Disassembly of Dendritic Micellar Containers Due to Protein Binding

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · MARCH 2010

Impact Factor: 12.11 · DOI: 10.1021/ja100746d · Source: PubMed

3 AUTHORS, INCLUDING:

Malar Azagarsamy
University of Colorado at Boulder
29 PUBLICATIONS **535** CITATIONS
SEE PROFILE

Volkan Yesilyurt
Massachusetts Institute of Technology
0 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Published in final edited form as:
J Am Chem Soc. 2010 April 7; 132(13): 4550–4551. doi:10.1021/ja100746d.

Disassembly of Dendritic Micellar Containers due to Protein Binding

Malar A. Azagarsamy, **Volkan Yesilyurt**, and **S. Thayumanavan***
Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

A promising approach to develop promising drug delivery vehicles is to harness nano-scale materials with stimuli responsive properties, as these have the potential to enhance drug efficacy and mitigate side effects. Temperature, pH, light and magnetic field are among the most frequently used stimuli in these approaches.[1] Utilizing biological stimuli such as proteins for this purpose has been geared up recently,[2] taking advantage of the overexpression of certain proteins at diseased cell sites. While these recent reports are mainly based on enzymatic actions of proteins, molecular systems that respond to non-enzymatic proteins are underexplored and are of great significance.[3] For example, responsive assemblies based on receptor-ligand binding interactions are of great interest, because: *(i)* these strategies can be applied to a much larger variety of proteins; *(ii)* overexpression of non-enzymatic proteins is highly relevant to a variety of diseases. With this motivation, we report here a dendrimeric system that responds to protein-ligand binding interactions.

The large, yet well-controlled architecture makes dendrimers an interesting class of molecules for a variety of applications.[4] In particular, amphiphilic dendrimers have become extremely attractive, because *(i)* these can sequester hydrophobic guest molecules in an aqueous milieu;[5,6] (*ii*) they exhibit guest encapsulation properties at low critical aggregation concentrations (CACs).[5,6] Recently, we reported on a distinct class of amphiphilic dendrimers in which every repeating unit in the dendritic molecule contains both lipophilic (hydrophobic) and hydrophilic functionalities. Owing to the orthogonal placement of these amphiphilic units, these molecules were able to form micelle-type and inverse micelle-type assemblies in polar and apolar solvents, respectively.[6,2b] Unlike classical amphiphilic dendrimers, our biaryl dendrimers aggregate to form micellar assemblies and this aggregation is primarily responsible for the hydrophobic guest encapsulation properties of these molecules. Hence, we envisioned that the deaggregation of these molecules would cause the micellar assemblies to disassemble, resulting in the release of encapsulated guest molecules. In fact, we have demonstrated such a possibility with enzymatic reactions recently.[2b] To execute the deaggregation through protein-ligand binding interactions, we incorporated a ligand functionality that binds to a specific protein. We hypothesized that dendrimer-protein binding caused by the ligand-receptor interaction would dramatically alter the hydrophilic-lipophilic balance (HLB) of the dendrimer molecule. This is based on the fact that water-soluble globular proteins have hydrophilic surface and the binding event replaces a small ligand functionality with a large hydrophilic protein. We conceived that this HLB change could cause the dendrimer to loose its ability to assemble. It then follows that this disassembly would also affect the release of sequestered guest molecules from the micellar interiors in response to protein binding (Figure 1).

* thai@chem.umass.edu .

Supporting Information Available: Experimental details, NMR, DLS and Fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

To test this hypothesis, we targeted dendrons (**G0-G2**) shown in Chart 1. In these dendrons, the decyl chain acts as the lipophilic unit and pentaethylene glycol (PEG) was chosen as the hydrophilic unit to reduce nonspecific interactions. Biotin, which is well known for its strong interaction with avidin was chosen as the ligand functionality. Biotin was incorporated onto the dendron by "click" chemistry, as this allows for easier future ligand variations.[7] We first studied the micellar properties of the dendrons (**G1-G2**) with pyrene as the spectroscopic probe.[7] The CACs of **G1** and **G2** were found to be 4.5 and 1.2 μM respectively, as typically observed for our amphiphilic dendrimers compared to their small molecule counterparts.[2b] The small molecule **G0** dendron exhibited poor water solubility, presumably due to the lack of the pentaethyleneglycol unit.

