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Short Communication

Binding of nanoparticle receptors to peptide α-helices using amino acid-functionalized nanoparticles‡

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Abstract: Nanoparticles provide large surface areas and controlled surface functionality and structure, making them excellent scaffolds for peptide recognition. A family of nanoparticles has been fabricated by amino acid functionalization to afford tailored surfaces. These particles are complementary to a tetraaspartate peptide (TAP) featuring cofacial anionic functionality when in the α-helical conformation. The functional groups present on these nanoparticle surfaces provide a tool to investigate the contribution of various noncovalent interactions at the nanoparticle–peptide interface. The ability of these particles to enforce the folding of the peptide into an α-helix was explored, demonstrating high helicity induction with particles featuring dicationic amino acids such as lysine or histidine, and little or no helix stabilization with hydrophobic amino acid termini. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

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Keywords: nanoparticle; amino acid; scaffold; α-helix; recognition

INTRODUCTION

Protein–protein interactions are driven by a combination of noncovalent interactions and shape complementarities [1]. The interaction of a helical segment with a shallow cleft on the binding partner is a particularly prevalent motif [2]. One important example is represented by the p53–HDM2 protein pair, where the hydrophobic residues on helical surface of p53 bind to a deep hydrophobic cleft of HDM2 (human double minute 2) [3]. Given the importance of this recognition motif, targeting of α-helices by synthetic receptors is crucial for biomedical applications [4]. A variety of synthetic receptors have been developed to bind short peptides and stabilize them into α-helical structure. These strategies include the use of cyclodextrin dimers [5], metal ions [6] and guanidinium-based scaffolds [7,8]. The challenge, however, arises in promoting the folding of a short peptide fragment into α-helix in completely aqueous solvent [9–11].

The size and ready functionalization of nanoparticles make this system an attractive scaffold for the creation of receptors for biomacromolecules [12–15]. We have extended the use of functionalized nanoparticles to assist helicity induction. In initial studies, trimethyl ammonium functionalized gold nanoparticles were used to stabilize a tetraaspartate peptide (TAP) into an α-helical conformation in aqueous medium [16]. We have further demonstrated that this recognition can be utilized to catalyze native peptide ligation [17]. In contrast to biomacromolecular systems, however, these particles lacked structural diversity. Therefore, we synthesized a series of cationic nanoparticles via functionalization with naturally occurring L-amino acids to explore the effect of head-group structure on recognition of peptide α-helices (Figure 1). The presence of structural diversity in amino acids provides ready access to a library of nanoparticles for probing the correlation between surface functionality and capability of inducing helicity [18,19].

MATERIALS AND METHODS

Materials

All chemicals were bought from Aldrich and used as received. Solvents were purchased from Pharmco–Aaper and used without further distillation, except dichloromethane and toluene which were purified according to the literature procedures. Flash column chromatography was performed over silica gel (SiO_2, particle size 40–63 μm) for purification of the synthesized compounds.

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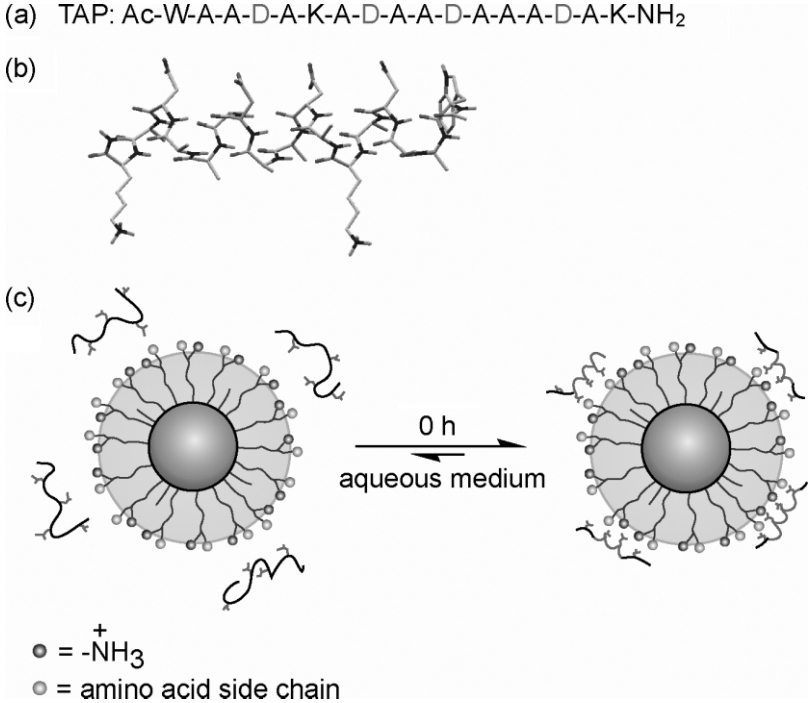

