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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 μ M) with increasing amount of GNPs (0–6 μ M) 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,

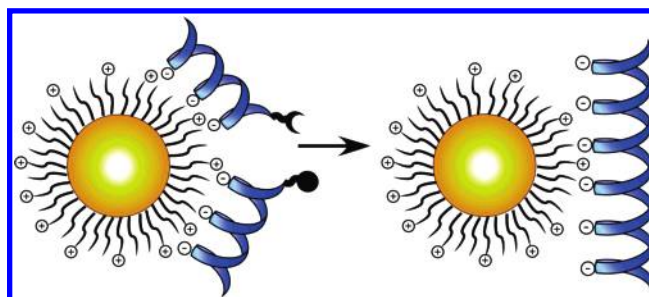


Figure 1. The design of functionalized, cationic GNPs as a template for peptide ligation.

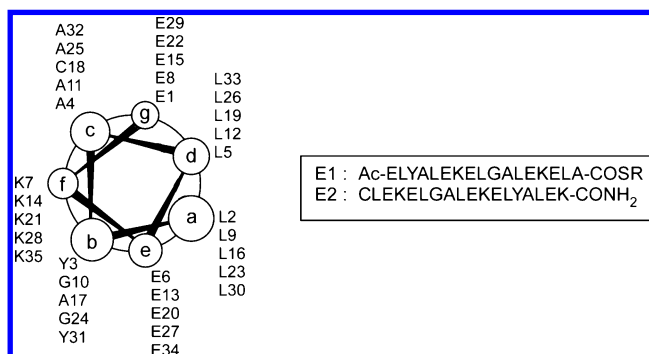


Figure 2. A helical wheel diagram for the peptide E1E2 and the sequence of its fragments, E1 and E2 (R = CH₂CH₂CO₂Et).

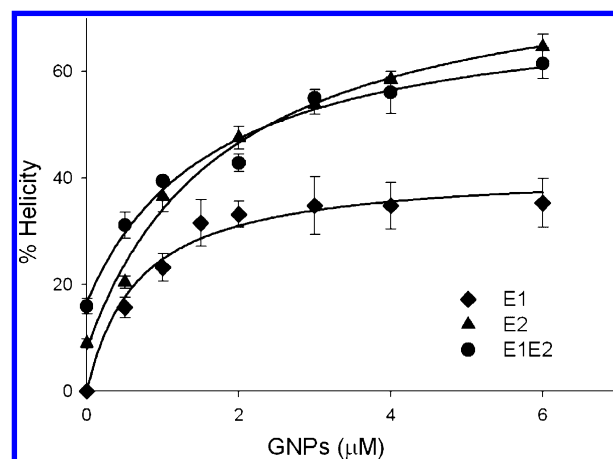


Figure 3. Helicity of E1E2, E1, and E2 (15 μ M) with added cationic GNPs in 5 mM phosphate buffer at pH 7.4.

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

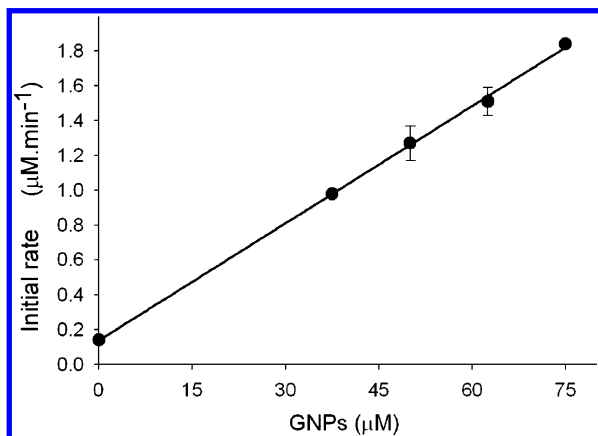
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Table 1. Thermodynamic Parameters for Complexation of Cationic GNPs and Peptides^a

peptides	K_a (10^6) M^{-1}	ΔH kcal mol^{-1}	ΔS cal $K^{-1} mol^{-1}$
E1	6.7 ± 1.0	-68.5 ± 1.6	-194.5
E2	4.1 ± 0.4	-94.6 ± 1.5	-282.0
E1E2	1.5 ± 0.1	-143.2 ± 3.7	-443.5

^a ITC measurements were carried out with peptide (40 μM) at 30 °C in 5 mM phosphate buffer at pH 7.4 with added GNP.

**Figure 4.** Initial rate of E1E2 production versus the concentration of GNPs.

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.¹

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 the ionic 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 ability of the GNPs to bring together the fragments E1 and E2 and promote their ligation. The ligation experiments were performed with 250 μM of the fragments, in phosphate buffer at pH 7.4, with increasing amounts of cationic GNPs. A reducing environment, necessary for Kent's chemical ligation, was obtained by using 0.3% ethyl 3-mercaptopropionate v/v. Product formation in the reactions was monitored with time by analytical HPLC, and initial rates of reaction were obtained (Figure 4).

The data demonstrate a clear increase in the rate of production of E1E2 with addition of cationic GNPs as compared to the control reaction with no GNPs. Increasing the amount of GNPs induced a faster production rate, thereby demonstrating the ability of the

cationic GNPs to act as a template for ligation. It is possible that at a much higher concentration of the cationic GNPs the ligation rate would begin to decrease, as each peptide fragment bound to a separate nanoparticle, but this was not observed under the conditions used in these experiments. A control reaction was carried out in which GNPs (37.5 μM) that were functionalized with 11-mercaptopoundecanoic acid (negatively charged) were added to the ligation reaction. No increase in the production of E1E2 was observed as compared to the background reaction, presumably because of electrostatic repulsion between the anionic GNPs and peptide fragments. In an alternative control reaction tetramethylammonium bromide (1 mM) was added to the ligation reaction to determine the effect of monomeric cations on the ligation reaction. Again there was no observable increase in product formation over the control ligation reaction, demonstrating the role of templation in the catalysis. While the template effect is expected to be the predominant source of catalysis, other factors arising from the supra-molecular association of the peptide fragments and particle could also contribute to the increased ligation rate, including decreased pK_a values for the cysteine thiol and the terminal, protonated amine of E2 in the presence of the cationic nanoparticles, and possible stabilization of the anionic transition state of the ligation reaction by the cationic GNPs.

In summary, we have successfully designed a system whereby functionalized gold particles promote the association and ligation of peptide fragments. The complementary electrostatic interactions between the peptide and the GNPs are presumably the major cause for templation and subsequent ligation. Significantly, this study highlights the utility of nanoparticle surfaces for mediating supra-molecular coupling reactions that can be extended toward other relatively large scaffolds that require appropriate positioning of the reactive centers. This system may also serve as a simplified model for prebiotic conditions in which small charged inorganic particles may have assisted the polymerization of early biopolymers.¹⁰

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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>.

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