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Protein-Induced Supramolecular Disassembly of Amphiphilic Polypeptide Nanoassemblies

Mijanur Rahaman Molla, Priyaa Prasad, and S. Thayumanavan*

Department of Chemistry, University of Massachusetts Amherst, Amherst, Massachusetts 01003, United States

Supporting Information

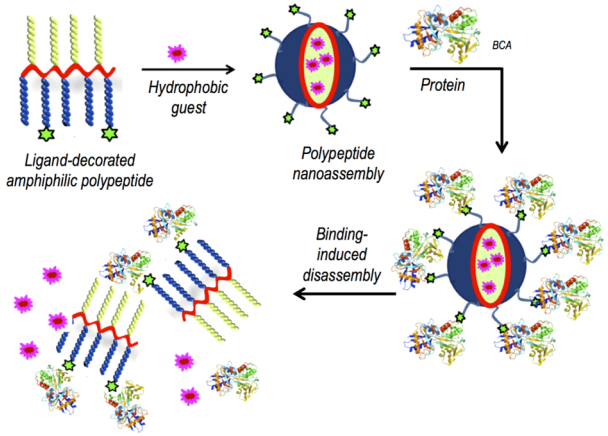
ABSTRACT: Mimicking noncovalent interaction based processes in nature has been an important goal of supramolecular chemistry. Here, we report on amphiphilic polypeptides that self-assemble to form nanoscale supramolecular assemblies and are programmed to disassemble in response to a specific protein. Benzenesulfonamide and carbonic anhydrase have been chosen as the ligand and protein, respectively, to demonstrate this possibility. Since the amphiphilic nanoassembly sequesters hydrophobic guest molecules, the protein-specific disassembly event provides a protein-sensitive molecular release as well. We envision that the binding induced disassembly and guest release might open up new opportunities for the next generation of supramolecular assemblies for protein-specific delivery and diagnostics.

The holy grail of supramolecular chemistry has been to design artificial molecules and molecular assemblies that mimic nature's ability to execute specific processes through precisely engineered intermolecular forces.[1] Nature has evolved specific interactions among macromolecules as an important recognition strategy to execute most of the biological processes. For example, proteins specifically interact with partner proteins to trigger biological events,[2] with nucleic acids to transcribe the genetic code,[3] and with carbohydrates to regulate cellular processes such as cell−cell communication.[4] In most cases, the binding event causes a conformational change in the partner macromolecule to activate one of the binding partners to trigger a biological cascade. Inspired by these processes that are central to the existence of biological systems, we were interested in designing artificial peptide-based supramolecular assemblies that respond to a specific protein binding event and cause a discernible cascade of events.

We chose peptide-based nanoassemblies because they can be functionally diverse with precise functional group placements within the scaffold.[5] As a first step, we were interested in a minimalist biomimetic design with peptide scaffolds. Accordingly, we have designed amphiphilic polypeptides, where the driving force for the nanoassembly formation is simply driven by hydrophobic forces. We also designed these peptide-based assemblies by derivatizing a peptide homopolymer with the requisite functionalities for achieving a nanoassembly that can specifically bind to a protein. In our molecular design, we utilize poly-L-glutamic acid, where a percentage of the carboxylic acid moieties are functionalized with hydrophobic units to render the polypeptide amphiphilic. Although this polymer itself is amphiphilic and self-assembles, we further derivatized the remaining carboxylic acid groups with oligoethylene glycol (OEG) moieties in order to avoid nonspecific, electrostatic interactions with proteins. To further ensure specific binding with a targeted protein, a small percentage of the OEGs also contain protein-specific ligand moieties.

The key design hypothesis is that the amphiphilic polypeptide, mentioned above, would self-assemble to present a protein-specific ligand on the hydrophilic face of an amphiphilic nanoaggregate in water. We then envisage that the protein binding event would cause a rather large change in the self-assembled structure.[6] Note that the protein binding causes a change in the hydrophilic face that presents the sulfonamide moiety to one that presents the hydrophilic surface of a rather large protein. This event should cause a significant change in the hydrophilic−lipophilic balance (HLB) of the amphiphile, resulting in supramolecular disassembly (Scheme 1). We chose carbonic anhydrase as the model protein because of its disease relevance and because noncovalent ligands for carbonic anhydrase are well-established.[7] Specifically, bovine carbonic anhydrase II (bCA-II) with a pI of 5.4 and a molecular weight of 30 kDa was used as the protein for this study.

Scheme 1. Schematic Representation of the Protein Binding Induced Disassembly of a Polypeptide Nanoassembly



The motivations to explore polypeptides as the scaffolds for binding induced disassembly using carbonic anhydrase are multifold. Two of these factors emerge as rather significant ones.