Next, we investigated the protein-ligand binding induced guest release from the micellar interiors. For this purpose, we used extravidin, a neutral form of avidin with minimal nonspecific interactions. When a 25 μM solution of **G1** was exposed to increasing concentrations of extravidin for 3h, we observed decrease in the emission intensity of pyrene (Figure 2a), indicating the release of pyrene from the micellar assemblies. To test whether this decrease in pyrene fluorescence is indeed due to the biotin-extravidin interactions, extravidin was added to a solution of our parent dendron **G1-control,** which lacks the biotin functionality (Chart 1).[6b] With this assembly, we did not observe any significant decrease in the fluorescence of pyrene (Figure 2a), supporting our hypothesis that the guest release in **G1** is in fact due to protein-ligand interaction.[7] Furthermore, to find whether the guest release is selective to extravidin, we exposed **G1** to proteins of varying pI values, *viz*., thrombin, pepsin and chymotrypsin (ChT) and observed only a small % of guest release (Figure 2a), probably due to non-specific interactions. This lack of significant guest release provides further evidence that protein-ligand binding is the most likely reason for the guest release in extravidin case.[7] Next, we tested whether a similar guest release and disassembly can also be affected in the **G2** dendron. Exposure of 25 μM **G2** to extravidin resulted in only 30% release as compared to 42% release with **G1**.[7] This is presumably because the higher generation dendron provides a more tightly packed assembly. It also remains that the lower CAC of **G2** allows us to decrease the starting concentration. Indeed, when 5 μM concentration of **G2** was used, a release of about 45% was obtained. Here too, the guest release was found to be selective to extravidin (Figure 2b).[7]

Two features are noteworthy. (*i*) The guest release was entirely concentration dependant. For example, in the case of **G1**, addition of 2 μM extravidin caused only 18% release and no further release was observed. Further release was observed, only upon subsequent addition of extravidin (Figure 2a), indicating the concentration-dependent release characteristics. (*ii*) When we exposed **G1** and **G2** solutions straightaway to 10 μM extravidin, we observed an immediate release of about 30% and 40% in 1h for **G1** and **G2** respectively and an additional 10% release in 5h (Figure 2c). But the direct addition of proteins other than extravidin did not result in any significant release of pyrene.[7] These results once again imply that the guest release is controlled by the concentration of protein and eventually affords almost the same amount of release, regardless of whether protein was added in small portions or all at once. (*iii*) The release observed in these cases is only about 45%. This could be due to the ability of the hydrophobic chains of our monomeric state of the dendrimer to withhold some amount of pyrene.

Finally, it is important to ask whether the guest release is really due to micellar disassembly? In order to address this, we investigated the transformation in the size of these micellar assemblies upon addition of protein using dynamic light scattering (DLS) technique. The initial size of **G1** assembly was found to be around 300 nm. Upon addition of 10 μM extravidin, the size decreased to 10 nm corresponding to size of the protein itself (Figure 2d), while no such decrease in size observed for other proteins.[7] This dramatic decrease in size supports our

hypothesis that the protein binding indeed causes the micellar disassembly, which then results in the guest release. Similar size decrease was also observed with **G2**, which further strengthens our disassembly hypothesis.[7]

In summary, we have demonstrated that dendrimer-based amphiphilic nanocontainers can be disassembled in response to an engineered ligand-receptor interaction. Our working hypothesis for the disassembly is based on the alteration in the HLB caused by the binding event. We have shown that such a disassembly event is selective to the protein for which the dendrimer is engineered. We also demonstrate that these disassembly events can cause a concurrent release of the guest molecules. The supramolecular disassembly based on ligand binding outlined here could provide design basis for novel protein responsive drug delivery and biosensing systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank NIGMS of the National Institutes of Health for support.

