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Organic Thin Layer of Molecular Gel-forming Glutamide Lipid on Silica Particles for Practical Application to Molecular Recognition

Makoto Takafuji,[1,2] Thiraporn Charoenraks,[1] Jin Izumi,[3] and Hirotaka Ihara*[1,2]
[1]*Department of Applied Chemistry and Biochemistry, Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555*
[2]*Kumamoto Institute for Photo-electro Organics (Phoenics), 3-11-38 Higashimachi, Kumamoto 862-0901*
[3]*Institute of Resource Development and Analysis, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555*

(Received November 10, 2011; CL-111098; E-mail: ihara@gpo.kumamoto-u.ac.jp)

Molecular gel-forming glutamide lipid-grafted porous silica particles (Sil-SiD*g*) are designed and prepared as the stationary phase in high-performance liquid chromatography (HPLC) for molecular shape recognition. It was proven that the glutamide lipids formed a highly oriented structure on the silica surface and showed thermally reversible phase transition like self-assembled molecular gels even on the silica surface. Unprecedented excellent results were achieved in the separation of tocopherols (Vitamin E) using Sil-SiD*g* as the stationary phase in HPLC analysis.

Molecular gels are soft materials consisting of self-assembling small molecules that form highly oriented structures through intermolecular interactions.[1] The structures are similar to the lipid membrane structure of a biological cell. The ordering of small molecules in such self-assemblies is effective to recognize guest molecules through efficient intermolecular interactions. Self-assembling integrated materials have been investigated with the viewpoint of developing molecular recognition systems.[2] We have reported that dialkyl L-glutamide derivatives form fibrous supramolecular assemblies and show unique properties based on their highly oriented structures in aqueous and organic media.[3] To realize molecular recognition in high-performance liquid chromatography (HPLC) stationary phases using molecular gels, we introduced a dialkyl L-glutamide derivative onto a porous silica surface.[4] In our previous work, the dialkyl L-glutamide derivative was immobilized onto the silica surface by coupling with an amine group which was grafted onto the silica surface in advance using a silane coupling reagent (3-aminopropyltrimethoxysilane). We concluded that the grafted lipids may not form highly oriented structures on the surface because no phase transition was observed and the alkyl chains of the lipid formed a *gauche* conformation rather than a *trans* conformation in the temperature range 10 to 50 °C. However, this stationary phase showed extremely high selectivity toward polycyclic aromatic hydrocarbons (PAHs) and biorelated molecules such as nucleic acid constituents. In order to more effectively utilize the molecular gels from glutamide-derived molecules as the stationary phase in HPLC, it is preferable to graft the self-assembling glutamide-derived molecules onto a silica surface with a highly ordered state. To this end, we introduced strategic molecular design for self-assembling glutamide-derived molecules as follows (chemical structures are shown in Figure 1): (1) Introduction of an alkoxysilyl group to the glutamide-derived molecule for direct grafting onto the silica surface, since direct grafting via a silane coupling agent is profitable to the introduction of large amounts of small molecules on the solid surface. (2) In order to reduce the perturbation to the oriented structure by the solid silica surface, a long spacer group was inserted between the glutamide moiety and the alkoxysilyl terminal group that was used for grafting onto the silica.

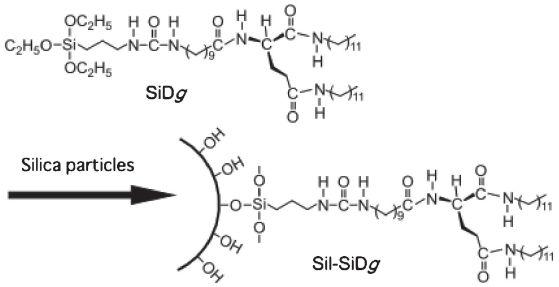

Figure 1. Chemical structure of L-glutamide-derived lipid-grafted porous silica particles (Sil-SiD*g*).

