Journal of Controlled Release 177 (2014) 11–19

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Self-assembled peptide nanoparticles as tumor microenvironment activatable probes for tumor targeting and imaging

Ying Zhao *,[1], Tianjiao Ji [1], Hai Wang, Suping Li, Yuliang Zhao **, Guangjun Nie ***

CAS Key Laboratory for Biomedical Effects of Nanomaterials & Nanosafety, National Center for Nanoscience and Technology (NCNST), 11 Beiyitiao, Zhongguancun, Beijing 100190, China

ARTICLE INFO

Article history:
Received 17 September 2013
Accepted 31 December 2013
Available online 10 January 2014

Keywords:
Peptides
Self-assembly
pH-sensitive activation
Tumor microenvironment
Tumor imaging

ABSTRACT

Design of specific and sensitive imaging probes for targeting tumor microenvironment holds great promise to achieve precise detection and rapid responsiveness to neoplastic tissues. Dysregulated pH, one of the most remarkable hallmarks of tumor microenvironment, can be considered as a good specific trigger for the design of broad-spectrum and local-environment responsive imaging probes. However, the current existing design strategies for pH-responsive systems are insufficient to meet the needs for a rapid and tumor-specific diagnosis. Here we reported a novel biomimetic nanostructure based on oligopeptide self-assembly that can quickly switch into dissociated stage with active fluorescence property from self-assembled stage with quenched fluorescence activity when encountering a subtle pH-change in tumor microenvironment (pH 6.8 *vs.* 7.4). This oligopeptide-assembly is examined as tumor microenvironment activatable probes for both intratumoral and intravenous *in vivo* tumor imaging. Through the distinct fluorescent intensities, it is validated that the acidic tumor microenvironment can activate stronger fluorescence signals. The tailor-made self-assembled oligopeptide nanomaterials have the potential for efficient and specific *in situ* diagnosis of various solid tumors with a weakly acidic microenvironment, which is expected to be of crucial importance for clinical tumor diagnostics.

1. Introduction

The expanding capabilities of nanotechnology, especially in targeted delivery of therapeutic and imaging agents, enable novel and improved approaches to treat various tumors [1,2]. Although the first generation of more than 40 nanotechnology-enabled products has reached the clinic [3], the design of synthetic nanoparticles capable of effectively overcoming biological barriers to access core area of tumor tissue is still a formidable challenge [4,5]. The interstitial transport of nanoparticles is limited by the dense extracellular matrix, cell packing, and high interstitial fluid pressure in solid tumors, so that most nanoparticles accumulate in the perivascular region, unable to distribute homogeneously throughout the tumors [4,6]. The intratumoral delivery of nanoparticles is, by nature, the bottleneck of nanocarrier-based drug delivery. Targeting the stromal tumor components surrounding tumor cells, termed tumor microenvironment [7,8], therefore, is emerging as a feasible strategy to improve the effectiveness of nanoparticle-based cancer diagnosis and therapy.

Intelligent design of specific and sensitive imaging nanoprobes specifically targeting tumor microenvironment holds great promise to achieve accurate detection and rapid responsiveness to neoplastic tissues [9]. Some recent reports showed that nanomaterial-enabled approaches were developed for tumor imaging *via* recognizing and targeting specific components of tumor microenvironment [10–14]. However, because tumor microenvironment is composed of various cell types, complicated extracellular matrix and soluble factors and the biophysicochemical differences between tumor and normal tissues may be subtle, it is extremely challenging to achieve specific and sensitive responses to a subtle stimulus, such as a molecular binding event or a specific trigger from local environmental changes, in a broad-spectrum manner for majority of tumor types.

