

Lipid Membrane Analogues. Specific Retention Behavior in Comb-Shaped Telomer-Immobilized Porous Silica Gels

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Lipid membranes are extremely important as media for producing various biofunctions. Therefore, immobilization of their functions expands their possible applications. The polymerization of lipids¹⁻⁷ is a good technique for stabilizing lipid membranes, but polymerization of lipids often suppresses the membrane fluidity. On the other hand, Kunitake et al.⁸ and Regen et al.^{9,10} have developed lipid membrane systems containing polyions as counterions. In this technique, the membrane stability was remarkably increased without damaging its unique properties. Okahata et al. found that quartz-crystal microbalances covered with polyion-complexed lipid membranes could be used as sensors in various applications.^{11,12}

The purpose of the present study is to introduce and immobilize lipid membrane properties on stationary phases for liquid chromatography. In this purpose, polyion-complexed lipid membranes are not only unstable to utilize as stationary phases, but also provide chromatographically undesirable ionic interaction with solutes. On the other hand, lipid-immobilized silica gels were obtained by amidation of aminated silica gels with a dimyristoylphosphatidylcholine derivative containing a terminal carboxylic group.¹³ Such chemically bonded lipids are more stable than polyion-complexed lipid membranes in the chromatography process but still possess disadvantages similar to polymerized lipids. Therefore, it is necessary to develop a new approach for immobilization of lipid membranes. In this study, we synthesized a comb-shaped acrylate telomer containing a methoxysilanol group at one side of the terminal group, which was then chemically introduced into porous silica gels (Figure 1). We report that this telomer does not form bilayer membranes in water but shows unique physicochemical properties analogous to lipid bilayer membranes.

The comb-shaped telomer was prepared by telomerization of octadecyl acrylate and (3-mercaptopropyl)-trimethoxysilane in ethanol.¹⁴ The structure and polymerization degrees of the telomer obtained were determined by NMR spectroscopy.¹⁵ The polymerization degree determined agreed with the values estimated by the molecular weights obtained by gel permeation chromatography (GPC) using a Shodex KF-803 column. These analyses show that the average molecular weight of ODA_n was closely related to the molar ratio in the telomerization.¹⁶ The telomers prepared are represented by ODA_n, where *n* is the average polymerization degree.

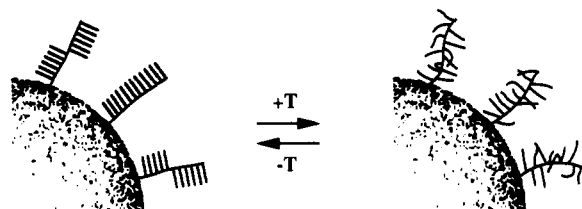
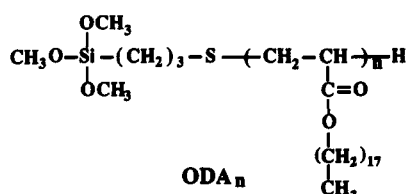


Figure 1. Schematic illustration of comb-shaped telomer-immobilized silica gels. The telomer undergoes phase transition on silica gels.

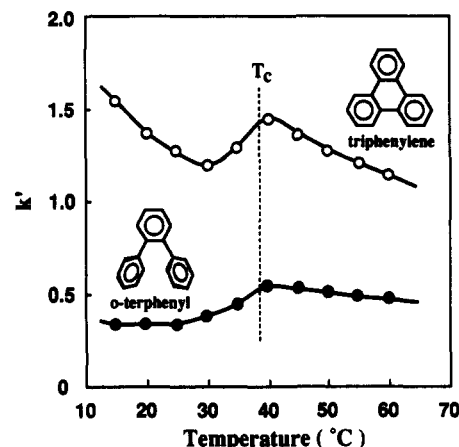


Figure 2. Temperature dependencies of the capacity factor (*k'*) with the Sil-ODA₂₈ column. Conditions: sample triphenylene (○), *o*-terphenyl (●); mobile phase, methanol; flow rate, 0.5 mL min⁻¹.

