Self-assembly of supra-amphiphile of azobenzene-galactopyranoside based on dynamic covalent bond and its dual responses

Tian-Nan Wang, Guang Yang, Li-Bin Wu, Guo-Song Chen *

The State Key Laboratory of Molecular Engineering of Polymers and Department of Macromolecular Science, Fudan University, Shanghai 200433, China

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ABSTRACT

In this paper, dynamic covalent bond has been employed to construct supra-amphiphile of carbohydrate for the first time. In neutral environment, the molecule was fabricated by reacting a hydrophobic building block bearing benzoic aldehyde with a hydrophilic building block bearing hydrazine to form a sugar-containing supra-amphiphile based on acylhydrazone bond. The obtained azobenzene-galactopyranoside (Azo-Gal) supra-amphiphile self-assembled to fibrillar structure in water, which showed dual responses to UV light and pH.

1. Introduction

Supra-amphiphiles [1] refer to amphiphiles that are constructed by dynamic covalent bonds [2,3] or non-covalent interactions such as hydrogen bonding, π–π interaction, host-guest interaction and charge transfer interaction [4–6]. Generally, the hydrophilic part and the hydrophobic part can be easily linked together via these dynamic connections, which avoids tedious syntheses. Moreover, the supra-amphiphiles are easier to be manipulated compared with amphiphiles fabricated by covalent bonds due to the dynamic nature of the linkage. They undergo self-assembly and disassembly processes in a controllable way with stimuli-responsive properties [7,8].

Carbohydrates play an important role in a variety of biological processes, such as cell adhesion, proliferation, differentiation, recognition, inflammation and the immune response [9]. They are able to form hydrogen bonds, which makes them become important building blocks in supramolecular chemistry [10]. However, few works have been done to incorporate carbohydrates into supra-amphiphiles [11]. Zhang and co-workers constructed the first example of sugar-containing supra-amphiphile, i.e. supramolecular glycolipid based on host-enhanced charge transfer interaction [12]. However, as far as we know, supra-amphiphile of carbohydrate has not been achieved on the basis of dynamic covalent bond.

Herein, for the first time, construction of azobenzene-galactopyranoside (Azo-Gal) supra-amphiphile based on acylhydrazone dynamic covalent bond formed by the aldehyde of the azobenzene-containing hydrophobic domain (Azo-CHO) and the hydrazine of the sugar moiety (Gal-N2H4) was reported (Fig. 1). Aciylhydrazone bond is stable in neutral or alkaline environment, but it can be hydrolyzed under acidic condition. Besides, azobenzene is utilized as a model hydrophobic moiety since it is a wildly used building block for self-assembly and forms reversible complex with α-CD. The Azo-Gal supra-amphiphile self-assembles into fiber in μm scale, which is featured by light and pH responsiveness.

2. Experimental

2.1. Materials and experiments

Ethyl 3-hydroxypropanoate was purchased from Maya Chemical and used as received. D-Galactose was purchased from Bangcheng Chemical and used as received. Trichloroacetontriile, dichloromethane (DCM) and N,N-dimethylformamide (DMF) were distilled before use. Unless specially mentioned, all other chemicals were purchased from J&K Chemical and used as received. The reactions were monitored and the Rf values were determined using analytical thin-layer chromatography (TLC). The TLC plates were visualized by immersion into 5% sulfuric acid solution in ethanol followed by heating using hot air heater.
Column chromatography was carried out on silica gel (200–300 mesh).

$^1$H NMR and $^{13}$C NMR spectra were recorded on an AVANCE III HD 400 MHz spectrometer. The matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) measurement was performed using a Perspective Biosystem Voyager DE-STR MALDI-TOF MS (Applied Biosystems, Framingham, MA). Transmission electron microscopy (TEM) images were taken on Tecnai G2 operating at 200 kV. Atomic force microscope (AFM) was carried out on a Bruker Multimode VIII SPM equipped with a J scanner. Dynamic light scattering studies (DLS) were conducted using Zetasizer Nano-ZS from Malvern Instruments. UV–vis spectroscopy was recorded in a conventional quartz cell (light path 10 mm) on a Perkin–Elmer Lambda 35 spectrophotometer.

