Fluorescence quenching of triazatruxene-based glycocluster induced by peanut agglutinin lectin

Ke-Rang Wang a,b,*, Hong-Wei An a,b, Dan Han a,b, Feng Qian a,b, Xiao-Liu Li a,b,*

1. Introduction

Carbohydrate–protein interactions play important roles in a wide variety of biological processes, for instance, cell growth regulation, adhesion, cancer cell metastasis, cellular trafficking, differentiation and infection by pathogens such as protein toxins and viruses, and the immune response [1–3]. However, monosaccharide–protein interactions are often extremely weak [4,5]. In order to enhance the binding efficiency in biological systems, synthetic glycoclusters as multivalent model systems have been employed to investigate the carbohydrate–protein interacting events [6–8]. Among them, fluorescent glycoclusters possessing both a reporting group (fluorescent dye) and a combination-recognition moiety (carbohydrate ligands) are of great importance in the studies of carbohydrate–protein interactions and biosensor applications because of their intrinsic fluorescence properties, high sensitivity to minor external stimuli, and good biocompatibility [9–12].

In particular, quenching the fluorescence of multivalent glycoclusters upon binding to lectins has been frequently studied [9–12]. For example, Bunz and coworkers synthesized a mannose-substituted poly(para-phenylene) unit which showed a fluorescence quenching effect upon interacting with Concanavalin A (Con A) [13]. Liu and coworkers reported that water-soluble poly(p-phenylene)s bearing mannoses, exhibited the quenching of its fluorescence upon binding with Con A [14]. Very recently, we reported multivalent glycoclusters based on chiral self-assemblies constructed by lactose- and mannose-functionalized perylene bisimide derivatives [15,16], multivalent glycodendrimer based on 18 peripheral mannose-functionalized perylene bisimides [17], and a triazatruxene-based glycocluster as fluorescence enhancers to sense Con A [18]. In this paper, we report a novel lactose-functionalized triazatruxene derivative, which exhibited fluorescence quenching properties upon binding to peanut agglutinin (PNA) lectin.

2. Experimental

2.1. Instruments

1H NMR and 13C NMR spectra were recorded on a Bruker 600 spectrometer. HRMS was performed on an ABI 4700 Proteomics Analyzer. Turbidity assay of the ligand–protein binding was measured on a Shimadzu UV-3600 spectrophotometer with quartz micro-cuvette (120 μL volume, 1 cm path length). Fluorescence spectra were collected on an F-7000. Circular dichroism spectra were recorded on an MOS-450.

2.2. Synthesis

**Compound 3:** Compound 1 (170 mg, 0.2 mmol) and compound 2 (635 mg, 0.9 mmol) were dissolved in THF (35 mL), and an
aqueous solution of CuSO$_4$•5H$_2$O (4.1 mg, 0.2 mmol) and sodium ascorbate (6.0 mg, 0.3 mmol) were added. The mixture was stirred for 5 h at 50 °C. The solvent was evaporated under vacuum. The residue was purified by silica-gel column chromatography using CH$_2$Cl$_2$/CH$_3$OH (30/1, v/v) as the eluent to give the product as a white solid (500 mg) with yield of 84%. Mp 101.3–102.1 °C; $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 1.96 (s, 9H, –COCH$_3$), 1.98 (s, 9H, –COCH$_3$), 2.03 (s, 9H, –COCH$_3$), 2.05 (s, 9H, –COCH$_3$), 2.06 (s, 9H, –COCH$_3$), 2.13 (s, 9H, –COCH$_3$), 2.16 (s, 9H, –COCH$_3$), 2.90 (s, 3H), 2.97 (s, 3H), 3.54 (m, 18H), 3.61 (m, 9H), 3.77 (t, 3H, $J$ = 9.6 Hz), 3.87 (t, 6H, $J$ = 6.0 Hz), 3.99 (t, 6H, $J$ = 6.0 Hz), 4.08 (m, 6H), 4.15 (m, 6H), 4.42–4.52 (m, 12H), 4.60 (s, 6H), 4.89 (dd, 3H, $J$ = 8.4 Hz, 9.6 Hz), 4.97 (dd, 3H, $J$ = 3.6 Hz, 10.2 Hz), 5.10 (dd, 3H, $J$ = 7.8 Hz, 10.2 Hz), 5.14–5.20 (m, 9H), 5.35 (d, 3H, $J$ = 2.4 Hz), 6.68 (t, 3H, $J$ = 7.2 Hz, Ar–H), 7.45 (t, 3H, $J$ = 7.2 Hz, Ar–H); 13C NMR (150 MHz, CDCl$_3$): $\delta$ 24.9 (m, 18H), 30.2 (m, 9H), 37.7 (m, 9H), 46.27, 49.89, 60.76, 61.74, 64.46, 66.60, 67.76, 69.12, 69.57, 69.72, 70.46, 70.70, 70.82, 70.95, 71.31, 72.48, 72.85, 76.06, 100.36, 101.05, 103.47, 110.70, 120.29, 121.67, 123.09, 123.16, 123.71, 138.80, 141.23, 144.96, 169.01, 169.57, 169.59, 170.02, 170.10, 170.26, 170.29; HRMS (MALDI-TOF): Calcd. for C$_{135}$H$_{174}$N$_{12}$O$_{63}$: 2972.0814, found: 2972.0264.