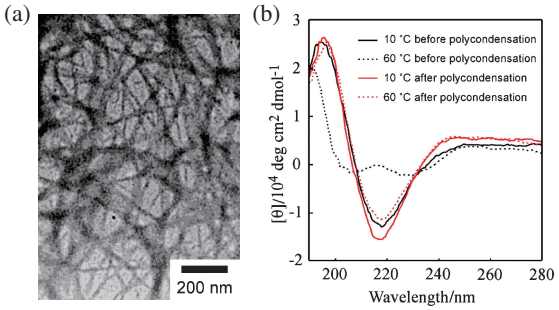

Figure 2. a) TEM image and b) circular dichroism spectra of SiD*g* in cyclohexane.

The self-assembling, glutamide-derived molecule with the triethoxysilyl head group via the long-spacer group (SiD*g*) was synthesized using a modification of a previously reported procedure.[5] SiD*g* formed a self-assembled molecular gel with fibrous morphological features in various organic solvents such as benzene and cyclohexane (Figure 2a). The highly oriented structure of the molecular gel of SiD*g* can be discerned by spectroscopic observation. In cyclohexane solution, circular dichroism (CD) signals were observed at 196 (positive) and 218 (negative) nm at 10 °C (Figure 2b), indicating that the amide bonds of the SiD*g* molecules formed structures with an S-chiral arrangement. These CD signals were weakened at temperatures above the characteristic gel-to-sol phase-transition temperature, T_g (T_g in cyclohexane is 41.5 and 64.6 °C in the heating process), because of disordering of the SiD*g* molecules. The amide bonds

surrounding the glutamide moieties of SiD*g* are important in the formation of molecular gels in which sequential intermolecular hydrogen bonds form a highly oriented structure. The CD spectra prove that the chirally ordered structure of the glutamide moiety of SiD*g* persisted even after the polycondensation of the alkoxysilyl groups. Furthermore, approximately the same CD signal was observed at the temperature above the original T_g (after polycondensation, no phase transition was observed in the temperature range of 10 to 70 °C).

SiD*g* was mixed with an ethanol suspension of porous silica particles (YMC silica, average diameter: 5 μm, pore size: 12 nm, specific surface area: 330 $m^2 g^{-1}$) and stirred at 60 °C for 1 day. After washing with ethanol three times, the silica particles were collected by filtration and dried in vacuo. The diffuse reflectance Fourier transform infrared (DRIFT) spectrum of the obtained particles (Sil-SiD*g*) showed typical adsorption peaks of SiD*g* at 2920, 2859 (ν_{C-H}), 1684 ($\nu_{C=O(urethane)}$), 1635 ($\nu_{C=O(amide)}$), and 1557 (δ_{N-H}) cm^{-1}. Furthermore, the C/N ratio obtained from the elemental analysis of Sil-SiD*g* was 7.75 (C% = 24.03, N% = 3.10), which is almost equal to the calculated C/N value of the polycondensed SiD*g*, 7.55 (calcd for $C_{44}H_{86}N_5O_7Si$). These results indicate that SiD*g* is directly immobilized onto the surface of the porous silica particles through siloxane bonds. The amount of grafted SiD*g* on the silica particles was calculated to be 34.1 wt % using a carbon amount in Sil-SiD*g* of C% = 24.03. The grafting density of organic phase on the silica surface can be calculated from the carbon percentage by using the equations given in the previous report.[6] The grafting density of SiD*g* (2.47 μmol m^{-2}) is lower than that of the polymeric octadecylated silica stationary phase used in this study (3.40 μmol m^{-2}) (p-ODS, Shodex C18P 4E (C% = 17.5)). However, given that the SiD*g* molecule has two alkyl chains, it was concluded that SiD*g* was densely grafted onto the silica surface. Furthermore the average pore size of silica particles (12 nm) is larger than the molecular length of SiD*g* (4.6 nm, calculated by HyperChem). SiD*g* molecules were possibly grafted on the surface of the pores inside. Differential scanning calorimetry (DSC) and solid-state cross-polarization/magic angle spinning (CP/MAS) ^{13}C-nuclear magnetic resonance (^{13}C NMR) spectroscopy were used to determine the physical state of the SiD*g* molecules on the silica surface. DSC of Sil-SiD*g* was conducted in a methanol–water (8:2) suspension, which was used as the mobile phase for the subsequent HPLC analysis. Predictably, DSC thermograms showed an endothermic peak at 52 °C (with a shoulder at 43 °C) and an exothermic peak at 32 °C in the heating and cooling processes, respectively (Figure 3a); these behaviors were thermally reversible. Since no endothermic or exothermic peaks were observed in polycondensed SiD*g*, the physical state of the dialkylated-glutamide moiety in Sil-SiD*g* was probably different from that in SiD*g*.

