Controlling Supramolecular Complex Formation on the Surface of a Monolayer-Protected Gold Nanoparticle in Water

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ABSTRACT: A combination of hydrophobic and electrostatic interactions drives the self-assembly of a large number of small molecules on the surface of a monolayer-protected gold nanoparticle. The hydrophobic interactions originate from the insertion of an aromatic unit in the hydrophobic part of the monolayer. This is evidenced by a shift in the emission wavelength of the fluorogenic probe upon binding. Up to around 35 small molecules can be simultaneously bound to the monolayer surface at micromolar concentrations in water. It is shown that an understanding of the supramolecular interactions that drive complex formation on the monolayer surface provides unprecedented control over the supramolecular chemistry occurring on the surface. By taking advantage of the different kinds of noncovalent interactions present in different probes, it is possible to displace one type of surface-bound molecule from a heteromeric surface selectively. Finally, it is also possible to catch and release one type of surface-bound molecule selectively.

INTRODUCTION

The self-assembly of large supramolecular structures in water is an attractive approach for systems able to interact with biotargets such as proteins, oligonucleotides, and even cells. Self-assembly permits straightforward access to nanosized objects with minimal synthesis effort. Additional advantages include error correction during self-assembly and the adaptive behavior of the resulting structure with respect to the target or external stimuli (pH, temperature, metal ions, ...). Various supramolecular aggregates, such as micelles, rods, peptide amphiphiles, supramolecular polymers, and monolayer-protected nanoparticles, have been shown to be excellent structures for the recognition and sensing of biotargets. In particular, monolayer-protected gold clusters (Au MPCs) have attracted an enormous amount of attention because of their high stability even at nanomolar concentrations and their well-defined discrete dimensions. The use of noncovalent interactions for assembling the small molecules on the monolayer surface in principle permits spontaneous adaptability to a target. A critical issue in this approach is the high affinity of the small molecules for the monolayer surface because complex formation should occur under saturation conditions even at low micromolar concentrations in water. Our studies have mainly involved Au MPC 1·Zn2+, which is a gold nanoparticle (d = 1.8 ± 0.4 nm) covered with hydrophobic C9-thiols terminated with a 1,4,7-triazacyclononane (TACN)·Zn2+ headgroup. This diameter implies that approximately 70 thiols are present on the surface of each nanoparticle. To ensure binding, small molecules were selected with the prerequisite of having a patch of negative charges in the form of either carboxylates or phosphates. Nonetheless, these studies provided clues that the binding affinity of the probes for the monolayer surface was not just caused by electrostatic interactions but also by interactions with the hydrophobic part of the monolayer. In general, many examples of supramolecular complex formation in water driven by a combination of hydrophobic and electrostatic interactions have been reported. Here, we provide compelling evidence that hydrophobic interactions play an important additional role in the binding of small molecules to Au MPC 1·Zn2+. The presence of a hydrophobic interaction permits a reduction of the number of negative charges in the probe without comprising the formation of the multivalent structure under saturation conditions. It is shown that the

Special Issue: Interfacial Nanoarchitectonics

Received: October 31, 2012
Revised: December 17, 2012
Published: December 21, 2012

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dx.doi.org/10.1021/la304316z | Langmuir 2013, 29, 7180–7185
possibility of regulating the binding strength of the probes gives an element of control over the supramolecular chemistry that takes place on the monolayer surface (selective exchange or catch and release of surface-bound small molecules).

Figure 1. Schematic representation of the formation of a multivalent supramolecular system and structures of the probes.

Figure 2. (a) Fluorescence intensities upon the addition of increasing amounts of dATP<sub>MANT</sub> to a solution of Au MPC<sub>1</sub>·Zn<sup>2+</sup> ([TACN·Zn<sup>2+</sup>] = 10 μM, [HEPES] = 10 mM, pH 7.0, T = 25 °C). On the right, the image shows the emission spectrum after the first additions. (b) Fluorescence intensities at 425 nm (red) and 448 nm (blue) as a function of the concentration of dATP<sub>MANT</sub>. (c) Maximum in the MANT emission wavelength (nm) as a function of the concentration of dATP<sub>MANT</sub> (red) superimposed on the fluorescence intensity at 448 nm (blue) as a function of the concentration of dATP<sub>MANT</sub>.