Figure 1 (a) Amino acid sequence of the peptide, (b) Side view of the peptide, (c) Complexation between peptide and amino acid-functionalized nanoparticles in aqueous medium.

Synthesis of the Peptide

The peptide (TAP) was synthesized using conventional solid-phase peptide synthesis technique based on Fmoc chemistry. A reverse-phase HPLC was performed to purify the crude peptide. Molecular weight of the peptide was determined to be 1672.6 from electrospray mass spectrometry (expected 1672.648).

Synthesis of the Nanoparticles

The thiol ligands were synthesized according to recently reported literature procedures [20]. The synthesized thiols were incorporated onto nanoparticles via place-exchange reaction with 1-pentanethiol protected gold colloids (Au_C_5, core diameter = ~2 nm). In a typical procedure, Au_C_5 (50 mg) and the thiols (100 mg) were dissolved separately in dichloromethane. The solutions were purged with argon for 30 min and then they were mixed together. After 48 h, the precipitated particles were washed with dichloromethane to get rid of free ligands. The particles were characterized by NMR in D_2O, showing absence of any free ligands and incorporation of the foreign thiols. The use of excess thiol provided efficient exchange; there was no residual peak of the native thiols in NMR spectra.

Circular Dichroism Experiments

A mixture of 15 μM peptide and 4 μm nanoparticles was prepared in AcOH/NaOH buffer (5 mM, pH 5) and taken in an 1-mm quartz cuvette. After 5-min incubation at 25 °C, the spectra were collected from 250 nm to 190 nm on a Jasco 720 spectrophotometer. Average of three scans was recorded by scanning at a rate of 20 nm/min with response of 8 s. The final spectra were obtained after subtracting the blank (4 μm nanoparticles without any peptide in the buffer to compensate for any CD signature from the nanoparticle ligands), and fitted into secondary structure algorithm CDSSTR (protein ref. set 7 comprising of 49 proteins) using DICHROWEB [21].

RESULTS AND DISCUSSION

Design of the Peptide

The targeted peptide, TAP consists of 17 amino acid residues, and it was designed such that four aspartic acids reside at alternating i, i + 3, and i + 4 positions. This strategy helps to place the carboxylate groups in a cofacial manner and hence fosters the recognition by nanoparticle surface (Figure 1). Additionally, *C*- and *N*-terminal were capped by amidation and acetylation, respectively, to reduce helix-macrodipole effect.

Fabrication of Amino Acid-Functionalized Nanoparticles

A family of nanoparticles was fabricated by tailoring the monolayer with four different amino acids to provide structurally diverse surfaces. The amino acid-conjugated ligands were synthesized in a straightforward procedure (Scheme 1). Trityl thiol was reacted with 11-bromoundecanol to yield tritylmercaptoundecanol. The alcohol end was conjugated with free carboxylic acid of amino acid via DCC (*N*,*N*-dicyclohexylcarbodiimide) coupling. The protecting groups were removed by trifluoroacetic acid (TFA) to

