


 Cite this: *RSC Adv.*, 2017, 7, 41686

 Received 30th June 2017
Accepted 22nd August 2017

DOI: 10.1039/c7ra07244a

rsc.li/rsc-advances

Thixotropic stiff hydrogels from a new class of oleoyl-D-glucamine-based low-molecular-weight gelators†

 Yutaka Ohseido,^a Masashi Oono,^d Kowichiro Saruhashi,^d Hisayuki Watanabe^{cd} and Nobuyoshi Miyamoto^a

A series of new compounds comprising oleoyl, amino acid and D-glucamine moieties were synthesised using mild reaction conditions, and their hydrogelation ability was evaluated. The gels exhibited improved stiffness and thixotropic properties dependent on the chemical structure of the amino acid linker and the number of potential hydrogen bonding sites.

Introduction

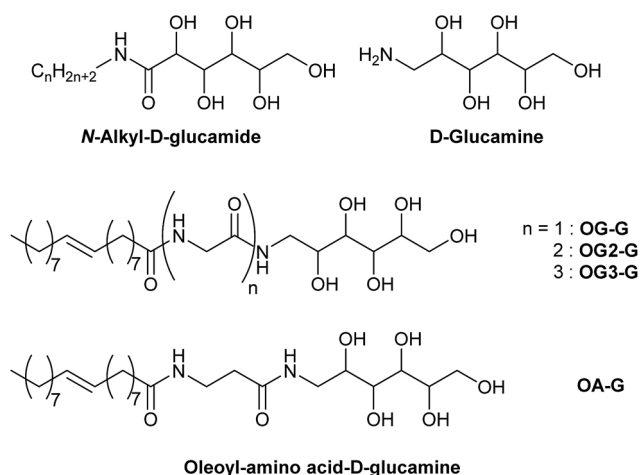
Low-molecular-weight gelators (LMWGs) have received much attention owing to their ability to be tuned by chemical synthesis to form soft matter (*i.e.* molecular gels) with desired properties.^{1,2} In the last two decades, there have been numerous attempts to synthesise molecular gels comprising LMWGs and organic (organogel) or aqueous (hydrogel) media for soft matter applications in fields such as medical science and optoelectronics.^{3,4} Therefore, LMWGs that form stimuli-responsive molecular gels have been extensively studied.⁵ To improve the mechanical properties of gels, the relationship between thixotropic behaviour, *i.e.* mechanically reversible sol-to-gel transitions, and their network structure has been extensively studied.⁶ In particular, thixotropic behaviour is required for injectable and ointment-type gels for biomaterials in medical applications.³

For the creation of molecular hydrogels, fibre micelle and hydrogel formation of *N*-alkylaldonamides such as *n*-octyl- and *n*-dodecyl-D-glucamides was extensively studied.⁷ These molecules, which can be obtained by simple amidation, are efficient hydrogelators. Several widely studied derivatives of *N*-alkylaldonamides exhibit unique nano- and micro-metre scale architectures such as tubular micelles.^{7–11} The original

N-alkylaldonamides tend to crystallise in aqueous solution, unless a surfactant is added to the hydrogel system.⁹

Recently, we reported that the onset of thixotropic behaviour in molecular organogels can be induced simply by mixing two alkyl-chain substituted homologues of commercially available hydrazides,^{12a,b} amides,^{12c,e,h} ureas^{12d} and hydrogelators^{12f,g} such as *N*-alkyl-D-glucamides. Although mixing induced thixotropy is observed in other systems,^{13,14} newer gelators that form functional gels are needed to promote the study of molecular gels.

In this study, we evaluate the potential of a new class of D-glucamine derivatives for their hydrogelation abilities. Our design of the hydrogelator constitutes three structural motifs: a D-glucamine moiety (head), an amino acid (linker), and an oleoyl moiety (tail). The three moieties are linked by two amide bonds (OG-G, OG2-G, OG3-G, and OA-G, Scheme 1). We consider that the oleoyl group, which is more hydrophobic than a stearoyl group and the long alkyl chain present in surfactants,



Scheme 1 Chemical structures of oleoyl-D-glucanamide derivatives.

^aDepartment of Life, Environment and Materials Science, Fukuoka Institute of Technology, 3-30-1, Wajiro-Higashi, Higashi-ku, Fukuoka 811-0295, Japan. E-mail: josedo@bene.fit.ac.jp; Fax: +81 92-606-3977; Tel: +81 92-606-3977

^bComprehensive Research Organization, Fukuoka Institute of Technology, 3-30-1, Wajiro-Higashi, Higashi-ku, Fukuoka 811-0295, Japan

^cGlobal Innovation Center, Kyushu University, 6-1 Kasuga-koen Kasuga-city, Fukuoka 816-8580, Japan

^dNissan Chemical Industries, Ltd., 2-10-1 Tsuboinishi, Funabashi, Chiba 274-8507, Japan

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7ra07244a



can facilitate gelation of water owing to its increased hydrophobicity relative to linear alkyl chains.¹⁵ Furthermore, the covalent amide bond linking the components can provide better chemical stability than the corresponding esters moieties present in other gelators. Finally, the hydrogen bonding ability of the molecules can be chemically tuned by introducing glycine(s) or its homologue as the linker between the oleoyl and D-glucamine groups.

Experimental

Materials and methods

β -Alanine (99%), D-glucamine (95%), glycine (99%), glycyglycine (99%), glycyglycyglycine (98%) and 1-hydroxybenzotriazole monohydrate (HOBt, 97%) were purchased from Tokyo Chemical Industry Co., Ltd. and used without further purification. All organic solvents, buffer aqueous solutions, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (water soluble carbodiimide (WSC)), hydrochloric acid, oleoyl chloride (97%) and sodium hydroxide were purchased from Wako Pure Chemical Industries, Ltd. and used without further purification. Water was deionised with an Elix UV 3 Milli-Q integral water purification system (Nihon Millipore K.K.). Phosphate buffered saline (1× PBS (–)) was obtained by diluting 10× PBS (–) buffer (Wako Pure Chemical Industries, Ltd.) with water.

¹H-NMR and ¹³C-NMR spectra were acquired using an AVANCE 500 (500 MHz, Bruker BioSpin K.K.) spectrometer. Mass spectra were recorded on an LTQ Orbitrap spectrometer (ESI FTMS, Thermo Fisher Scientific K.K.) or a 3100 Mass detector equipped with e2695 Separations module and an s2489 UV/Visible detector (Nihon Waters K.K.). Elemental analysis was performed with a JM10 elemental analyzer (J-SCIENCE LAB CO., Ltd.).

