Published in final edited form as:

Chem Commun (Camb). 2012 September 21; 48(73): 9138–9140. doi:10.1039/c2cc00072e.

Increasing Materials' Response to Two-photon NIR Light via Self-immolative Dendritic Scaffolds

Nadezda Fomina^a, Cathryn L. McFearin^a, and Adah Almutairi^{a,b,c,d,*}

^aSkaggs School Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, California 92093, United States

^bDepartment of NanoEngineering, University of California at San Diego, La Jolla, California 92093, United States

^cMaterials Science and Engineering, University of California at San Diego, La Jolla, California 92093, United States

^dBiomedical Sciences Programs, University of California at San Diego, La Jolla, California 92093, United States

Abstract

Photoactivation using two photons of NIR allows non-invasive biological manipulation. We applied the principle of dendritic amplification to improve materials' sensitivity to NIR light. Light induced uncaging or release of *L*-glutamic acid was 2.8 fold higher when incorporating 4-bromo-7-hydroxycoumarin (Bhc) with self-immolative dendrimers compared with Bhc directly conjugated to *L*-glutamic acid.

Photoactivation using near infrared (NIR) light has been recognised as an attractive new avenue for non-invasive delivery of bioactive molecules to target sites.¹ Two-photon excitation and uncaging provides excellent spatial and temporal resolution for this method. However, the development of new two-photon sensitive organic materials with sufficiently high two-photon uncaging action cross-sections is still a challenge. Despite a large amount of research dedicated to improving the two-photon action cross section of organic protecting groups, the progress has been rather slow due to the inability to predict the cross-sections for two-photon excitation and the quantum yields of uncaging based on the chromophore structure.² The first caging group generally applicable for two-photon uncaging of biologically important molecules with an action cross section of 1 GM at 740 nm, was 4-bromo-7-hydroxycoumarin (Bhc).³ The largest two-photon uncaging action cross-section reported to date is 10 GM,⁴; however, this caging group is obtained through multi-step synthesis and its cytotoxicity has not been evaluated. Therefore the field has achieved a 10 fold enhancement in the action cross-section of biologically relevant two-photon uncaging groups over a thirteen year period.

Although more efficient two-photon caging groups are being developed, alternative strategies of increasing sensitivity to two-photon NIR would be attractive. One way to increase two photon sensitivity that has never been done before is to design a self-immolative^{5–7} dendrimer that translates a single triggering event into a cascade of molecular rearrangements to release multiple terminal groups. Self-immolative dendritic structures have been applied to create stimuli-responsive prodrugs^{8,9} and in analytical assays^{10–12}.

aalmutairi@ucsd.edu. Tel.: 18 58 822 2942. Fax: 18 58 200 3815.

[†]Electronic Supplementary Information (ESI) available: experimental details. See DOI: 10.1039/b000000x/

Here we demonstrate that the principle of dendritic amplification may be applied to create materials with improved sensitivity to two-photon NIR light. Conjugation of the known and well-studied two-photon caging group, 4-bromo-7-hydroxycoumarin, to a self-immolative quinone-methide based dendrimer results in a 3-fold higher amount of uncaged *L*-glutamic acid (LGA) compared to Bhc directly conjugated to LGA.

The structures of the zero (**G0**), first (**G1**), and second (**G2**) generation quinone-methide based dendrimers incorporating the Bhc photo triggering group and 1, 2, and 4 caged molecules of LGA, respectively, are shown in Figure 1. LGA was chosen as a model small bioactive molecule commonly used in two-photon uncaging studies.^{13–16} Compound **G0** was used as a reference, since it has been shown to release LGA upon two-photon excitation.¹⁶ We anticipated that the branched structure of **G1** and **G2** allows for more incorporation of caged molecules and should result in 2- and 4-fold enhanced release of LGA relative to **G0** upon a given dose of NIR stimulation.

The synthesis of the dendrimers is outlined in Scheme 1. The zero generation dendrimer was synthesised by reacting compound **1**¹⁷ with LGA dimethyl ester hydrochloride, followed by sequential removal of the methoxymethyl and methyl esters by acid- and base-catalysed hydrolysis, respectively. The **G1** and **G2** dendrimers were synthesised by a convergent route: compound **3**¹⁸ was reacted with LGA dimethyl ester hydrochloride to afford intermediate **4**. After removal of the BOC protecting group, reacting **4** with **1** installed the triggering group. Finally, sequential deprotection of **5** afforded **G1**. Similarly, **G2** was obtained by reacting **4** with **3** followed by installation of Bhc photo triggering group.