### 2.2. Synthesis

The synthesis details of A1–A3 and G1–G3 are shown in Supporting information.

Synthesis of Azo-CHO: A2 (130 mg, 0.67 mmol), A3 (461 mg, 1 mmol) were added to acetonitrile and the mixture was refluxed overnight. After cooling down to room temperature, the solvent was evaporated under vacuum and the crude product was purified by column chromatography using EtOAc/petroleum ether (1:2) as eluent to yield 0.3 g (42.5%) of A4 as a white solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.39 (dd, 1H, $J = 3.4$, 1.1 Hz), 5.18 (dd, 1H, $J = 10.5$, 7.9 Hz), 5.01 (dd, 1H, $J = 10.5$, 3.4 Hz), 4.52 (dd, 1H, $J = 8.0$ Hz), 4.19–4.10 (m, 5H), 3.96–3.81 (m, 2H), 2.65–2.56 (m, 2H), 2.15 (s, 3H), 2.05 (dd, 1H, $J = 0.8$ Hz), 1.98 (s, 3H), 1.27 (t, 3H, $J = 7.1$ Hz). $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 171.02, 170.39, 170.25, 170.15, 169.46, 101.53, 70.85, 70.65, 68.64, 67.00, 65.54, 61.24, 60.58, 34.83, 20.71, 20.66, 20.58, 14.17.

Synthesis of Gal-N$_2$H$_4$: A mixture of G4 (0.7 g, 1.56 mmol), 85% hydrazine hydrate (0.59 g, 15.7 mmol) in ethanol was stirred at ambient temperature for 1 d until the complete disappearance of the raw material of G4 and appearance of the product (using the TLC to monitor the reaction process). The mixture was concentrated and was purified by column chromatography using water/acetonitrile (1:2) as eluent to yield 0.2 g (48.8%) of Gal-N$_2$H$_4$ as a white solid. $^1$H NMR (400 MHz, D$_2$O): $\delta$ 4.26 (d, 1H, $J = 7.9$ Hz), 4.01 (dt, 1H, $J = 10.8$, 6.0 Hz), 3.85–3.77 (m, 2H), 3.64 (qd, 2H, $J = 11.7$, 6.1 Hz), 3.54 (ddd, 2H, $J = 13.4$, 8.9, 3.8 Hz), 3.37 (dd, 1H, $J = 9.9$, 7.9 Hz), 2.42 (t, 2H, $J = 6.0$ Hz). $^{13}$C NMR (101 MHz, D$_2$O): $\delta$ 172.78, 102.83, 75.10, 72.64, 70.60, 68.57, 65.80, 60.95, 34.36. Maldi-TOF MS: calcd. for C$_{13}$H$_{22}$N$_2$O$_7$: 266.11, found 266.29 (289.29 Na$^+$).

### 2.3. Preparation of Azo-Gal supra-amphiphile

Mixing Azo-CHO and Gal-N$_2$H$_4$ together in the molar ratio of 1:1 in DMSO for 3 days until the complete formation of Azo-Gal and then stored as original solution. Ultrasound or heating accelerates the formation speed.

### 2.4. Self-assembly of Azo-Gal supra-amphiphile

Deionized water (8 mL) was added into the DMSO solution of Azo-Gal (1 mL, 1 mg/mL) dropwise by using a syringe pump at the rate of 20 mm/h under vigorous stirring. Then the solution was dialyzed (MWCO 1000) against deionized water to remove the extra DMSO. Concentration of the solution was fixed at 0.1 mg/mL by adding extra deionized water.