Dysregulated pH is emerging as one of the most remarkable hallmarks of tumor microenvironment in almost all tumor types, compared to normal tissues. Overproduction of lactic acid due to the hypoxic environment and fast metabolic rates of tumor cells are the major contributors to a weakly acidic environment around tumor tissues, which in turn may facilitate cancer cell progression by promoting proliferation, migration and invasion [15]. Such a distinct feature has made weakly acidic pH (pH ~ 6.7–7.1), a specific trigger for design of broad-spectrum and local-environment responsive imaging probes. Hence, nanoparticles with designed features to detect a weakly acidic pH stimulus and respond accordingly by structural or conformational changes with a detectable readout will allow rapid, specific and sensitive detection at the diseased areas, no need to be internalized by tumor cells.

pH-responsive systems have been extensively developed for cancer therapy and imaging. However, most of them function at more acidic pH range, such as pH 4–6, and such intelligent systems may be suitable for

* Corresponding author. Tel.: +86 10 82545662; fax: +86 10 62656765.
** Corresponding author. Tel.: +86 10 82545570; fax: +86 10 62656765.
*** Corresponding author. Tel.: +86 10 82545529; fax: +86 10 62656765.
E-mail addresses: zhaoying@nanoctr.cn (Y. Zhao), zhaoyl@nanoctr.cn (Y. Zhao), niegj@nanoctr.cn (G. Nie).
[1] These authors contributed equally to this work.

0168-3659/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jconrel.2013.12.037

intracellular delivery (the acidic vesicles-mediated drug release), but not for tumor microenvironment triggered response (a subtle pH alteration) [16–20]. Furthermore, most existing pH-sensitive nanoparticles based on long chain polymers showed a relatively slow response to pH stimulus from 10 h to several days [20–22]. Therefore, these design strategies may be insufficient to meet the needs for a rapid and tumor environment-responsive diagnosis. As intelligent pH-responsive nanoparticles, a rapid response is one of the most important advantages. Long response time may lead to their low tumor targeting efficacy due to re-entry blood circulation. Recently, some self-organized pH sensitive nanoparticles composed of polymers conjugated with pH-cleavable linkers or functional groups with pH-inducible charge conversion were designed [23–29], which showed certain sensitivity to the subtle changes in pH mimicking the difference between tumor microenvironment and normal tissues. Despite some progress achieved, further development is urgently needed to consolidate the pH activatable strategy for tumor microenvironment targeting and imaging. For example, conjugating functional molecules at fixed location with controllable ratios and manner in polymer chains, and purification of the functionalized polymers with biocompatibility and low or no toxicity are difficult to be executed.

Self-assembled oligopeptide nanoparticles can address these challenges. Firstly, oligopeptides are easily obtained by a solid-phase peptide synthesis (SPPS) method. Secondly, oligopeptides provide various reactive groups in the side chains for effective cross-linking and chemical modification. Such rich chemistry for multi-functionality can be used for biomimetic or de novo designs to ensure the modification ratios and location and the product purity under tight control. Lastly, pH-sensitive disassembly of the oligopeptide nanostructure could render a rapid response to local stimuli compared to the long chain polymer nanoparticles, and the resulting low molecular weight constitutes can more readily diffuse within tumor tissues. Although extensive research efforts have been devoted to the design and fabrication of novel biomimetic nanomaterials through peptide self-assembly [30–33], tailored-design of intelligent oligopeptide nanostructures specifically recognizing and responding weakly acidic microenvironment (pH ~ 6.7–7.1) of tumor tissues are challenging [34]. We hypothesize that self-assembled nanostructures based on short peptides combined with chemical modifications with non-peptide functional groups may endow great opportunities for the design of tumor microenvironment rapidly responsive imaging probes with enhanced biocompatibility. The use of short peptides as the major building blocks to construct pH-activatable probes targeting tumor microenvironment through self-assembly process has, to the best of our knowledge, seldom been reported.

Here we reported a novel biomimetic nanostructure based on oligopeptide self-assembly that can quickly switch into dissociated stage with active fluorescent property from self-assembled stage with quenched fluorescence activity when changing local pH. An assembly/disassembly was achieved by packing an energy pair (donor and receptor) into a peptide assembled nanostructure, a swift response from a self-assembled supramolecular nanoparticle (quenched state of the probes due to proximity of a fluorescent dye and its quencher) into disassembled peptide molecules (activated state when encountering a pH trigger), which corresponds to a change in their surface charge (Fig. 1).