The gelation tests were performed using the vial inversion method. A crystal of gelator was placed in a vial with a solvent at a set concentration (wt%) and capped. The vial was heated in a dry bath of 100 °C until the crystal of gelator was dissolved. Gelator solution was left for 1 h at room temperature, and gelation was checked with naked eyes by inverting the vial.

The thixotropic behaviour was evaluated using the vial inversion method. The hydrogel in vial was shaken and collapsed by a vortex genie (Scientific Industries, Inc). Then the obtained sol was left for the set time at room temperature, the recovery of the gel state from the sol state was checked with naked eyes by inverting the vial.

Thermal analysis was performed with an EXSTAR6000 differential scanning calorimeter DSC (Seiko Instruments Inc.) using an Ag-made closable sample pan. Tgel → sol and Tsol → gel of gels were determined as extrapolated onset temperatures from the DSC curves.

SEM images were recorded with a SU-8000 scanning electron microscope (Hitachi High-Technologies Corporation) at 1.0 kV; the SEM sample was vacuum dried and placed on a conductive tape on the SEM sample stage. Pt, as a conductive material, was used as a coating on the sample (Pt coating is 10 nm thick). The xerogel for SEM sample was obtained from freeze-drying of the hydrogel at the concentration of critical gelation concentration by using dry ice/acetone as a refrigerant.

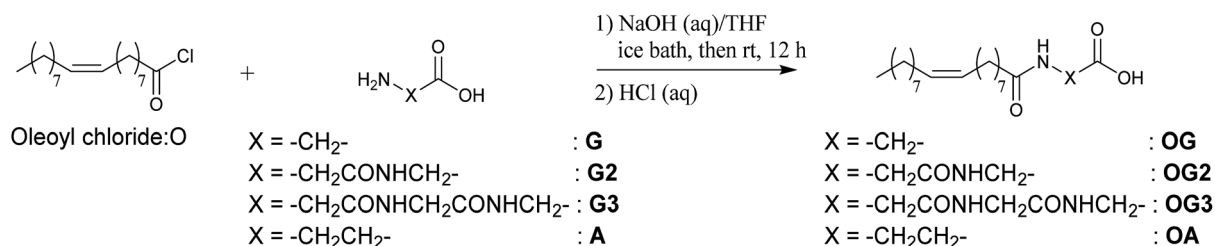
Rheological measurements of frequency sweep were performed with an MCR-301 rheometer (Anton Paar Japan K.K.) with a parallel plate (8 mm diameter) at a gap of 0.50 mm and γ of 0.01% (measurement temperature: 25 °C). Rheological measurements of strain sweep were performed with an MCR-301 rheometer with a parallel plate (8 mm diameter) at a gap of 0.50 mm and constant angular frequency 1 rad s^{−1} (measurement temperature: 25 °C). For rheological measurements, the hydrogel sample was applied onto the parallel plate and sample stage (the overflow gel was swept). The hydrogel sample for rheological measurements was placed on a parallel plate and a sample stage (the overflow gel was swept). Step-shear measurement was carried out by applying normal strain (strain amplitude 0.01% and frequency 1 Hz) and large strain (shear rate 3000 s^{−1} for 0.1 s), repeatedly.

X-ray diffraction data were recorded on a D8 Discover X-ray diffractometer (Bruker AXS K.K.) with CuK α at 26 °C (the sample was filled in a quartz glass capillary tube of 1 mm diameter).

Infrared spectroscopy was performed with an FT/IR-620 (JASCO Corporation) using the ATR method (ZnSe prism).

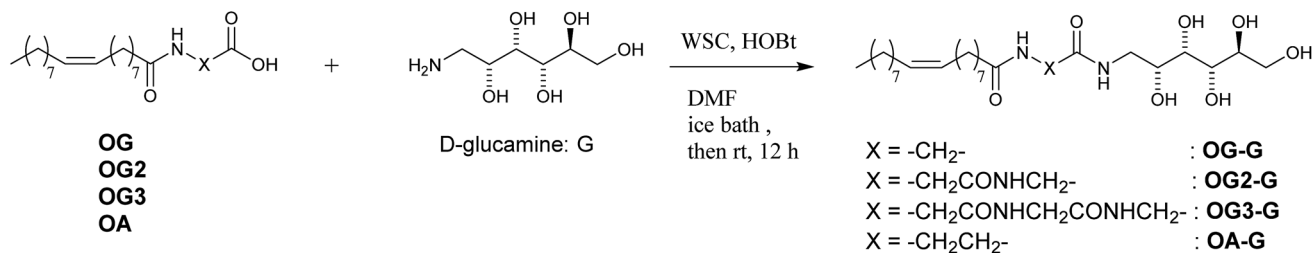
Synthesis of OG-G, OG2-G, OG3-G and OA-G

Synthesis of oleoyl amino acid derivatives (general synthesis 1, Scheme 2). Oleoyl amino acids were synthesized following the reported method.^{15,16} Oleoyl chloride (13.29 mmol) in absolute THF (20 mL) was added slowly under N₂ atmosphere over a 30 min period, with a dropping funnel, in an aqueous NaOH (2 M) solution (40 mL) of amino acid (19.94 mmol), which was immersed in ice bath. The solution, along with the generated white precipitate, was stirred in an ice bath for an additional hour, and then at rt for 12 h. Subsequently, water (10 mL) was



Scheme 2 Synthesis of oleoyl amino acid derivatives.





Scheme 3 Coupling of oleoyl amino acids with D-glucamine.

added to dissolve the precipitate, and then aq. HCl (3 M, 20 mL) was added to reduce the pH of the solution to <2. The generated white precipitate was filtered, rinsed with water, and subsequently dried *in vacuo*. The dried precipitate was recrystallised from MeOH to yield the oleoyl amino acids.