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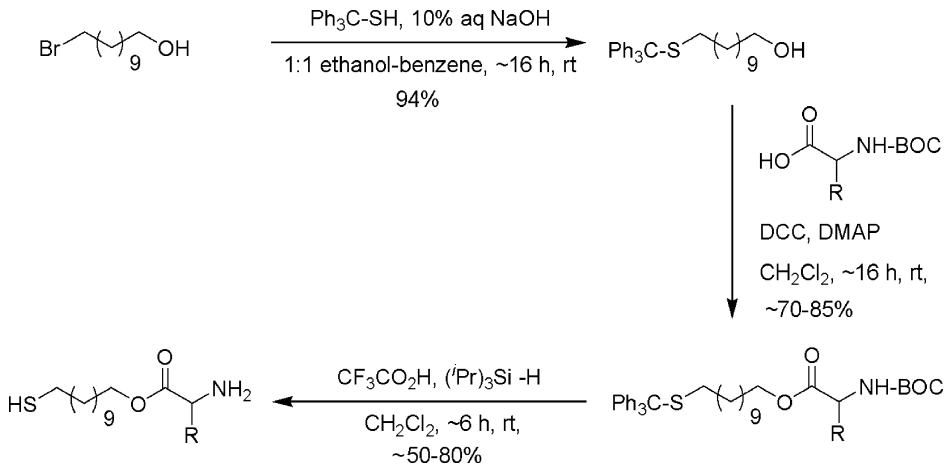


Scheme 1 Synthetic protocol for conjugating amino acids to the ligands.

deprotect the thiol and amine moieties at the termini. The synthesized ligands were grafted onto the particle surface *via* the Murray place-exchange reaction [22] with 1-pentanethiol protected gold nanoparticles (Scheme 2).

Circular Dichroism Study

First, the solution structure of the peptide alone was studied in 5 mM AcOH/NaOH buffer (pH 5) using circular dichroism (CD). The strong negative peak around 200 nm indicates that the peptide itself adopts a random coil conformation (Figure 2). Next, the ability of amino acid-functionalized nanoparticles to induce helicity was investigated by incubating the nanoparticles (4 μM) with the peptide (15 μM) in AcOH/NaOH buffer (5 mM, pH 5). The characteristic peaks of α-helix, maximum at 192 nm and minima at 208 nm and 222 nm, were prominent in the CD signal for peptide–NP_L-Lys complex (Figure 2).

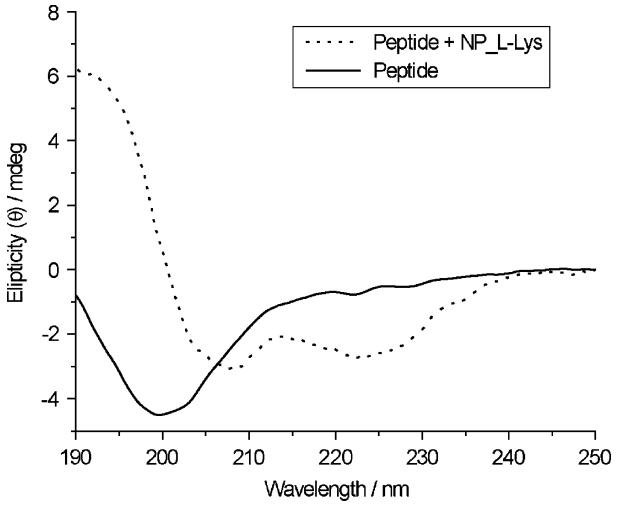
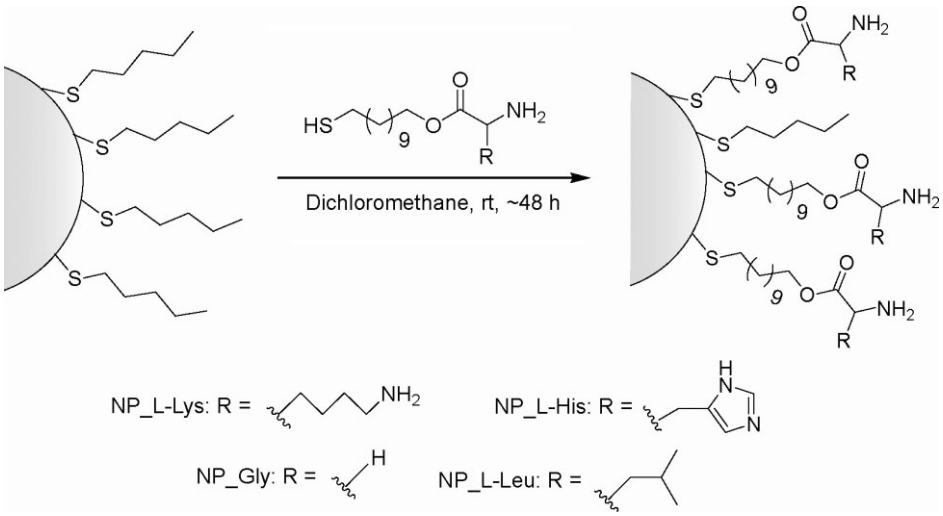


