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Dual stimuli-sensitive dendrimers: Photothermogenic gold nanoparticle-loaded thermo-responsive elastin-mimetic dendrimers

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ABSTRACT

Dendrimers are synthetic macromolecules with unique structures that can work as nanoplatforms for both photothermogenic gold nanoparticles (AuNPs) and thermosensitive elastin-like peptides (ELPs) with valine-proline-glycine-valine-glycine (VPGVG) repeats. In this study, photothermogenic AuNPs were loaded into thermo-responsive elastin-mimetic dendrimers (dendrimers conjugating ELPs at their periphery) to produce dual stimuli-sensitive nanoparticles. Polyamidoamine G4 dendrimers were modified with acetylated VPGVG and $(VPGVG)_2$, and the resulting materials were named ELP1-den and ELP2-den, respectively. The AuNPs were prepared by the reduction of Au ions using a dendrimer-nanotemplated method. The AuNP-loaded elastin-mimetic dendrimers exhibited photothermal properties. ELP1-den and ELP2-den showed similar temperature-dependent changes in their conformations. Phase transitions were observed at around 55 °C and 35 °C for the AuNP-loaded ELP1-den and AuNP-loaded ELP2-den, respectively, but not for the corresponding PEGylated dendrimer. In contrast to the AuNP-loaded PEGylated dendrimer, AuNP-loaded ELP2-den readily associated with cells and induced efficient photocytotoxicity at 37 °C. The cell association and the photocytotoxicity properties of AuNP-loaded ELP2-den could be controlled by temperature. These results therefore suggest that dual stimuli-sensitive dendrimer nanoparticles of this type could be used for photothermal therapy.

1. Introduction

Considerable research efforts have been directed toward the production of smart nanomaterials capable of responding to external stimuli, which could be applicable to personalized nanomedicine. Radio frequency ablation is clinically approved as a local heating system, which can be used to administer hyperthermia therapy (thermotherapy). Photo-irradiation is another form of stimulus which is being used in an increasing number of clinical applications. With this in mind, thermo- and photo-responsive materials could be readily applied to biomedical applications using conventional instruments [1]. Thermosensitive polymers have been used as temperature-dependent drug delivery systems, and there have been many reports in the literature pertaining to the use of these polymers for the temperature-controlled release and cellular uptake of drug molecules [2,3]. Poly(N-isopropylamide) (PNIPAM) is a typical thermosensitive polymer, which undergoes a phase transition at 32 °C. Naturally-occurring thermosensitive materials are more suitable for biomedical applications than synthetic polymers such as PNIPAM. Elastin is a temperature-sensitive protein with a highly ordered structure, which can change from a hydrophilic random coil to a hydrophobic β-turn with increasing temperature [4,5]. Elastin-mimetic materials have been applied to thermosensitive biomaterials [5]. Gold nanoparticles (AuNPs) have attracted considerable attention during the last decade because of their photosensitive properties, which are quite different from those of bulk gold and gold atoms [6]. AuNPs have been applied as sensors for the detection of a variety of different biomolecules because the photosensitivity of these AuNPs can be affected by the size and the shape of the molecules [6,7]. Moreover, AuNPs exhibit photo-inducible heat-generating properties as a consequence of localized surface plasmon resonance (LSPR). Based on these

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properties, AuNPs can be used to kill specific cells by generating heat under light irradiation conditions in a process known as photothermal therapy [6–8].

Dendrimers are highly branched macromolecules with controllable size, chemical structure and surface properties, making them quite different from linear polymers. Furthermore, dendrimers can be used to encapsulate and even modify a wide variety of different compounds and metal nanoparticles because of the interior space and the multiple functional groups positioned on their periphery [9–11]. Based on these properties, dendrimers represent potent nanoplatforms for the design of unimolecular multifunctional nanoparticles. Dual stimuli-sensitive dendrimers can be also designed by adding two different kinds of stimuli-responsive material to a single dendrimer molecule, and dendrimers of this type can be useful for the construction of increasingly sophisticated systems.

