J|A|C|S
COMMUNICATIONS

Published on Web 08/14/2004

Recognition and Stabilization of Peptide α-Helices Using Templatable Nanoparticle Receptors

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α-Helices are an important secondary structural motif, playing a ubiquitous role in mediation of numerous protein−protein surface interactions. Rational design of synthetic scaffolds for specific and selective recognition of α-helices, however, remains a challenging goal. This challenge primarily stems from the large and elongated surface area presented by an α-helix, as well as the correct matching of surface topology in terms of the hydrophobic and electrostatic complementarity. Several successful strategies for recognition and binding of α-helical units have made use of synthetic scaffolds.[1] However, quantification studies demonstrating recognition and stabilization of peptide α-helices through the use of various receptors have revealed that significant stabilization and affinity to complementary peptide surfaces in completely aqueous systems is difficult to achieve.[1a,b]

A promising approach for increased receptor affinity to biomolecules is through templation of the host to the surface of the target protein. This has been achieved via strategies such as template polymerization[2] through molecular surface[3] and gel[4] imprinting and also through metal imprinting[5] of enzymes. In earlier studies, we have demonstrated templation of gold mixed monolayer protected clusters (MMPCs) to a small-molecule flavin target.[6] The ability of nanoparticle monolayers to template, coupled with a large surface area they provide for target binding, makes them tools for biomolecular surface recognition and, hence, potential receptors to target α-helices. Here, we report the use of trimethylammonium-functionalized MMPCs to show (a) binding and significant stabilization of a tetra-aspartate peptide in completely aqueous solution and (b) templation of the host monolayer to the peptide surface through monolayer reorganization as evidenced by increase in helicity during incubation.

Peptide **1** was used to test the ability of MMPC **2** in recognition and stabilization of α-helices. Tetra-aspartate peptide **1** has the aspartate residues distributed at alternating *i*, *i* + 3, and *i* + 4 positions, providing a reasonably cofacial presentation of carboxylates suitable for recognition by the colloid surface (Figure 1). The peptide was synthesized[7] to include an N-terminal Trp to assist concentration determination[8] and to provide direct evidence for the binding of the peptide to the nanoparticle surface. Additionally, C- and N-terminal capping were introduced to reduce helix macrodipole effect.

Trp fluorescence was monitored to investigate the binding of the peptide to the nanoparticle.[9] Quenching of Trp fluorescence due to the gold upon binding to the MMPC monolayer has been observed in our previous studies.[10] The cofacial orientation of the Trp to the aspartate-binding surface (Figure 1b) enhances the sensitivity of the binding studies due to its proximity to the gold core, allowing a greater fluorescence quenching to occur.

As expected, addition of the nanoparticle to the peptide resulted in a proportional decrease in the fluorescence intensity, confirming binding of the peptide to the receptor surface (Figure 2a).

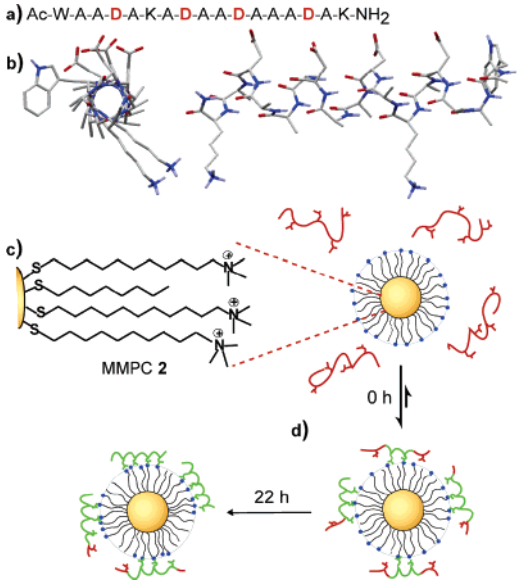

Figure 1. (a) Peptide **1** sequence. (b) End and side view of the tetraaspartate peptide. (c) MMPC **2** monolayer composition. (d) Schematic representation of the peptide binding to MMPC surface showing relative sizes of MMPC (2-nm core diameter) and peptide helix.

The binding of the peptide to the nanoparticle was investigated through CD titrations of MMPC **2** (0−10 μM) with peptide **1** (15 μM). This resulted in a significant increase in α-helicity (minima at 208 and 222 nm).[7] The resulting helicity[11] from each addition was analyzed[12] and plotted (Figure 2b). The overall helicity of the peptide in absence of the nanoparticle was found to be <4%. However, addition of the nanoparticles results in a significant increase in helicity (up to ∼60%) displaying a large affinity[13] of the peptide for the nanoparticle that results in a large stabilization (>15-fold) of the peptide α-helix. The overall helicity can be divided into regular and distorted helix (due to fraying at either or both ends).[14] Curve fitting of the CD data[15] indicates that increasing the nanoparticle receptor concentration results in a greater overall helicity over the peptide length compared to a more pronounced distortion of the helix at lower MMPC concentrations. The binding process displayed a certain amount of selectivity, as addition of negatively charged carboxylate-functionalized MMPCs to the peptide demonstrated no variation in the initial helicity.