The DSC thermogram of ODA₂₈ showed a sharp endothermic peak (peak-top temperature, *T*_c = 49 °C) with a shoulder at 42–47 °C.¹⁷ Polarity microscopic observation indicated that the main peak was assigned to the liquid crystal-to-isotropic phase transition. The liquid crystalline state was observed in a temperature range of 43–47 °C. Phase transitions were also observed in a methanol dispersion, accompanied by a slight *T*_c shift to a lower temperature by about 2 deg.

The ODA₂₈ was immobilized on porous silica gels (YMC SIL-120-S5) by mixing in tetrachloromethane at 80 °C. The elemental analysis showed that 22 wt % of ODA₂₈ was introduced on the silica gels. Successive washing of the resulting gels with chloroform showed no change in weight. In addition, the immobilized ODA₂₈ showed phase transition in the temperature range 32–44 °C (*T*_c, 39 °C) in a methanol dispersion. This *T*_c is about 8 deg lower than that of ODA₂₈ itself. These results indicate that ODA₂₈ was chemically bonded to the silica gels via Si–O–Si bondings.

Silica-supported ODA₂₈ (Sil-ODA₂₈) was packed into a stainless steel column (4.6-mm i.d. × 100 mm), and the liquid chromatographic property was examined using methanol as an eluent. Complete separation of the alkylbenzene mixture was obtained in the column. The elution order of alkylbenzenes¹⁸ agreed with the order observed in octadecylated silica gels (ODS, Inertsil, GL Sciences, Inc.) commonly used as reversed-phase liquid chromatography packings. This indicates that the separation mode includes hydrophobic interaction between the bonded phase and the solutes. The performance of the Sil-ODA₂₈ column was unique in that it was characterized by the separation of the mixture of triphenylene and *o*-terphenyl. As shown in Figure 2, the Sil-ODA₂₈ column showed much higher retention capacity (*k'*)¹⁹ for triphenylene than for *o*-terphenyl. In addition, the Sil-

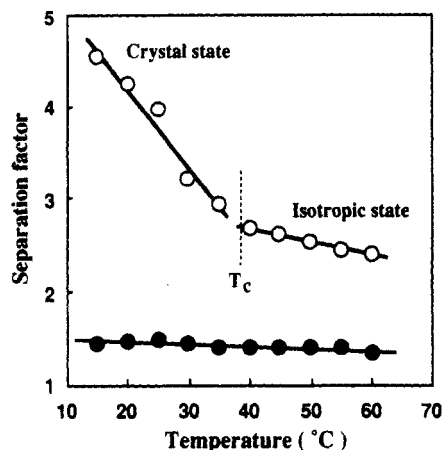


Figure 3. Temperature dependencies of separation factor (α) between triphenylene and *o*-terphenyl with Sil-ODA₂₈ (O) and ODS (●) columns. Conditions: mobile phase, methanol; flow rate, 0.5 mL min⁻¹.

ODA₂₈ column showed remarkable temperature dependencies with respect to the retention capacity (Figure 2). The k' -temperature plots show a distinct jump between 30 and 40 °C. This temperature range almost exactly matches that of the phase transition from crystal to isotropic states in Sil-ODA₂₈ in methanol. Temperature dependencies were also observed for the separation factor.¹⁹ According to Figure 3, the separation factor is much higher in the crystal state than in the isotropic state. On the other hand, the ODS showed a small retention capacity ($k' = 0.85$ and 0.72 at 15 and 45 °C) for triphenylene. In addition, the separation factors were very small and no similar jump was observed as shown in Figure 3. Therefore, the enhancement of retention capacity at the crystal state temperature cannot be explained only by the hydrophobic interaction between the bonded phase and the solute. It is known that planar compounds such as triphenylene are more strongly incorporated to a highly oriented medium than nonplanar compounds such as *o*-terphenyl.^{20,21} It is presumed that Sil-ODA₂₈ recognizes the molecular planarity as well as hydrophobicity.

In conclusion, comb-shaped telomers showed critical phase transition from crystal to isotropic states on the silica gels. Telomers in the crystal state showed remarkably a high retention capacity for a planar compound. Thus, just as cholesterol is easily incorporated into lipid membranes, it was observed that this property induces strong retention for cholesterol²² in the telomers as well.

