J|A|C|S COMMUNICATIONS

Published on Web 05/26/2010

Surface-Functionalizable Polymer Nanogels with Facile Hydrophobic Guest Encapsulation Capabilities

Ja-Hyoung Ryu, Siriporn Jiwpanich, Reuben Chacko, Sean Bickerton, and S. Thayumanavan*

Department of Chemistry, University of Massachusetts at Amherst, Amherst, Massachusetts 01003

Received March 19, 2010; E-mail: thai@chem.umass.edu

Chemically cross-linked, water-soluble polymer nanoparticles constitute a promising scaffold in therapeutic delivery applications, offering potential to circumvent stability issues.[1] However, these polymeric nanoparticles or nanogels face certain complications, as they are prepared by microemulsion or inverse microemulsion methods.[2,3] These methods are relatively complex and require multiple purification steps to remove not only unreacted monomer but also the surfactant materials that were used to stabilize the emulsion. When a water-soluble polymer nanoparticle is desired, inverse microemulsion based synthesis is the preferable method. Note that the continuous phase in the inverse microemulsion (water-in-oil emulsion) method is based on a lipophilic solvent and therefore cannot be used to encapsulate hydrophobic guest molecules during nanoparticle formation. An attractive alternative to forming polymer nanoparticles is to collapse a limited number of polymer chains. Such methods have been previously reported but require ultrahigh dilution conditions or inverse addition conditions,[4,5] which limit capabilities for guest molecule incorporation.

In this paper, we report on a facile method that allows for the design and syntheses of polymer nanoparticles under nonemulsion conditions. The versatility of these polymer nanoparticles has been further demonstrated by showing that (*i*) guest molecules can be easily incorporated noncovalently within the nanoparticles; (*ii*) the noncovalently encapsulated guest molecules can be released in response to a biologically relevant stimulus; and (*iii*) the surfaces of these particles are functionalizable. For a stimuli-responsive functional group, we targeted a disulfide bond, since these bonds are susceptible to biochemical reductants such as glutathione (GSH), thioredoxin, and peroxiredoxin.[6] We have previously reported a synthetic methodology in which a pyridyl disulfide (PDS) side chain functionality was used as a handle for incorporating thiol-based functional groups onto polymers.[7] The basis for this methodology is the higher reactivity of the pyridyl disulfide bonds with thiols as compared to other disulfide functionalities, which is facilitated by the release of a stable 2-thiopyridone byproduct. We envisioned taking advantage of this reactivity of the PDS functionality to effect cross-linking in polymer chains. Our hypothesis involves the addition of a deficient amount of dithiothreitol (DTT), which is known to cleave PDS bonds with great efficiency. When a deficient amount of DTT is added, a corresponding small percentage of PDS groups will be converted to free thiols. These free thiols would then react with an equivalent amount of the remaining PDS functionalities to create disulfide bonds, which would effectively cross-link the polymer chains, independent of whether the process is intrachain or interchain. We further envisioned that because the PDS functionalities are relatively hydrophobic, they would be collapsed in the aqueous phase. If this were the case, we hypothesized that polymer nanoparticles would be obtained upon treatment of our polymers with a deficient amount of DTT, without the need for ultrahigh dilution preparative conditions (Figure 1). We also envisaged that the hydrophobic interior in the aggregate would provide an opportunity to encapsulate lipophilic guest molecules prior to cross-linking.

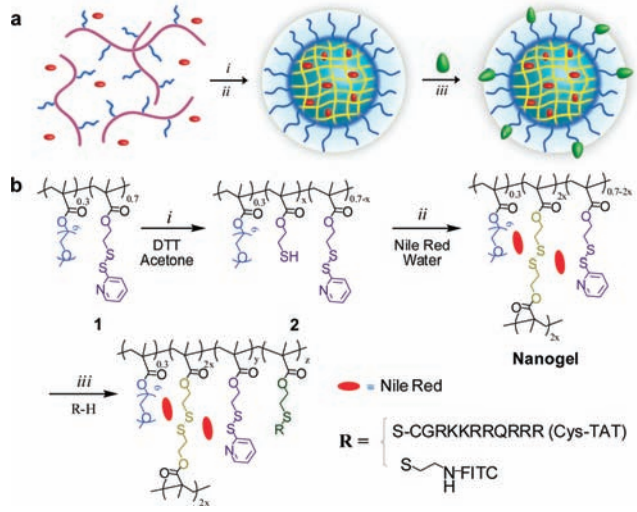

Figure 1. Design and synthesis of the polymer nanoparticles. (a) Schematic representation of the preparation of biodegradable nanogels with surface modification. (b) Structure of the polymer and nanogel. (*i*) Cleavage of specific amount of PDS group by DTT. (*ii*) Nanogel formation by inter/intrachain cross-linking. (*iii*) Surface modification of nanogels with thiol-modified Tat peptide or FITC.

