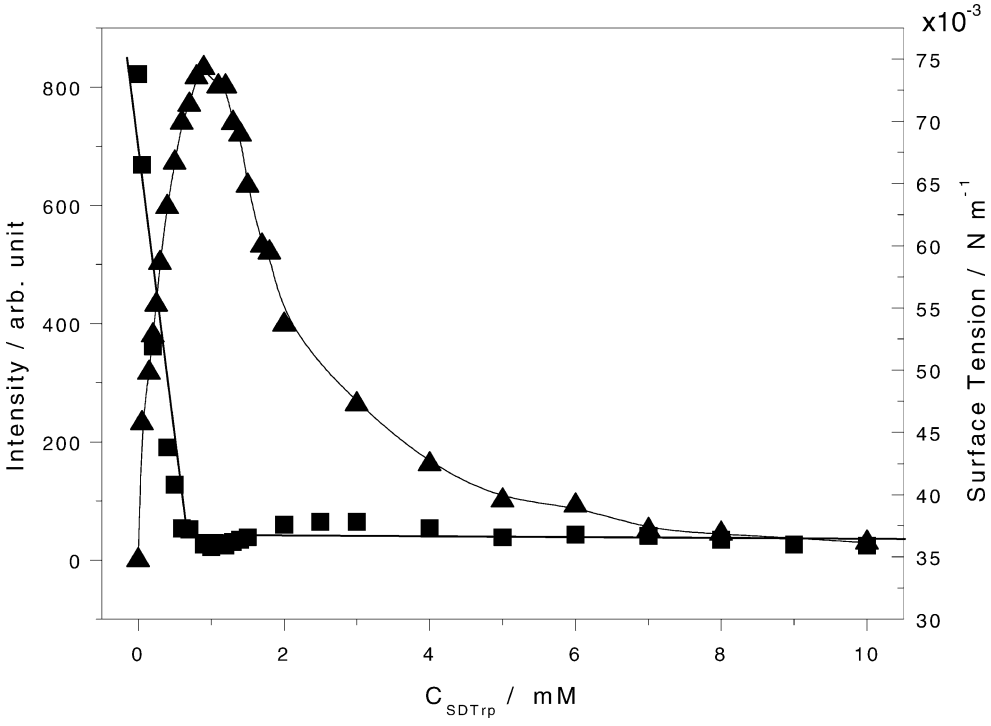

Fig. 2 Fluorescence intensity (*filled triangle*) and surface tension (*filled square*) vs concentration of SDTrp aqueous solution at room temperature

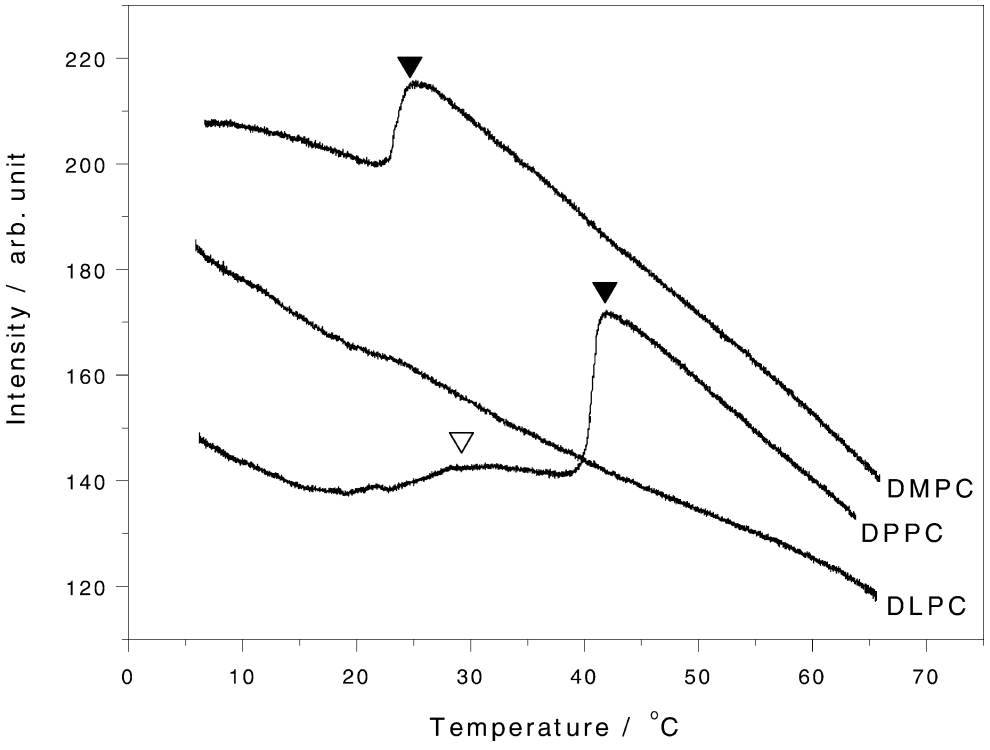

Fig. 3 Temperature dependencies of the fluorescence intensity for different SDTrp/liposome mixtures. The concentrations of all lipids and SDTrp were 6.5 and 0.25 mM, respectively

the tryptophan residue. On the other hand, in the liquid crystalline phase, SDTrp molecules are homogeneously distributed within the liposome membrane.