Coupling of oleoyl amino acids with D-glucamine (general synthesis 2, Scheme 3). The synthesis of **OG-G** is described here as an example. The synthesis of other gelators followed a similar protocol. Both D-glucamine and water-soluble carbodiimide (5.26 mmol each) were added under an atmosphere of N₂ to the solution of oleoyl amino acid, **OG** (4.78 mmol) and 1-hydroxybenzotriazole monohydrate (5.26 mmol) in absolute DMF (20 mL) at 0 °C. The solution was stirred in an ice bath for 1 h (the solution became opaque), and then at rt for 12 h (the opaque solution became gel-like). DMF was removed *in vacuo* and the white residue was recrystallised twice in MeOH to yield **OG-G** (yield 71.4%, white crystal).

OG. **OG** (white crystals) was prepared from oleoyl chloride and glycine (**G**) according to general synthesis 1 in 76.5% yield. ¹H-NMR (500 MHz, DMSO-*d*₆, TMS, δ, ppm): 8.11 (t, 1H, *J* = 5.8 Hz), 5.33 (t, 2H, *J* = 4.7 Hz), 3.72 (d, 2H, *J* = 6.0 Hz), 2.10 (m, 2H, *J* = 7.4 Hz), 1.99 (m, 4H), 1.50 (m, 2H), 1.27 (m, 20H), 0.86 (t, 3H, *J* = 6.8 Hz). LC-MS (ESI): calcd for C₂₂H₃₈NO₃, 339.28; found *m/z* = 338.2 [M – H][–].

OG2. **OG2** (white crystals) was prepared from oleoyl chloride and glycerylglycine (**G2**) according to general synthesis 1 in 63.0% yield. ¹H-NMR (500 MHz, DMSO-*d*₆, TMS, δ, ppm): 8.14 (t, 1H, *J* = 5.7 Hz), 8.05 (t, 1H, *J* = 5.8 Hz), 5.33 (t, 2H, *J* = 5.2 Hz), 3.75 (d, 2H, *J* = 6.0 Hz), 3.73 (d, 2H, *J* = 6.0 Hz), 3.70 (d, 2H, *J* = 5.7 Hz), 2.12 (t, 2H, *J* = 7.4 Hz), 1.99 (m, 4H), 1.51 (m, 2H), 1.25 (m, 20H), 0.86 (t, 3H, *J* = 6.8 Hz). LC-MS (ESI): calcd for C₂₂H₄₀N₂O₄, 396.30; found *m/z* = found 395.2 [M – H][–].

OG3. **OG3** (white crystals) was prepared from oleoyl chloride and glycerylglycylglycine (**G3**) according to general synthesis 1 in 37.8% yield. ¹H-NMR (500 MHz, DMSO-*d*₆, TMS, δ, ppm): 8.20 (t, 1H, *J* = 6.0 Hz), 8.13 (t, 1H, *J* = 5.7 Hz), 8.07 (t, 1H, *J* = 5.7 Hz), 5.33 (t, 2H, *J* = 5.2 Hz), 3.75 (d, 2H, *J* = 5.7 Hz), 3.71 (d, 2H, *J* = 5.7 Hz), 2.12 (t, 2H, *J* = 7.4 Hz), 1.48 (m, 2H), 1.25 (m, 20H), 0.86 (t, 3H, *J* = 6.9 Hz). LC-MS (ESI): calcd for C₂₃H₄₁N₃O₅, 439.30; found *m/z* = 438.3 [M – H][–].

OA. **OA** (white crystals) was prepared from oleoyl chloride and β-alanine (**A**) according to general synthesis 1 in 67.5% yield. ¹H-NMR (500 MHz, DMSO-*d*₆, TMS, δ, ppm): 7.86 (t, 1H, *J* = 5.4 Hz), 5.33 (t, 2H, *J* = 4.7 Hz), 3.21 (q, 2H, *J* = 6.6 Hz), 2.35 (m, 2H, *J* = 6.9 Hz), 2.00 (m, 4H), 1.51 (m, 2H), 1.24 (m, 20H),

0.86 (t, 3H, *J* = 6.9 Hz). LC-MS (ESI): calcd for C₂₁H₃₉NO₃, 353.29; found *m/z* = 352.2 [M – H][–].

OG-G. **OG-G** (white crystals) was synthesized in 71.4% yield by coupling **OG** with D-glucamine following the protocol described in general synthesis 2. ¹H-NMR (500 MHz, DMSO-*d*₆, TMS, δ, ppm): 8.00 (t, 1H, *J* = 5.7 Hz), 7.70 (t, 1H, *J* = 5.5 Hz), 5.31 (t, 2H, *J* = 5.5 Hz), 4.78 (d, 1H, *J* = 4.7 Hz), 4.50 (d, 1H, *J* = 5.7 Hz), 4.40 (d, 1H, *J* = 6.3 Hz), 4.37 (t, 1H, *J* = 5.4 Hz), 4.31 (d, 1H, *J* = 6.6 Hz), 3.71 (m, 2H, *J* = 5.7 Hz), 3.59 (m, 3H), 3.49 (m, 1H), 3.38 (m, 2H), 3.27 (m, 1H), 3.04 (m, 1H), 2.11 (d, 2H, *J* = 7.6 Hz), 1.97 (m, 4H), 1.53 (m, 2H), 1.24 (m, 20H), 0.85 (t, 3H, *J* = 6.6 Hz). ¹³C-NMR (125 MHz, DMSO-*d*₆, TMS, δ, ppm): 173.38, 170.10, 130.43, 130.38, 72.82, 72.44, 70.68, 64.21, 42.94, 42.85, 36.08, 32.16, 30.06, 30.00, 29.73, 29.63 (4C), 29.56, 29.49, 27.52, 27.46, 26.01, 22.96, 14.72. LC-MS (ESI): calcd for C₂₆H₅₀N₂O₇, 502.36; found *m/z* = 503.37 [M + H]⁺. Elemental anal. calcd (%) for C₂₆H₅₀N₂O₇: C, 62.12; H, 10.03; N, 5.57. Found: C, 62.07; H, 10.01; N, 5.52.

