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Enzyme-Triggered Disassembly of Dendrimer-Based Amphiphilic Nanocontainers

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Stimuli-responsive drug delivery systems have gained significant attention in recent years because their controlled release characteristics have resulted in enhanced efficacy at the site of action.[1] Most of these systems respond to stimuli such as temperature, pH, light, magnetic fields, and ionic strength.[2] An attractive class of responsive materials would involve molecular designs that respond to biological stimuli[3] such as proteins, since overexpression of proteins, especially enzymes, has frequently been implicated in the diseased state of cells. Here, we disclose our findings on an enzyme-responsive dendrimer assembly.

Dendrimers are interesting as macromolecular structures for a variety of applications because of their globular shape and the high degree of control over their size.[4] In the area of enzyme-responsive systems, a novel class of dendrimers called "self-immolative dendrimers" has been reported, in which all covalently appended drug/active molecules are released by a single enzymatic trigger at the dendritic core.[5] A useful complement to this approach involves noncovalently sequestering the guest molecules and releasing them in response to an enzymatic trigger. This approach is attractive for two reasons: (*i*) hydrophobic guests are encapsulated by the water-soluble dendrimers, and therefore, the hydrophobicity of the molecule does not affect the fidelity of the approach; (*ii*) guest molecules, such as drugs, need not be converted to prodrugs, and thus, the molecular design is simplified and the applicability can be rather broad. We report here the enzymatic disassembly of dendrimer-based micelles. Dendritic micellar assemblies are also appealing because (*i*) these macromolecules exhibit low critical aggregation concentrations (CACs) and high stabilities relative to their small-molecule counterparts[6,7] and (*ii*) systematic control over the molecular weight of the dendrimer through generational variations provides an opportunity for differential release rates.

We recently reported a distinct class of amphiphilic biaryl dendrimers that form micelle-like and inverse-micelle-like structures in polar and apolar solvents, respectively.[7] The fact that hydrophilic−lipophilic balance (HLB) is crucial for the formation of micellar assemblies of these dendrimers presents an opportunity for disassembly by disturbance of the HLB in response to an enzymatic trigger. Unlike classical amphiphilic dendrimers,[6] our facially amphiphilic dendrimers form micellar assemblies by aggregation of several dendritic molecules as a result of the orthogonal placement of hydrophilic and lipophilic units in every repeat unit of the dendrimer. To enzymatically trigger the disassembly of these assemblies, we installed enzyme-cleavable ester moieties as lipophilic units. We hypothesized that enzymatic cleavage of these lipophilic units would render the dendrimer hydrophilic and cause deaggregation. Since the hydrophobic container property of these dendrimers in water is predicated on the micelle-like aggregation, the enzyme-triggered deaggregation event should result in a concomitant release of the sequestered hydrophobic guest molecules (Figure 1).

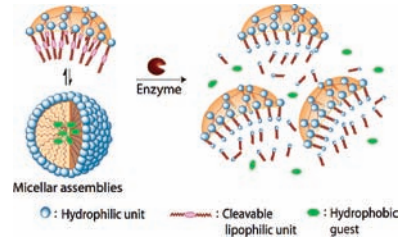
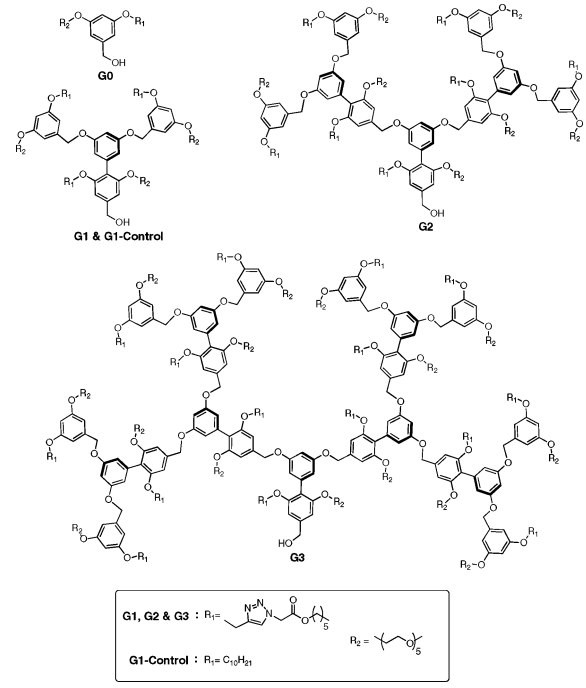


Figure 1. Schematic representation of enzyme-induced disassembly of dendritic micellar assemblies and guest release.

