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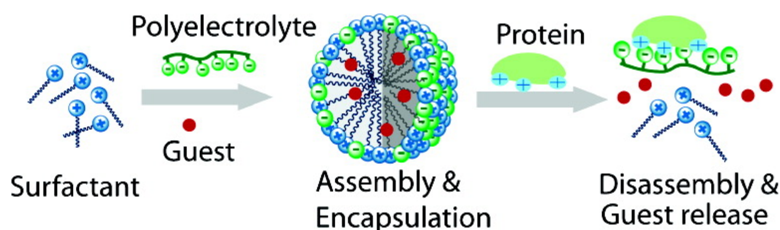

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Disassembly of Noncovalent Amphiphilic Polymers with Proteins and Utility in Pattern Sensing

Elamprakash N. Savariar, Suhrit Ghosh, Daniella C. González, and S. Thayumanavan*

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

Received January 8, 2008; E-mail: thai@chem.umass.edu

Supramolecular assemblies from surfactant molecules are of interest because of their container properties in aqueous solutions (e.g., micelles and vesicles).[1] Polymeric versions of these assemblies have been extensively explored due to their greater stability and the lower critical aggregation concentrations (CAC).[2] We have recently reported a class of amphiphilic homopolymers that exhibit certain unique supramolecular characteristics.[3] The scope of these polymer assemblies will be greatly enhanced if we are also able to obtain these amphiphilic structures through noncovalent interactions.[4] The approach is even more enticing for applications, such as sensing, if these supramolecular structures can be disassembled in response to biologically relevant analytes.[5] Here, we report on the formation and disassembly of noncovalent amphiphilic structures. We then demonstrate the utility of these noncovalent processes in pattern generation for protein sensing.

Sensing through pattern generation is promising due to the simpler molecular design principles, compared to the typical lock and key sensor.[6] However, pattern generation does require numerous synthetic receptors. Hence, it is interesting to develop strategies that either simplify the assembly of the receptors or reduce the need for multiple receptors. In this direction, we recently introduced an approach that uses a single receptor scaffold, but with multiple fluorescent transducers for pattern recognition of metalloproteins.[7] This strategy is useful for analytes that are inherently capable of quenching the fluorescence of the transducers. An attractive alternative would be to develop a method for analytes that are not necessarily electronically complementary to the transducers. Generating patterns using protein-induced disassembly not only provides this opportunity but also reduces the synthetic complexity even further because these are assembled from its components noncovalently.

The basis for our hypothesis is as follows: when polyelectrolytes complex to complementary small molecule surfactants, the combination should provide supramolecular assemblies with apolar interiors that can sequester hydrophobic guest molecules in water. Since polymeric amphiphiles typically exhibit lower CAC, it is reasonable to expect the polymer−surfactant complex to exhibit lower CACs than the small molecule surfactant by itself.[8] At a concentration between these two CACs, the guest molecules will be released if the interaction between the polymer and the surfactant is interrupted (Figure 1a). This is because the surfactant by itself is not capable of forming a micelle at this concentration. Since polyelectrolytes are known to effectively bind globular proteins, the protein binding should cause the release of the guest molecules.

To probe the possibility of a decrease in the CAC upon polymer complexation, we investigated the onset of micellization of the complex between the polymer poly(potassium acrylate) (PPA) and the surfactant cetyltrimethylammonium bromide (CTAB) using the environment-dependent emission spectrum of pyrene as the probe.[9] We were gratified to find that the CAC of the PPA−CTAB complex is more than an order of magnitude lower than CTAB itself (4×10^{-5} M vs 1.3×10^{-3} M).[9]

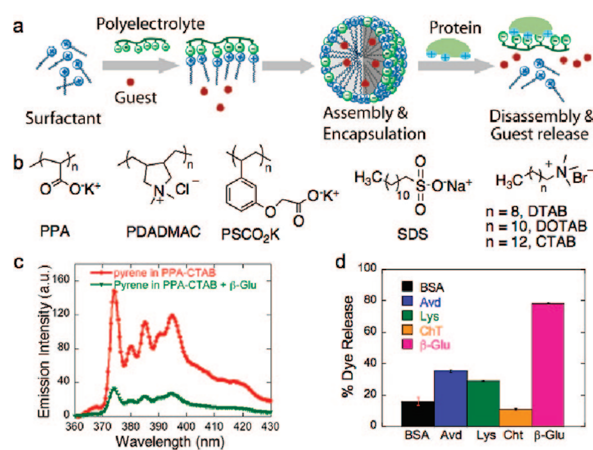

Figure 1. (a) Schematic of the assembly and disassembly upon protein binding. (b) Structures of polymers and surfactants used in the study. (c) Fluorescence spectra of pyrene. (d) Differential dye release upon adding various proteins from PPA−CTAB.[9]

Next, we were interested in finding whether protein binding to the polymer will result in releasing the guest molecule from the hydrophobic interiors of the complex. For this purpose, we utilized the PPA−CTAB complex at 1.0×10^{-4} M concentration and tested the disassembly of the pyrene-loaded complex by β-glucosidase (β-Glu). We indeed found that emission intensity of pyrene decreased upon adding the protein; at 8 μM of β-Glu, 78% dye release was observed (Figure 1c). Note that β-Glu by itself is not capable of quenching pyrene emission.

In order to test our hypothesis, we characterized the polymer−surfactant complex by TEM. TEM image of the PPA−CTAB complex clearly showed the presence of spherical particles of about 40 nm in radius. However, no discernible assemblies are observed upon adding β-Glu (Figure 2a,b). These results are further supported by DLS. A radius of 51 nm was noted for the PPA−CTAB complex, and the size was reduced to 3 nm upon adding β-Glu, which is most likely from the protein or the protein−polymer complex.[9] Note that the discrepancy in size between TEM and DLS is understandable because the measurement is done directly in solution with DLS while the solution was laid on a surface with TEM. The key feature is that the formation of the complex and its disassembly in response to proteins is clear.