OG2-G. **OG2-G** (white crystals) was synthesized in 81.9% yield by coupling **OG2** with D-glucamine following the protocol described in general synthesis 2. ¹H-NMR (500 MHz, DMSO-*d*₆, TMS, δ, ppm): 8.08 (m, 2H), 7.71 (s (br), 1H), 5.32 (t, 2H, *J* = 5.4 Hz), 4.78 (s (br), 1H), 4.50 (d, 1H, *J* = 5.4 Hz), 4.40 (d, 1H, *J* = 6.0 Hz), 4.34 (t, 1H, *J* = 5.0 Hz), 4.29 (d, 1H, *J* = 6.0 Hz), 3.70 (m, 4H, *J* = 3.5 Hz), 3.59 (m, 3H), 3.49 (m, 1H), 3.42 (m, 2H), 3.29 (m, 1H), 3.06 (m, 1H), 2.15 (d, 2H, *J* = 7.6 Hz), 1.92 (m, 4H), 1.54 (m, 2H), 1.25 (m, 20H), 0.86 (t, 3H, *J* = 6.6 Hz). ¹³C-NMR (125 MHz, DMSO-*d*₆, TMS, δ, ppm): 173.3, 169.82, 169.36, 130.07, 130.04, 72.43, 72.05, 70.20, 63.79, 42.62, 42.54, 35.65 (2C), 31.75, 29.63, 29.57, 29.53, 29.31, 29.20 (3C), 29.14, 29.06, 27.10, 27.05, 25.57, 22.54, 14.36. LC-MS (ESI): calcd for C₂₈H₅₃N₃O₈, 559.38; found *m/z* = 560.39 [M + H]⁺. Elemental anal. calcd (%) for C₂₈H₅₃N₃O₈: C, 60.08; H, 9.54; N, 7.51. Found: C, 59.78; H, 9.48; N, 7.41.

OG3-G. **OG3-G** (white crystals) was synthesized in 69.5% yield by coupling **OG3** with D-glucamine following the protocol described in general synthesis 2. ¹H-NMR (500 MHz, DMSO-*d*₆, TMS, δ, ppm): 8.13 (t, 1H, *J* = 5.7 Hz), 8.10 (t, 1H, *J* = 5.7 Hz), 8.02 (t, 1H, *J* = 5.7 Hz), 7.70 (t, 1H, *J* = 5.4 Hz), 5.33 (t, 2H, *J* = 5.7 Hz), 4.77 (d, 1H, *J* = 4.4 Hz), 4.50 (d, 1H, *J* = 5.4 Hz), 4.41 (d, 1H, *J* = 6.0 Hz), 4.36 (t, 1H, *J* = 5.4 Hz), 4.29 (d, 1H, *J* = 6.6 Hz), 3.72 (td, 6H, *J* = 9.9 Hz, *J* = 5.6 Hz), 3.59 (m, 3H), 3.48 (m, 1H), 3.42 (m, 2H), 3.29 (m, 1H), 3.06 (m, 1H), 2.16 (d, 2H, *J* = 7.3 Hz), 1.95 (m, 4H), 1.52 (m, 2H), 1.24 (m, 20H), 0.86 (t, 3H, *J* = 6.9 Hz). ¹³C-NMR (125 MHz, DMSO-*d*₆, TMS, δ, ppm): 173.19, 170.13,



169.55, 169.31, 130.08, 130.05, 72.43, 72.02, 70.21, 63.79, 42.54 (2C), 35.64 (3C), 31.74, 29.62, 29.57, 29.52, 29.30, 29.20 (3C), 29.13, 29.06, 27.10, 27.05, 25.57, 22.54, 14.37. LC-MS (ESI): calcd for $C_{30}H_{56}N_4O_9$, 616.40; found $m/z = 617.4 [M + H]^+$. Elemental anal. calcd (%) for $C_{30}H_{56}N_4O_9$: C, 58.42; H, 9.15; N, 9.08. Found: C, 58.56; H, 9.18; N, 8.99.

OA-G. OA-G (white crystals) was synthesized in 72.5% yield by coupling OA with D-glucamine following the protocol described in general synthesis 2. 1H -NMR (500 MHz, DMSO- d_6 , TMS, δ , ppm): 7.81 (m, 1H), 7.70 (m, 1H), 5.33 (t, 2H, $J = 5.0$ Hz), 4.74 (s (br), 1H), 4.47 (d, 1H, $J = 5.0$ Hz), 4.37 (d, 1H, $J = 5.7$ Hz), 4.33 (s (br), 1H), 4.22 (d, 1H, $J = 6.3$ Hz), 3.57 (m, 3H), 3.49 (m, 1H), 3.40 (m, 2H), 3.22 (m, 3H), 3.05 (m, 1H), 2.24 (d, 2H, $J = 7.1$ Hz), 2.00 (m, 6H), 1.48 (m, 2H), 1.27 (m, 20H), 0.86 (t, 3H, $J = 6.6$ Hz). ^{13}C -NMR (125 MHz, DMSO- d_6 , TMS, δ , ppm): 172.95, 171.59, 130.48, 130.47, 73.01, 72.61, 72.41, 70.45, 64.23, 42.87, 36.40, 36.24, 36.19, 32.14, 30.02, 29.97, 29.92, 29.70, 29.57, 29.54, 29.46, 29.44, 27.49, 27.45, 26.13, 22.94, 14.77. LC-MS (ESI): calcd for $C_{27}H_{52}N_2O_7$, 516.38; found $m/z = 517.37 [M + H]^+$. Elemental anal. calcd (%) for $C_{27}H_{52}N_2O_7$: C, 62.76; H, 10.14; N, 5.42. Found: C, 62.72; H, 10.11; N, 5.47.

Results and discussion

Molecules containing glycine (OG-G, OG2-G, and OG3-G) and β -alanine (OA-G) as the linkers are synthesised by condensation of *N*-glucamine with alkyloylamino acid in DMF under mild conditions. After DMF is removed *in vacuo*, recrystallisation affords the pure compound. Note that column chromatography is unnecessary for the purification. Elemental analysis and comparison with the predicted values further confirmed the purity. The alkyloylamino acids used in the above condensation are synthesised from the appropriate amino acid and the alkyloyl chloride in aqueous NaOH.¹⁶