References

(1)(a). Gillies ER, Jonsson TB, Fréchet JMJ. J. Am. Chem. Soc 2004;126:11936. [PubMed: 15382929] (b) Meng F, Zhong Z, Feijen J. Biomacromolecules 2009;10:197. [PubMed: 19123775] (c) Li Y, Lokitz BS, McCormick CL. Angew. Chem. Int. Ed 2006;45:5792. (d) Wang G, Tong X, Zhao Y. Macromolecules 2004;37:8911.

(2)(a). Thornton PD, McConnell G, Ulijn RV. Chem. Commun 2005:5913. (b) Azagarsamy MA, Sokkalingam P, Thayumanavan S. J. Am. Chem. Soc 2009;131:14184. [PubMed: 19757790] (c) Amir RJ, Zhong S, Pochan DJ, Hawker CJ. J. Am. Chem. Soc 2009;131:13949. [PubMed: 19739628] (d) Shamis M, Lode HN, Shabat D. J. Am. Chem. Soc 2004;126:1726. [PubMed: 14871103]

(3)(a). Takaoka Y, Sakamoto T, Tsukiji S, Narazaki M, Matsuda T, Tochio H, Shirakawa M, Hamachi I. Nat. Chem 2009;1:557. (b) Yamaguchi N, Zhang L, Chae B, Palla CS, Furst EM, Kiick KL. J. Am. Chem. Soc 2007;129:3040. [PubMed: 17315874]

(4)(a). Grayson SM, Fréchet JM. J. Chem. Rev 2001;101:3819.(b) Newkome, GR.; Moorefield, CN.; Vögtle, F. Dendrimers. 2nd Edition. Wiley-VCH; 2001.

(5)(a). Hawker CJ, Wooley KL, Fréchet JMJ. J. Chem. Soc., Perkin Trans 1993;1:1287. (b) Newkome GR, Moorefield CN, Baker GR, Johnson AL, Behera RK. Angew. Chem., Int. Ed 1991;30:1176.

(6)(a). Vutukuri DR, Basu S, Thayumanavan S. J. Am. Chem. Soc 2004;126:15636. [PubMed: 15571373] (b) Aathimanikandan SV, Savariar EN, Thayumanavan S. J. Am. Chem. Soc 2005;127:14922. [PubMed: 16231948]

(7). See Supporting Information for the details.

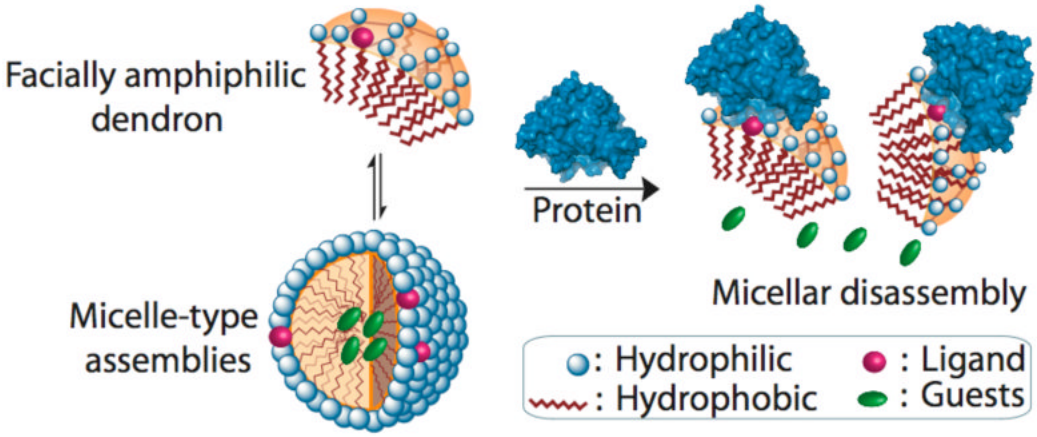

Figure 1.
Schematic of the protein-ligand binding induced disassembly of dendritic micellar assemblies and guest release.