In this study, we have focused on the development of dual stimuli-sensitive nanomaterials that are capable of responding to both temperature and light, because materials of this type could be readily employed in conventional therapeutic systems. It was envisaged that dual stimuli-sensitive nanobiomaterials of this type could be designed using elastin-mimetics and AuNPs. Lemieux et al. previously reported the modification of AuNPs with an elastin-like peptide (ELP), and the resulting ELP-modified AuNPs exhibited thermosensitivity only under acidic conditions because the carboxyl groups of the ELP were exposed at the periphery [12]. Our group has prepared elastin-mimetic dendrimers by conjugating acetyl-terminal ELPs to the terminal groups of polyamidoamine (PAMAM) dendrimers. The resulting elastin-mimetic dendrimers exhibited temperature-responsive changes in their conformation and underwent a phase transition under physiological conditions in a manner similar to that of natural elastin [13,14]. In this study, AuNPs were loaded into elastin-mimetic dendrimers to prepare dual stimuli-sensitive nanoparticles for use in biomedical applications. AuNPs exhibit photosensitive properties, and elastin-mimetic dendrimers have thermosensitive properties under physiological conditions. The use of elastin-mimetic dendrimers can be advantageous for size-controlled and one-pot preparations. Notably, size-controlled AuNPs with a narrow size distribution have been produced using a nanotemplating method with dendrimers [15], and it was envisaged that this strategy could be adapted to our elastin-mimetic dendrimers. AuNP-loaded elastin-mimetic dendrimers were prepared by reducing Au (III) ions inside elastin-mimetic dendrimers using a one-pot procedure. The photo-responsive properties and the size of the AuNPs loaded into the elastin-mimetic dendrimers were analyzed by ultraviolet–visible (UV–Vis) spectrometry, scanning transmission electron microscopy (STEM) and photothermal measurements. The thermosensitive properties of the AuNP-loaded elastin-mimetic dendrimers were analyzed by circular dichroism (CD) spectrometry and dynamic light scattering (DLS). AuNP-encapsulated dendrimers have been used in a broad range of applications, including catalytic reactions, stimuli-responsive drug delivery systems and imaging devices, because dendrimers can work as functional vehicles for AuNPs [16–18]. Given that photothermal therapy has been identified as a potential application of AuNP-loaded elastin-mimetic dendrimers, the main aim of the current study was to evaluate the photocytotoxicity of AuNP-loaded elastin mimetic dendrimers at different temperatures.

2. Experimental

2.1. Materials

An ethylenediamine core polyamidoamine (PAMAM) dendrimer of generation 4 (G4) and propidium iodide (PI) were obtained from Sigma-Aldrich Japan (Tokyo, Japan). Opti-MEM without phenol red was obtained from Invitrogen (Carlsbad, USA). 3-(4,5-Dimethyl-2-thiazioryl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was obtained from Dojindo Laboratories (Kumamoto, Japan). Gold (III) chloride hydrate ($HAu(III)Cl_4{\cdot}4H_2O$) and calcein-acetoxymethyl ester (calcein-AM) were obtained from Nacalai Tesque (Kyoto, Japan).

2.2. Preparation of AuNP-loaded elastin-mimetic dendrimers

Ac-VPGVG- and Ac-$(VPGVG)_2$-bearing PAMAM G4 dendrimers (ELP1-den and ELP2-den) were synthesized, as described in our previous reports [13,14]. AuNP-loaded ELP-dens were prepared according to our previous report [19,20]. Briefly, 55 equiv. of $HAuCl_4$ to dendrimer (2 mM, 1.755 mL) was added to an aqueous solution of ELP-dens (2.1 μM, 30 mL). Then, 275 equiv. of $NaBH_4$ (150 mM) dissolved in 0.3 M NaOH (117 μL) was added to the mixture with vigorous stirring at room temperature for 1 h. Purification and concentration of AuNP solutions were performed by ultrafiltration with Amicon Ultra 3000 Da (Merck KGaA, Darmstadt, Germany) if necessary. The concentrations of AuNP-loaded dendrimers after the centrifugation were estimated by measuring absorbance at 520 nm.

2.3. Characterization of AuNPs

Absorption spectra were measured between 400 and 800 nm with a Jasco Model V-630 spectrometer (Jasco Co., Tokyo, Japan). The absorption spectra of the $HAuCl_4$ solution were measured in the absence and presence of ELP2-den and $NaBH_4$ at wavelengths in the range of 200–500 nm, both before and after the ultrafiltration process. High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images were acquired by a TITAN^3 G2 60–300 microscope (FEI, Hillsboro, OR, USA) equipped with a corrector for the spherical aberration of the probe-forming lens. Transmission electron microscopy (TEM) images were collected by JEM-3010 microscope (JEOL Ltd., Tokyo, Japan) using negative films. Both microscopes were operated at an accelerating voltage of 300 kV.

2.4. Photothermal measurement

The sample solution (3 mL) (55 μM Au atom) was irradiated by GBM100KH (λ = 532 nm, 4.00 A (*ca.* 1 W), Katokoken Co., Ltd., Kanagawa, Japan). The sample temperature was measured by SK-1250MC (Sato Keiryoki MFG. Co., Ltd., Tokyo, Japan) immersed in the solution of the cell.

2.5. CD measurement

CD spectra were measured with a J-820 spectropolarimeter (Jasco Co., Tokyo, Japan), according to our previous reports [13,14]. Briefly, the sample solution (0.05 mg/mL ELP-den, 10 mM phosphate buffer) was added to a 0.1-cm path length cell. Before measuring CD spectra, the sample solutions were incubated at the designated temperature for 5 min. CD spectra were recorded from 190 to 260 nm at a scan speed of 50 nm/min. The resulting spectra were processed by the simple moving average method.

2.6. DLS measurement

DLS measurements were performed by ELSZ-DN2 (Otsuka Electronics, Osaka, Japan), according to our previous reports [14]. Briefly, sample solutions (1 mg/mL ELP-den) in phosphate buffer (10 mM; pH 7.4) containing 150 mM NaCl were prepared. Before measuring DLS, the sample solutions were filtered with a 0.45-μm filter. The hydrodynamic diameter was measured between 15 °C