Figure 2 CD spectra of the peptide alone and peptide–nanoparticle complex in 5 mM AcOH/NaOH buffer (pH 5).



Scheme 2 Incorporation of amino acid-conjugated thiols onto nanoparticles through place-exchange reaction.

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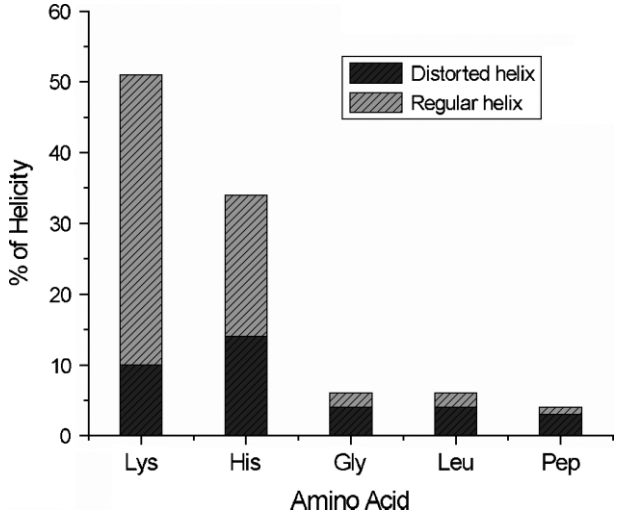

Figure 3 DICHROWEB analysis to compare the ability of helix stabilization by different amino acid-conjugated nanoparticles.

The collected CD spectra were analyzed using DICHROWEB to compare the capability of nanoparticles in promoting the conformational change from random coil into α-helix. The overall helicity was determined by combining the regular helix and distorted helix obtained from DICHROWEB analysis. The results clearly indicate that the extent of helicity induction in this system depends on cationic charge density on the nanoparticle surface (Figure 3). Among all the nanoparticles, NP_L-Lys showed maximum helix stabilization (up to 52%) because of enhanced electrostatic interaction governed by the side-chain ammonium group of lysine. NP_L-His stabilizes the α-helix moderately (35%), presumably because of partial protonation of the imidazole group of histidine side chain at experimental conditions. However, NP_Gly and NP_L-Leu did not assist the folding of the peptide into α-helix, indicating that decrease in cationic charge density or introduction of hydrophobic moiety prevents the recognition of α-helices (see supporting information for CD spectra of nanoparticle–peptide complexes Figure S1).

CONCLUSIONS

In summary, we have demonstrated α-helix stabilization in aqueous medium using nanoparticles featuring amino acid-terminated side chains. The extent of helicity induction can be tuned by tailoring the structure of the ligands comprising the monolayer of the nanoparticles. In particular, it was found that dicationic nanoparticles were much more effective at stabilizing the helical structure of the adsorbed peptides than monovalent analogs. These results suggest that dendritic structures may be very efficient at templating helicity, an approach currently under investigation.

Supplementary Material

Supplementary electronic material for this paper is available in Wiley InterScience at: http://www.interscience.wiley.com/jpages/1075-2617/suppmat/

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