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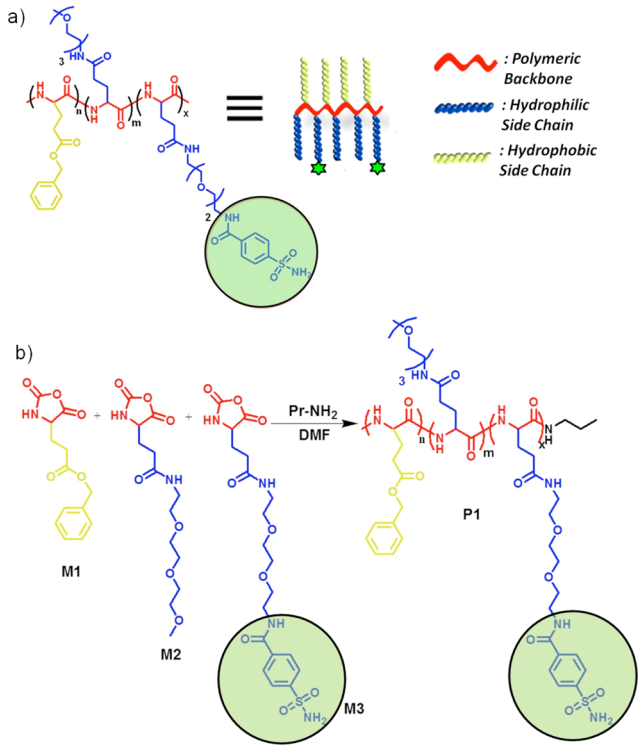
From the mechanistic possibility, prior work has shown that one needs multivalent protein−ligand interactions to cause the binding-induced disassembly.[6a] This requirement is rather limiting, as most biologically relevant proteins do not have multiple binding sites. Therefore, demonstration of binding induced disassembly with carbonic anhydrase, where the protein contains a single ligand binding site, would be a critical finding to expand the repertoire of this strategy. Second, we were interested in polypeptides, not only because they ultimately provide the opportunity to introduce diverse functional groups through sequencing but also because its backbone is biodegradable.

The targeted amphiphilic random copolypeptide is made of three different substituted glutamic acid monomers (**M1**, **M2**, and **M3**, Scheme2). Benzenesulfonamide was chosen as ligand

Scheme 2. (a) Chemical Structure of Polymer P1 and Corresponding Cartoon; (b) Schematic Presentation of the Final Step of P1 Synthesis



because of its well-established, high binding affinity toward carbonic anhydrase.[7] The polymer **P1** was synthesized from NCA monomers as shown in Scheme 2. FTIR analysis of **P1** showed complete disappearance of two characteristic peaks of the monomers at 1850 and 1782 cm^{-1} (Figure S1). NMR revealed that the experimental ratio of the monomers in **P1** was about 0.48:0.37:0.15. Gel permeation chromatography (GPC) shows that M_n and dispersity were 3.2 kDa and 1.2, respectively (Figure S2).

Next, we investigated the self-assembly features of the polypeptide **P1** by evaluating its ability to act as host for a hydrophobic guest (DiI) in aqueous medium (Figure 1d). DiI is not soluble in water unless a hydrophobic pocket is provided. The emission from DiI increases with increase in **P1** concentration, although initial DiI concentration was kept constant in all solutions (Figure 1a). The CAC of **P1** was calculated to be about 31 μM (Figure 1b). Spectroscopic

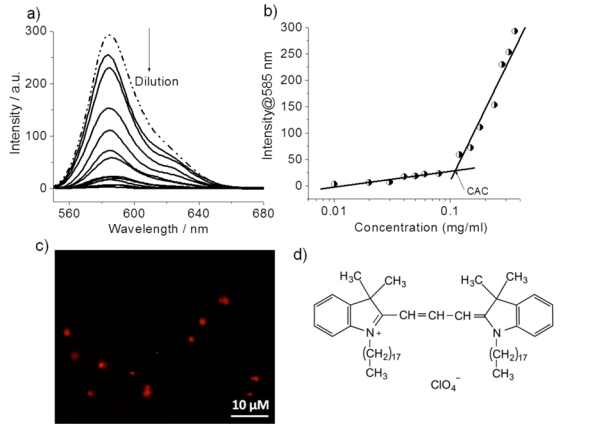


Figure 1. (a) Emission spectra of DiI encapsulated in the **P1** micelle in varying concentration. (b) Variation of λ_{max} (585 nm) of DiI as a function of **P1** concentration. (c) Optical fluorescence microscopy (OPM) image of DiI encapsulated **P1** micelle. (d) Chemical structure of DiI dye molecule.

evidence of DiI encapsulation was also justified by OPM images, showing red-emitting spherical particles (Figure 1c).