The solid-state CP/MAS ^{13}C NMR spectroscopy of Sil-SiD*g* showed two well-resolved peaks at 33 and 30 ppm, which correspond to *trans* and *gauche* conformations of alkyl chains, respectively. As shown in Figure 3b, the alkyl chains of Sil-SiD*g* show a predominantly *trans* conformation with a high-field small peak indicating a *gauche* conformation at temperatures below phase transition, but show a predominantly *gauche* conformation with a low-field shoulder indicating a *trans* conformation at temperatures above phase transition. These results suggest that SiD*g* molecules form highly oriented

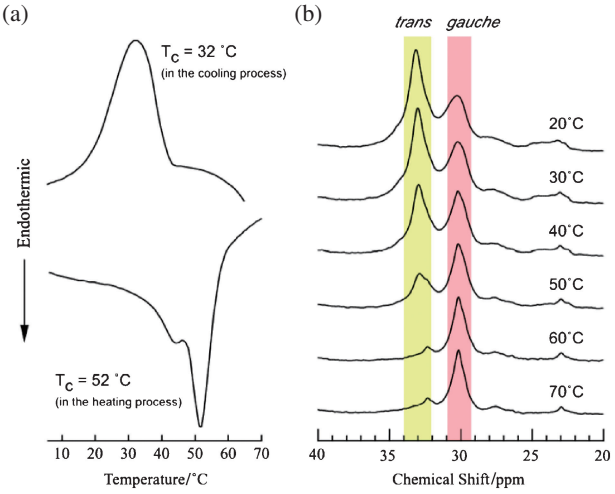

Figure 3. a) DSC thermograms of Sil-SiD*g* in methanol–water mixture (8:2) and b) solid-state CP/MAS ^{13}C NMR spectra of Sil-SiD*g*.

structures even on the silica surface and that ordered–disordered phase transition can occur on the solid surface. SiD*g*, in which the glutamide moiety and a reactive triethoxysilyl group are connected with a long spacer group, effectively forms highly oriented structures on the silica surface. We successfully introduced self-assembling glutamide molecules onto the solid surface in an ordered state by appending two strategic molecular designs. However, the phase-transition behavior of SiD*g* on the silica surface defies our prediction from the results in bulk solution. Perturbation of the oriented structures by the silica surface would be less than that from the siloxane polymer network in the fibrous aggregates. Further investigation is needed to clarify the ordered state of the lipid in the fibrous aggregates and on the surface of the silica.

The Sil-SiD*g* was packed into a stainless steel column (4.6 mm × 150 mm) and evaluated as a reversed-phase HPLC (RP-HPLC) stationary phase. A series of four-ring planar PAH isomers with different length to breadth ratios (L/B ratio) and planar/nonplanar solutes was used to evaluate the shape-selective retention behavior of Sil-SiD*g*. Retention factors and separation factors for PAHs examined in this study are summarized in Table 1. The retention order of the four-ring PAHs with different L/B ratios in Sil-SiD*g* is distinctly different from that in conventional octadecylated-silica (ODS) at 20 °C. Sil-SiD*g* showed higher selectivity for triphenylene, which has a lower L/B ratio, compared with naphthacene, which has a higher L/B ratio. Further study of the molecular shape selectivity of Sil-SiD*g* was carried out with terphenyl isomers as structural isomers and stilbene isomers as geometric isomers. The elution orders of these isomers in Sil-SiD*g* are the same as those in ODS, but the selectivities are much higher in Sil-SiD*g*. Evidently Sil-SiD*g* recognizes planar and circular (low L/B ratio) molecules. Similar elution orders for these molecular sets can be found in poly(vinylpyridine)-grafted silica (Sil-VP_n),[7] as we have reported. Poly(vinylpyridine) was grafted onto the silica surface with a terminal at one side and exists in an amorphous state. However, the pyridine moieties of the VP_n polymers can interact with the silica surface through hydrogen bonds[6] that

