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Peptide Ligation Catalyzed by Functionalized Gold Nanoparticles

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Recently, the capability of trimethylammonium functionalized gold nanoparticles (GNPs) to promote the folding of a negatively charged peptide into an α-helix was established.1 This design allowed favorable electrostatic interactions between the nanoparticle and the peptide when the negatively charged residues were positioned in a cofacial manner along the helix and was responsible for the assisted folding observed. In this paper, we demonstrate the use of such functionalized GNPs to template the assembly of peptide fragments and promote their ligation.

GNPs provide several advantageous attributes that make them versatile scaffolds for biomolecular surface recognition through complementary supramolecular interactions.2 These receptors have been used for numerous applications in biological systems ranging from the control of protein structure and function to light “triggered” gene delivery.3,4 Some studies have also focused on the use of organic monolayer-protected gold nanoparticles for catalysis of reactions that involve cleavage of bonds.5,6 However, the use of nanoparticles to assist bond forming reactions is relatively unexplored7 and supramolecular catalysis has not been demonstrated. In our current study, we demonstrate the use of electrostatic interactions to bring peptide fragments together on the nanoparticle surface to catalyze a coupling reaction (Figure 1).

In previous studies, a self-replicating peptide, E1E2, was designed to be responsive to pH,8 as it has a high level of glutamic acid residues and is only helical at acidic pH. Templation of its fragments, E1 and E2, and subsequent replication also occurs at acidic pH. As with the previous tetraaspartate peptide,1 we envisioned that the GNPs would bind to and promote the helicity of the E1 and E2 fragments at neutral pH, thereby acting as a template to assist their ligation to E1E2. The ligation of E1 and E2 would be realized via Kent’s native chemical ligation, where E1 contains a thioester at its C-terminus and E2 a cysteine at its N-terminus (Figure 2).9

Circular dichroism spectroscopy was used to evaluate the ability of the functionalized, cationic GNPs to induce helicity in the fragments and full-length peptide product. The helicity of E1E2, E1, and E2 (15 μM) with increasing amount of GNPs (0–6 μM) in phosphate buffer (pH 7.4) was assessed. The results demonstrated a significant increase in α-helicity for the three peptides with added GNPs (Figure 3). Maximum helical contents of 62% and 64% were achieved for E1E2 and E2, respectively, with a lower helical content overall for E1 (35%). Previous studies had shown that E1E2 was 85% helical at pH 4,8 somewhat higher than that observed at neutral pH with GNPs. This may be attributed to the curvature of the nanoparticle surface which may impede the generation of a higher helical content owing to the comparable sizes of E1E2 (~5 nm length) and the nanoparticle scaffold (~6 nm diameter). However, at pH 4, the fragments E1 and E2 were only 20% helical, significantly lower than that observed in the current experiment.

A Job titration was conducted using CD to assess the maximum number of E1E2 peptides bound to the cationic GNPs. The maximum helicity was observed at a 0.8 molar fraction of E1E2 to
GNPs, which correspond to a stoichiometry of ~4 peptides per GNP (See Supporting Information). These data are quite similar to the stoichiometry reported for GNPs and the previously studied 17 amino acid residue.

Isothermal titration calorimetry was used to probe the affinity of each peptide for the cationic GNPs; E1 was found to bind the tightest, followed by E2 and E1E2 (Table 1). In principle, this binding process would be enthalpically favorable because of theionic interactions between the negatively charged peptides and the cationic GNPs, but entropically disfavored because of the formation of an ordered helical structure from an unordered, random coil peptide. An examination of the thermodynamic parameters, however, indicates a more complex scenario. For instance, one would have predicted that the binding of E1 to GNPs would have been more enthalpically favorable as compared to E2, owing to the higher net negative charge for E1 (−3) as compared to E2 (−1) at pH 7.4. The opposite is observed, however, perhaps due to additional hydrophobic interactions that may occur with the Leu residues of the more ordered, helical E2 peptide and the lipophilic groups on the GNPs. It is true that the binding of the least helical peptide, E1, to the GNPs is the most entropically favored, presumably due to less reorganization of its structure upon binding. Overall, one may conclude that entropic changes are more significant than enthalpic changes for the binding of these peptides to GNPs.

Having demonstrated that the cationic GNPs bind to and template the folding of the E1E2 peptide and its fragments, we next explored the specificity of peptide binding to GNPs. An examination of the thermodynamic parameters, however, indicates a more complex scenario. For instance, one would have predicted that the binding of E1 to GNPs would have been more enthalpically favorable as compared to E2, owing to the higher net negative charge for E1 (−3) as compared to E2 (−1) at pH 7.4. The opposite is observed, however, perhaps due to additional hydrophobic interactions that may occur with the Leu residues of the more ordered, helical E2 peptide and the lipophilic groups on the GNPs. It is true that the binding of the least helical peptide, E1, to the GNPs is the most entropically favored, presumably due to less reorganization of its structure upon binding. Overall, one may conclude that entropic changes are more significant than enthalpic changes for the binding of these peptides to GNPs.

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Supporting Information Available: Peptide characterization, Job plot of CD data, ITC data, and experimental protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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