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Recognition-Adsorption of Artificial Liposomes onto Glass Surface Modified with Octadecylsilyl Monolayer

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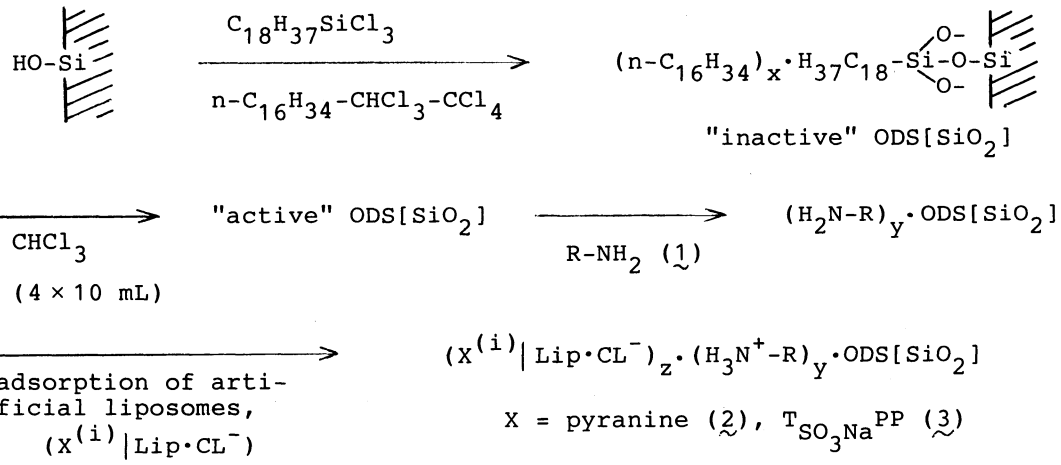
Octadecylsilyl(ODS) modified glass support having R-NH_2 in the template cavity on ODS, $(H_2N\text{-}R)_y\cdot ODS[SiO_2]$, adsorbed negatively charged artificial liposomes, $(X^{(i)}|Lip\cdot CL^-)$, opening a way to a new unique supramolecular assembly system.

Current increasing attention to artificial liposomes stemms from unique physical properties or chemical reactions of ordered molecular aggregates, the access to which has been supported by the progress of physicochemical measurements such as electron microscopy, light scattering methods, etc.[1-3] Now we wish to report a new supramolecular assembly system, in which artificial liposomes of microheterogeneous system are linked to a glass(solid) support through designed octadecylsilyl monolayer.

We have shown recently that octadecylsilyl(ODS) monolayer covalently bound onto a glass support, $ODS[SiO_2]$ (Scheme 1), has acquired molecular binding ability by extracting out the co-implanted template guest molecule from the ODS monolayer.[4] For example, $ODS[SiO_2]$ which was prepared by extracting out n-hexadecane from the monolayer (see Scheme 1), strongly adsorbed guest molecules having long, thin hydrophobic tail such as chlorophyl a, vit-K_1, K_2, or E.[4]

Such a "recognition-active" $ODS[SiO_2]$ monolayer was employed to incorporate an amine, N-(3,6,9-trioxaheptacosyl)ethylene diamine (1), in the molecular binding



Scheme 1.

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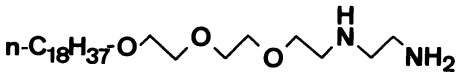
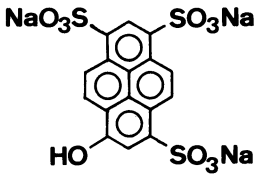
cavity on ODS monolayer, affording $(H_2N\text{-}R)_y\cdot ODS\text{-}[SiO_2]$ monolayer (Scheme 1), the surface of which is strongly basic due to 1.



1

Thus, the "active" $ODS[SiO_2]$, which was obtained by soaking "inactive" $ODS[SiO_2]$ in $CHCl_3$ (4 × 10 mL), was treated with a 2.1 mmol dm^{-3} solution of 1 in MeOH-H_2O(3:2) at room temperature for 10 min. The surface of $(H_2N\text{-}R)_y\cdot ODS[SiO_2]$ became hydrophilic due to 1, whereas the unmodified $ODS[SiO_2]$ monolayer was strongly hydrophobic. The surface modification was made so that over 90% of the host binding sites (ca. 3.5/100 $Å^2$ apparent surface area)[4] are occupied by 1, based on the adsorption equilibrium constant of ca. 6500 mol^{-1} dm^3 for 1.[5]



2

The $(H_2N\text{-}R)_y\cdot ODS[SiO_2]$ monolayer thus obtained was soaked in a solution of negatively charged artificial liposomes, $(pyranine^{(i)}|Lip\cdot CL^-)$[6,7] containing pyranine, 2 (8-hydroxypyrene-1,3,6-trisulfonate trisodium, 20 mmol dm^{-3}) in their interior aqueous phase. The artificial liposomes were prepared from a lipid mixture of lecithin (EL) - cardiolipin (CL, from bovine heart) = 80:20 (w/w) (see Ref. 7 for details), and these were shown to be single-walled liposomes by electron microscopy.[7]

