

Short communication

Dioctadecyl L-glutamide-derived lipid-grafted silica as a novel organic stationary phase for RP-HPLC

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Abstract

Dialkyl L-glutamide-derived lipids have been found to form supramolecular assemblies and to show specific properties based on their highly ordered structures in aqueous and organic media. To use these unique properties to create molecular recognition systems, dioctadecyl L-glutamide-derived lipid-grafted porous silica particles (Sil-DSG) were newly prepared and used as a stationary phase in reversed phase liquid chromatography. Compared with conventional ODS (octadecylated silica), the Sil-DSG column showed remarkably higher selectivity for polycyclic aromatic hydrocarbons (PAHs). Especially, Sil-DSG recognized the molecular linearity and planarity of PAHs. Suspension state ^1H NMR and ^{13}C CP/MAS-NMR spectroscopies showed that the alkyl chains on the Sil-DSG are in *gauche* form and their mobility is strongly restricted at room temperature. This paper discusses higher selectivity of Sil-DSG with a carbonyl π -benzene π interactions.

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Keywords: Lipid membrane analogue; Highly-ordered structure; Carbonyl- π interaction; Polycyclic aromatic hydrocarbons; Geometrical isomers; Molecular-shape selectivity

1. Introduction

In biological membranes, lipid bilayers play key roles in the selective permeation and transition of substrates and molecules [1,2]. These functions of biological membranes would be powerful tools for analytical chemistry and separation engineering because the membranes are based on highly oriented structures. Therefore, we have developed the poly(octadecylacrylate) derivatives as lipid membrane analogues and grafted them onto silica for application to HPLC. These new modified silica particles showed unique separation behaviors with ordered-to-disordered phase transitions of long alkyl chains [3]. In particular, extremely high selectivity toward polycyclic aromatic hydrocarbons (PAHs) was observed in the ordered state [4–7], and the combination of chromophoric diastereomerizing reagents yields better selectivity for enantiomers than in ordinary ODS [8–10].

Our detailed investigations indicate that the highly ordered structure induced the orientation of the carbonyl groups that work as π - π interaction sources with solute molecules. The aligned carbonyl groups are effective for recognizing the molecular planarity and linearity of PAHs through multiple π - π interactions [11–13]. Similarly, some ordinary ODS also recognize the molecular shapes of PAHs [14–19], and their selectivity is related to both the length and density of the alkyl chains immobilized on ODS [20]. However, it is clear that the mechanism separating Sil-ODA_n differs essentially from that of ODS, because ODS has no π -electron source. Based on these facts, we propose a novel organic phase (Fig. 1) as a lipid membrane analogue. The molecular design is based on the fact that dialkyl L-glutamide-derived amphiphilic lipids form nano-tubes [21–23], -helices [21–24], and -fibers [25] based on bilayer structures in water, and on the fact that intermolecular hydrogen bondings among the amide moieties contribute to self-assembly [25]. Similar self-organization has been realized by lipophilic derivatives of L-glutamide even in organic solvents [26–31]. The uniqueness of these

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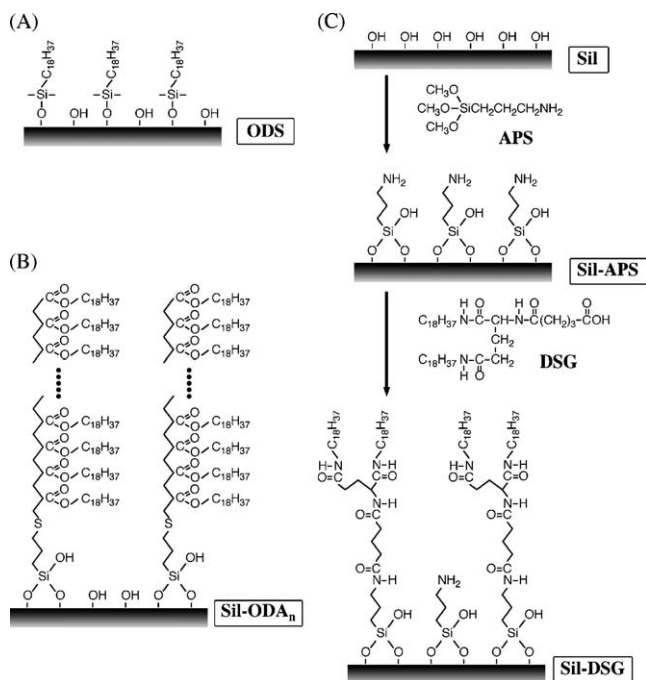


