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RESEARCH PAPER

Study of Efficiency of Coupling Peptides with Gold Nanoparticles

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Abstract: Fluorescence spectroscopy was used to investigate the efficiency of coupling peptides to gold nanoparticles via 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride-*N*-hydroxysuccinimide (EDC-NHS). In this study, the effects of experiment conditions including the buffer solution (PBS, HEPES, Tris-HCl, and borate buffer solution), pH (6.5–9.0) and concentrations of buffer solution (10, 25, 40 and 50 mM), concentrations of NHS and EDC (from 0.2 M NHS-0.01 M EDC to 1.0 M NHS-0.5 M EDC), ratios of NHS to EDC (0, 0.5, 1.0, 2.0 and 2.5), and the coupling reaction time (4, 8, 12, 24 and 36 h) on the coupling efficiency were investigated and the optimized conditions were developed. These results indicated that the optimal experimental conditions were: pH 7.0 and 25 mM of the HEPES buffer solution, the concentrations ratios of NHS to EDC of 2:1 (0.4 M NHS-0.2 M EDC), and coupling reaction time of 24 h.

Key Words: 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; *N*-Hydroxysuccinimide; Gold nanoparticles; Peptides

1 Introduction

The conjugation of peptides or proteins to gold nanoparticles have a huge applications in chemistry, biology, medicine, and so on, because gold nanoparticles have unique optical properties, such as surface plasma resonance absorption and resonance light scattering, the surface that can be easily modified with ligands containing functional groups and excellent biocompatibility[1–3]. The conjugation of peptides to gold nanoparticles were applied into biomimetic systems for imunorecognition[4], transcription factor[5,6], enzyme mimic[7], and protein inhibitor[8]. Usually, the capture of peptides on the surface of gold nanoparticles via 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride-*N*-hydroxysuccinimide (EDC-NHS) activation involve a two-step procedure. Firstly, thiol-containing alkane carboxylic acids are bonded onto gold nanoparticles' surface via Au-thiol chemistry to form carboxylic acid terminated monolayer. Secondly, the terminated carboxylic acids are conjugated with peptides or proteins via EDC-NHS coupling to form amide bond[5,6,9,10]. EDC-NHS reactions have some advantages, such as mild reaction conditions, outstanding biocompatibility, little influence on biomolecules, and much cleaner products[11]. However, quite different coupling conditions including concentrations of NHS and EDC, buffer solutions, and pH were used by different groups[12–15]. It was reported that activation conditions of carboxylic group-containing polymer and products by EDC-NHS reactions depend on the polymer[16,17]. The morphology of the substrate also effects on the efficiency of EDC-NHS coupling[13]. However, conjugation of peptides or proteins to gold nanoparticles by EDC-NHS coupling still has some challenges because of the instability of gold nanoparticles in the process of coupling, the hydrolysis of intermediate, and lower coupling efficiency[13,17]. So, it is necessary to carry out a systematic investigation on coupling peptides onto gold nanoparticles because of the

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scientific significance of peptide-gold conjugations. The reaction between carboxylic acid and amino groups by EDC-NHS coupling from the initial intermediate of *O*-acylisourea to stable intermediates goes through the following three pathways: (1) EDC reacts with carboxylic acid to generate the intermediate, *O*-acylisourea, which can be readily hydrolyzed to reproduce carboxylic acid; (2) *O*-acylisourea is converted to NHS-ester by reaction with NHS which can increase the stability of active intermediates and the NHS-ester is more easier to react with amino group of peptide or protein to form amide bond; (3) both NHS-ester and *O*-acylisourea can couple with amino-containing peptides or proteins to generate the biomolecule-immobilized surface product (Fig.1). The intermediates in this process are readily hydrolyzed, which require experimental procedures as simple as possible.