Cleavage of Bhc photo triggering groups by NIR light was measured by HPLC. One mM solutions of the dendrimers in PBS pH 7.4 were irradiated at 740 nm for 30 min. The irradiated solutions were injected into HPLC with 4-hydroxy-benzoic acid-*n*-hexyl ester as an internal standard and chromatograms were recorded at 280 nm. The fraction of the cleaved triggering groups was calculated by integrating the peaks of the dendrimers relative to the peak of the internal standard before and after NIR exposure. As expected, photo triggered removal of Bhc group was of similar efficiency across the dendrimer series and varied between 47 and 53% (Table 1). The rearrangement of the quinone-methide dendrimers upon this phototrigger was followed by HPLC-MS (Figure 2).

Irradiated solutions were incubated at 37°C and aliquots were removed periodically to determine the concentrations of the intermediates (Figure 2, **IM 1** for **G1** and **IM 21** and **IM 22** for **G2**) by integrating the peaks of the single ions, $m/z = 628$ (for **IM 1** and **IM 22**, $z = 1$) and $m/z = 795$ (for **IM 22**, $z = 2$), respectively. The signal from **IM 1** gradually decreases within 96 hours, consistent with the previously published degradation kinetics of quinone-methide dendrimers,¹⁹ while **IM 21** remains in solution for 120 hours (Figure 2). The build-up of **IM 22** in solution over time and its subsequent disappearance after 196 hrs confirms the end-to-end degradation of **G2**. The total efficiencies of **G1** and **G2** degradation were calculated to be 99% and 84.4% after 96 and 196 hours, respectively. The increase in **IM 1** and **IM 22** over time in non-irradiated **G1** and **G2** solutions results from partial degradation via dark hydrolysis and is taken into account when calculating the total efficiencies of **G1** and **G2** degradation. Based on this result, the expected amplification of the amount of uncaged LGA was 1.98 for **G1** and 3.37 for **G2**.

The release of caged LGA was quantified using an Amplex Red enzymatic assay. After irradiation with NIR light for 30 min, the 1 mM solutions of **G0**, **G1**, and **G2** were diluted with PBS (pH 7.4) to 7, 3.5 and 1.75 μ M, respectively, to ensure that the final concentration of caged LGA was the same across the series. The solutions were incubated at 37°C and aliquots were taken out at the specified time periods and analysed for LGA (Figure 3). **G0**

released 31 μg of LGA upon irradiation and this served as the benchmark to determine the amplification factors for **G1** and **G2**. The kinetics of release by **G1** and **G2** were consistent with the degradation profiles of the intermediates (Figure 2). Thus, **G1** released 51 μg of the caged acid over 96 hrs, while **G2** showed release within 196 hrs (Table 1) with an induction period of 48 hrs, yielding 88 μg of LGA. The control solutions also showed some release of LGA after prolonged incubation (over 100 hrs), pointing to non-specific hydrolysis.

The results collected via the Amplex Red assay reveal that, indeed, the amount of LGA released after 30 min of exposure to NIR light is 1.63-fold higher for **G1** and 2.80-fold higher for **G2**, compared to **G0**, demonstrating that chemical amplification through self-immolative constructs is a useful strategy to increase the materials' response to NIR light. However, the amplification is slightly lower than the expected values of 1.98 and 3.37. We investigated whether the byproducts of the dendrimers' degradation, namely, 4-bromo-7-hydroxycoumarin, 2,6-bis-(hydroxymethyl)-*p*-cresol and 1,3-dimethyl-2-imidazolidinone,^{6, 17} interfered with the enzymatic assay and ruled that out as a cause (Figure S1). Because photobleaching is enhanced in the two-photon regime compared to single photon excitation²⁰, one possible explanation for the lower than expected amplification is photochemical side reactions that occur upon exposure to NIR light and consume the starting material but do not lead to the desired liberation of the intermediates **IM1** and **IM 21**. Appearance of side products was observed by HPLC, but their structures could not be deduced.

Conclusions

This work demonstrates that the principle of dendritic amplification may be used to increase the responsiveness of materials to two-photon excitation using well-studied triggering groups. The results obtained with release of the small molecule LGA should be translatable to uncaging of macromolecules or even to the nano scale. It is important to note that for higher generation dendrimers the overall degradation process is slower because rearrangement reactions occur in series. This is a useful strategy for increasing overall biological response to light and achieving sustained release. For the uncaging of neurotransmitters, however, designing self-immolative dendrimers with faster kinetics of disassembly is of critical importance. Our current efforts are focused on incorporating these dendritic amplifiers into hydrogel nanocarriers that can be remotely activated to release their cargo upon exposure to NIR light.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the NIH Directors New Innovator Award (1 DP2 OD006499-01) and King Abdul Aziz City of Science and Technology (KACST) for financially supporting this study.