References and Notes

- (1) Ihara, H.; Takafuji, M.; Hirayama, C.; O'Brien, D. F. *Langmuir* 1992, 8, 1548.
- (2) O'Brien, D. F.; Ramaswami, R. In *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; J. Wiley & Sons: New York, 1989; Vol. 17, pp 108-135.
- (3) O'Brien, D. F.; Liman, U. *Angew. Makromol. Chem.* 1989, 166/167, 2761.
- (4) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* 1988, 27, 113.
- (5) Regen, S. L. In *Liposomes: From Biophysics to Therapeutics*; Ostro, M. J., Ed.; Marcel Dekker: New York, 1987; p 73.
- (6) Fendler, J. H. *Science* 1984, 223, 888.
- (7) Kunitake, T.; Nakashima, N.; Takarabe, K.; Nagai, M.; Tsuge, A.; Yanagi, H. *J. Am. Chem. Soc.* 1981, 103, 5945.
- (8) Kunitake, T.; Tsuge, A.; Nakashima, N. *Chem. Lett.* 1984, 1783.
- (9) Fukuda, H.; Diem, T.; Stefely, J.; Kezdy, F. J.; Regen, S. L. *J. Am. Chem. Soc.* 1986, 108, 2321.
- (10) Regen, S. L.; Shin, J.; Yamaguchi, K. *J. Am. Chem. Soc.* 1984, 106, 2446.
- (11) Okahata, Y.; Ebato, H.; Taguchi, K. *J. Chem. Soc., Chem. Commun.* 1987, 1363.
- (12) Okahata, Y.; Shimizu, O. *Langmuir* 1987, 3, 1171.
- (13) Pidgeon, C.; Venkataram, U. V. *Anal. Biochem.* 1989, 176, 36.
- (14) The synthetic procedure of ODA₂₈ is as follows: octadecyl acrylate and (3-mercaptopropyl)trimethoxysilane (30:1 in the molar ratio) were dissolved in ethanol. Azobis[isobutyronitrile] (0.1 wt % for monomers) was added to the solution at 80 °C. The mixture was stirred for 6 h at 80 °C under a N₂ gas atmosphere. The white precipitates obtained were gathered by filtration, washed successively with methanol and acetone, and dried in vacuo.
- (15) ¹H NMR chemical shifts of ODA_n in CDCl₃ were follows: $\delta = 0.75$ (SiCH₃), 1.87 (CHCO), 3.58 ppm (SiOCH₃), 4.15 ppm (C(=O)OCH₂).
- (16) The molecular weight of ODA₂₈ determined by NMR spectroscopy is 9.3×10^3 . GPC analysis of ODA₂₈ showed $M_w = 1.3 \times 10^4$, $M_n = 7.4 \times 10^3$, and $M_w/M_n = 1.76$.
- (17) DSC thermograms of the dry samples of ODA₂₈ (5 mg) were obtained using a heating rate of 2 °C min⁻¹ with Seiko I & E SSC-580 with a DSC-10 instrument.
- (18) The chromatograph includes a JASCO 880 PU pump and a Shimadzu UV-visible photodiode array SPD-M6A. A 5- μ L aliquot of the sample dissolved in methanol was injected through a Rheodyne Model 7125 injector. Chromatography was carried out at a flow rate of 0.5 mL min⁻¹. The elution order of the alkylbenzene mixture is as follows: benzene, toluene, ethylbenzene, butylbenzene, hexylbenzene, and octylbenzene.
- (19) The capacity factor (k') was determined by $(V_e - V_0)/V_0$, where V_e is an elution volume (for the column volume) of solutes and V_0 is 65 vol % as a void volume. The separation factor (α) was estimated by the ratio of capacity factors.
- (20) Tanaka, N.; Hashizume, K.; Araki, M. *J. Chromatogr.* 1987, 400, 33.
- (21) Hirayama, C.; Ihara, H.; Nagaoka, S.; Shono, T. *Chem. Lett.* 1992, 971.
- (22) The k' values for cholesterol with the Sil-ODA₂₈ column were 3.90 and 1.98 at 15 and 45 °C, respectively.