Table 1. Retention factors (k) and separation factors (α) of four-ring PAHs and terphenyl isomers[a]

Elute	L/B	Sil-SiD*g*				p-ODS	
		20 °C		60 °C		20 °C	
		k	α (1/α)	k	α (1/α)	k	α
Triphenylene	1.12	9.69	— (1.00)	3.40	— (1.00)	5.89	—
Benz[*a*]anthracene	1.58	6.10	0.63 (1.59)	3.34	0.98 (1.02)	6.72	1.14
Chrysene	1.72	6.55	0.68 (1.47)	3.79	1.12 (0.89)	6.90	1.17
Naphthacene	1.89	5.34	0.55 (1.82)	3.11	0.92 (1.09)	9.37	1.60
o-Terphenyl	1.11	2.15	—	1.20	—	1.72	—
m-Terphenyl	1.47	4.54	2.11	1.68	1.40	2.92	1.70
p-Terphenyl	2.34	18.30	8.51	4.47	3.73	3.68	2.14
cis-Stilbene	—	3.26	—	1.98	—	2.57	—
trans-Stilbene	—	8.43	2.58	3.98	2.01	3.05	1.19

[a]Mobile phase: methanol-water (8:2). Flow rate: 1.0 mL min^{-1}.

reduce the mobility of the polymers. We predicted that such quasi-solidified polymers on the silica surface bring effective interaction for disc-like solutes resulting in an elution order opposite to that of the ODS phase. In the case of Sil-SiD*g*, highly oriented SiD*g* induces the specific alignment of the glutamide moieties that contain π-electron sources. Probably, the aligned amide bonds recognize the molecular shapes of PAH isomers. Interestingly, the selectivities for terphenyl isomers and stilbene isomers in Sil-SiD*g* are reduced at 60 °C, but are still higher than those in the polymeric ODS phase at 20 °C. As mentioned above, the alkyl chains of SiD*g* formed a *gauche* conformation at 60 °C, which is above the phase-transition temperature. Therefore, the glutamide moieties contribute to the recognition of the molecular shapes of PAH isomers, even above the phase-transition temperature. This is probably because of the effect of the accumulation of glutamide moieties on the surface of the silica particles.

Unique selectivity based on the molecular assembly from the glutamide-derived lipid can be also expressed by tocopherol isomers. Tocopherols constitute the major component of the vitamin E group and consist of four homologues, namely, α-, β-, γ-, and δ-tocopherol, exhibiting different bioactivities. The isomers differ structurally only in the substitutional groups of the benzene ring. Complete separation, especially the separation of γ- and δ-tocopherol, is problematic when using the conventional ODS stationary phase because of the similarities in the stereostructure and hydrophobicity of the isomers. As shown in Figure 4, Sil-SiD*g* successfully performed the baseline separation of tocopherol isomers. Such a precise separation of tocopherol isomers can be achieved by the formation on the surface of the silica particles of an organic thin layer of highly oriented molecular gel. The hydroxy and ether groups of the tocopherol isomers can interact with the amide bonds around the glutamide moiety through hydrogen bonds; the benzene ring of the isomers can interact with the glutamide moiety through carbonyl–π interactions. It can be postulated that highly oriented glutamide moieties on the silica surface enhance the selectivity of stereoisomers and structural analogs. Probably, the stereohindrance around the glutamide moiety determines the interaction with specifically aligned structures of the guest molecules.