### 3. Results and discussion

#### 3.1. Preparation of the Azo-Gal supra-amphiphile

First, the two new precursors of Azo-Gal, Azo-CHO and Gal-N$_2$H$_4$ were synthesized separately. As shown inScheme 1, Azo-CHO was prepared via four steps. First, 4-hydroxybenzaldehyde reacted with 1,2-dibromoethane in the presence of K$_2$CO$_3$ affording compound A1. Then A1 was treated with dimethylamino hydrochloride in the presence of K$_2$CO$_3$ to afford compound A2. Meanwhile, compound A3 was synthesized by reacting 4-phenylazophenol with 1,12-dibromododecane in the presence of K$_2$CO$_3$. In the end, through the reaction between A2 and A3, the final product Azo-CHO was formed. On the other hand, Gal-N$_2$H$_4$ was synthesized via five steps following the classical glycosylation strategy. First all of the hydroxy groups of galactose were protected by acetyl groups. After selectively deprotecting the acetyl group on the anomeric carbon followed by reacting with trichloroacetonitrile, galactosyl trichloroacetimidate was synthesized. Compound G4 was prepared via glycosylation of ethyl 3-hydroxypropionate with the galactosyl trichloroacetimidate and was transformed to the final product of Gal-N$_2$H$_4$ with hydrazine hydrate in ethanol.

After mixing the synthesized Azo-CHO and Gal-N$_2$H$_4$ in DMSO, the dynamic covalent bond formed, which was confirmed by $^1$H NMR. The spectrum of the mixture of Azo-CHO and Gal-N$_2$H$_4$ in...
equimolar amount showed obvious differences from those of the two compounds themselves (Fig. 2). When the mixing time was increased, intensity of the signal at 9.91 ppm corresponding to the aldehyde group in Azo-CHO decreased gradually. Concomitantly a new peak at 11.2 ppm gradually became stronger, indicating formation of the acylhydrazone bond [13]. After addition of 5.0 μL DCl solution (20 wt% in D₂O), the signal of the aldehyde group at 9.91 ppm appeared again with disappearance of the peak at 11.2 ppm, indicating hydrolyzation of the acylhydrazone bond. Then, upon changing the condition back to basic by adding 2.75 μL NaOD solution (40 wt% in D₂O), the dynamic covalent bond was regenerated with the reappearance of the acylhydrazone peak at 11.2 ppm as well as the disappearance of the aldehyde peak at 9.91 ppm. This result fully supported the formation of acylhydrazone bond and its response to pH. In addition, the acylhydrazone bond formation was also supported by Maldi-TOF MS result (Fig. S1 in Supporting information).

3.2. Self-assembly of the Azo-Gal supra-ampiphile

The amphiphilic Azo-Gal was assembled by selective solvent method. The obtained assemblies were first characterized by TEM and AFM. One dimensional fibrillar structure was observed with their length in micrometer scale and their width in nanometer scale under TEM (Fig. 3a). As shown in the AFM image in Fig. 3b, height of the fibers was found around 150 nm. This result indicates that the fiber was formed by multilayers of the Azo-Gal molecules. Under TEM and AFM, the fibers were found in similar size and morphology with relatively sharp endings, indicating the morphology was quite stable and reproducible. Finally, DLS was performed proving existence of the fibers in aqueous solution (Fig. S2 in Supporting information).

![Fig. 2](image-url)  
**Fig. 2.** 1H NMR spectra (400 MHz, DMSO-d₆) of (a) Azo-CHO, (b) Gal-N₂H₄, (c)–(g) mixtures of (a) and (b) in the molar ratio of 1:1 with an increasing ultrasound time, (h) after addition of DCl to (g), (i) after addition of NaOD to (h).

![Fig. 3](image-url)  
**Fig. 3.** (a) TEM and (b) AFM images of the self-assembled Azo-Gal supra-ampiphile.
3.3. Dual-stimuli responsiveness of the self-assembly

