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COMMUNICATION

# Characterization of the Physicochemical Properties of the Micelles of Platelet-Activating Factor ($C_{18:0}$)

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**ABSTRACT**

*The purpose of this study was to clarify the physicochemical properties of the micelles of platelet-activating factor (PAF; $C_{18:0}$). The critical micelle concentration (CMC) of PAF ($C_{18:0}$) was determined (0.20 μM) using fluorescence techniques. The fluidity and the micropolarity of the PAF ($C_{18:0}$) micelle were similar to those of the micelle of stearoyl lysophosphatidylcholine*.

**Key Words:** *Critical micelle concentration; Fluidity; Micropolarity; Platelet-activating factor; Stearoyl lysophosphatidylcholine.*

## INTRODUCTION

Platelet-activating factor (PAF), 1-alkyl-2-acetylglycerylphosphocholine, is a group of biologically potent active phosphoglycerides with actions more diverse than those of eicosanoids (1). PAF exhibits a variety of biological activities, including activation of platelets (2), neutrophils (3), bronchoconstriction (4), hyperpermeability in peripheral veins (5), hypotension (6), and cardiac dysfunction (7). Because these biological activities of PAF are extremely potent, it is generally accepted that PAF is a mediator of inflammation (8) and plays important roles in the pathology of thrombosis, asthma, or hypotension in shock (9–11). Since the discovery of the PAF, a great deal of attention has been focused on alkyl ether phospholipids and their effects on the host defense system (12).

Many of the effects of PAF on biological systems are related to the amphiphilic character of the molecules. The surface activities of PAF, as well as other physical characteristics in solution, have been studied by a number of workers (13–16). However, most reports deal with PAF ($C_{16:0}$), and little attention for PAF ($C_{18:0}$) has been given to the fundamental properties of the micelles.

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In this study, we focused on the physicochemical properties of PAF ($C_{18:0}$). The critical micelle concentration (CMC) of PAF ($C_{18:0}$) was determined using the fluorescence probe of 1-anilinonaphthalene-8-sulfonate. In addition, the fluidity and the micropolarity around the hydrocarbon region of the micelles were determined by the fluorescence techniques. These features of the micelles were compared with those of stearoyl lysophosphatidylcholine.

## EXPERIMENTAL

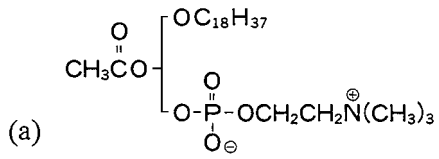
### Materials

1-*o*-Octadecyl-2-*o*-acetyl-*sn*-glycero-3-phosphocholine [PAF ($C_{18:0}$)] and L-α-stearoyl lysophosphatidylcholine (SlysoPC) were purchased from Sigma Chemical Company, Limited (St Louis, MO) (Fig. 1; Table 1). 1-Anilinonaphthalene-8-sulfonate (ANS) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Wako Pure Industrial Limited (Osaka, Japan). Nile red (NR) was purchased from Lambda Company, Limited (Graz, Austria).

### Methods

#### Determination of the Critical Micelle Concentration of Platelet-Activating Factor ($C_{18:0}$) Micelle

Fluorescence techniques were employed to determine the CMCs for PAF ($C_{18:0}$) and SlysoPC in water. The method used was according to the report by Nakagaki et al. (17) and used a spectrophotometer (model F-4500, Hitachi Co., Ltd., Tokyo, Japan). ANS was used to label