Our polymer is based on an oligoethyleneglycol unit as the hydrophilic functionality, to render the polymer water-soluble, and the PDS-derived thioethylmethacrylate as the cross-linkable functionality. Random copolymer **1**, containing 30% of the oligoethyleneglycol methacrylate and 70% of the PDS-derived methacrylate, was prepared by reversible addition−fragmentation chain transfer (RAFT) polymerization. Cross-linked particles were synthesized from this polymer (10 mg mL^{-1}) by adding 20 mol % of DTT with respect to the number of PDS functionalities in the polymer. Note that there would be residual PDS functionalities in these gels, providing useful handles for introducing ligands on the nanogel surfaces (*vide infra*). We characterized the structures obtained from these reactions by transmission electron microscopy (TEM) and dynamic light scattering (DLS). DLS studies reveal that the structures obtained are ∼190 nm in size (Figure 2a). TEM images revealed well-defined spherical structures with slightly smaller diameters than those observed in DLS, which is attributed to the possible swelling of the nanoparticles in water. We were able to obtain two very different particle sizes by concentration change in our methodology. Upon decreasing the concentration of copolymer **1** to below 0.5 wt % in water, we obtained 16 nm nanoparticles after DTT induced cross-linking.[8]

Next, to investigate the possibility of encapsulating a hydrophobic guest molecule within the interiors of these nanogels, we carried out the DTT-based cross-linking reaction in the presence of Nile

10.1021/ja102316a

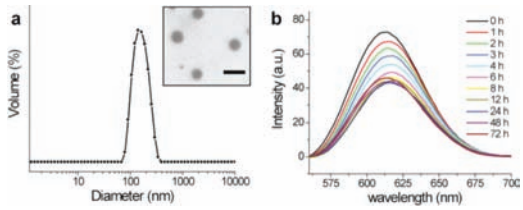

Figure 2. (a) Size distribution of the nanogel (1 mg mL^{-1}) in water by DLS. Inset is a TEM image of the nanogel (scale bar is 200 nm). (b) Change in fluorescence emission intensity of Nile red encapsulated inside nanogels in the presence of 10 mM GSH at pH 7.4.

red, a hydrophobic dye. In order for a carrier to be effective, guest molecules should be released in response to a biologically relevant trigger. We hypothesized that GSH can induce release of loaded dyes through cleavage of the cross-linking disulfide bonds. For this purpose, we added different concentrations of GSH (10 μM and 10 mM) into dye-loaded nanogel solutions and investigated the release of Nile red by tracing the decrease of the hydrophobic dye's spectral emission intensity caused by its insolubility in aqueous media.[8] At low GSH concentrations (10 μM), corresponding to those commonly observed outside the cell and within the blood plasma, little dye release was observed. In contrast, high concentrations of GSH (10 mM), corresponding to those found inside the cell, induced significant dye release (Figure 2b).

We anticipated that our gels would be relatively nontoxic, because they are made from biocompatible oligoethyleneglycol components as surface displays in a methacrylate backbone. The nanogels indeed exhibit high cell viability and no concentration-dependent toxicity up to a nanogel concentration of 1 mg mL^{-1} (Figure 3a). This result indicates that the nanogel material is nontoxic and thus a potential candidate for biological applications. We were also interested in testing our hypothesis that we can achieve surface functionalization of these nanogels by thiol−disulfide exchange reactions with the unreacted PDS groups. These PDS groups can be reacted with thiol-containing compounds, allowing for chemoselective ligand modification. To test this, nanogel solutions (1 mg mL^{-1}) were treated with either excess fluorescein isothiocyanate (FITC) or thiol-modified FITC (1 mg mL^{-1}). Figure 3b shows a significant difference between the two samples; while thiol-modified FITC treated nanogels exhibited very strong fluorescein emission, the bare FITC treated nanogels solution showed very little emission, suggesting that the nanogels were covalently functionalized with thiol-modified FITC by disulfide linkage and

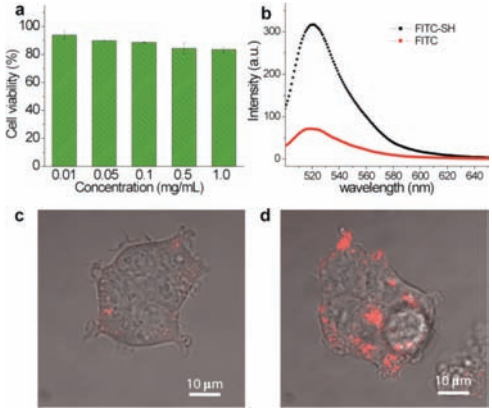

Figure 3. (a) In vitro toxicity of empty nanogels with 293T cells after 24 h incubation. (b) The emission spectra of nanogels (1 mg mL^{-1}) treated with thiol-modified FITC (FITC-SH) and with FITC. Representative confocal microscopy images of MCF-7 cells incubated with the unmodified nanogels (c) and Tat-SH treated nanogels (d).