2. Materials and methods

2.1. Materials

3-Diethylaminopropyl isothiocyanate (DEAP), anhydrous dimethylformamide (DMF), N-methyl morpholine (NMM), and 4-dimethylaminopyridine (DMAP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Alexa Fluor 488 Carboxylic Acid, Succinimidyl Ester (A488-SE) and black hole quencher-1 (BHQ-1 Carboxylic Acid, Succinimidyl Ester) were obtained from Life Technologies (Carlsbad, CA, USA) and Biosearch Technologies (Novato, CA, USA), respectively. Protected peptide conjugate KS5-DEAP2 ([CH_3CONH]-K(DEAP)SKSK(DEAP)-[$CONH_2$]) was synthesized and purified by ChinaPeptides (Shanghai, China). Water used in the experiment was double-distilled (DW) and all other chemicals were used as received without any purification.

2.2. Synthesis of A488 and BHQ-1 conjugated peptide KS5-DEAP2

The peptide conjugates were prepared by conjugating pH-stable fluorescence dye, Alexa Fluor 488 (A488, ex/em: 494 nm/519 nm) and BHQ-1 (abs. 534 nm) to pH-sensitive peptide conjugate KS5-DEAP2. Peptide conjugate KS5-DEAP2 ([CH_3CONH]-K(DEAP)SKSK(DEAP)-[$CONH_2$]) was synthesized using standard solid-phase Fmoc peptide chemistry. Then, A488-SE (12 μmol) was coupled to the primary amine of the central lysine residue on KS5-DEAP2 (5 μmol) in DMF (250 μL) containing NMM (65 μmol) and DMAP (3.2 μmol) at room temperature in the dark under stirring for 2 h. The product (A-pep) was purified to >90% by reversed-phase high performance liquid chromatography (RP-HPLC; Kromasil 100-5C18, Eka Chemicals, Separation Products, SE-445 80 Bohus, Sweden): 10% to 50% acetonitrile containing 0.1% TFA *versus* DW containing 0.1% TFA over 20 min at a flow rate of 4.0 mL min^{-1}. For quencher conjugation, the preparation process was similar with the above dye conjugation. Briefly, the BHQ-1 (12 μmol) was coupled to the primary amine of the central lysine residue on KS5-DEAP2 (5 μmol) in DMF (250 μL) containing NMM (65 μmol) and DMAP (3.2 μmol) under stirring at room temperature in the dark overnight. The product (B-pep) was also purified by RP-HPLC. The mass of product was analyzed with Microflex LRF matrix-assisted laser desorption ionization with time of flight (MALDI-TOF) mass spectrometer (Bruker Daltonics Inc., USA).

2.3. Preparation of hybrid peptide nanoparticles in aqueous solution

A-pep (5 nmol) and B-pep (0, 2.5, 5, 10 nmol) were dissolved together in DMF (1 mL), and the solution was kept at room temperature with strong stirring for about 1 h. Subsequently, 400 μL DW was slowly added to the solution under strong stirring through a syringe within 2 h. The solution became slightly turbid, indicating the happening of self-assembly. After being stirred over night at room temperature, the solution was dialyzed against Tris·HCl buffer (pH 7.4) under the room temperature to remove DMF for 24 h. The Tris buffer was changed every hour for the first 6 h, and subsequently every 6 h. The obtained solution was filtered and then set to 10 mL to get an A-pep concentration about 0.5 μM. Finally, a series of products of hybrid peptide nanoparticles (A/B ratio = 2:1, 1:1, 1:2, and A-pep alone) was obtained.

2.4. Dynamic light scattering (DLS) test

The particle size distribution of the products (A/B ratio = 1:1) at pH 7.4 or pH 6.8 in Tris·HCl buffer was measured at room temperature by a ZetaSizer Nano series Nano-ZS (Malvern Instruments Ltd., Malvern, UK) equipped with a He–Ne Laser beam at a wavelength of 633 nm and a fixed scattering angle of 90°. Prior to the test, the sample solution was stabilized at room temperature for 2 h.

2.5. Transmission electron microscopy (TEM) observation

The morphology of the hybrid products was confirmed using a Transmission Electron Microscopy (Tecnai G2 F20 U-TWIN, FEI, USA). In brief, the samples were prepared by casting a solution of the hybrid peptide product (A/B ratio = 1:1, pH 7.4 or 6.8) on a copper grid, allowed to stand on the grid for 90 s. Filter paper was then used to wick away residual sample and liquid. One drop of 1% (w/w) aqueous uranyl acetate (negative stain) was then placed on the grid, allowed to