Versatile PEG-derivatized phosphine oxide ligands for water-dispersible metal oxide nanocrystals†

Hyon Bin Na,[a] In Su Lee,[b] Heonjin Seo,[c] Yong Il Park,[a] Jung Hee Lee,[d] Sang-Wook Kim*[c] and Taeghwan Hyeon*[a]

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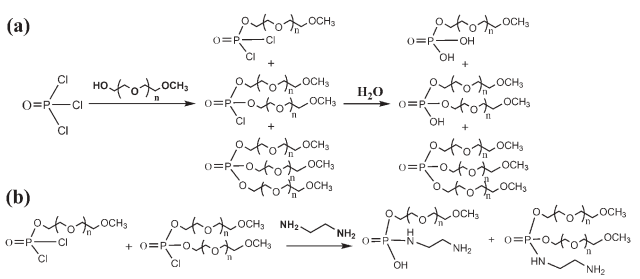
We report the simple synthesis of poly(ethylene glycol) (PEG)-derivatized phosphine oxide ligands for water-dispersible metal oxide nanocrystals.

Metal oxide nanocrystals have been used in a variety of applications, including nano-electronics, biological imaging, and clinical treatments.[1] In particular, superparamagnetic iron oxide nanocrystals have been used in many biomedical applications such as magnetic resonance imaging (MRI) contrast enhancement, hyperthermia, drug delivery, and cell separation.[2–5] A key requirement for the successful use of these nanocrystals in biomedical applications is their good dispersity, colloidal stability in biological media and low toxicity. Recently, various methods for synthesizing high-quality metal oxide nanocrystals in organic solvents with improved monodispersity and crystallinity compared to those prepared in aqueous solution have been reported.[6] However, such nanocrystals are water-immiscible and not sufficiently stable for biomedical applications. Therefore, the development of a method for modifying the surface of these nanocrystals in order to endow them with water compatibility is essential for extensive biomedical applications.[7] Quite recently, several surface modification methods of rendering magnetic ferrite nanocrystals water-dispersible have been reported. Most of these studies focused on passivating the nanocrystals with silica or polymer shells through rather sophisticated procedures.[8] In this context, this study designed novel PEG-derivatized phosphine oxide (PO-PEG) ligands with biocompatible poly(ethylene glycol) (PEG) tail groups and a surface coordinating phosphine oxide head group for the purpose of displacing the hydrophobic ligands on the surface of the as-synthesized metal oxide nanocrystals, thereby stabilizing them in aqueous media. The synthesis of PO-PEG ligands and the modification of the nanocrystals with them are extremely facile and, thus, practical for the large-scale preparation of well-dispersed and highly concentrated aqueous dispersions of nanocrystals. In addition, this procedure can be adapted to provide active functional groups that are capable of conjugating with probing or targeting agents on the surface of nanocrystals.

Scheme 1 shows the synthesis of PO-PEGs. In a typical procedure, phosphoryl trichloride ($POCl_3$) was added to a THF solution containing three equivalents of poly(ethylene glycol) methyl ether (mPEG). The resulting mixture was then stirred at room temperature for 1 day. Evacuation of the resulting product mixture at 100 °C for 12 h yielded a colorless gel. Matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometric and ^{31}P NMR analysis revealed the formation of the intended PO-PEGs. MALDI-TOF mass spectrometric data showed that the products were a mixture of unreacted mPEG and three PO-PEG ligands that were derivatized with one, two and three equivalents of mPEG, respectively (Fig. 1). The magnified part of the MALDI-TOF mass spectrum of the mono-mPEG derivatized PO-PEGs showed peaks in between the peaks from the unreacted mPEG (ESI†). The ^{31}P NMR spectrum in $CDCl_3$ showed three major peaks near 0 ppm and another peak at −12.48 ppm. These peaks were different from the $POCl_3$ peak at 4.09 ppm (Fig. 2). The peak at −12.48 ppm disappeared when the NMR spectrum was obtained in D_2O, demonstrating that the peak originated from the chlorinated phosphine. Generally, it is expected to be very hard to obtain an exclusively trisubstituted product from the reaction of all three chlorides of $POCl_3$ with a long chain primary alcohol.[9] As described above, the products were a mixture of mono-, bi-, and trisubstituted derivatives and the relative ratios of these three products were dependent on the molecular weight of mPEG (ESI†). The fraction of trisubstituted product increased when mPEG reactant with a low molecular weight was used.

Monodisperse magnetite (Fe_3O_4) nanocrystals were synthesized from the thermal decomposition of a Fe–oleate complex and stabilized with oleic acid. The nanocrystals were used to examine



Scheme 1 Synthesis of (a) PO-PEGs and (b) amine-functionalized PO-PEGs.