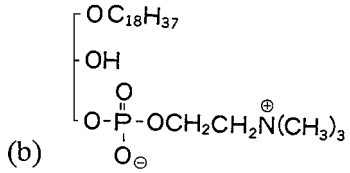




**Figure 1.** Chemical structure of the (a) PAF ($C_{18:0}$) and (b) SlysoPC.

***Table 1***

*Comparison of the Physicochemical Properties of PAF ($C_{18:0}$) and SlysoPC*

| | PAF ($C_{18:0}$) | SlysoPC |
|---|---|---|
| Critical micellization concentration (μM) | 0.20 | 0.25 |
| Fluorescence anisotropy at 25°C (DPH probe) | 0.082 | 0.072 |
| Emission maximum at 25°C (nm) (NR probe) | 631 | 632 |

the hydrophobic interior of the micelles in water. The dye has previously been used to study the CMC values of anionic, zwitterionic, and nonionic surfactants (18) and exhibits a strong environment-dependent blue shift, a high quantum yield, and low fluorescence in water.

The samples were prepared as follows. A known amount of ANS in tetrahydrofuran (5 μl to 5 ml sample volume; final concentration of ANS 1 μM) was added to the samples, and the samples were mixed thoroughly. The spectra were determined with excitation at 356 nm and emission at 480 nm.

#### Fluidity of Hydrocarbon Regions in the Micelles

The fluidity of hydrocarbon regions in PAF ($C_{18:0}$) and SlysoPC micelles was determined at 25°C using a fluorescence polarization technique (1,6-diphenyl-1,3,5-hexatrine [DPH] probe) as reported by Iwamoto et al. (19). DPH was added at 1 mol% of total lipids. The excitation and emission wavelengths used were 360 nm and 428 nm, respectively. The steady-state fluorescence anisotropy $r_s$ is defined by the following equation:

$$r_s = (I_{VV} - C_f \cdot I_{VH})/(I_{VV} + 2C_f \cdot I_{VH})$$

where $I$ is the fluorescence intensity, and subscripts V and H indicate the vertical and horizontal orientations of excitation (first) and analysis (second) polarizers, respectively. $C_f$ ($= I_{HV}/I_{HH}$) is the grating correction factor.

#### Determination of the Micropolarity Around Nile Red in the Micelles

The micropolarity of hydrocarbon regions in PAF ($C_{18:0}$) and SlysoPC micelles was determined using a fluorescence technique (NR probe). NR exhibits a strong environment-dependent blue shift, a high quantum yield, and low fluorescence in water (20,21). The fluorescence spec-

tra were measured on excitation at 549 nm at 25°C. The micropolarity of NR incorporated into the lipid aggregates was evaluated using the wavelength of maximum intensity of emission. For this determination, 3.8 mg of NR were dissolved in 10 ml of acetone (100 μM). Then, 5 μl of each solution were diluted with 5 ml of 10 mM PAF and SlysoPC aqueous solutions, methanol, ethanol, propanol, butanol, isobutanol, acetone, tetrahydrofuran, and acetonitrile. The wavelengths at the maximum fluorescence intensity of each solution were plotted against the polarity of each solvent (22). The micropolarity around the probe was determined using this relationship.

## RESULTS AND DISCUSSION

### Determination of the Critical Micelle Concentration of the Micelles

When PAF ($C_{18:0}$) was present at a concentration above the CMC, there was a large increase in the emission intensity of ANS and a blue shift of about 40 nm in the peak wavelength. It is generally observed that surfactant solutions show sharp changes in physical properties such as density, refractive index, surface tension, equivalent conductance, solubility, and the like when the CMC is reached (23).

Figure 2 shows the enhancement of fluorescence intensity of ANS at 460 nm as a function of the concentration of the lipids. Two straight lines can be drawn through the experimental points; the intersections (0.20 μM and 0.25 μM) are taken as the concentrations above which PAF ($C_{18:0}$) and SlysoPC form micelles, indicating that the CMCs of PAF ($C_{18:0}$) and SlysoPC are 0.20 μM and 0.25 μM, respectively. The CMC value of SlysoPC (0.25 μM) studied (24) was estimated to be 0.25 μM, and our result was similar to the reported value.