Quite short contact time was enough for the liposome adsorption. Thus, when soaked for 1 min, the modified monolayer exhibited a strong fluorescence at 500 nm (400 nm excitation), which is characteristic to pyranine (Fig. 1a), after removing tiny droplets from the surface by using a filter paper. By contrast, no or little fluorescence was observed for $(H_2N\text{-}R)_y\cdot ODS[SiO_2]$, when it was treated with $(pyranine^{(i)}|Lip)$ liposomes containing no cardiolipin (CL) (see Fig. 1b),

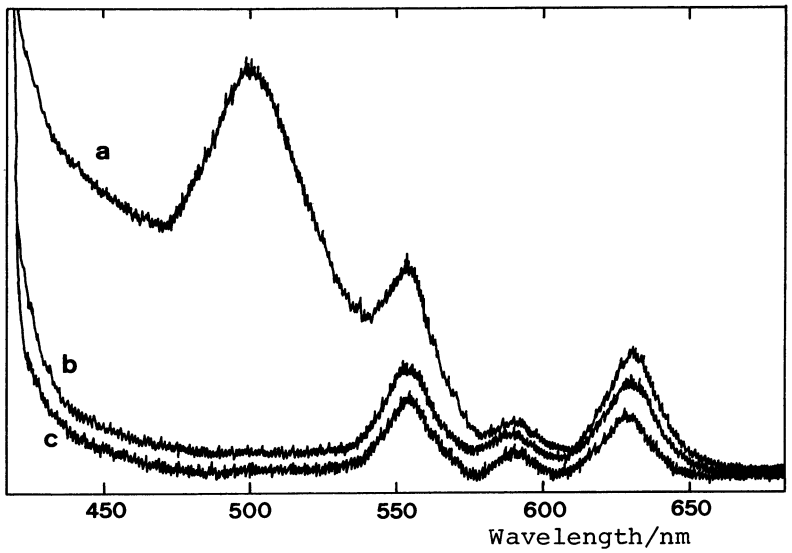


Fig. 1. Fluorescence spectra of (a) $(pyranine^{(i)}|Lip\cdot CL^-)_z\cdot(H_3N^+\text{-}R)_y\cdot ODS[SiO_2]$, (b) $(H_2N\text{-}R)_y\cdot ODS[SiO_2]$ treated with $(pyranine^{(i)}|Lip)$ containing no CL, and (c) $ODS[SiO_2]$. Excitation 400 nm.

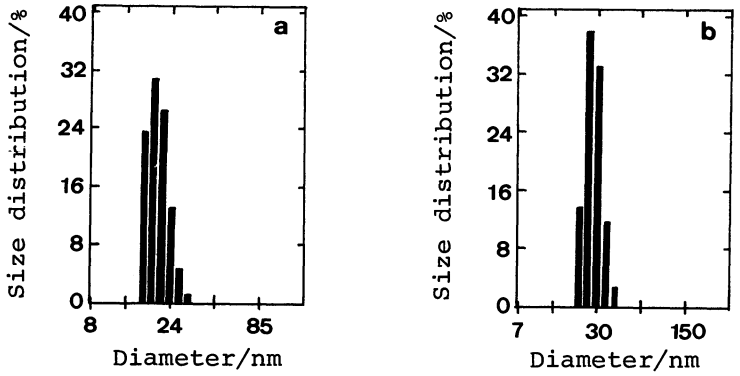


Fig. 2. Size distribution of artificial liposomes determined by dynamic light scattering(DLS) method. (a) (pyranine$^{(i)}$|Lip·CL$^-$) before adsorption. (b) desorbed (pyranine$^{(i)}$|Lip·CL$^-$) solution.

indicating that the strongly acidic cardiolipin (CL) is necessary for the present liposome adsorption.

The liposome-adsorbed glass was soaked in excess water for a prolonged time (5 min). The glass was no longer fluorescent, and its surface became hydrophobic again, suggesting that the liposomes desorbed most probably only together with R-NH_2 (1). Dynamic light scattering (DLS) measurement indicated a particle size distribution of 28 ± 5 nm for the solution of desorbed liposomes (Fig. 2b). Therefore, liposome fusion is not serious under the present condition of adsorption-desorption, since the intact artificial liposomes (before adsorption) exhibited 20 ± 3 nm size distribution (Fig. 2a).

To investigate the adsorption behavior of liposomes in detail, artificial liposomes, ($T_{SO_3Na}PP^{(i)}$|Lip·CL$^-$) containing tetrasodium meso-tetraphenylporphyrin-tetrasulfonate ($\underset{\sim}{3}$, 2.5 mmol dm^{-3} in water of pH 7) were adsorbed to $(H_2N\text{-}R)_y$·ODS[SiO_2] in a similar manner as described. A Soret absorption band due to $T_{SO_3Na}PP$ ($\underset{\sim}{3}$) entrapped in the interior aqueous phase appeared at 420 nm for the modified glass support (Fig. 3), again supporting the liposome adsorption.