Fig. 1. Structures of stationary phases.

self-assemblies can be explained by ordered-to-disordered transition, phase separation behavior [26], and enhancement of chirality [22,29,30]. In this communication, we present the first example of L-glutamide-derived lipid-grafted silica and their chromatographic properties in an RP-HPLC mode.

2. Experimental

2.1. Materials

Glutaric anhydride and triethylamine were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan) and Nacalai Tesque Inc. (Kyoto, Japan), respectively, and used as received. 3-Aminopropyltrimethoxy silane was purchased from Azmax Co. (Chiba, Japan) and diethylphosphorocyanide as a condensing agent was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Porous silica particles (YMC-GEL) were purchased from YMC Co. Ltd. (Kyoto, Japan) whose average diameter, pore size and surface area are 5 μm , 120 \AA and 339 $\text{m}^2 \text{g}^{-1}$, respectively. An ordinary monomeric ODS column (Inertsil ODS-3, GL Science Inc., Tokyo, Japan), polymeric ODS column (Shodex Silica C18-P 4E, Showa Denko K.K., Tokyo, Japan) and Sil-ODA₂₅ [3–11] were used as references. The surface areas and the chain density of these packing materials are shown in Table 3.

2.2. Synthesis of L-glutamide-derived lipid

N,N'-Didodecyl-L-glutamide (3 g, 4.7 mmol), which was synthesized according to our previous report [32],

glutaric anhydride (0.9 g, 7.9 mmol), and triethylamine (0.9 ml, 6.5 mmol) were mixed in chloroform (300 ml) for 4 h at ambient temperature. The solution was concentrated and recrystallized from ethanol: yield (3.1 g, 87%); mp 128–130 °C, IR (KCl) 3288, 2917, 2849, 1730, 1637 cm^{-1} . Anal. calculated for $\text{C}_{45}\text{H}_{89}\text{N}_3\text{O}_5$: C, 72.3; H, 11.7; N, 5.5. Found: C, 72.0; H, 11.6; N, 5.5. The chemical structure of dioctadecyl L-glutamide lipid (DSG) was determined by ^1H NMR and IR spectroscopies and by elemental analysis.

2.3. Grafting of lipid onto silica surface

3-Aminopropyltrimethoxysilane (APS, 0.1 ml) and 3.0 g of porous silica particles (YMC-GEL), were stirred in toluene at reflux temperature for 20 h. The APS-introduced silica particles (Sil-APS) were washed with toluene, ethanol, and diethyl ether, dried in vacuo. And Sil-APS (3.0 g) was then coupled with dioctadecyl L-glutamide lipid (3.0 g, 3.9 mmol) in dry THF by using diethylphosphorocyanide (1.5 g, 9.6 mmol) as a condensing agent. After mixing at 60 °C for 1 day, the particles were collected and washed with hot THF, hot chloroform, ethanol, and diethyl ether, and then dried in vacuo to obtain Sil-DSG (Fig. 1). The amount of DSG on the silica was determined by elemental analysis.

2.4. ^1H and ^{13}C NMR spectroscopic measurements of the stationary phase

The lipids on the silica particles were evaluated by ^1H and ^{13}C NMR spectroscopic measurements. All NMR spectra were measured by a Unity Inova AS400 (Varian Inc., Palo Alto, CA, USA) in a static magnetic field of 9.4 T using a GHX nanoprobe for suspension state NMR and a solid probe for CP/MAS-NMR. The suspensions were prepared by adding 100 μl of deuterium methanol (CD_3OD) to 10 mg of each silica particle. Tetramethylsilane (TMS) and hexamethyldisilane (HMS) were used as references for chemical shift and intensity (peak area), respectively. Normalization was carried out depending on the amount of grafted octadecyl moieties.

