Biomaterials 35 (2014) 3480–3487

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Cell-selective intracellular drug delivery using doxorubicin and α-helical peptides conjugated to gold nanoparticles

Hyejin Park, Hiroshi Tsutsumi, Hisakazu Mihara*

Department of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta-cho 4259-B40, Midori-ku, Yokohama 226-8501, Japan

ARTICLE INFO

Article history:
Received 16 December 2013
Accepted 30 December 2013
Available online 15 January 2014

Keywords:
Cell internalization
Drug delivery
Nanoparticles
Peptides

ABSTRACT

Cell penetrating peptides (CPPs), which can enter a cell through the cell membrane, have potential research applications in the fields of drug delivery, gene therapy, and cancer therapy. However, CPPs are associated with problems such as low cell selectivity, low cell penetrating activity, and cell toxicity. To overcome the disadvantages of CPPs, we constructed a drug delivery system by developing 25 nm gold nanospheres (GNSs) conjugated to four α-helical CPPs from our peptide library. We examined the applicability of this cell-selective drug delivery system by evaluating its cell-penetrating and cell death activities and comparing them with those activities of the TAT peptide. Using the 25 nm GNS, we obtained higher cell death induction activity by the anti-cancer drug doxorubicin compared with our previous study using a 41 nm GNS. After entering the cell, the peptide-conjugated 25 nm GNS accumulated around the cell nucleus. High cell selectivity by α-helical CPP sequences was also demonstrated. Our results indicate that these α-helical peptide and 25 nm GNS conjugates are useful elements in an efficient cell-selective drug delivery system.

1. Introduction

Intracellular drug delivery systems have been used as efficient approaches for studies of human disease care, including cancer therapy. However, several factors are required for the successful application of these systems: 1) high internalization efficiency, 2) a high payload of drugs, 3) non-degradation of the drugs in extracellular space, and 4) low toxicity and immunogenicity. Viral vectors and non-viral vectors such as liposomes and cell-penetrating proteins and peptides have been extensively studied for intracellular drug delivery [1–5]. Among these materials, cell-penetrating peptide (CPP)-based intracellular delivery has several advantages, including ease of synthesis and the possibility of sequence modification [6,7]. Typical CPPs, such as TAT, show high cell-penetrating (CP) activity; however, these peptides have low cell selectivity, which is an important factor, especially for cancer therapy applications.

Organic fluorescent dyes have been widely used for molecular imaging in chemical and cellular biology. However, they are associated with problems such as insolubility and photobleaching [8–10]. Nanometer-sized particles, such as gold and silver nanoparticles, quantum dots, and magnetic particles, have been studied in the fields of biosensing, bioimaging, and drug delivery, with a focus on their *in vivo* applications [11–14]. Gold nanospheres (GNSs) possess advantages such as high biocompatibility, high solubility, and high bioconjugation ability, as well as physical properties such as strong absorption and scattering. Therefore, GNSs have been well utilized in the delivery of various molecules, including DNA, siRNA and protein, and in the molecular imaging of cells and tissues.

Owing to the possibility of modifying the sequences of CPPs, we constructed a library of 16-amino acid peptides that showed different CP activities in four cell lines [15–19]. Previously, we selected two peptide sequences from this library, conjugated them to a 41 nm GNS (P-GNS41), and evaluated their CP activities and cell death activity by an anti-cancer drug, doxorubicin (DOX), conjugation in three cell lines [19]. To achieve greater cell death activity than our previous research, a nanoprobe should accumulate around/in the cell nucleus and should have a high DOX release rate from the GNS surface. Therefore, recent studies have employed nuclear targeting using, for example, such as nuclear localization signal (NLS) peptides [20–24] and pH-sensitive materials [25–27]. Smaller-sized GNSs have also been reported to pass through the nuclear membrane [28–30]. In this study, we examined the feasibility of using 25 nm GNS instead of 41 nm GNS [19] for nuclear targeting. We constructed five nanoprobes conjugated to either the

* Corresponding author. Tel.: +81 45 924 5756; fax: +81 45 924 5833.
E-mail address: hmihara@bio.titech.ac.jp (H. Mihara).

0142-9612/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.12.094

Name	Sequences	GNS conjugate
RF	GLKKLA**R**L**F**HKLLKLGC-NH2	RF-PEG-GNS25
RA	GLKKLA**R**L**A**HKLLKLGC-NH2	RA-PEG-GNS25
EF	GLKKLA**E**L**F**HKLLKLGC-NH2	EF-PEG-GNS25
EA	GLKKLA**E**L**A**HKLLKLGC-NH2	EA-PEG-GNS25
TAT	GRKKRRQRRRGC-NH2	TAT-PEG-GNS25

Fig. 1. Sequences of RF, RA, EF, EA, and TAT peptides and peptides conjugated with GNS.

TAT peptide or one of four peptides from the 16-amino acid peptide library (P-GNS25) (Fig. 1). These five nanoprobes were coated with PEG (P-PEG-GNS25) to maintain the high stability during long incubations for nuclear targeting. P-PEG-GNSs are expected to be taken up by the cell via endocytosis, and the P-PEG-GNSs in endosomes or lysosomes may then migrate to the nucleus. Therefore, a pH-sensitive DOX-PEG is synthesized using a hydrazone linkage and conjugated to a 25 nm GNS (P-DOX-PEG-GNS25) to allow the controlled release of DOX from the vesicles under pH 5 (Fig. 2) [31–33]. We demonstrated a more efficient cell-selective drug delivery system than that of P-GNS41 by examining the CP activities of five constructed P-PEG-GNS25 nanoprobes and the cell death activities of P-DOX-PEG-GNS25 and then comparing the results with the activities of the TAT peptide.