Free $T_{SO_3Na}PP$ in a 2.7 mmol dm^{-3} solution also adsorbed to $(H_2N\text{-}R)_y$·ODS[SiO_2] surface. In this case, however, the Soret absorptivity observed (Abs = 0.33) corresponds to ca. 2×10^{14} $T_{SO_3Na}PP$ molecules/cm^2 apparent surface area (ca. 2 molecules/100 Å^2), and the adsorbed $T_{SO_3Na}PP$ remained on the surface even after the repeated-soaking in water for a prolonged time, in a sharp contrast with the adsorption-desorption behavior observed for the liposome case. Quantitative discussion on the recognition ability of the present modified ODS monolayer will appear in our forth-coming full length article.

The most probable way for the present liposome adsorption is the strong electrostatic interaction between the negatively charged liposome and the positively charged ODS surface. These results may open a way to a new supramolecular assembly system having a unique molecular contact between artificial liposomes and

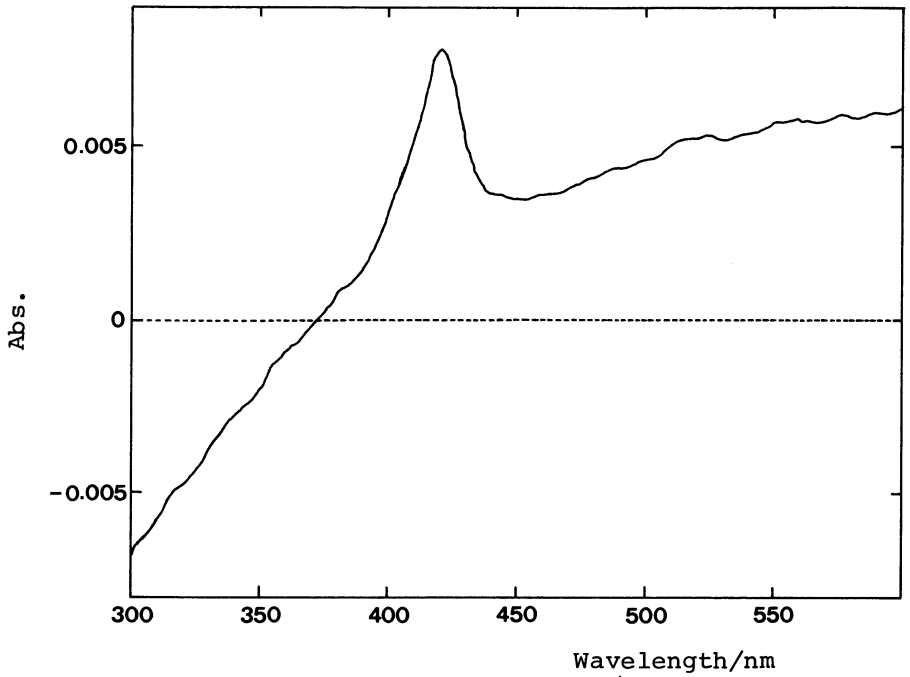


Fig. 3. Electronic absorption spectrum of $(T_{SO_3Na}PP^{(i)}|Lip\cdot CL^-)_z\cdot(H_3N^+-R)_y\cdot ODS[SiO_2]$ measured by a multi-channel photo-diode array detector. 100 times data accumulation of 100 ms scanning. $ODS[SiO_2]$ was employed as the reference sample.

designed membrane on the solid support.

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References

1) R. Wagner, "Introduction to Biological Membrane," John Wiley & Sons, New York (1980), p.6 ; A.D. Bangham, M.M. Standish, and J.C. Watkins, J. Mol. Biol., 13, 238 (1965).
2) N.A. Mazer and G.B. Benedek, J. Phys. Chem., 80, 1075 (1976) ; M. Corti and V. Degiorgio, Chem. Phys. Lett., 53, 237 (1978).
3) D. Chapman, R.M. Williams, and B.D. Ladbrook, Chem. Phys. Lipids, 1, 445 (1967).
4) I. Tabushi, K. Kurihara, K. Naka, K. Yamamura, and H. Hatakeyama, Tetrahedron Lett., 28, 4299 (1987).
5) Determined by a competitive binding of $\underset{\sim}{1}$ vs. vit-K_1 toward the host binding sites on "active" $ODS[SnO_2]$.
6) I. Tabushi and S. Kugimiya, J. Am. Chem. Soc., 107, 1859 (1985).
7) I. Tabushi, T. Nishiya, M. Shimomura, T. Kunitake, H. Inokuchi, and T. Yagi, J. Am. Chem. Soc., 106, 219 (1984).

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