2.5. HPLC

The lipid-grafted silica (Sil-DSG) was packed into a stainless steel column (250 mm \times 4.6 mm I.D.). Chromatographic-grade solvents were used to prepare mobile phase solutions, and PAH standards were obtained either from Sigma-Aldrich or Fluka Chemika-Biochemika. HPLC was performed with a PU-980 intelligent HPLC pump (JASCO Co., Japan), an MD 2010 plus multi-wavelength UV detector (JASCO Co., Tokyo, Japan), and a Rheodyne 7125 injection valve (Rheodyne, Rohnert Park, CA, USA). The temperature of the column was controlled by a U-620 column oven (Sugai, Tokyo, Japan). A typical chromatogram with a Sil-DSG packed column is shown in Fig. 2. The theoretical plate number and asymmetric factor (at 5%

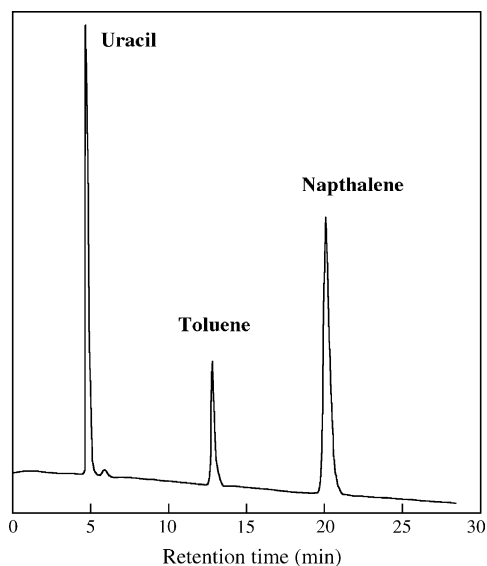


Fig. 2. Chromatogram for a mixture of uracil, toluene and naphthalene with Sil-DSG. Mobile phase: methanol–water (70:30), temperature: 25 °C, flow rate: 0.5 ml min⁻¹.

height) for naphthalene are 13 600 and 1.28, respectively. We used this Sil-DSG column for all experiments.

3. Results and discussion

3.1. Dioctadecyl L-glutamide-derived lipid (DSG)

DSG was prepared from *N*-benzyloxycarbonyl-L-glutamic acid through dealkylation, debenzyloxycarbonylation, and ring-opening reaction with glutaric anhydride. The DSG formed gel in organic solvents such as chloroform and THF and exhibited a thermo-reversible gel-to-sol phase transition. These phenomena are typical of self-assembled organogels.

3.2. Grafting of DSG onto silica

DSG [33] was coupled with the amine group of 3-aminopropyltrimethoxysilane-grafted silica (Sil-APS) to obtain DSG-grafted silica (Sil-DSG). Table 1 shows the elemental analyses of Sil-APS and Sil-DSG. The C/N value, 3.11, indicates that about two and a half of the methoxy groups of APS were consumed for silanation to silica and/or for cross-linking. The following grafting process with DSG increased the carbon percentage from 8.41 to 20.1 wt.%. These values correspond to 2.34 APS-mmol g⁻¹ and

0.21 DSG-mmol g⁻¹, respectively. This indicates that about 90% of the amine groups remained after the grafting process. The amounts of grafted alkyl chains are 2.13 μmol/m² (C: 11.7%) in Sil-DSG, 1.72 μmol/m² (C: 14.0%) in monomeric ODS, 3.40 μmol/m² (C: 17.5%) in polymeric ODS and 2.63 μmol/m² (C: 15.7%) in Sil-ODA₂₅, respectively.

The suspensions of Sil-DSG in methanol and ethanol showed no phase transition by thermal analysis using a differential scanning calorimetry (DSC). In previous papers, we have reported that the phase transition of poly(octadecyl acrylate) was observed even after the terminal group was grafted onto the silica surface (Sil-ODA_n) [4]. This is due to small perturbation on the silica surface because only one side of the terminal group is chemically bonded, allowing the polymer main chain to remain flexible. In the case of Sil-DSG, the lipid was connected directly to the surface, and thus the mobility is strongly restricted. The physical state of DSG on silica is discussed with NMR spectroscopy later.