Compound 4: Compound 3 (297 mg, 0.1 mmol) was dissolved in MeOH (20 mL), a solution of sodium hydroxide (2.0 mL, 8 mmol) was added, and the reaction mixture was stirred at room temperature until the disappearance of the starting material. The mixture was neutralized with aqueous hydrochloric acid (2 mol/L). The mixture was filtered and washed with MeOH. The solid was dissolved in water, the solution was put in a standard alkaline solution of CuSO$_4$•5H$_2$O (4.1 mg, 0.2 mmol) and sodium ascorbate (6.0 mg, 0.3 mmol) were added. The mixture was stirred for 5 h at 50 °C. The solvent was evaporated under vacuum. The residue was purified by silica-gel column chromatography using CH$_2$Cl$_2$/CH$_3$OH (30/1, v/v) as the eluent to give the product as a white solid (500 mg) with yield of 84%. Mp 101.3–102.1 °C; $^1$H NMR (600 MHz, DMSO-d$_6$): $\delta$ 1.96 (s, 9H, –COCH$_3$), 1.98 (s, 9H, –COCH$_3$), 2.03 (s, 9H, –COCH$_3$), 2.05 (s, 9H, –COCH$_3$), 2.06 (s, 9H, –COCH$_3$), 2.13 (s, 9H, –COCH$_3$), 2.16 (s, 9H, –COCH$_3$), 2.90 (s, 3H), 2.97 (s, 3H), 3.54 (m, 18H), 3.61 (m, 9H), 3.77 (t, 3H, $J$ = 9.6 Hz), 3.87 (t, 6H, $J$ = 6.0 Hz), 3.99 (t, 6H, $J$ = 6.0 Hz), 4.08 (m, 6H), 4.15 (m, 6H), 4.42–4.52 (m, 12H), 4.60 (s, 6H), 4.89 (dd, 3H, $J$ = 8.4 Hz, 9.6 Hz), 4.97 (dd, 3H, $J$ = 3.6 Hz, 10.2 Hz), 5.10 (dd, 3H, $J$ = 7.8 Hz, 10.2 Hz), 5.14–5.20 (m, 9H), 5.35 (d, 3H, $J$ = 2.4 Hz), 7.35 (t, 3H, $J$ = 7.2 Hz, Ar–H), 7.45 (t, 3H, $J$ = 7.2 Hz, Ar–H), 7.53 (s, 3H, Triaz-H), 7.75 (d, 3H, $J$ = 7.8 Hz, Ar–H), 8.33 (d, 3H, $J$ = 7.8 Hz, Ar–H); 13C NMR (150 MHz, CDCl$_3$): $\delta$ 20.49, 20.56, 20.61, 20.74, 20.83, 46.27, 49.89, 60.76, 61.74, 64.46, 66.60, 67.76, 69.12, 69.57, 69.72, 70.46, 70.70, 70.82, 70.95, 71.31, 72.48, 72.85, 76.06, 100.36, 101.05, 103.47, 110.70, 120.29, 121.67, 123.09, 123.16, 123.71, 138.80, 141.23, 144.96, 169.01, 169.57, 169.59, 170.02, 170.10, 170.26, 170.29; HRMS (MALDI-TOF): Calcd. for C$_{135}$H$_{174}$N$_{12}$O$_{63}$: 2972.0814, found: 2972.0264.