In this work, one pot method was adopted, in other words, EDC and NHS were simultaneously added into the mixture of rhodamine B dye labelled SHELKLKLKL peptide and 11-mercaptoundecanoic acid modified gold nanoparticles. 20-nm Gold nanoparticles were synthesized according to previous reports[18–21], which were verified by transmission electron microscope and UV-visble spectrophotometry The experimental conditions was investigated for coupling rhodamine B dye labelled peptides of SHELKLKLKL which was combined with nanomaterials to have hydrolase activity[22]. The coupling reaction efficiency was further investigated by measuring the fluorescence intensity of the conjugations. Experiment conditions including the buffer solution, pH value and concentrations of buffer solution, concentrations of NHS and EDC, concentration ratios of NHS to EDC, and the coupling reaction time were investigated and the optimized conditions were proposed in this study.

2 Experimental

2.1 Chemicals

1-Ethyl-3-(3-(dimethylamino) propyl) carbodiimide) (EDC, 99%) was purchased from J & K Scientific Ltd. (Beijing, China). *N*-Hydroxysuccinimide (NHS, 98%) was purchased from Aladdin Co. Ltd. (Shanghai, China). Auric acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Trisodium citrate (98%) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). Peptides of the rhodamine B dye labeled N-SHELKLKLKL-C were purchased from Bootech BioScience & Technology Co. Ltd. (Shanghai, China).

2.2 Synthesis of gold nanoparticles

Gold nanoparticles were prepared according to the well-established citrate reduction method[18–21]. 100 mL aqueous $HAuCl_4$ (0.25 mM) was prepared in a 250 mL flask. The solution was brought to boil while being stirred, and 2.374 mL of 5% aqueous sodium citrate was added. The reaction was kept for 20 min until a wine red color solution was obtained, indicating the reaction was completed. 1 mL anhydrous ethanol solution of 11-mercaptoundecanoic acid (MUA) was added to the gold nanoparticles solution (4 mL) and incubated for 24 h, and then the un-reacted MUA was removed to obtain the MUA modified gold nanoparticles.

2.3 EDC-NHS coupling reactions

In a reaction, a solution of the peptide labelled by rhodamine B (20 μL, 10 mg mL^{-1}) was added to 1 mL gold nanoparticles

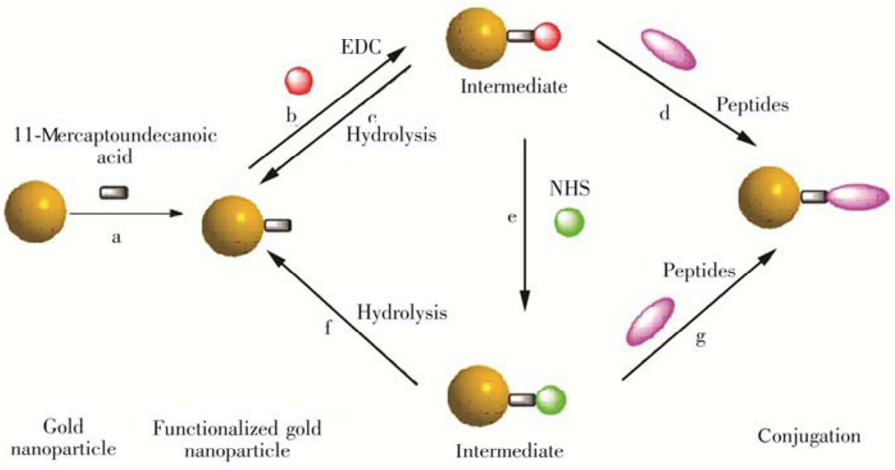

Fig.1 Mechanism of coupling peptides to gold nanoparticles. (a) Gold nanoparticles are modified with 11-mercaptoundecanoic acid. (b) The carboxylic acid on modified gold nanoparticles reacts with EDC to produce the first intermediate of *O*-acylisourea. (c) The unstable *O*-acylisourea is hydrolyzed to regenerate carboxylic acid. (d) The unstable *O*-acylisourea reacts with peptide to form the conjugation. (e) The unstable *O*-acylisourea is converted to NHS-ester by reaction with NHS. (f) The NHS-ester can be hydrolyzed to regenerate carboxylic acid. (g) The NHS-ester inclines to react with peptide to form the conjugation.