3.3. Selectivity for polycyclic aromatic hydrocarbons

It is known that ordinary ODS stationary phases recognize the hydrophobicity of elutes, but Sil-ODA_n recognizes aromaticity through carbonyl-π interaction instead [4–11]. Furthermore, we have clarified that the alkyl chains of Sil-ODA_n form highly oriented structures in the crystalline state. Thus the aligned carbonyl groups enhance molecular-shape selectivity for PAHs through multiple π-π interactions. Table 2 shows the retention factors (*k*) and separation factors (*α*) of various polycyclic aromatic hydrocarbons (PAHs) with monomeric ODS, polymeric ODS, Sil-ODA₂₅, and Sil-DSG columns. It seems that the retention behavior of Sil-DSG obeys a reversed phase mode like ODS, but it should be noted that the separation factor is much higher in Sil-DSG than in ODS: e.g., α_{pentacene/dibenz[a,c]anthracene} are 11.6 and 2.19, respectively.

To understand this unusually enhanced selectivity of Sil-DSG, the temperature dependences were examined using naphthalene and chrysene as elutes that have the same numbers of carbon atoms and π-electrons but different molecular shapes. As shown in Fig. 3, the selectivity was low and slightly decrease with temperature increasing in monomeric ODS (*α* = 1.1–1.2) and polymeric ODS (*α* = 1.3–1.6). It has been described that the shape selectivity for PAHs is strongly influenced by the surface coverage of alkyl chain [14] and better selectivity is usually observed in polymeric phase among the commercial ODS columns because higher surface coverage is achieved in polymeric system. On the other hand, Sil-ODA₂₅ showed remarkable temperature dependence with high selectivity. The selectivity decrease with temperature increasing similar to other columns, but nonlinear change was observed around 40 °C which is attributed to the phase transition between ordered and disordered states of the ODA₂₅ moiety (DSC measurement of Sil-ODA₂₅ showed an endothermic peak in the range of 30–47 °C, with the highest

Table 1
Elemental analysis of Sil-APS and Sil-DSG

	C (%)	H (%)	N (%)	C/N
Sil-APS	8.41	2.22	2.70	3.11
Sil-DSG	20.1	3.81	2.98	6.74

Table 2
Selectivity of monomeric and polymeric ODS, Sil-ODA₂₅ and Sil-DSG stationary phases for polycyclic aromatic hydrocarbons (PAHs)

	L/B	log P	monomeric ODS		polymeric ODS		Sil-ODA _n		Sil-DSG	
			k	α	k	α	k	α	k	α
1 Benzene	1.10	2.28	0.81	1.80	0.56	2.00	0.23	1.91	0.11	2.55
2 Naphthalene	1.24	3.21	1.46		1.12		0.44		0.28	
3 Anthracene	1.57	4.38	3.00		3.00		1.30		1.10	
4 Triphenylene	1.12	5.28	5.43	1.08	5.89	1.14	3.15	1.35	2.94	1.33
5 Benz[a]anthracene	1.58	5.42	5.84		6.72		4.24		3.91	
6 Chrysene	1.72	5.40	5.84		6.90		4.63		4.82	
7 Naphthacene	1.89	5.71	6.91	1.27	9.37	1.59	8.62	2.74	11.12	3.78
8 Dibenz[a, c]anthracene	1.24	6.47	0.73		1.31		0.57		2.02	
9 Dibenzo[a, h]anthracene	1.79	6.60	0.75		1.46		0.75		3.74	
10 Pentacene	2.23	7.25	1.60	2.19	4.95	3.78	5.55	9.74	23.50	11.6

Mobile phase: methanol–water (9:1) for benzene, naphthalene, anthracene and four rings PAHs, ethanol for five rings PAHs. Temperature: 20 °C.