In conclusion, a dialkyl glutamide-derived lipid with a triethoxysilyl head group, which can form a molecular gel in various organic solvents, has been successfully grafted onto porous silica particles. The lipid undergoes a condensation reaction with the silanol groups on the silica surface to form Sil-SiD*g*. The alkyl chains of the grafted lipid molecules showed ordered–disordered phase transitions in the suspension state. When Sil-SiD*g* was evaluated as an RP-HPLC stationary phase, it showed much better selectivity for aromatic hydrocarbon isomers than conventional ODS stationary phase. Furthermore, excellent results were achieved in the separation of four tocopherol isomers (Vitamin E). We propose that the unique selective separations of Sil-SiD*g* are due to both the highly oriented structure of the alkyl chains and the assembled glutamide moieties that interact through hydrogen bonds. Further investigation is necessary to understand the unique molecular shape selectivities of Sil-SiD*g*.

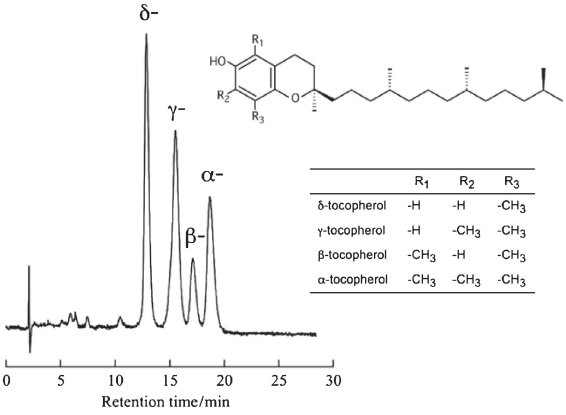

Figure 4. Chromatogram for tocopherol isomers with Sil-SiD*g*. Mobile phase: methanol–water (9:1), Flow rate: 1.0 mL min^{-1}, Column temperature: 20 °C.

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References

1 a) P. Terech, R. G. Weiss, *Chem. Rev.* **1997**, *97*, 3133. b) J. H. van Esch, B. L. Feringa, *Angew. Chem., Int. Ed.* **2000**, *39*, 2263. c) H. Ihara, M. Takafuji, T. Sakurai, in *Encyclopedia of Nanoscience and Nanotechnology*, ed. by H. S. Nalwa, American Scientific Publishers, Stevenson Ranch, CA, **2004**, Vol. 9, pp. 473–495. d) T. Shimizu, M. Masuda, H. Minamikawa, *Chem. Rev.* **2005**, *105*, 1401. e) R. Oda, in *Molecular Gels: Materials with Self-Assembled Fibrillar Networks*, ed. by R. G. Weiss, P. Terech, Springer, Berlin, **2006**, pp. 577–612.
2 a) S. Ong, H. Liu, X. Qiu, G. Bhat, C. Pidgeon, *Anal. Chem.* **1995**, *67*, 755. b) A. Taillardat-Bertschinger, P.-A. Carrupt, F. Barbato, B. Testa, *J. Med. Chem.* **2003**, *46*, 655.
3 a) H. Ihara, T. Sakurai, T. Yamada, T. Hashimoto, M. Takafuji, T. Sagawa, H. Hachisako, *Langmuir* **2002**, *18*, 7120. b) M. Takafuji, Y. Kira, H. Tsuji, S. Sawada, H. Hachisako, H. Ihara, *Tetrahedron* **2007**, *63*, 7489.
4 M. M. Rahman, M. Takafuji, H. R. Ansarian, H. Ihara, *Anal. Chem.* **2005**, *77*, 6671.
5 M. Takafuji, N. Azuma, K. Miyamoto, S. Maeda, H. Ihara, *Langmuir* **2009**, *25*, 8428.
6 H. R. Ansarian, M. Derakhshan, M. M. Rahman, T. Sakurai, M. Takafuji, I. Taniguchi, H. Ihara, *Anal. Chim. Acta* **2005**, *547*, 179.
7 a) H. Ihara, M. Fukui, T. Mimaki, A. Shundo, W. Dong, M. Derakhshan, T. Sakurai, M. Takafuji, S. Nagaoka, *Anal. Chim. Acta* **2005**, *548*, 51. b) U. G. Gautam, A. Shundo, M. P. Gautam, M. Takafuji, H. Ihara, *J. Chromatogr., A* **2008**, *1189*, 77.