A decrease in the fluorescence intensity with increasing temperature was observed for all the liposome systems above T_m. To clarify this behavior, the temperature dependency of the fluorescence intensity at 360 nm of a 0.25 mM SDTrp aqueous solution was measured. At this concentration, SDTrp molecules existed as monomers. It was found that the fluorescence intensity decreased steadily with increasing temperature in the aqueous solution also (data not shown). In the case of the aqueous solution of the peptide of C-terminal tryptophan, it has been reported that quantum yield decreases with increasing temperature [16, 17]. This behavior indicates that the observed decrease in the quantum yield of SDTrp in liposome systems above T_m is a property of SDTrp and not directly indicative of the properties of the liposomes.

Conclusions

Based on the results obtained in this study, we propose that the formation of domains of SDTrp within the liposome membranes occurs as schematically shown in Fig. 4. SDTrp molecules form a kind of domain in the gel phase, below T_m, of each liposome system. In this phase, tryptophan residues are condensed within a certain area, resulting in fluorescence quenching. On the other hand, in the liquid crystalline phase, above T_m, SDTrp molecules are homogeneously dispersed; therefore, the fluorescence of the tryptophan residue is increased. In accordance, it is suggested that SDTrp molecules are effective in detecting domain formation within liposome membranes and that temperature plays a key role in controlling domain formation of *N*-dodecanoyl-L-tryptophan within liposomes.

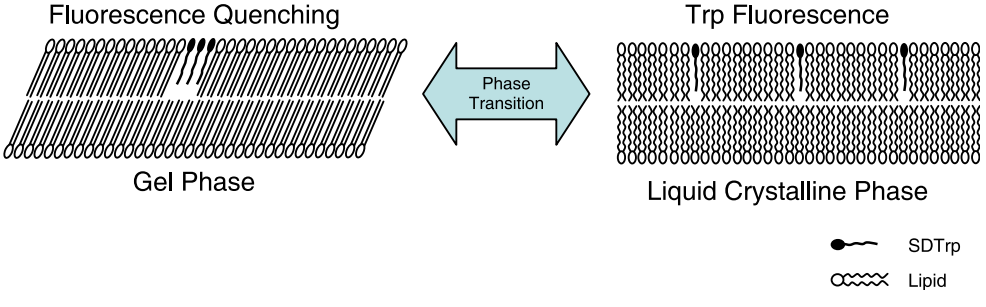

Fig. 4 Phase-dependent domain association model

Acknowledgments The authors thank Professor S. Miyagishi and Associate Professor A. Ohta of Kanazawa University for their invaluable advice on the synthesis of *N*-dodecanoyl-L-tryptophan. Thanks are due to Professor Peter Walde of ETH Zurich for reading the manuscript and making a number of useful suggestions. The work was partly supported by a Grant-in-Aid for Scientific Research (Nos. 15206089, 16686046, 16760635, and 17656268) from the Ministry of Education, Science, Sports, and Culture of Japan, a grant from the 21st Century COE program "Creation of Integrated EcoChemistry" of Japan Society for the Promotion of Science (JSPS).

References

1. Simons K, Ikonen E (1997) Nature 387:569
2. Silvius JR (2003) Biochim Biophys Acta 1610:174
3. Aussenac F, Tavares M, Dufourc E (2003) J Biochem 42:1383
4. Veiga MP, Goñi FM, Alonso A, Marsh D (2000) Biochemistry 39:9876
5. Harms GS, Sonnleitner M, Schutz GJ, Gruber HJ, Schmidt T (1999) Biophys J 77:2864
6. Nagami H, Umakoshi H, Shimanouchi T, Kuboi R (2004) Biochem Eng J 21:221
7. Schrader W, Kaatze U (2001) J Phys Chem B 105:6266
8. Yasuhara K, Ohta A, Asakura Y, Kodama T, Asakawa T, Miyagishi S (2005) Colloid and Polymer Sci 283:987
9. Cowgill RW (1967) Biochim Biophys Acta 133:6
10. Miyagishi S, Nishida M (1978) J Colloid Interface Sci 65:380
11. MacDonald RC, MacDonald RI, Menco BP, Takaeshita K, Subbarao NK, Hu L (1991) Biochim Biophys Acta 1061:297
12. Takayama M, Itoh S, Nagasaki T, Tanimizu I (1977) Clin Chim Acta 79:93
13. Ladbrooke BD, Chapman D (1969) Chem Phys Lipids 3:304
14. Weiner MC, White SH (1992) Biophys J 61:434
15. Müller M, Zschörnig O, Ohki S, Arnold KJ (2003) Membr Biol 192:33
16. Lee J, Robinson GW (1984) J Chem Phys 81:1203
17. Brancaleon L, Crippa PR, Diemmi D (1995) Biopolymers 36:723