**RESULTS AND DISCUSSION**

**Electrostatic and Hydrophobic Interactions Drive Complex Formation on the Monolayer Surface.** We decided to study the interaction of probe dATP<sub>MANT</sub> (2′-deoxy-
3′-O-((N′-methylanthraniloyl)adenosine-5′-O-triphosphate) with Au MPC 1-Zn\(^{2+}\) for several reasons. First, previous studies had already revealed a high affinity of ATP and its fluorescent analogue ATP\(_{f}\) for Au MPC 1-Zn\(^{2+}\).\(^{15,23}\) Second, dATP\(_{MANT}\) has an apolar aromatic unit attached to the deoxyribose that seemed perfectly placed to penetrate the monolayer. Third, the N-methylanthraniloyl (MANT) unit is fluorescent (λ\(_{ex}\) = 355 nm, λ\(_{em}\) = 448 nm), which is essential for studying the binding to Au MPC 1-Zn\(^{2+}\). In addition, the MANT fluorophore is sensitive to its environment,\(^{24}\) and it is reported that the emission wavelength shifts 10–20 nm to shorter wavelengths in apolar environments.\(^{25}\) Thus, the interaction between dATP\(_{MANT}\) and Au MPC 1-Zn\(^{2+}\) was studied by means of a fluorescence titration taking advantage of the ability of gold nanoparticles to quench the fluorescence of bound fluorophores.\(^{26}\) All studies were performed in aqueous buffer (\([\text{HEPES}] = 10 \text{ mM}\) at pH 7.0) with TACN headgroup concentrations equal to 10 ± 1 μM.\(^{27}\) A detailed analysis of the obtained spectra gave interesting insights into the nature of the complex formation. The first additions of dATP\(_{MANT}\) resulted only in a minor fluorescence emission with a maximum at around 425 nm (Figure 2a). For these concentrations, the emission maximum and intensity were determined after subtracting the contribution from the signal at 407 nm owing to the Raman scattering of water (Supporting Information). Up to a concentration of around 2.5 μM dATP\(_{MANT}\), a linear increase in the (weak) emission intensity at this wavelength was observed (Figure 2b). Importantly, the observed maximum at 425 nm (23 nm below the expected wavelength) is a strong indication that the MANT fluorophore is inserted into the apolar monolayer. In fact, further additions of dATP\(_{MANT}\) resulted in a strong increase in fluorescence emission with a maximum at 448 nm corresponding to the (expected) emission of free dATP\(_{MANT}\). As reported previously, from a plot of the fluorescence intensity at 448 nm as a function of the concentration of dATP\(_{MANT}\), the surface saturation concentration (SSC) of the probe could be determined in a straightforward manner (SSC\(_{dATP_{MANT}}\) = 3.2 μM). The SSC defines the number of molecules bound to the monolayer surface at saturation. The observation that the change in the maximum emission wavelength neatly occurs upon reaching the SSC confirms that it originates from a change in the environment of the MANT fluorophore (Figure 2c). It is interesting that just before reaching the SSC a small blue shift in the emission maximum is observed to 420 nm. This could point to an organizational process occurring on the surface to permit the accommodation of a major number of probes.

The above experiments suggest that the MANT fluorophore is inserted into the monolayer. To obtain more information, we measured the interaction between dATP\(_{MANT}\) and Au MPC 1-Zn\(^{2+}\) under conditions where no binding was observed for ATP\(_{f}\) (2-aminopurine riboside-5′-O-triphosphate).\(^{28}\) λ\(_{ex}\) = 305 nm, λ\(_{em}\) = 370 nm). ATP\(_{f}\) is structurally very similar to dATP\(_{MANT}\) but lacks the MANT fluorophore (Figure 1).
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significance between dATP and dAMP. The peculiar high affinity of analogous compound AMP. Also for dADP, a shift in the emission wavelength was observed during the fluorescence titration (Supporting Information). So whereas for the ATP probe the presence of Zn2+ (or, more generally, a positive charge) in the monolayer is essential to obtaining a significant SSC, these experiments show that this is much less relevant for dATP. Although the SSC in itself does not provide information on the strength of the interaction (such information is obtained from the competition experiments described below), these results are indicative of a difference in the binding mode between ATP and dATP. For that reason, we extended our studies to dADP and dAMP. Interestingly, notwithstanding the reduced negative charge, both compounds still bound Au MPC 1:Zn2+ under saturation conditions at a headgroup concentration of 10 μM (Figure 4a).

In particular for dAMP, having just one phosphate group, this is impressive, especially considering the relatively low affinity of analogous compound AMP. Also for dADP and dAMP, a shift in the emission wavelength was observed during the fluorescence titrations, indicating that within the MANT series the difference in emission wavelength progressively decreased (Supporting Information, 23 nm for dATP, 20 nm for dADP, and 11 nm for dAMP). This is tentatively ascribed to a different position of the MANT fluorophore in the hydrophobic part of the monolayer. It is important to note that a reduced size of the probe is accompanied by an increase in SSC (3.2 μM for dATP, 4.2 μM for dADP, and 4.8 μM for dAMP). For dAMP, this means a binding stoichiometry of 1 probe for each 2 TACN-Zn2+ complexes. With an estimated 70 thiols covering the gold nanoparticle, this implies that at saturation around 35 dAMP probes are localized on the surface.