that the observed fluorescence is not due to noncovalent surface binding of the dye molecules.[8] To further investigate surface modification possibilities, nanogel solutions (0.1 mg mL^{-1}) containing the hydrophobic dye, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), were treated with a modified cell penetrating peptide, Tat-SH (0.1 mg mL^{-1}) with a *C*-terminal cysteine, for 24 h. These solutions were then incubated with MCF-7 human breast cancer cell lines for just 2 h. The cell internalization efficiency of the unmodified nanogels (control) and Tat-SH treated nanogels were then examined by confocal microscopy. As shown in Figure 3c and 3d, the internalization of Tat-SH modified nanogels occurred much more readily than that observed with the control gels, confirming the effectiveness of using the remaining PDS groups to simply modify the nanogel surface. This presents a clear method for incorporating ligands onto the polymer nanoparticles and thus achieves specificity to pathogenic cells using chemoselective disulfide chemistry.

In summary, we have demonstrated a simple, emulsion-free method for the preparation of biocompatible nanogels that provides the ability to encapsulate hydrophobic guest molecules using intra/intermolecular disulfide formation of PDS containing polymers. Since disulfide bonds are biodegradable in a reducing environment, these nanoparticles hold great potential as intracelluar drug delivery vehicles. The release of guest molecules can be induced by external stimuli. Additionally, we have demonstrated that the surfaces of these nanoparticles can be efficiently functionalized under mild conditions. Taken together, the nanogel formation using the self-cross-linking polymers and corresponding method of surface modification open a new avenue for enhanced cytosolic drug delivery and establish a novel approach to creating polymer nanogels for a range of biomedical applications from drug delivery to biosensing. Fine control over the nanogel size and the release kinetics, along with target specific drug delivery, are the focus of current work in our laboratories.

Acknowledgment. We thank DARPA for support and NSF-NSEC, and NSF-MRSEC at UMass for infrastructure.

Supporting Information Available: Synthetic procedures and characterizations of the nanogel. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kabanov, A. V.; Vinogradov, S. V. *Angew. Chem., Int. Ed.* **2009**, *48*, 5418–5429. (b) Oh, J. K.; Drumright, R.; Siegwart, D. J.; Matyjaszewski, K. *Prog. Polym. Sci.* **2008**, *33*, 448–447. (c) Kakizawa, Y.; Harada, A.; Kataoka, K. *J. Am. Chem. Soc.* **1999**, *121*, 11247–11248. (d) Aliyar, H. A.; Hamilton, P. D.; Remsen, E. E.; Ravi, N. *J. Bioactive Compat. Polym.* **2005**, *20*, 169–181. (e) Fang, H.; Zhang, K.; Shen, G.; Wooley, K. L.; Taylor, J. *Mol. Pharm.* **2009**, *6*, 615–626.

(2) (a) Bachelder, E. M.; Beaudette, T. T.; Broaders, K. E.; Dashe, J.; Fréchet, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 10494–10495. (b) Sisson, A. L.; Steinhilber, D.; Rossow, T.; Welker, P.; Licha, K.; Haag, R. *Angew. Chem., Int. Ed.* **2009**, *48*, 7540–7545. (c) Talsma, S.; Babensee, J.; Murthy, N.; Williams, R. *J. Controlled Release* **2006**, *112*, 271–279.

(3) Oh, J. K.; Siegwart, D. J.; Lee, H.-i.; Sherwood, G.; Peteanu, L.; Hollinger, J. O.; Kataoka, K.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2007**, *129*, 5939–5945.

(4) (a) Kadlubowski, S.; Grobelny, J.; Olejniczak, W.; Cichomski, M.; Ulanski, P. *Macromolecules* **2003**, *36*, 2484–2492. (b) Jiang, J.; Thayumanavan, S. *Macromolecules* **2005**, *38*, 5886–5891.

(5) (a) Cherian, A. E.; Sun, F. C.; Sheiko, S. S.; Coates, G. W. *J. Am. Chem. Soc.* **2007**, *129*, 11350–11351. (b) Harth, E.; Horn, B. V.; Lee, V. Y.; Germack, D. S.; Gonzales, C. P.; Miller, R. D.; Hawker, C. J. *J. Am. Chem. Soc.* **2002**, *124*, 8653–8660.

(6) (a) Heffernan, M.; Murthy, N. *Ann. Biomed. Eng.* **2009**, *37*, 1993–2002. (b) Bernkop-Schnürch, A. *Adv. Drug Delivery Rev.* **2005**, *57*, 1569–1582.

(7) Ghosh, S.; Basu, S.; Thayumanavan, S. *Macromolecules* **2006**, *39*, 5595–5597.

(8) See Supporting Information for details.

JA102316A