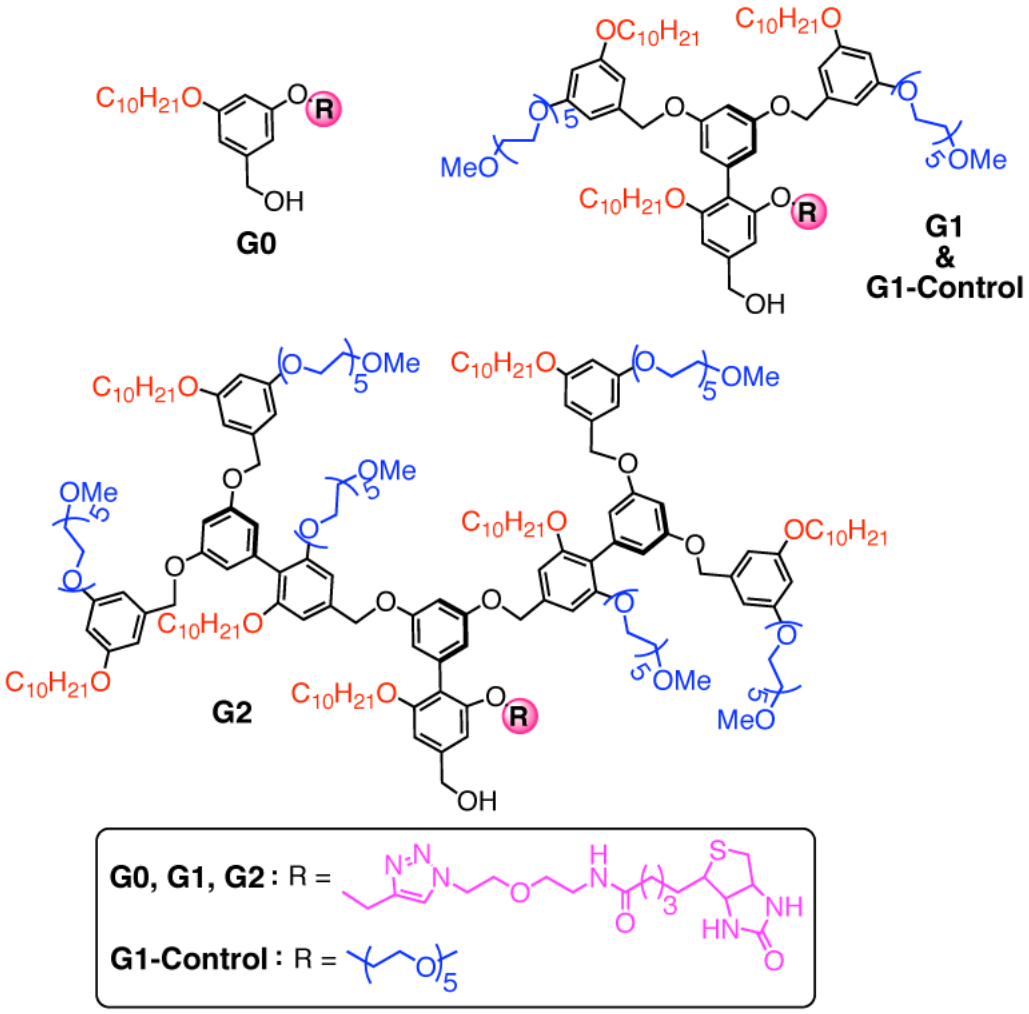

Chart 1.
Structure of ligand-functionalized self-assembling facially amphiphilic dendrons