3. Results and discussion

As shown in Scheme 1, the lactose-modified triazatruxene-glycocluster 4 was synthesized by a “click” reaction of propargylated triethyleneglycol derivative 1 [18] with 2-azide hepta-O-acetyl-β-D-lactoside 2 [19] in the presence of substoichiometric amount of CuSO$_4$ and sodium ascorbate in homogeneous THF/water to afford the fully substituted star-shaped triazatruxene derivative 3 in 85% yield. And then, deprotection of the acetyl groups in mannosylated analog 3 under standard conditions (NaOH, MeOH/H$_2$O) furnished the triazatruxene-based glyocluster 4 in 90% yield. These compounds were characterized by NMR and HRMS analyses. Benefiting from the grafts of lactose, compound 4 possesses good water-solubility.

The binding effects of the multivalent lactose-modified triazatruxene 4 with PNA lectin were studied by fluorescence spectroscopy. It is well known that PNA lectin selectively binds to mannose residues [20]. As shown in Fig. 1, a progressive decrease of the fluorescence emission intensity at 393.6 nm was observed as the PNA concentration increased, but with no obvious shift of the emission maximum. The observed fluorescence quenching of compound 4 was mainly due to a static quenching process, in which the PNA lectin and compound 4 formed a ground state complex. Once generated, the excited-state was efficiently quenched after excitation [21,22]. The Stern–Volmer relationship offers a simple way to extract the binding constant. From these spectral data, the Stern–Volmer quenching constant of compound 4 binding with PNA was calculated as 5.8 × 10$^5$ mol$^{-1}$ L by fitting the experimental data of the maximum fluorescence changes (Fig. 1 inset).
Furthermore, the specific and selective binding interactions of compound 4 with various proteins were investigated by fluorescence spectroscopy (Fig. 2). Upon addition of 5 equiv. of PNA, the fluorescence of compound 4 was quenched by about 73%. However, titration of Con A and bovine serum albumin (BSA) into the solution of compound 4 produced no significant change of the fluorescence. These results clearly indicated that the lactose functionalized triazatruxene-glycocluster 4 selectively binded to PNA, resulting in the quenching of the fluorescence.

Circular dichroism (CD) spectroscopy of PNA was used to elucidate the secondary structural changes that take place upon binding with compound 4. The spectrum for PNA showed a single negative band at about 225 nm (Fig. 3), which is a hallmark for β-sheet rich proteins [23]. Upon adding compound 4, the intensity of the CD signal showed a progressive decrease, indicating the loss of the secondary structure because of the formation of cross-linked complexes [24,25]. And the CD signal clearly showed bathochromical shifts, which indicated that there was a significant disturbance in the PNA conformation [23].

A turbidity assay was used to monitor the formation of cross-linked complexes in real time [26]. Upon adding compound 4, the turbidity showed an immediate increase (Fig. 4), indicating potential carbohydrate-protein binding interactions. In order to demonstrate that the observed turbidity increase is due to carbohydrate-protein interactions, a large excess of lactose was added to inhibit glycocluster–PNA binding. With the addition of lactose, a substantial decrease in turbidity was observed (Fig. 4). The disappearance of turbidity clearly indicated that the observed increase in optical density was a result of carbohydrate–protein interactions [27,28]. In addition, as the control experiments, the turbidity of the mixture of 4 with Con A and BSA exhibited no significant changes. This observation suggests that no binding to Con A or BSA occurred and is consistent with the results from the fluorescent experiments (Fig. 2). These results showed that the triazatruxene-based glycocluster exhibited specific and selective binding properties with PNA.
4. Conclusion

A novel triazatruxene-based fluorescent glyocluster 4 was designed and synthesized, and its structure was fully characterized by NMR and HRMS analyses. Benefiting from the grafts of lactose moieties, compound 4 exhibited specific and selective binding with PNA, which were investigated by fluorescence spectroscopy, CD spectroscopy, and a turbidity assay. A concentration-dependent quenching of the fluorescence upon binding to PNA was found, with a Stern–Volmer quenching constant of $5.8 \times 10^{2}$ mol$^{-1}$ L.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 21002020 and 21172051) and the Hebei Natural Science Foundation (Nos. B2011201052 and B2012201041).

References