peak at 42 °C in a methanol–water (45:55) suspension). Similar nonlinear changes were observed in the mixtures of *trans*-stilbene, *cis*-stilbene, triphenylene, and *o*-terphenyl at same temperature range [4]. We have concluded that the selectivity enhancement of Sil-ODA_n is brought about by the ordering of the organic phase. Compared with Sil-ODA_n, Sil-DSG and ODS exhibit no phase transitions in the

temperature range of 10–60 °C, and no bending was observed in those stationary phases, but the selectivity of Sil-DSG was extremely higher than that of ODS or Sil-ODA_n, regardless of the fact that the alkyl chain density of Sil-DSG (2.13 μmol/m²) is lower than that of polymeric ODS (3.40 μmol/m²) and Sil-ODA_n (2.63 μmol/m²). Therefore, the alkyl chain density cannot explain the high selectivity of Sil-DSG. We have described how that the carbonyl groups interact with aromatic elutes through π–π interactions that are stronger (1.87 kcal mol⁻¹ in HCHO–benzene) than those of CH–π (0.57 kcal mol⁻¹ in CH₄–benzene) [8] or those of benzene π–benzene π (0.49 kcal mol⁻¹) [34], and the aligned carbonyl groups in Sil-ODA_n are effective for enhancing the shape selectivity of PAHs. Furthermore, since the α values are unchanged after 0.01% trifluoroacetic acid is added to the mobile phase, the selectivity is independent of the remaining amine groups on the silica. These facts strongly suggest that the higher selectivity of Sil-DSG is brought about by the carbonyl groups of the L-glutamide moiety. In support of this hypothesis, when acetone as an inhibitor for π–π interactions was added to the mobile phase, the α value for naphthalene/chrysene decreased, as shown in Table 3. A similar reduction was observed in Sil-ODA₂₅, but was not observed in monomeric and polymeric ODS. These results support the importance of the carbonyl groups of DSG in enhancing molecular-shape selectivity of PAHs.

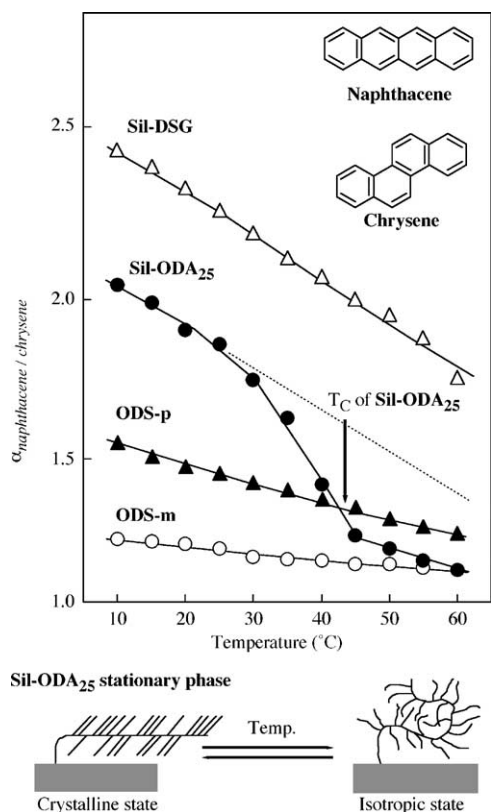


Fig. 3. Temperature dependencies of $\alpha_{\text{naphthalene/chrysene}}$ with monomeric ODS, polymeric ODS, Sil-ODA₂₅ (phase transition behavior is schematically illustrated) and Sil-DSG columns. Mobile phase: methanol–water (90:10), flow rate: 1 ml min⁻¹.

3.4. Separation mechanism of Sil-DSG

Suspension state ¹H NMR spectroscopy in CD₃OD and ¹³C CP/MAS NMR spectroscopy in a solid state were carried out. Fig. 4 shows typical ¹³C CP/MAS NMR spectra of ODS, Sil-ODA₂₅, and Sil-DSG. The peaks around 33 and 30 ppm are assigned to a *trans*-conformation and a *gauche* form of the methylene carbons, respectively [35]. These assignments allow us that the long alkyl chains in monomeric ODS and Sil-DSG are in the *gauche* form at 20 °C, whereas those in polymeric ODS and Sil-ODA₂₅ are in *trans*-conformation. In

Table 3
Effects of addition of acetone to mobile phase on the separation factors ($\alpha_{\text{naphthalene/chrysene}}$)

	Carbon (%)	Surface area (m ²)	Surface coverage ($\mu\text{mol/m}^2$)	$\alpha_{\text{naphthalene/chrysene}}$	
				Methanol/water (90:10)	Methanol/acetone/water (70:20:10)
Monomeric ODS	14.0	450	1.72	1.20	1.20
Polymeric ODS	17.0	300	3.40	1.50	1.50
Sil-ODA ₂₅	15.7	300	2.63	2.02	1.91
Sil-DSG	11.7	340	2.13	2.42	2.31