To ascertain whether initial binding of the peptide to the nanoparticle surface was driven by complementary electrostatic interactions, the change in helicity on addition of nanoparticle (5 μM) to a 15-μM peptide **1** solution (which directly correlated with binding of the peptide) was monitored through CD spectroscopy in the presence of varying salt concentrations. As expected, increase in the salt concentration from 0 to 5 mM resulted in a more than 2-fold decrease in helicity, demonstrating attenuated binding at

10.1021/ja047719h CCC: $27.50

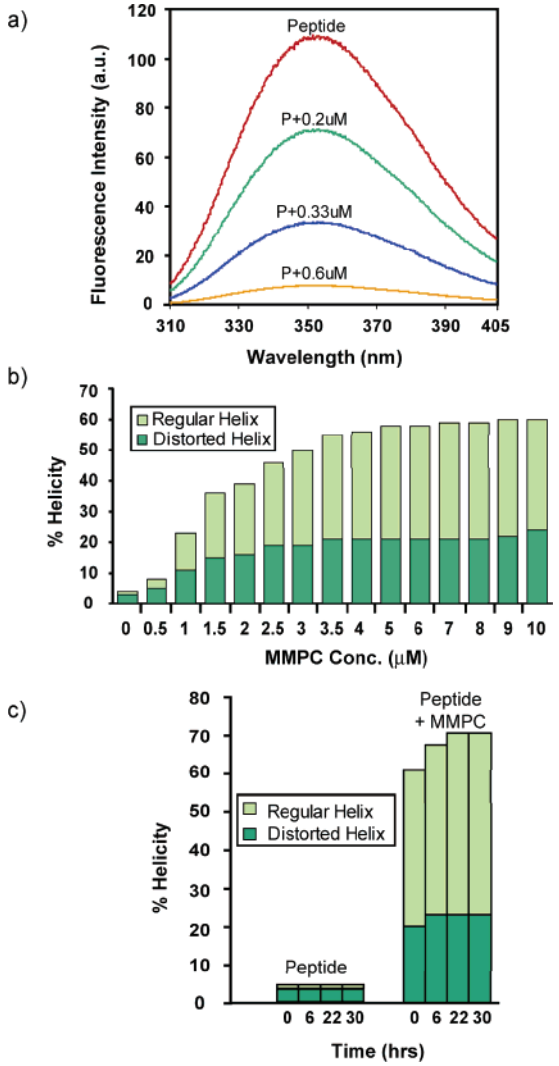

Figure 2. (a) Fluorescence spectra on addition of nanoparticle to a 1-μM peptide **1** solution. The fluorescence spectra were normalized for absorbance due to the gold by control studies using noninteracting nanoparticles. (b) Increase in peptide helicity on addition of nanoparticle receptors to a 15-μM peptide solution. (c) Increase in helicity over time on incubation of 15-μM peptide **1** solution with MMPC **2**, demonstrating receptor templation.

higher ionic strength.[7,16] A Job[17] CD titration was conducted between the host colloid and the peptide to determine the stoichiometry of the association. Maximum signal change for the Job titration was observed at >0.8 mol fraction of the peptide **1**, indicative of ~1:4 MMPC **2**-to-peptide **1** complexation.[7]

To investigate time dependence of peptide helicity, and hence templation, the peptide was incubated with the trimethylammonium-functionalized nanoparticles, and helicity was determined as a function of time. Increased incubation time (22 h) displayed ~20% increase in helicity, indicating templation of the nanoparticle monolayer to the peptide surface through monolayer reorganization[6] (Figure 2c). This demonstrates the ability of MMPCs to template to large surface areas, providing additional stabilization through increased favorable interactions.

In summary, we have demonstrated that trimethylammonium-functionalized MMPCs effectively recognize and stabilize a tetra-aspartate peptide in water. It is observed that while initial electrostatic complementarity mediates binding, further stabilization is achieved through additional favorable interactions on templation of nanoparticle to the peptide surface. This enhances the potential utility of MMPCs in helix recognition, which can be applied toward targeting helical domains in proteins with significant affinity and selectivity. Additionally, the ability of the monolayer to template to the peptide surface provides a potent tool to generate protein surface-specific designed MMPCs through strategies such as monolayer cross-linking.[18] Such studies are underway and will be reported in due course.

Acknowledgment. This research was supported by the National Institutes of Health (GM 62998). We are grateful for assistance from Ken Rotondi and Nicholas O. Fischer for pictorial representation.

Supporting Information Available: Synthesis and characterization of peptide **1**, experimental procedure for CD, fluorescence and NMR and CD spectra of nanoparticle + peptide, and NMR results. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) The CD spectra were analyzed by the secondary structure program CDSSTR. For all spectra, the normalized mean root square deviation (NMRSD) values were <0.02 displaying excellent agreement of the data with the curve fitting program.

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JA047719H