### Fluidity of the Hydrocarbon Regions of the Micelles

The fluidity of the hydrocarbon regions of the micelles was determined by the fluorescence polarization method using DPH as a hydrophobic probe. The fluorescence anisotropy values $r_s$ of PAF ($C_{18:0}$) and SlysoPC micelles at 25°C were 0.082 and 0.072, respectively, indicating that the fluidities of the hydrocarbon regions of PAF ($C_{18:0}$) and SlysoPC micelles are similar.

### Micropolarity Around Nile Red in the Micelles

The micropolarity around NR in the micelles was determined using the emission maximum of NR. It has been reported that the fluorescence characteristics of NR depend on the micropolarity around the probe, and it is located in a hydrophobic region in the lipid aggregates (20,21). Therefore, it is expected that the emission maximum of NR in the lipid aggregates will provide informa-

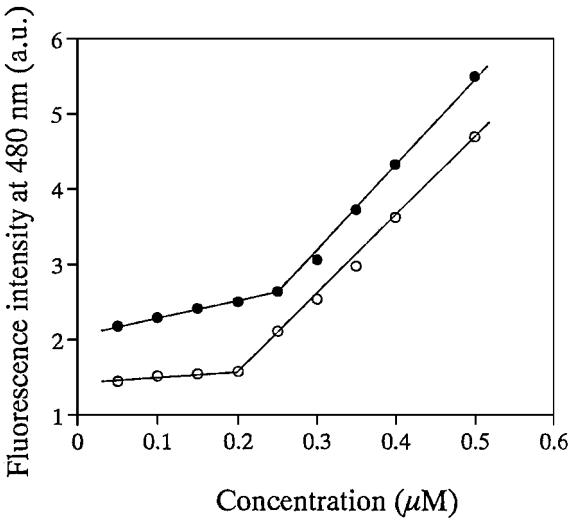



**Figure 2.** Fluorescence titrations of ANS with ○ PAF ($C_{18:0}$) and ● SlysoPC at 25°C. The wavelengths for excitation and emission were 356 nm and 480 nm, respectively. The concentration of ANS was 1 μM.

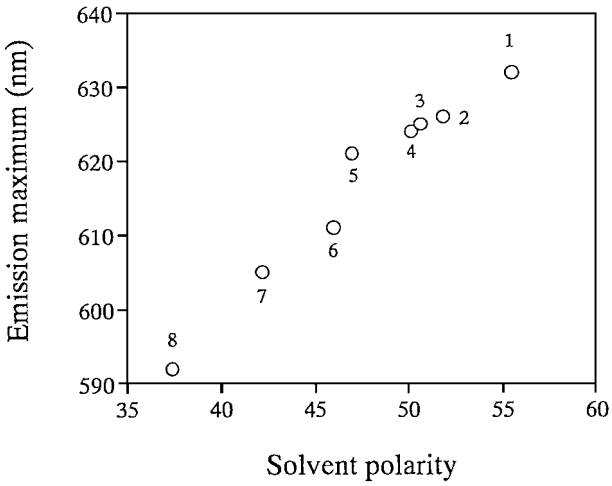



**Figure 3.** Relationships between solvent polarity and emission maximum of NR (100 nm) at 25°C: 1, methanol; 2, ethanol; 3, propanol; 4, butanol; 5, isobutanol; 6, acetone; 7, tetrahydrofuran; 8, acetonitrile.

tion on the micropolarity around the hydrocarbon chains. Figure 3 shows the relationship between solvent polarity and emission maximum of NR at 25°C. The emission maximum of PAF ($C_{18:0}$) and SlysoPC micelles were 631 and 632 nm, respectively, indicating that the micropolarity around the probe in the micelles is similar and comparable to that of methanol.

## CONCLUSIONS

The physicochemical properties of the micelles of PAF ($C_{18:0}$) were investigated. The CMC of PAF ($C_{18:0}$) was determined (0.20 μM) using fluorescence techniques (ANS probe). The fluidity of the PAF ($C_{18:0}$) micelle was similar to that of the micelle of SlysoPC. The micropolarity around NR for the micelles of PAF ($C_{18:0}$) and SlysoPC were also similar and comparable to that of methanol.