The hydrogelation ability of these new compounds are examined at various pH values and in the presence of salts to obtain the critical gelation concentration (CGC) (Fig. 1, S1, S2, ESI† and Table 1). These newly synthesised compounds, OG2-G and OG3-G, can gelate water in a pH range from 2 to 10, as well as in the presence of a high concentration of NaCl (1 M) and phosphate buffered saline (PBS), which is generally used in

biological studies. In Table 1, OG-G showed hydrogelation, except with aq. NaCl and PBS, and OA-G formed a hydrogel in deionised water and carbonate buffer. The CGCs in buffer solutions become higher than those in deionised water. Furthermore, in higher salt concentrations (1 M NaCl), the CGCs become higher than those in buffer solutions; the presence of salt and acid or base may change the polarity of aqueous solutions, making hydrogelation difficult by disturbing intermolecular hydrogen bonding between gelators. Although hydrogelation in deionised water and buffered solutions occurs at moderate CGCs for all gelators, the gel is maintained for over 6 months without onset of crystallisation. *N*-alkyl-D-glucamines, compounds where the polar head is directly connected to the alkyl tail (*i.e.* in the absence of an amino acid linker), demonstrate poor gel stability in the absence of a surfactant. Therefore, the better gel stability observed in this study is attributed to the presence of the peptide moiety in these molecules. In addition, it is thought that the balance of hydrogen bonding (involving the peptide moieties) and hydrophobicity of gelators play an important role in hydrogelation. The better CGC observed in the case of OG2-G and OG3-G may be the result of a higher number of hydrogen bonding sites when compared OG-G and OA-G. Moreover, the changes in the CGC with the introduction of a single methylene group (OA-G has one more methylene unit than OG-G) show that an appropriate chemical structure can enhance the gelation properties in this family of LMWGs (OA-G hydrogels showed inferior CGCs due to increased hydrophobicity in this system). Additionally, the CGC of OG-G hydrogels are superior to those of SG-G (stearoylamido-D-glucamine),¹⁵ likely due to the increased hydrophobic interactions of the oleoyl group.

The rheometric results (Fig. 2) show the existence of a gel state, similar to that observed in polymer gels.¹⁷ Because of an increase in the applied stress, the relationship between storage modulus, G' , and the loss modulus, G'' , changed relative to $G' > G''$ systems.¹⁷ In the frequency sweep, a pseudo plateau with $G' > G''$ (gel state) is observed. On comparing the response of the hydrogels to applied shear stress, stable values with a modulus of a higher magnitude (10^4 to 10^5 Pa) was observed compared to the SG-G analogue with the same number of alkyl carbons (stearoyl group). The only exception was for the weak hydrogels formed with OA-G, likely caused by unbalanced hydrophobicity resulting in a weaker gel fibre or network. In the stiffness of hydrogels, OG-G and OG3-G showed similar stiffness; OG2-G

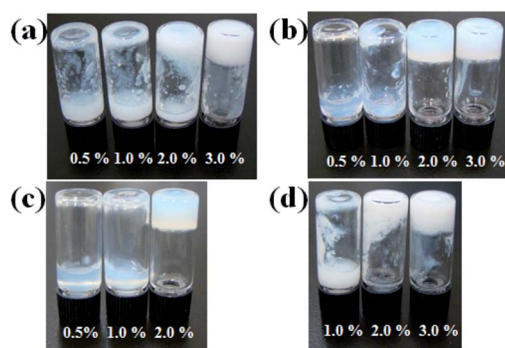


Fig. 1 Evaluation of hydrogelation in deionised water: (a) OG-G, (b) OG2-G, (c) OG3-G, and (d) OA-G.

Table 1 Critical gelation concentrations (CGCs) of hydrogelators

Solvent	OG-G	OG2-G	OG3-G	OA-G	SG-G
Deionised water	3.0 ^d	2.0 ^d	2.0 ^d	3.0 ^e	3.0 ^e
Oxalate buffer ^a	4.0 ^d	3.0 ^d	3.0 ^d	5.0 ^d	5.0 ^g
Phosphate buffer ^b	4.0 ^d	3.0 ^d	3.0 ^d	5.0 ^f	5.0 ^g
Carbonate buffer ^c	4.0 ^d	3.0 ^d	3.0 ^d	5.0 ^d	5.0 ^g
1 M NaCl aq.	5.0 ^f	4.0 ^d	4.0 ^d	5.0 ^f	5.0 ^f
PBS(−) ⁱ	5.0 ^f	3.0 ^d	3.0 ^h	5.0 ^f	5.0 ^f

^a pH = 1.68. ^b pH = 6.86. ^c pH = 10.11. ^d Turbid gel. ^e Clear gel. ^f Crystal gel. ^g Partial gel. ^h Opaque gel. ⁱ PBS: phosphate buffered saline. The CGC values are expressed as wt%.



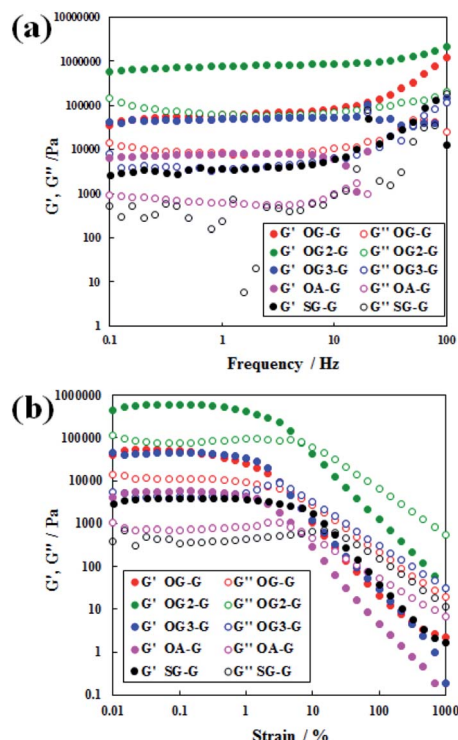


Fig. 2 Dynamic rheological properties of hydrogels formed with OG-G (3.0 wt%), OG2-G (2.0 wt%), OG3-G (2.0 wt%), and OA-G (3.0 wt%): (a) frequency sweep and (b) strain sweep.

formed the stiffest hydrogel, likely due to balanced hydrophobicity and hydrogen bonding yielding a stronger gel fibre or network. In recent studies of molecular gel systems, higher G' values near 10^5 Pa were obtained by Yu (over 10^6 Pa for 1.5% (w/v) organogels),¹⁸ Steed (over 10^5 Pa for 1% (w/v) organogels)¹⁹ and Shinkai (over 10^4 Pa for 1 mM organogels).²⁰ Importantly, our molecular hydrogel systems demonstrated a similar degree of high stiffness to those shown in the studies of Yu, Steed and Shinkai. This indicates that a stiff and stable hydrogel can be obtained by introducing an oleoyl group, instead of stearoyl chain, and glycine linker between the polar head and alkyl tail of *N*-alkyl-D-glucamines (note that the structure of glycine is more rigid than that of β -alanine).