The above experiments provide the preliminary proof-of-principle for the disassembly of polymer−surfactant complexes using proteins. However, to generate the targeted analyte (protein)-dependent patterns, the binding affinity of the polymer to different proteins has to be necessarily different. We therefore tested the release of pyrene from the PPA−CTAB complex with four other

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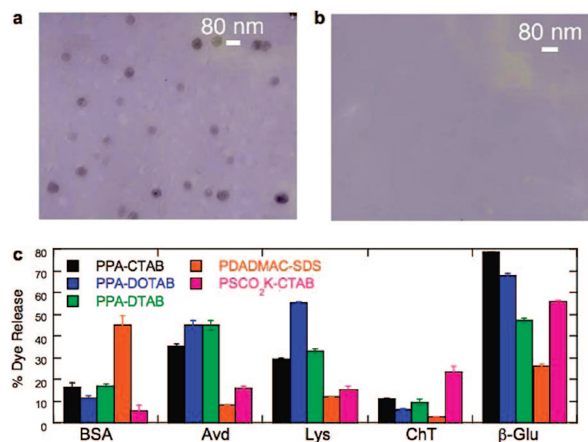

Figure 2. TEM images of PPA−CTAB (a) before and (b) after adding β-Glu. (c) Protein-dependent arrays from guest release at 8 μM concentration of the proteins

randomly chosen proteins, bovine serum albumin (BSA), lysozyme (Lys), Avidin (Avd), and chymotrypsin (ChT), at the same concentration. Consistent with our expectations, these proteins did respond differently at identical concentrations (Figure 1d). To demonstrate a successful design for pattern generation, the trend in response to variations in analytes should have some predictability since this provides the guidelines for molecular design. However, it is also necessary that there are unpredictable subtleties which are necessary for the generation of analyte-dependent fingerprints. Our system satisfies both criteria. For example, Avd and BSA have similar molecular weights but differ in charge with the pIs of 10 and 4.8, respectively. The observed dye release was indeed more for the cationic Avd compared to the anionic BSA. Similarly, Lys (pI 11) and ChT (pI 8.8) are both positively charged but differ significantly in molecular weights. As expected, only about 11% dye release was observed with ChT, compared to 29% with Lys. On the other hand, even though β-Glu is a negatively charged protein, the obtained dye release was much more than any of the other proteins tested. It is understandable nonetheless that a negatively charged protein can bind to a similarly charged polymer because proteins are polyampholytes and polymers are polyelectrolytes.[10] Also, note that protein sizes also seem to play a role in these polymer binding events.[7] This provides the requisite unpredictability and therefore the opportunity for analyte-dependent fingerprints.

Two other features add to this complexity: (*i*) the released guest molecules can bind to the hydrophobic cavities of the protein. This results in a lack of decrease in fluorescence from pyrene. In our experiments, BSA is the only protein that exhibits significant pyrene binding.[9] (*ii*) In addition to the polymer, it is also possible that the charged surfactant molecules have some binding affinity toward the proteins. This was observed with β-Glu, more than any other protein tested.[9] These features, combined with the polyampholytic nature of proteins, auger well to provide protein-dependent patterns.

To provide multiple receptors that respond differently to proteins, we resorted to variations in (*i*) charge of the polymer; (*ii*) hydrophobicity of the polyelectrolyte, and (*iii*) hydrophobicity of the surfactant. To test the first variation, we used the cationic polymer poly(diallyldimethylammonium chloride) (PDADMAC) and SDS as anionic surfactant. The response to the protein from this complex was very different. The highest pyrene release was observed for BSA, followed by β-Glu, Lys, Avd, and ChT.[9]

To test the second variation, we replaced PPA with $PSCO_2K$ in the polymer−CTAB complex and tested the dye release. $PSCO_2K$ is more hydrophobic than PPA, and therefore, we expected the interaction with proteins to be different. We found that the responses were indeed different. For example, the lowest dye release was observed with BSA (5%), whereas ChT exhibited 23% dye release.[9]

Finally, we compared the dye release from the PPA−CTAB complex with those of PPA with DOTAB and DTAB. Note that the hydrophobic chain length of the surfactants changes from C16 to C12 and C10 as we go from CTAB to DOTAB and DTAB, respectively (Figure 1b). We hypothesized that the decrease in CAC and thus the stability of the supramolecular assembly will vary with the hydrophobicity of the surfactant combinations. As expected, this variation also provided differential release response to the proteins tested.[9]

The most important question is do all these variations provide significantly different responses to generate patterns for different proteins? These do indeed. The combination of responses from each of these variations is plotted for each protein and is shown in Figure 2c. With just five different combinations of polymers and surfactants, we were able to demonstrate distinct patterns for each of the five proteins studied. Considering that both polymers and surfactants are very easily accessible and are noncovalently assembled, generating multiple receptor and therefore several data points is viable.

In summary, we have shown that (*i*) CACs of polymer−surfactant complexes can be significantly lower than the surfactant itself; (*ii*) by dissociating the complex at an intermediate concentration, the assembly can be disrupted to affect guest release; (*iii*) by varying the structures of the polymer and the surfactant, differential responses to various proteins can be achieved; and (*iv*) this method does not require the analyte to be electronically complementary to the fluorescent transducer, where the analyte itself quenches the fluorescence. The simplicity of the design due to the fully noncovalent nature of the receptor assembly makes this approach highly versatile.

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

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