The formation of an amphiphilic aggregate with an average hydrodynamic diameter of 190−200 nm from **P1** was confirmed using dynamic light scattering (DLS) (Figure 2a). Transmission

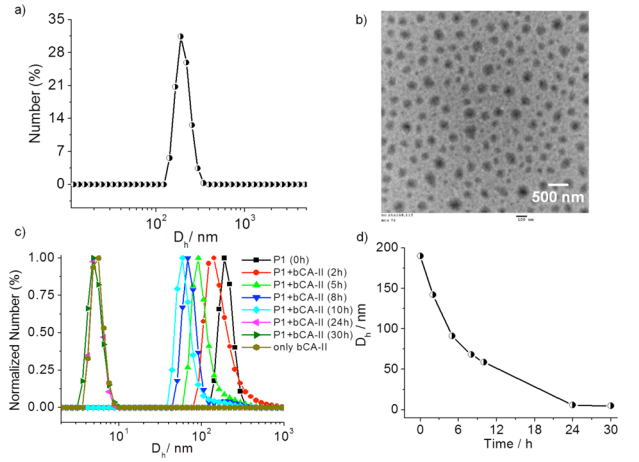


Figure 2. (a) DLS of **P1** aggregates in water (concentration of **P1** = 50 μM). (b) TEM image of **P1** aggregates. (c) Time-dependent DLS profile of **P1** in the presence of bCA-II protein. (d) Size variation of the particle with time.

electron microscopy (TEM) analysis showed the presence of spherical assemblies in the range of 170−180 nm (Figure 2b), which are slightly lower than those from DLS. This difference is likely due to the shrinkage of the particles in the dry state[8] or due to overestimation of the size of the particles in DLS as it also includes hydration shells around the particles.

To investigate whether the specific interaction between the ligand and the complementary protein would cause significant changes to the aggregation state of the assembly from **P1**, an aqueous solution of **P1** (50 μM) was treated with bCA-II (30 μM). The size evolution of the aggregates was monitored over 30 h by DLS. Upon addition of protein, the size decreased from ~200 to ~5 nm, which is close to the size of the bCA-II protein by itself (Figure 2c,d). However, no size change was observed over the same time period in the absence of protein (Figure S3).

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These data provided the indication that is consistent with our binding induced disassembly hypothesis, illustrated in Scheme 1. It is however important to show that this presumed binding-induced disassembly is indeed due to a specific ligand−protein interaction. For this purpose, we used noncomplementary proteins such as pepsin, bovine serum albumin (BSA), and lysozyme. These proteins were selected as noncomplementary proteins for their diversity in pI values since this is often the source of nonspecific interactions (pI = 1.0 (pepsin), 4.8 (BSA), and 11 (lysozyme)). Indeed, we noticed that there was no change in size of the aggregates in solution over 30 h for any of these proteins (Figures 3a,b and S4). To further validate that the

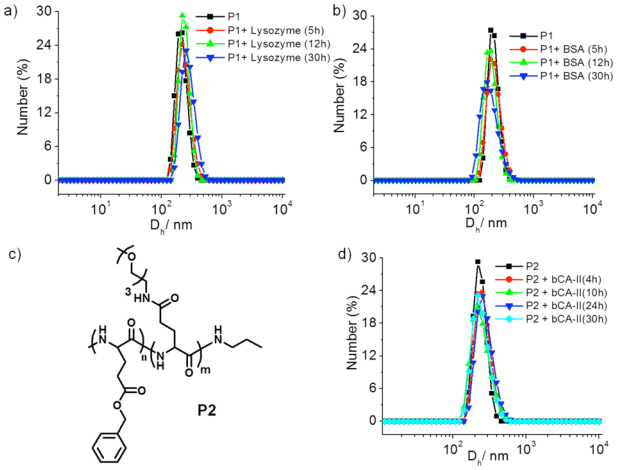

Figure 3. DLS of **P1** aggregates in the presence of (a) lysozyme and (b) BSA (concentration of **P1** = 50 μM). (c) Chemical structure of **P2**. (d) Time-dependent DLS of **P2** in the presence of bCA-II protein.

observed disassembly is due to specific protein−ligand interactions, we synthesized a control polypeptide **P2**, which does not contain the complementary sulfonamide ligand (Figure 3c). **P2** also forms assemblies similar to those from the ligand-bearing polymer **P1** (Figure S5). The size of the aggregates from **P2** was found to be ∼140 nm by both DLS and TEM. When an aqueous solution of **P2** is treated with bCA-II, the size did not change over 30 h (Figure 3d), again supporting the hypothesis that the observed size change is due to binding-induced disassembly caused by a specific ligand−protein interaction.