Temperature-dependent data of sol-to-gel and gel-to-sol changes of the hydrogels measured by differential scanning calorimetry (DSC, Fig. S3 and Table S1, ESI[†]) show that similar ΔH values are involved in these reversible transitions. Sol-to-gel and gel-to-sol transition temperatures of hydrogels increase with the increase in the number of glycine repeat units, *i.e.* from OG-G to OG3-G, probably due to an increase in the interaction between glycine moieties, in addition to the increased molecular weight. Furthermore, the effect on thermal transitions by introduction of a methylene group in OA-G is similar to the addition of a second glycine unit, as in OG2-G. Considering the results of the gelation test, the transition temperatures may reflect the fibre properties, rather than the network properties of gel in this system (Table S1[†]).

Investigations into the mechanical properties of hydrogels show reveal thixotropic behaviour, even at the CGC. For

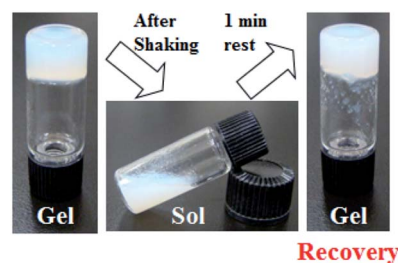


Fig. 3 Thixotropic behaviour of hydrogel formed with OG3-G (2.0 wt%).

example, re-gelation of a shaken OG3-G (2.0 wt%) hydrogel is clearly observed (Fig. 3). Evaluation of the qualitative thixotropic behaviour of hydrogels is performed with rheometry (Fig. 4, full version shown in Fig. S4, ESI[†]). The hydrogels show better recovery for G' than G'' even after being repeatedly subjected to a large deformation shear. Higher recoveries are observed for the stiffer and more stable hydrogels obtained from OG-G, OG2-G, and OG3-G compared to OA-G and SG-G¹⁵ hydrogels; an observation that may result from the introduction of the hydrogen bonding amino acid moieties and more hydrophobic oleoyl group that can facilitate the rebuilding of a damaged network. In addition, thixotropic behaviour was observed for OG3-G 3.0 wt% PBS hydrogel (Fig. S5, ESI[†]), whereas OG2-G 3.0 wt% PBS hydrogel did not show such behaviour. Further investigations on thixotropic properties of hydrogels, including other types of aqueous solutions, are underway. The results indicate that these hydrogels will be applicable in the healthcare and medical fields.

Scanning electron microscope (SEM) images of xerogels prepared by freeze-drying the corresponding hydrogels at the CGC show a network structure composed primarily of sub-micrometre thick fibres (Fig. 5). Considering that freeze-drying promotes the bundling of fibres, it is possible that the hydrogels consist of a finer network than what is observed in xerogels. Subsequently, these networks in the hydrogel can

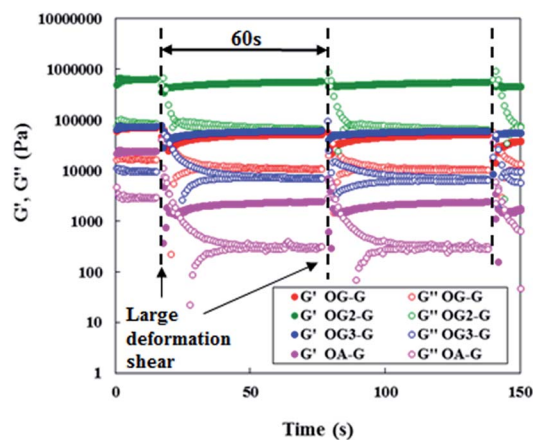


Fig. 4 Periodical step-shear test results for OG-G (3.0 wt%), OG2-G (2.0 wt%), OG3-G (2.0 wt%), and OA-G (3.0 wt%) hydrogels.²¹ For full version see Fig. S4, ESI[†].



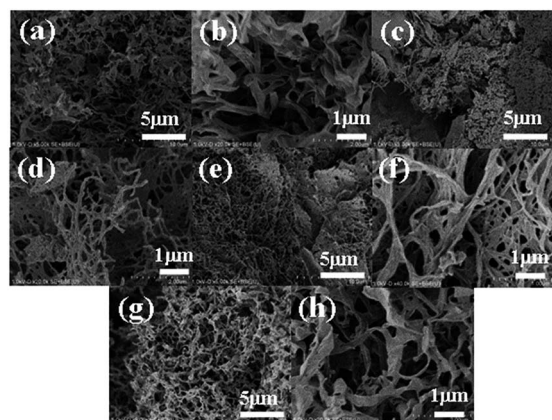


Fig. 5 SEM images of xerogels prepared from the corresponding hydrogels: (a and b) OG-G (3.0 wt%), (c and d) OG2-G (2.0 wt%), (e and f) OG3-G (2.0 wt%), and (g and h) OA-G (3.0 wt%). The values in parenthesis denote the concentration of hydrogels before drying.

exhibit polymer-like functions, as demonstrated by the observed thixotropic behaviour.

To study the molecular packing structure in the fibre, X-ray diffraction (XRD) measurements were performed. The XRD results (Fig. 6a and b), when analysed in the context of the contour length of the gelators (Fig. 6c), suggests that the peak positions of hydrogels are different from those of corresponding xerogel samples and larger than the corresponding contour length of gelators (for example, 31.1 Å for OG-G). As previously shown, *N*-alkyl-*D*-aldonamide molecules with similar structures to the gelators in this study form fibre micelles and lamellar structures, based on the belayed structures of the molecules. By

extension, in the gel state, it is possible that the molecules of the gelators can form bilayered or lamellar structures in the fibre (Fig. 6d). Also, in the xerogels, gelator molecules can form bilayered or lamellar structures. As shown in the xerogels of OG-G, OG2-G, and OG3-G, gelator molecules can also form tight bilayered or lamellar structures likely due to the absence of water molecules, and the XRD peaks might occur from a larger periodic structure corresponding to the length of two gelators.

To investigate the interaction of gelators, IR measurements were carried out. As seen in the IR spectra of different states of gelator systems, hydrogen bonding of gelators may play an important role in the driving force of fibre formation (Fig. S6, ESI†).