[a]*National Creative Research Initiative Center for Oxide Nanocrystalline Materials and School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Korea. E-mail: thyeon@snu.ac.kr; Fax: +82 2 886 8457; Tel: +82 2 880 7150*
[b]*Department of Chemistry and Advanced Material Science, Kyunghee University, Yongin 446-701, Korea*
[c]*Department of Molecular Science and Technology, Ajou University, Suwon 443-749, Korea. E-mail: swkim@ajou.ac.kr; Fax: +82 31 219 2394; Tel: +82 31 219 2522*
[d]*Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, Korea*
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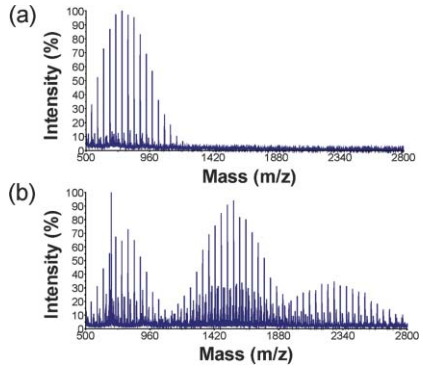

Fig. 1 MALDI-TOF mass spectra of (a) mPEG (M_n: 750) and (b) the resulting PO-PEGs.

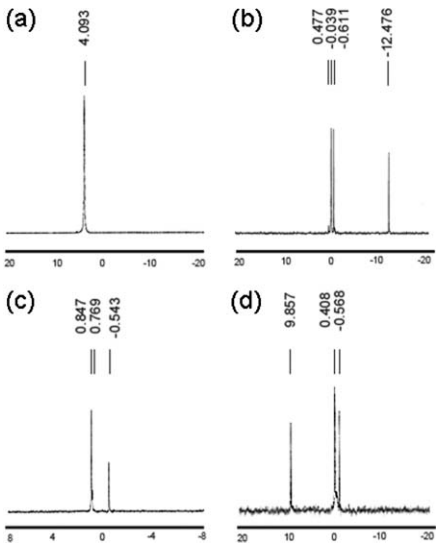

Fig. 2 ^{31}P NMR spectra of (a) $POCl_3$ in $CDCl_3$, (b) PO-PEGs in $CDCl_3$, (c) PO-PEGs in D_2O and (d) amine-functionalized PO-PEGs in $CDCl_3$.

the ligand exchange reaction with PO-PEG ligands to generate water-dispersible and biocompatible nanocrystals.[6] The ligand exchange reaction was carried out by mixing the Fe_3O_4 nanocrystals and PO-PEGs in THF. The resulting mixture was kept under vacuum at 150 °C for 1 h. The addition of water resulted in the production of a dark brown suspension with a white solid floating on the surface, which was believed to be oleic acid molecules displaced from the surface of the nanocrystals. The filtering of oleic acid afforded a transparent aqueous suspension of well-dispersed Fe_3O_4 nanocrystals. The unbound PO-PEGs were removed from the suspension by ultracentrifugation, column chromatography, or dialysis. TEM analysis shows that nearly no discernable change occurred after the ligand exchange process and no aggregation was observed in the dispersion (Fig. 3a,b). The hydrodynamic diameter of the ligand-exchanged Fe_3O_4 nanocrystals with a core diameter of 8 nm was 16 nm, as measured by light scattering, which confirmed no aggregation of the nanocrystals (ESI†). No degradation or aggregation was observed in the aqueous dispersion after keeping at ambient temperature for more than 3 months. Furthermore, the nanocrystals remained stable under various experimental conditions, such as several ultracentrifugations and size exclusion column purification. Ligand

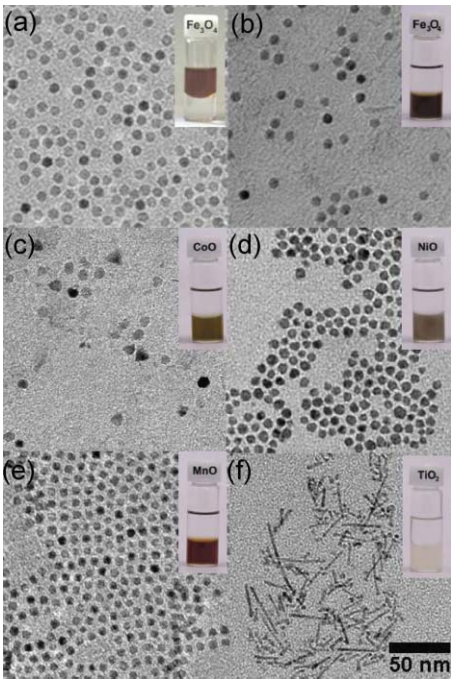

Fig. 3 TEM images of (a) as-synthesized Fe_3O_4 nanocrystals dispersed in hexane and (b) PO-PEG stabilized Fe_3O_4 nanocrystals. TEM images of PO-PEG stabilized metal oxide nanocrystals dispersed in water: (c) CoO, (d) NiO, (e) MnO, and (f) TiO_2. [Inset: photographs of metal oxide nanocrystal solutions (upper phase: hexane, lower phase: water)].

exchange reactions of PO-PEGs with various oxide nanocrystals were performed to demonstrate the general applicability of PO-PEGs as exchangeable ligands for transforming the oxide nanocrystals synthesized in organic media to water-dispersible nanocrystals. The ligand exchange reactions resulted in the transparent aqueous dispersions of various oxide nanocrystals. Fig. 3 shows TEM images and the corresponding photographs of the aqueous dispersions of nanocrystals of Fe_3O_4, CoO, NiO, MnO, and TiO_2, demonstrating no aggregation of nanocrystals. Furthermore, the facile synthetic procedure and simple ligand exchange reaction made it possible to produce water-dispersible and biocompatible oxide nanocrystals on a large-scale (ESI†).