In addition, the results with the control polypeptide **P2** also rules out a potential alternate explanation for disassembly in response to carbonic anhydrase. It is known that carbonic anhydrase has some esterase activity.[9] Therefore, it is possible that the observed disassembly in **P1** is due to the cleavage of the ester moiety in the hydrophobic part of the amphiphilic peptide. In this case, the disassembly is anticipated due to the resultant change in the hydrophilicity of the self-assembling peptide. The fact that the structurally identical polymer **P2**, except for the ligand presence, did not disassemble in the presence of bCA-II rules out this alternate hydrolysis based disassembly pathway (Figure 3d).

Since the protein binding causes a disassembly, it is possible that we can utilize this process to cause the guest molecules to be released. Since the hydrophobic guest is insoluble in water, it is likely that it will simply precipitate out of solution. To test this hypothesis, DiI-encapsulated **P1** (50 μM) was treated with bCA-II (30 μM), and the possible guest release of the DiI was monitored by absorption spectroscopy. Indeed, the absorbance of DiI decreased overtime in the presence of bCA-II, while there was no such change in the absence of bCA-II (Figure 4a,b).

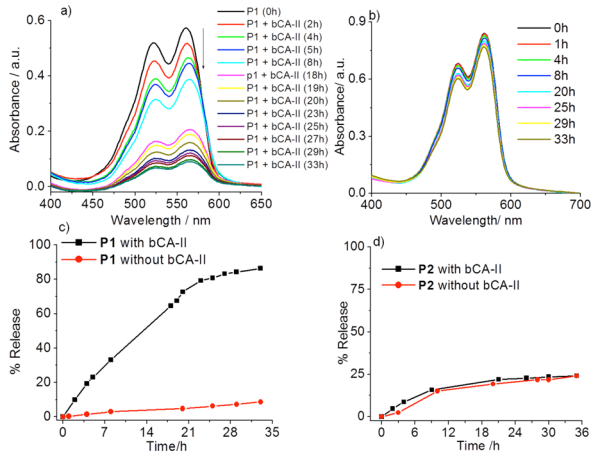

Figure 4. DiI release from **P1** micelle (a) in the presence and (b) in the absence of bCA-II protein (concentration of **P1** = 50 μM). (c) Plot of % release with time. (d) Plot of % release of DiI from control polymer **P2** in the presence and absence of bCA-II.

These observations indicate that the observed process is a protein-specific molecular release (∼85% release over 33 h, Figure 4c). To further test whether the process is specific to bCA-II, in a control experiment, we exposed the same solution to noncomplementary proteins with different surface charges, viz. pepsin, BSA, and lysozyme. The guest release was found to be relatively insignificant (Figure S6). Similarly, we also monitored the possible guest release from **P2** aggregate, and it was <20%. More importantly, the extent of guest release was found to be identical to that without the protein from this assembly over the same amount of time (Figures 4d and S7). These results indicate that the guest release from the **P1** assembly is indeed due to specific ligand−protein interaction induced disassembly.

Finally, since the degradable components of this polypeptide are considered biocompatible, we envisaged that the amphiphilic polypeptide itself might be biocompatible, i.e., not cytotoxic. To test this, we studied the *in vitro* cell viability of the polymer **P1** using an Alamar blue assay with HeLa cell lines and found the cells to be ∼80% viable even at 250 μg/mL of polymer solution (Figure S8).

In summary, we have designed and synthesized a polypeptide, the amphiphilic nature of which provides a nanoscale supramolecular assembly that can stably encapsulate hydrophobic guest molecules in aqueous media.[6] The polypeptide is engineered to present a protein-specific ligand in its hydrophilic face. We show that the binding interaction between the ligand moiety and the complementary protein causes the assembly to fall apart. This binding-induced disassembly has been shown to be specific to bCA-II and to cause release of guest molecules. The extent of guest release in response to protein binding was found to be substantial (∼85%). This feature, along with the simplicity of the synthetic route, highlights the utility of peptide-based assemblies for protein-induced supramolecular disassembly. Although responsive molecular assemblies have been consistently targeted for applications such as delivery and diagnostics,[10] systems that respond to protein activity are very limited. These are interesting because aberrant protein activity is the basis for all genetic diseases. While there have been significant efforts on systems that respond to enzymatic activity variations,[11]

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assemblies that respond to nonenzymatic proteins are very limited. The polypeptides, outlined here, are poised to make a significant impact in this area with their biocompatible, biodegradable, and high fidelity responsive disassembly characteristics.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic procedures and characterizations of the polymers. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b04285.

■ AUTHOR INFORMATION

Corresponding Author

*thai@chem.umass.edu

Notes

The authors declare no competing financial interest.

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