It is known that the trans and cis conformations of azobenzene can be transformed to each other by UV or visible light [14]. Previous works by us and other research groups suggested that the assembly of the azobenzene-containing molecules into nano-objects can be regulated by photo irradiation and vice versa [15–18]. So at first we expected possible morphology transformation of the fiber upon irradiation of UV light. UV–vis spectroscopy was first applied to investigate the photo responsiveness of the Azo-Gal supra-amphiphile (Fig. 4a). Upon UV irradiation at 365 nm for 0.5 h, the absorption band at around 325 nm of Azo-Gal in water decreased remarkably compared to that before irradiation, and concomitantly the band at around 430 nm increased slightly. The absorption band at 325 nm is ascribed to π–π* transition of trans-azobenzene while the 430 nm absorption band is attributed to n–π* transition of the cis one [19]. This result clearly shows the transformation of trans-isomer of the Azo-Gal supra-amphiphile to cis. After exposing the sample to ambient light, gradual increase of the absorption peak at 325 nm as well as decrease of peak at 430 nm were observed, suggesting the transformation of cis-isomer to trans. The above results proved that the reversible photo isomerization ability of azobenzene was remained in Azo-Gal. However, UV irradiation (365 nm, 600 s) did not induce any significant change of the fiber morphology, as shown in Fig. 4b. We suppose that after the assembly formed, the Azo-Gal supra-amphiphile packed quite compactly, thus the photo isomerization of azobenzene group had no obvious effect on the shape of self-assembled aggregates.

α-CD and azobenzene is a well-known host-guest pair in supramolecular chemistry. Only trans-azobenzene can be recognized by α-CD to form a stable complex, while the cis one could not [20,21]. In order to further explore the effect of α-CD on the self-assembly of Azo-Gal, TEM experiment was performed. After addition of α-CD in a molar ratio of 1:1 to Azo-Gal, the fibrillar aggregates disassembled (Fig. 4c). As shown in Fig. S3 in Supporting information, the 3H NMR clearly showed the formation of inclusion complex between α-CD and azobenzene. Before adding α-CD, the hydrophobic azobenzene group was in the core of the Azo-Gal assemblies, thus the signals of azobenzene group could hardly been observed. However, after the addition of α-CD in equal amount, signals of azobenzene group appeared as a result of the inclusion between α-CD and azobenzene and the disassembly of the self-assembled aggregates, which was consistent with literature [20]. Thus the decrease of hydrophobicity of the amphiphilic Azo-Gal supra-amphiphile after addition of α-CD induced the dissociation of the fiber. Moreover, when the above system was irradiated by UV light, it was found that the fibrillar structures formed again due to the dissociation of the α-CD/azobenzene complex (Fig. 4d).

As above described, the dynamic covalent bond used here is pH-responsive, which can be fabricated under neutral or alkaline conditions and hydrolyzed in acidic environment. To explore the pH responsiveness of the Azo-Gal assemblies, TEM and DLS experiments were carried out. When changing the self-assembled Azo-Gal solution to acidic condition (2 mL Azo-Gal solution, 20 μL 20 wt% HCl solution), the fibers transformed into nanospheres as a result of the hydrolysis of the acylhydrazone bond (Fig. 5a). Since the Gal-N2H4 has very good water solubility and cannot form aggregates in water itself, the corresponding spherical aggregates was formed by Azo-CHO. When adjusting the solution back to slightly alkaline (2 mL Azo-Gal solution, 10.92 μL 40 wt% NaOH solution), the morphology returned to fiber again because of the formation of the dynamic covalent bond (Fig. 5b). DLS results were in consistent with the observations under TEM (Fig. 5c).

![Fig. 4](image-url) (a) UV–vis spectra of Azo-Gal before and after 0.5 h irradiation with UV light (365 nm) as well as the time-dependent interconversion of cis- to trans-Azo-Gal under ambient light. The concentration of Azo-Gal is 0.05 mg/mL. TEM images of Azo-Gal: (b) (c) after UV irradiation with UV light (365 nm); (c) mixed with α-CD (1:1 molar ratio); (d) after irradiation with UV light (365 nm).

![Fig. 5](image-url) TEM images of Azo-Gal aggregates (a) in acidic environment and (b) then changing back to slightly alkaline environment; (c) DLS of Azo-Gal supra-amphiphile assemblies (blue), after adding HCl (red) and then after adding NaOH (black).
4. Conclusion

In summary, we have successfully constructed a dual-responsive Azo-Gal supra-amphiphile on the basis of acylyhydrazone dynamic covalent bond in water. The amphiphilic Azo-Gal self-assembled into fibers that can be regulated by addition of α-CD and pH (Scheme 2). This dual-responsive Azo-Gal supra-amphiphile will enrich the family of supra-amphiphiles and have potential applications in biomedical fields, such as drug delivery and controlled release.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2016.05.009.

References