Chart 1. Structures of Ester-Functionalized Amphiphilic Dendrons



The structures of dendrons **G0**−**G3** with a hexyl ester functionality as the lipophilic unit and pentaethylene glycol (PEG) as the hydrophilic unit are shown in Chart 1. PEG was chosen to avoid nonspecific interactions. The dendrimers were synthesized in a modular fashion, where the enzyme-sensitive functionalities were installed using the Huisgen 1,3-dipolar cycloaddition reaction.[8] Prior to testing the disassembly, we investigated the micellar behavior of these dendrons in water by encapsulating pyrene as a probe. CACs of these dendrons were determined using the concentration dependence of the excitation spectrum of pyrene.[8] As expected, the CAC of the small-molecule-surfactant **G0** dendron was found to be in the millimolar range, whereas dendrons **G1**−**G3** exhibited CACs of 4.3, 0.7, and 0.3 μM, respectively.

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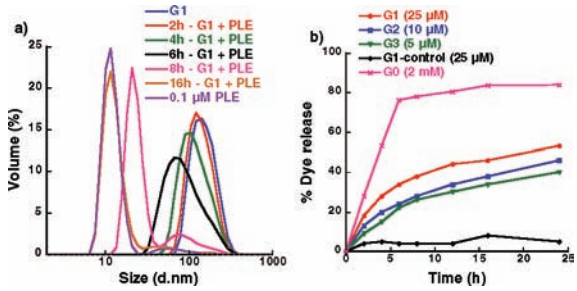

Figure 2. Disassembly of dendritic micellar assemblies upon exposure to PLE: (a) size evolution of the **G1** dendritic assembly using DLS; (b) % release of pyrene using fluorescence studies.

The enzyme-induced disassembly was first investigated using dynamic light scattering studies. The assembly size in a 25 μM solution of dendron **G1** was ~100 nm. Upon addition of a 0.1 μM solution of porcine liver esterase (PLE), we were gratified to find a systematic decrease with time in the size of the **G1** dendron assembly, which finally reached ~10 nm after 16 h (Figure 2a). The final size (10 nm) is identical to the size of the enzyme. This indicates that the final unaggregated water-soluble dendrons are not discernible by DLS. To further test whether the disassembly occurs solely because of the enzymatic hydrolysis of ester functionalities, PLE was added to a solution of the structurally similar dendron **G1-control** (Chart 1)[7b] that lacks ester functionalities. The lack of disassembly in this case supports the enzyme-specific disassembly of the **G1** micelle-like assembly.[8]

We were also interested in the generation dependence of the disassembly with respect to the relative kinetics of disassembly. We conceived that the size of the dendrons would have an effect on this kinetics. For comparison, we maintained identical concentrations of the ester functionalities in all generations. Upon exposure to PLE, the **G2** assembly size was reduced to 10 nm in 24 h, while **G3** took 36 h to reach the same size.[8] This generation dependence of the size evolution is possibly results because the ester functionalities are less accessible to the enzyme in the tightly packed higher-generation dendrons. In other words, the higher-generation dendrons sterically protect the ester functionalities from enzymatic degradation. The rapid decrease in the size observed for **G0** further supports this hypothesis.[8]

It is reasonable to expect that the disassembly would effect a concomitant release of any hydrophobic guest molecule sequestered within the micellar interior. To test this, PLE was added to the pyrene-encapsulated dendrons (**G0-G3**), after which a systematic decrease in pyrene fluorescence over time was observed (Figure 2b). The temporal evolution of the pyrene fluorescence indicates that the disassembly was indeed accompanied by release of the guest. Also, no pyrene release was observed with **G1-control**, as expected (Figure 2b).[8] Three features are noteworthy: (*i*) the rate of guest release systematically decreased with increasing dendron generation, which is consistent with the DLS data; (*ii*) the burst release observed for **G0** is also consistent with the DLS results; and (iii) the maximum dye release observed in the case of dendrons reached a plateau at ~50%. We attribute this to the possibility that the rather hydrophobic backbone of our biaryl dendrimers is capable of solvating a small amount of pyrene. This was supported by the significant pyrene encapsulation capability of the hydrolyzed **G2** carboxylic acid-containing dendron.[8]

In summary, we have designed dendrimer-based amphiphilic assemblies that can noncovalently sequester hydrophobic guest molecules and release these guests in response to an enzymatic trigger. This was achieved by incorporating enzyme-sensitive functionalities at the lipophilic face of the dendrons. This feature causes a change in the HLB when the enzyme is encountered, effecting disassembly and guest-molecule release. The noncovalent nature of the binding and release of the guest molecules is likely to further increase the repertoire of dendrimers in enzyme-responsive drug delivery systems and biosensors.

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

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(8) See the Supporting Information for details.

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