Conclusions

In conclusion, we have created a new family of oleoyl- and *D*-glucamine-based hydrogelators by introducing glycine-based linkers connecting the two moieties. The molecules, prepared under mild reaction conditions, form hydrogels that demonstrate thixotropic properties and improved stiffness compared to the stearyl analogue. These results suggest that *N*-alkylaldonamides can be chemically tuned to generate functional hydrogels by introducing an amino acid linker and an oleoyl group, rather than the stearyl group.¹⁵ Their thixotropic nature suggests that these hydrogels will have extensive applications in the healthcare and medical fields for use as biomaterials.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank Nissan Chemical Industries, Ltd. for financial and technical supports. A part of this work was supported by Research Center for Materials and Energy Devices of Fukuoka Institute of Technology (FIT-ME) (Strategic Research Foundation Grant-Aided Project for Private University from MEXT, Japan) as well as by Canon Foundation, KEKENHI (#24104005, #15K05610 and #15K05657) and Network Joint Research Center for Materials and Devices (#201507 and #20166009). A part of this work was also supported by the Nanotechnology Network Project (Kyushu-area Nanotechnology Network) of the MEXT, Japan. The SEM measurement was performed at the Center of Advanced Instrumental Analysis, Kyushu University. We are indebted to the Service Center of the Elementary Analysis of Organic Compounds, Kyushu University for elemental analysis. We would like to thank Enago (<http://www.enago.jp>) for the English language review.

Notes and references

- For books: (a) *Molecular Gels: Materials With Self-assembled Fibrillar Networks*, ed. R. G. Weiss and P. Terech, Springer Netherlands, Dordrecht, 2006; (b) *Functional Molecular*

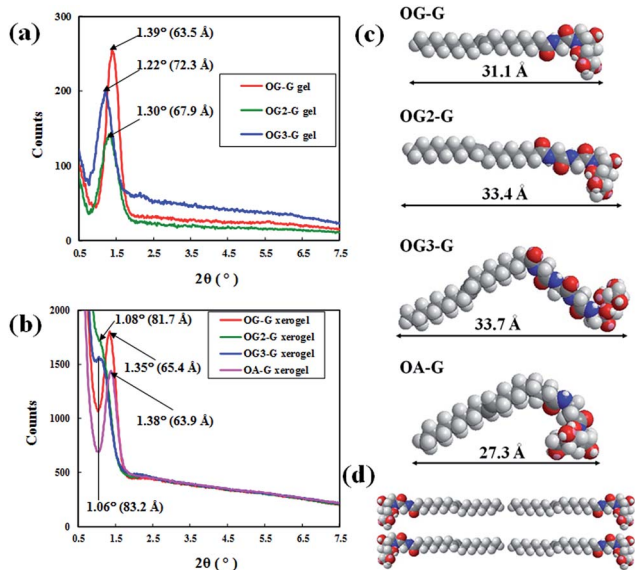


Fig. 6 XRD data of (a) hydrogels at CGC and (b) xerogels formed with OG-G, OG2-G, OG3-G, and OA-G, (c) contour length and the length of OG-G, OG2-G, OG3-G and OA-G obtained from MM2 calculation in ChemDraw and (d) schematic illustration of possible lamella packing structure of OG-G.