For many biomedical applications, nanocrystals need to have surface functional groups to conjugate with biomarkers and bioactive materials. These PO-PEGs can be endowed with reactive functional groups through a reaction with bifunctional reagents such as 1,2-ethylenediamine. To accomplish this, more than 50 equivalents of 1,2-ethylenediamine were added at room temperature to the solution that had been prepared from a reaction between phosphoryl chloride and two equivalents of mPEG at room temperature for 12 h. The resulting mixture was further stirred at room temperature for >12 h. The ^{31}P NMR spectrum of the product showed a new peak at 9.86 ppm in addition to the peaks at 0.41 and −0.57 ppm (Fig. 2d). This demonstrates the formation of the amine-functionalized PO-PEGs. The amine-functionalized water-dispersible Fe_3O_4 nanocrystals can be readily conjugated with fluorescein-5′-isothiocyanate (FITC) through a reaction between the amine group and the isothiocyanate moiety of the dye. Fig. 4a shows a photograph comparing the FITC-conjugated and the un-conjugated Fe_3O_4 nanocrystals under UV

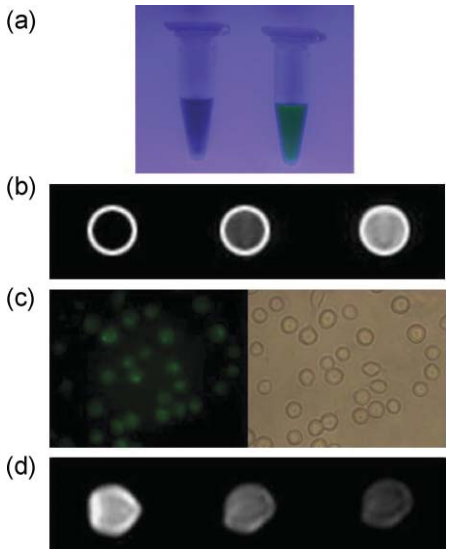

Fig. 4 (a) Photograph of un-conjugated (left) and FITC-conjugated (right) Fe_3O_4 nanocrystals under UV irradiation. (b) T_2-weighted MRI of 18 nm (left), 11 nm (middle), and 5 nm (right) Fe_3O_4 nanocrystals at 3.0 T. (c) Fluorescent image (left) and bright-field image (right) of cells labelled by FITC-conjugated Fe_3O_4 nanocrystals. (d) T_2-weighted MRI of labelled cells by Fe_3O_4 nanocrystals with various iron concentrations in media (left: 0, middle: 50 μM and right: 100 μM).

irradiation. As a representative biomedical application, MRI was performed using the water-dispersible Fe_3O_4 nanocrystals as a contrast enhancement agent. Fig. 4b shows the size-dependent magnetic resonance contrasting properties of the water-dispersible Fe_3O_4 nanocrystals stabilized with the PO-PEGs. The nanocrystals with core diameters of 18 nm, 11 nm and 5 nm at the same iron concentration of 300 μM showed spin–spin relaxation times (T_2) of 23 ms, 38 ms and 99 ms, respectively, demonstrating that the larger Fe_3O_4 nanocrystals exhibited larger T_2 effect.[3] The current water-dispersible and monodisperse Fe_3O_4 nanocrystals could find application in the MRI of lymph-nodes and the brain.[4]

The cellular toxicity of the water-dispersible Fe_3O_4 nanocrystals stabilized with PO-PEGs was tested (ESI†). The nanocrystals showed no appreciable toxicity to the cells, confirming that the PO-PEG stabilized oxide nanocrystals are suitable for various biomedical applications such as cellular labeling, cell-tracking, and biomedical imaging.[5] The fluorescence microscopic image (Fig. 4c) showed that the FITC-conjugated Fe_3O_4 nanocrystals were easily transfected into human breast cancer cells (SKBR-3) without any transfection agents. Furthermore, they were detected by MRI. Fig. 4d shows that the labeled cells had T_2 shortening effects, which were dependent on the concentration of Fe_3O_4 nanocrystals in the incubating media. This shows that the PO-PEG ligands are efficient stabilizing agents for oxide nanocrystals in their applications to cellular labeling and molecular imaging.

In summary, PEG-derivatized phosphine oxide (PO-PEG) ligands were synthesized from a simple reaction between $POCl_3$ and PEG. The PO-PEG ligands could be readily endowed with surface functional groups for the conjugation with biomarkers and bio-active materials. The overall synthetic process is quite simple and inexpensive, and is readily applicable to the large-scale production of water-dispersible and biocompatible oxide nanocrystals for various biomedical applications.

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