## REFERENCES


1. D. J. Hanahan, Annu. Rev. Biochem., 55, 483 (1986).
2. J. Benveniste, P. M. Hansen, and C. O. Conchrane, J. Exp. Med., 136, 1356 (1972).
3. J. O. Shaw, R. N. Pinchard, K. S. Ferrigini, L. M. McManus, and D. J. Hanahan, J. Immunol., 127, 1250 (1981).
4. B. B. Vergafitig, J. Lefort, M. Chignard, and J. Benveniste, Eur. J. Pharmacol., 65, 185 (1980).
5. D. M. Hamphrey, L. M. McManus, K. Satouchi, D. J. Hanahan, and R. N. Pinchard, Lab. Invest., 46, 422 (1982).
6. M. L. Blank, F. Snyder, L. W. Byers, B. Brooks, and E. E. Muirhead, Biochim. Biophys. Res. Commun., 90, 1194 (1979).
7. P. Bessin, J. Bonnet, D. Apfel, C. Soulaard, L. Desgroux, I. Pelas, and J. Benveniste, Eur. J. Pharmacol., 86, 403 (1983).
8. S. Saeki, F. Masugi, T. Ogihara, A. Otsuka, Y. Kumahara, K. Watanabe, K. Tamura, A. Akashi, and A. Kumagai, Life Sci., 37, 325 (1985).
9. S. Ohishi, K. Yamaki, M. Hayashi, S. Tsushima, and H. Nomura, Chem. Pharm. Bull., 34, 4896 (1986).
10. Y. Imura, Z. Terashita, and K. Nishikawa, Life Sci., 39, 111 (1986).
11. H. Takizawa, A. Ishii, S. Suzuki, J. Shiga, and T. Miyamoto, Int. Arch. Allergy Appl. Immunol., 86, 375 (1988).
12. W. E. Berdel, R. Andreesen, and P. G. Munder, *Phospholipids and Cellular Regulation*, Vol. 2, (J. F. Kuo, Ed.), CRC Press, Boca Raton, FL, 1985, pp. 41–73.
13. M. L. Blank, T. Lee, V. Fitzgerald, and F. Snyder, J. Biol. Chem., 256, 175 (1981).
14. P. Bette and A. Bienvenue, Agents Actions, 12, 701 (1982).
15. O. Convert, E. Michel, F. Heymans, and J. J. Godfroid, Agents Actions, 12, 706 (1982).
16. W. Kamp, G. Pieroni, R. N. Young, J. F. Seccmbe, and D. J. Hanahan, Chem. Phys. Lipids, 35, 49 (1984).
17. M. Nakagaki, H. Komatsu, and T. Handa, Chem. Pharm. Bull., 34, 4479 (1986).
18. E. De Vendittis, G. Parlato, and V. Bocchini, Anal. Chem., 115, 278 (1981).
19. K. Iwamoto, J. Sunamoto, K. Inoue, T. Endo, and S. Nojima, Biochim. Biophys. Acta, 691, 44 (1982).
20. P. Greenspan and S. D. Fowler, Lipid Res., 26, 781 (1985).
21. D. L. Sackett and J. Wolff, J. Anal. Biochem., 167, 228 (1987).
22. K. Dimorth and C. Reichardt, Liebg. Ann., 661, 1 (1963).
23. P. Mukerjee and K. J. Mysels, *Critical Micelle Concentrations of Aqueous Surfactant Systems*, National Standards Reference Data Series, Vol. 36, National Bureau of Standards, Washington, DC, 1971, p. 8.
24. K. Matsuzaki, T. Handa, K. Miyajima, Y. Mikura, H. Shimizu, and H. Toguchi, Chem. Pharm. Bull., 36, 4253 (1988).