Information on the relative affinity of the probes for the monolayer surface was obtained from a series of competition experiments between ATP and the MANT probes (Figure 4b). In these experiments, increasing amounts of the ATP competitor were added to a MANT probe bound to the surface of Au MPC 1:Zn2+, and the fluorescence emission from the released MANT probe was measured at 448 nm. These displacement experiments provide information on the relative affinity between ATP and the respective MANT probe. The displacement of 50% dAMP (1.75 μM) required 4.0 μM of ATP corresponding to a relative binding affinity of around 2 in favor of dAMP. The very similar binding affinity is remarkable considering the presence of just 2 negative charges in dAMP against 4 for ATP. Another remarkable observation is that the addition of up to 1 mM ATP just replaced 0.75 μM dAMP (25%) from the surface. A control experiment in which ATP and dADP were added in reverse order excluded the fact that these distributions were the result of kinetically slow exchange processes (Supporting Information).

Controlling the Properties of a Dynamic Heteromeric Surface. The ability to regulate the binding affinity of small molecules to the monolayer surface is a key element in the application of these supramolecular structures as responsive systems. The peculiar high affinity of the MANT probes offers some important possibilities that will be illustrated by two examples. The first is the possibility to exchange one component from a heteromeric surface exclusively. A heteromeric surface was simply generated by adding WDDD and dATP against 4 for ATP. Another remarkable observation is that the addition of up to 1 mM ATP just replaced 0.75 μM dAMP (25%) from the surface. A control experiment in which ATP and dADP were added in reverse order excluded the fact that these distributions were the result of kinetically slow exchange processes (Supporting Information).

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Previously studies had shown that in the absence of Zn2+ the SSC of ATP decreased from 2.5 to 0.8 μM at pH 7.0, illustrating the importance of the metal ion in having a high SSC (Figure 3). Furthermore, at pH 8.0 a very low SSC value of 0.2 μM was measured for ATP on Au MPC 1. On the contrary, dATP showed nearly no decrease in SSC at pH 7.0 (2.8 μM) in the absence of Zn2+ and still a significant value of 1.8 μM at pH 8.0 (Figure 3). It is also noted that at pH 8.0 a shift in the emission wavelength was observed during the fluorescence titration (Supporting Information). So whereas for the ATP probe the presence of Zn2+ (or, more generally, a positive charge) in the monolayer is essential to obtaining a significant SSC, these experiments show that this is much less relevant for dATP. Although the SSC in itself does not provide information on the strength of the interaction (such information is obtained from the competition experiments described below), these results are indicative of a difference in the binding mode between ATP and dATP. For that reason, we extended our studies to dADP and dAMP. Interestingly, notwithstanding the reduced negative charge, both compounds still bound Au MPC 1:Zn2+ under saturation conditions at a headgroup concentration of 10 μM (Figure 4a).

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MANT or tryptophan indicates that both probes are quantitatively bound to the monolayer surface. The addition of ADP as a competitor results in the exclusive displacement of WDDD from the surface as witnessed by the full recovery of the expected fluorescence of WDDD and the complete absence of fluorescence emission from dATP<sub>MANT</sub> (Figure 5a). This is significant progress compared to our previous studies in which a selectivity factor of just 7 between two different probes was achieved. The second example exploits the high affinity of dATP<sub>MANT</sub> for Au MPC 1 even in the absence of Zn<sup>2+</sup>. By taking advantage of a protocol reported recently, this generates the possibility to catch and release the WDDD probe exclusively from the same heteromeric surface used previously. Thus, the valency of the obtained supramolecular nanostructure.

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the ERC (StG-239898) and COST (CM0703 and CM0905) is acknowledged.

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(27) The concentration of TACN headgroups is determined as reported previously from kinetic titrations using either Zn(NO₃)₂ or Cu(NO₃)₂. The accuracy of the obtained concentration is 10 ± 1 μM.


(30) The 1:2 stoichiometry was independently confirmed by catalytic inhibition experiments using a protocol described before (Supporting Information).

(31) These values are approximated because the probe is present at a concentration slightly below the SSC to ensure the complete absence of initial fluorescence. Consequently, initial additions of ATP may result in surface binding without displacing the probe. This is also the reason that the initial points were excluded from the fitting of the dAMP<sub>SLM</sub> curve.