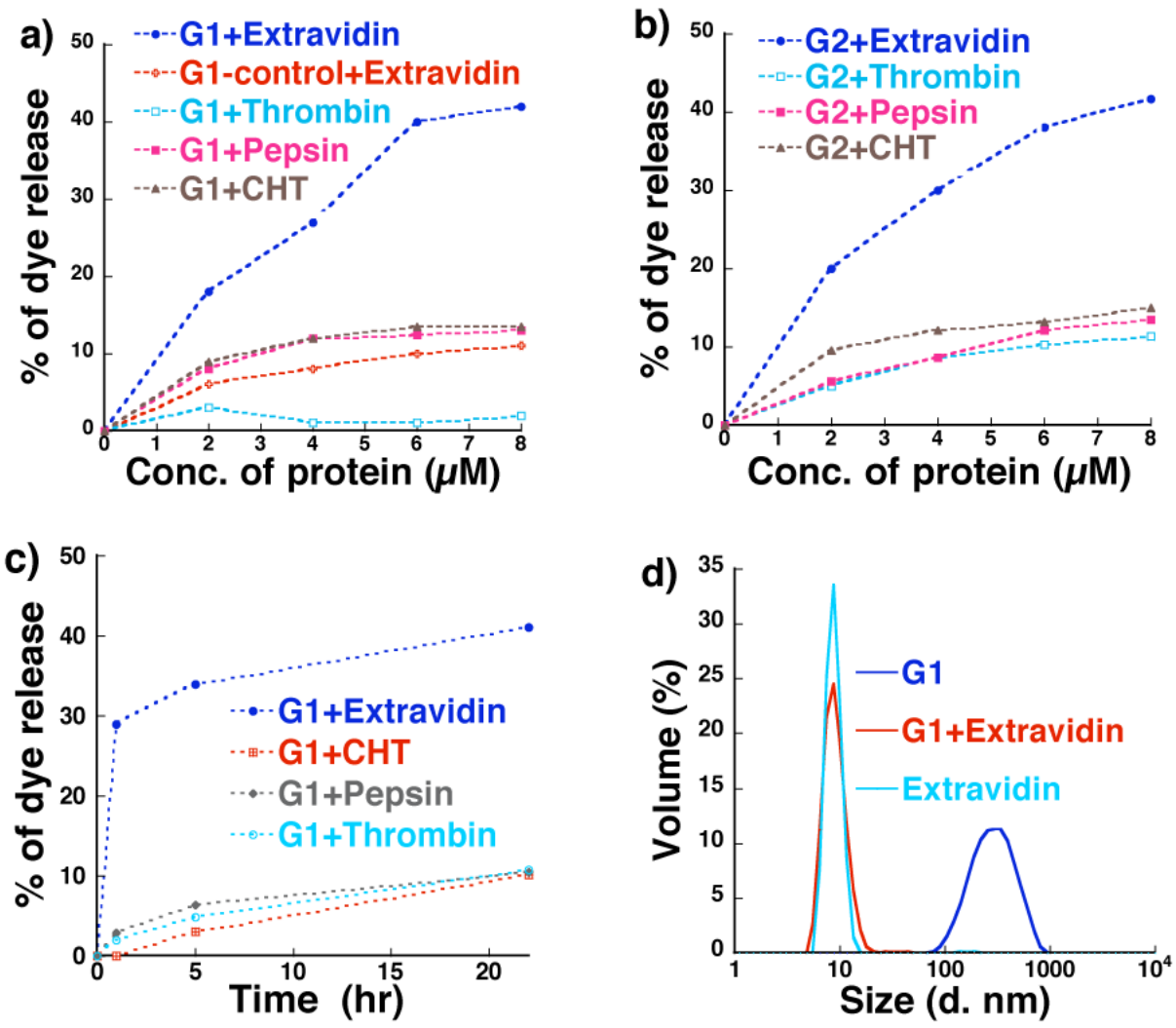

Figure 2.

Studies on the disassembly of **G1** (25 μM) and **G2** (5 μM) assemblies: Concentration dependent dye release (a) **G1**; (b) **G2**, (c) Direct addition of 10 μM of proteins to **G1**; (d) Size variation upon exposure to proteins (10 μM) by DLS.