2. Experiments

2.1. Materials

Amino acids and reagents for peptide synthesis were purchased from Watanabe Chemical, Kokusan Chemical and Hipep Laboratories. Gold (III) chloride trihydrate, penicillin-streptomycin (PS) and doxorubicin were obtained from Sigma–Aldrich. Trisodium citrate dihydrate was obtained from Koso Chemical. SH-PEG (Mw 2000) and HO-PEG-NH_2 (Mw 2000) were purchased from Nof Corporation. Dulbecco's modified eagle's medium (DMEM) was obtained from Wako Pure Chemicals Industries. OPTI-MEM was obtained from Gibco. Cell counting kit-8 (CCK-8) and DAPI were purchased from Dojindo Molecular Technologies. LysoTracker Green DND-26 was purchased from Invitrogen. SlowFade Gold Antifade Reagent was purchased from Life Technologies.

2.2. Gold nanosphere (GNS) synthesis

GNS was synthesized according to the Frens method [34]. After 50 mL of 0.03% gold (III) chloride trihydrate solution was started for boiling under reflux condition, added was 0.75 mL 3% trisodium citrate dihydrate solution as a reducing agent for 25 nm of GNS (1.5 nM). At this time, solution color was changed from yellow to colorless and then to red. The mixture was boiled for 20 min after color change and the heating was stopped. The diameter of the GNS was characterized by transmission electron microscopy (TEM, Hitachi H-7500 electron microscope) and UV–VIS spectroscopy. The absorption peak of 25 nm GNS was shown at 525 nm (Fig. S1 and 2a).

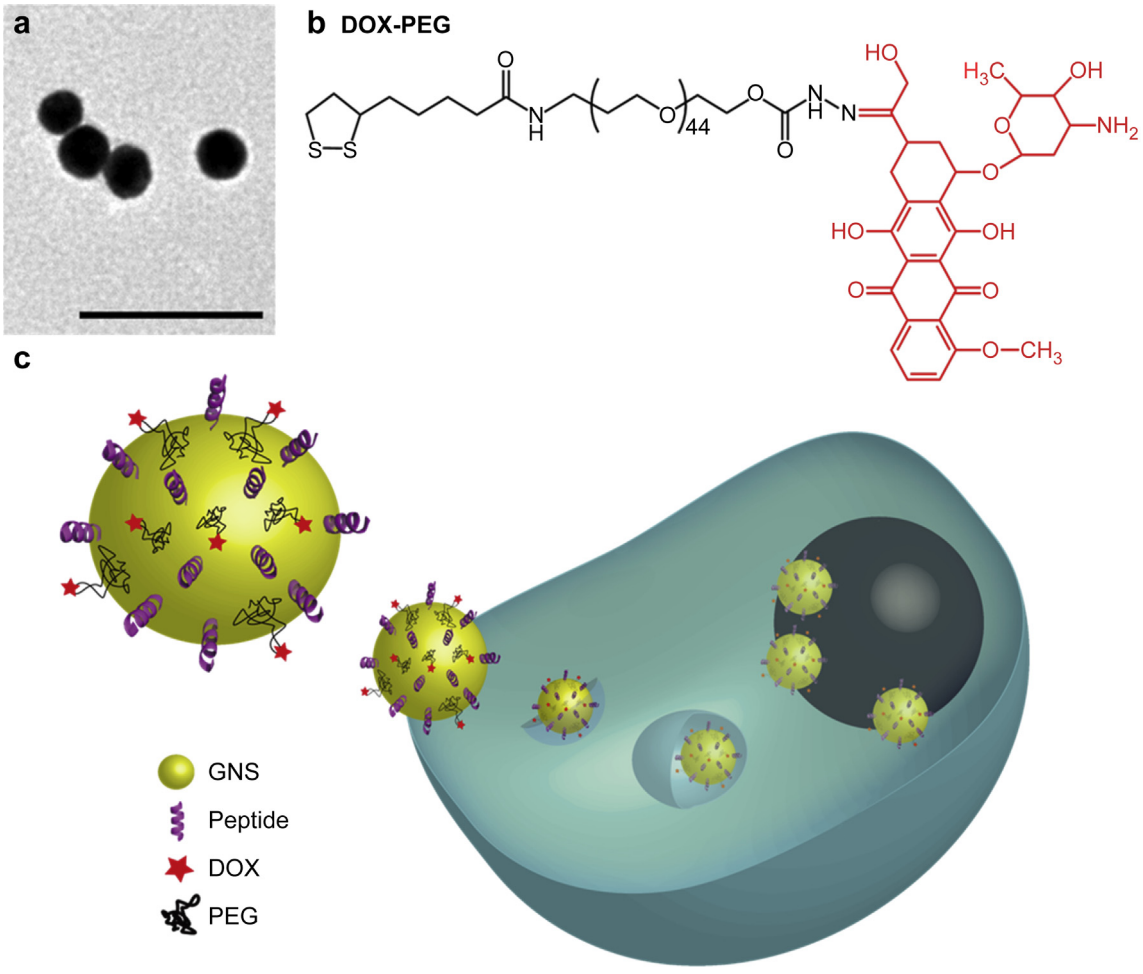

Fig. 2. a) TEM image of 25 nm GNS, scale bar: 100 nm, b) DOX-PEG having the pH-sensitive hydrazone linkage and the lipoic acid moiety for GNS conjugation and c) illustration of migration of peptides and DOX-PEG conjugated 25 nm GNS (P-DOX-PEG-GNS25) inside cell.