Temperature: 10 °C.

general, higher shape selectivity for PAHs is achieved in polymeric ODS rather than monomeric ODS. This has been explained by alkyl chain density and higher chain density brings about higher shape selectivity. The difference of alkyl chain conformation in monomeric and polymeric ODS is based on the fact that the mobility of alkyl chains are restricted by spacing of the neighboring bounded chains. These results indicate that the extremely high selectivity of Sil-DSG cannot be explained only by the alkyl chain density. Furthermore, a suspension ¹H NMR spectroscopy [36] showed almost no peak based on the alkyl methylene protons in each stationary

phase at 20 °C. However, the corresponding peak appeared clearly only in Sil-ODA₂₅ at temperatures above T_c . These results indicate that the mobility of alkyl chains in Sil-ODA₂₅ increases above T_c , but the mobility of alkyl chains in ODS and Sil-DSG are always restricted like a solid state. These NMR assignments, together with the chromatographic results, lead us to the following conclusions:

- (1) The carbonyl groups of Sil-DSG play a key role for selective separation of PAHs through carbonyl π -benzene π interaction. In the case of Sil-ODA_n, carbonyl groups can be oriented by forming a highly ordered structure of the side alkyl chains, and a thermally induced disordered-to-ordered phase transition in the alkyl chains is observed. Compared with Sil-ODA_n, Sil-DSG has no transition at 10–60 °C, and the alkyl chains are in disordered states. In spite of this, Sil-DSG shows remarkably higher selectivity than ODS or Sil-ODA_n. Therefore, it is expected that molecular-shape selectivity of Sil-DSG is enhanced through multiple carbonyl- π interactions, which can be promoted by orienting the carbonyl groups.
- (2) The mobility of alkyl moiety in Sil-DSG is strongly restricted, and no conformational change happens. This indicates that the carbonyl groups are rigidly immobilized on the silica surface and would form condensing thin layers. Probably, the inter- and/or intramolecular hydrogen bondings promote this assembly, which can be a driving force for multiple carbonyl π -benzene π interactions.

Further investigations will be done to consider the separation behavior of Sil-DSG and the results including chemical modification of stationary phase and spectroscopic measurements will be described in elsewhere.

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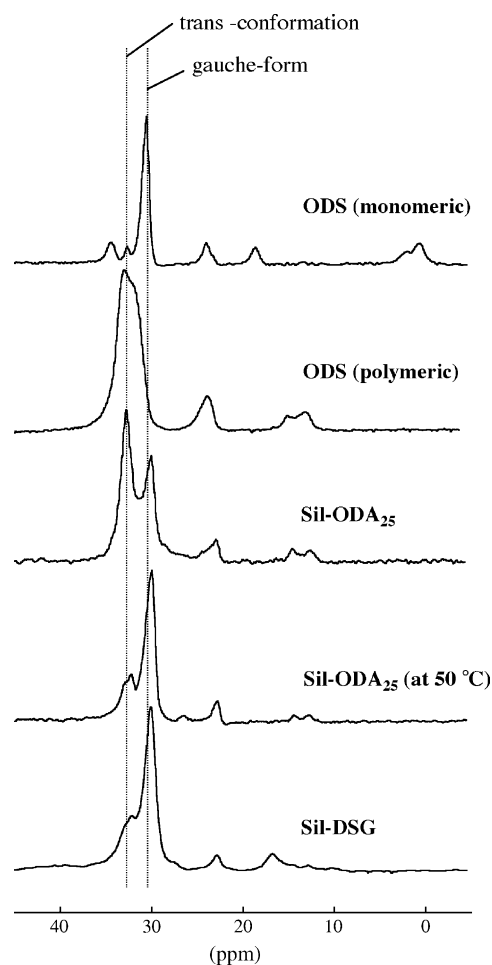


Fig. 4. ¹³C CP-MAS/NMR spectra of monomeric ODS, polymeric ODS, Sil-ODA₂₅ and Sil-DSG stationary phase. Temperature: 20 °C.

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