Notes and references

1. Raghavachari, R., editor. Near-Infrared Applications in Biotechnology. New York: 2001.
2. Gagey N, Neveu P, Benbrahim C, Goetz B, Aujard I, Baudin JB, Jullien L. J Am Chem Soc. 2007; 129:9986–9998. [PubMed: 17658803]
3. Furuta T, Wang SSH, Dantzker JL, Dore TM, Bybee WJ, Callaway EM, Denk W, Tsien RY. Proc Natl Acad Sci U S A. 1999; 96:1193–1200. [PubMed: 9990000]
4. Donato L, Mourrot A, Davenport CM, Herbivo C, Warther D, Léonard J, Bolze F, Nicoud J-F, Kramer RH, Goeldner M, Specht A. Angew Chem Int Ed. 2012;1002/anie.201106559

5. Szalai ML, Kevwitch RM, McGrath DV. J Am Chem Soc. 2003; 125:15688–15689. [PubMed: 14677927]
6. Amir RJ, Pessah N, Shamis M, Shabat D. Angew Chem Int Ed. 2003; 42:4494–4499.
7. de Groot FMH, Albrecht C, Koekkoek R, Beusker PH, Scheeren HW. Angew Chem Int Ed. 2003; 42:4490–4494.
8. Shamis M, Lode HN, Shabat D. J Am Chem Soc. 2004; 126:1726–1731. [PubMed: 14871103]
9. Sagi A, Segal E, Satchi-Fainaro R, Shabat D. Bioorg Med Chem. 2007; 15:3720–3727. [PubMed: 17416532]
10. Sella, E.; Shabat, D. Chem Commun (Cambridge, U K). 2008. p. 5701-5703.
11. Sella E, Shabat D. J Am Chem Soc. 2009; 131:9934. [PubMed: 19569684]
12. Avital-Shmilovici M, Shabat D. Bioorg Med Chem. 2010; 18:3643–3647. [PubMed: 20231097]
13. Bolze F, Gug S, Specht A, Bourgogne C, Goeldner M, Nicoud JF. Angew Chem Int Ed. 2008; 47:9525–9529.
14. Pettit DL, Wang SSH, Gee KR, Augustine GJ. Neuron. 1997; 19:465–471. [PubMed: 9331338]
15. Kantevari S, Matsuzaki M, Kanemoto Y, Kasai H, Ellis-Davies GCR. Nat Meth. 2010; 7:123–125.
16. Furuta T, Wang SSH, Dantzker JL, Dore TM, Bybee WJ, Callaway EM, Denk W, Tsien RY. Proc Natl Acad Sci U S A. 1999; 96:1193–1200. [PubMed: 9990000]
17. Fomina N, McFearn CL, Sermakdi M, Morachis JM, Almutairi A. Macromolecules. 2011; 44:8590–8597. [PubMed: 22096258]
18. Fomina N, McFearn C, Sermakdi M, Edigin O, Almutairi A. J Am Chem Soc. 2010; 132:9540–9542. [PubMed: 20568765]
19. Amir RJ, Danieli E, Shabat D. Chem--Eur J. 2007; 13:812–821. [PubMed: 17075925]
20. Patterson GH, Piston DW. Biophys J. 2000; 78:2159–2162. [PubMed: 10733993]

Figure 1.
Structures of the dendrimers bearing a coumarin photo trigger (blue) and caged LGA (red).

Figure 2. Disassembly of the intermediates generated by cleavage of Bhc with NIR light monitored by HPLC-MS. **A, G1, B-C, G2, D**, structures of the intermediates, **E**, schematic of dendrimer disassembly.

Figure 3.
Release of LGA from **G0**, **G1** and **G2** after NIR exposure.

Scheme 1.
Synthesis of **G0**, **G1** and **G2** dendrimers.

Table 1Exposure of **G0**, **G1** and **G2** to NIR light for 30 min.

Generation	% Photo triggered ^a	L-GA released μg ^b	Expected amplification factor	Experimental amplification factor
G0	47.6 (± 6)	31.1 (± 7)	1	1
G1	52.9 (± 2)	50.8 (± 5)	1.98	1.63
G2	46.7 (± 3)	87.0 (± 14)	3.37	2.80

^aDetermined by HPLC relative to an internal standard.^bThe values obtained by Amplex Red assay after 72 hrs (**G0**), 96 hrs (**G1**) and 192 hrs (**G2**) and corrected for controls.^cThe numbers were calculated from graphs in Figure 2 and corrected for controls.