- Gels*, ed. B. Escuder and J. F. Miravet, Royal Society of Chemistry, Cambridge, 2013.
- 2 For recent reviews: (a) A. Dawn, T. Shiraki, S. Haraguchi, S. Tamaru and S. Shinkai, *Chem. Asian J.*, 2011, **6**, 266; (b) R. G. Weiss, *J. Am. Chem. Soc.*, 2014, **136**, 7519; (c) S. S. Babu, V. K. Praveen and A. Ajayaghosh, *Chem. Rev.*, 2014, **114**, 1973; (d) M. A. Rogers and R. G. Weiss, *New J. Chem.*, 2015, **39**, 785; (e) Y. Ohseido, *Polym. Adv. Technol.*, 2016, **27**, 704; (f) M. Yamanaka, *Chem. Rec.*, 2016, **16**, 768; (g) H. Ihara, M. Takafuji and Y. Kuwahara, *Polym. J.*, 2016, **48**, 843; (h) D. B. Amabilino, D. K. Smith and J. W. Steed, *Chem. Soc. Rev.*, 2017, **46**, 2404; (i) R. Miao, J. Peng and Y. Fang, *Langmuir*, 2017, DOI: 10.1021/acs.langmuir.6b04655.
 - 3 (a) J. Boekhoven and S. I. Stupp, *Adv. Mater.*, 2014, **26**, 1642; (b) Y. Ohseido, *Gels*, 2016, **2**, 13; (c) H. Wang, Z. Feng and B. Xu, *Chem. Soc. Rev.*, 2017, **46**, 2421.
 - 4 (a) A. R. Hirst, B. Escuder, J. F. Miravet and D. K. Smith, *Angew. Chem., Int. Ed.*, 2008, **47**, 8002; (b) S. S. Babu, S. Prasanthkumar and A. Ajayaghosh, *Angew. Chem., Int. Ed.*, 2012, **51**, 1766.
 - 5 For recent examples of stimuli-responsive LMWG gels: (a) A. Dawn, T. Shiraki, H. Ichikawa, A. Takada, Y. Tahakashi, Y. Tsuchiya, L. T. N. Lien and S. Shinkai, *J. Am. Chem. Soc.*, 2012, **134**, 2161; (b) Y. Ohseido, M. Miyamoto, A. Tanaka and H. Watanabe, *New J. Chem.*, 2013, **37**, 2874; (c) H. Yu, Y. Liu, X. Chen, K. Liu and Y. Fang, *Soft Matter*, 2014, **10**, 9159; (d) P. Duan, N. Yanai, H. Nagatomi and N. Kimizuka, *J. Am. Chem. Soc.*, 2015, **137**(770), 1887; (e) A. Maity, F. Ali, H. Agarwalla, B. Anothumakkool and A. Das, *Chem. Commun.*, 2015, **51**, 2130; (f) J. Schiller, J. V. Alegre-Requena, E. Marqups-Léóez, R. P. Herrera, J. Casanovas, C. Alemán and D. D. Díaz, *Soft Matter*, 2016, **12**, 4361; (g) V. K. Pandey, M. K. Dixit, S. Manneville, C. Bucher and M. Dubey, *J. Mater. Chem. A*, 2017, **5**, 6211.
 - 6 For recent examples of thixotropic LMWG gels: (a) N. Yan, Z.-Y. Xu, K. K. Diehn, S. R. Raghavan, Y. Fang and R. G. Weiss, *Langmuir*, 2013, **29**, 793; (b) A. Baral, S. Roy, A. Dehsorkhi, I. W. Hamley, S. Mohapatra, S. Ghosh and A. Banerjee, *Langmuir*, 2014, **30**, 929; (c) M. Suzuki, Y. Hayakawa and K. Hanabusa, *Gels*, 2015, **1**, 81; (d) V. Ajay Mallia and R. G. Weiss, *Soft Matter*, 2016, **12**, 3665; (e) B. K. Srivastava and M. K. Manheri, *Chem. Commun.*, 2017, **53**, 4485; (f) N. Zanna, S. Focaroli, A. Merlettini, L. Gentilucci, G. Teti, M. Falconi and C. Tomasini, *ACS Omega*, 2017, **2**, 2374.
 - 7 (a) J. Fuhrhop, P. Schnieder, J. Rosenberg and E. Boekema, *J. Am. Chem. Soc.*, 1987, **109**, 3387; (b) J. Fuhrhop, P. Schnieder, E. Boekema and W. Helfrich, *J. Am. Chem. Soc.*, 1988, **110**, 2861; (c) J. Fuhrhop and C. Boettcher, *J. Am. Chem. Soc.*, 1990, **112**, 1768; (d) J. Fuhrhop, S. Svenson, C. Boettcher, E. Rössler and H. Vieth, *J. Am. Chem. Soc.*, 1990, **112**, 4307.
 - 8 T. Shimizu, M. Masuda and H. Minamikawa, *Chem. Rev.*, 2005, **105**, 1401.
 - 9 L. E. Buerkle, R. Galleguillos and S. J. Rowan, *Soft Matter*, 2011, **7**, 6984.
 - 10 S. Sun and P. Wu, *Soft Matter*, 2011, **7**, 6451.
 - 11 C. J. Capicciotti, M. Leclère, F. A. Perras, D. L. Bryce, H. Paulin, J. Harden, Y. Liu and R. N. Ben, *Chem. Sci.*, 2012, **3**, 1408.
 - 12 (a) Y. Ohseido, M. Miyamoto, H. Watanabe, M. Oono and A. Tanaka, *Bull. Chem. Soc. Jpn.*, 2013, **86**, 671; (b) Y. Ohseido, H. Watanabe, M. Oono and A. Tanaka, *Chem. Lett.*, 2013, **42**, 363; (c) Y. Ohseido, H. Watanabe, M. Oono and A. Tanaka, *RSC Adv.*, 2013, **3**, 5803; (d) Y. Ohseido, M. Oono, A. Tanaka and H. Watanabe, *New J. Chem.*, 2013, **37**, 2250; (e) Y. Ohseido, M. Taniguchi, M. Oono, K. Saruhashi and H. Watanabe, *RSC Adv.*, 2014, **4**, 35484; (f) Y. Ohseido, M. Oono, K. Saruhashi and H. Watanabe, *RSC Adv.*, 2014, **4**, 43560; (g) Y. Ohseido, M. Oono, K. Saruhashi, H. Watanabe and N. Miyamoto, *RSC Adv.*, 2014, **4**, 44837; (h) Y. Ohseido, M. Taniguchi, M. Oono, K. Saruhashi and H. Watanabe, *New J. Chem.*, 2015, **39**, 6482.
 - 13 For reviews of multi-component LMWG gels: (a) A. R. Hirst and D. K. Smith, *Chem.-Eur. J.*, 2005, **11**, 5496; (b) C. A. Dreiss, *Soft Matter*, 2007, **3**, 956; (c) L. E. Buerkle and S. J. Rowan, *Chem. Soc. Rev.*, 2012, **41**, 6089; (d) D. J. Cornwell and D. K. Smith, *Mater. Horiz.*, 2015, **2**, 279; (e) J. Raeburn and D. J. Adams, *Chem. Commun.*, 2015, **51**, 5170.
 - 14 For recent examples of multi-component LMWG gels: (a) W. Edwards and D. K. Smith, *J. Am. Chem. Soc.*, 2014, **136**, 1116; (b) E. R. Draper, E. G. B. Eden, T. O. McDonald and D. J. Adams, *Nat. Commun.*, 2015, **7**, 848; (c) E. Ressouche, S. Pensec, B. Isare, G. Ducouret and L. Bouteiller, *ACS Macro Lett.*, 2016, **5**, 244; (d) S. Onogi, H. Shigemitsu, T. Yoshii, T. Tanida, M. Ikeda, R. Kubota and I. Hamachi, *Nat. Chem.*, 2016, **8**, 743; (e) K. Hanabusa, T. Ueda, S. Takata and M. Suzuki, *Chem.-Eur. J.*, 2016, **22**, 16939; (f) E. R. Draper, M. Wallace, R. Schweins, R. J. Poole and D. J. Adams, *Langmuir*, 2017, **33**, 2387.
 - 15 For *N*-alkylamido-D-glucamine-based hydrogelators: Y. Ohseido, M. Oono, K. Saruhashi and H. Watanabe, *RSC Adv.*, 2014, **4**, 48554.
 - 16 Synthesis of alkylolamino acid: Y. M. Osornio, P. Uebelhart, S. Bosshard, F. Konrad, J. S. Siegel and E. M. Landau, *J. Org. Chem.*, 2012, **77**, 10583.
 - 17 G. M. Kavanagh and S. B. Ross-Murphy, *Prog. Polym. Sci.*, 1998, **23**, 533.
 - 18 Z. Xu, J. Peng, N. Yan, H. Yu, S. Zhang, K. Liu and Y. Fang, *Soft Matter*, 2013, **9**, 1091.
 - 19 J. A. Foster, R. M. Edkins, G. J. Cameron, N. Colgin, K. Fücke, S. Ridgeway, A. G. Crawford, T. B. Marder, A. Beeby, S. L. Cobb and J. W. Steed, *Chem.-Eur. J.*, 2014, **20**, 279.
 - 20 M. Shirakawa, N. Fujita, A. Takada and S. Shinkai, *Chem. Lett.*, 2014, **43**, 1330.
 - 21 In the measurements, there had been observed the oscillations of G' value in recovery process, so they seemed like to have two curves of G' in Fig. 4.

