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Hexanedioic acid mediated surface–ligand-exchange process for transferring $NaYF_4$:Yb/Er (or Yb/Tm) up-converting nanoparticles from hydrophobic to hydrophilic

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ABSTRACT

Water-soluble and carboxyl-functionalized up-converting rare-earth nanoparticles (UCNPs) are obtained via an efficient surface–ligand-exchange procedure. Hexanedioic acid molecules are employed to replace the original hydrophobic ligands in diethylene glycol solvent at high temperature. Various characterizations indicate the ligand-exchange process has negligible adverse effect on the quality of the UCNPs. The resulting hydrophilic UCNPs show small size, strong up-converting emission and high water stability. The specific molecular recognition capacity of avidin-modified hydrophilic UCNPs confirms that hydrophilic UCNPs are suitable for potential biological labeling.

1. Introduction

In the past few years, up-converting rare-earth nanoparticles (UCNPs) have shown great potential as a new class of fluorophores for biological and biomedical applications [1–3]. In comparison with conventional downconversion fluorescent materials, including organic dyes [4] and semiconductor quantum dots (QDs) such as CdS, CdSe and CdTe, etc. [5–7], the UCNPs have many advantages, such as high penetration depth and low background fluorescence due to excitation in near infrared and nearly absence of photo-damage under low power excitation [2,8–10].

Generally, synthesis of high-quality UCNPs is performed in organic solvents with surface passivation by long hydrocarbon chains of the surfactants. This nonaqueous method is capable of producing colloidal nanocrystals (NCs) with high crystalline, strong upconversion luminescence and narrow particle size distribution, comparing with the synthesis in aqueous solution [11–15]. However, insolubility of the NCs in water greatly limits their biological applications. Yet several strategies have been developed to transfer hydrophobic NCs into hydrophilic (such as Fe_2O_3, rare-earth NCs and QDs, etc.) [16–20]. Among which one is based on the encapsulation of hydrophobic NCs with amphiphilic polymer [21,22] or SiO_2 [23–26]. However, the additional layers of amphiphilic polymer or SiO_2 will dramatically increase the size of the particles which is not desirable for the biological applications, especially for fluorescence resonance energy transfer and in vivo applications [27,28]. Confronted with these challenges, Li et al. recently reported the directly oxidizing oleic acid ligand strategy to transfer UCNPs from hydrophobic to hydrophilic [20]. There are some limitation of this method, such as a relatively long reaction time and low yields, which remain to be solved.

Surface–ligand exchange is an alternative method, which is widely used in QDs. The mainly advantages of the method are simply and the process will not increase the size of the nanoparticles due to the small size of the exchange agents such as mercaptoacetic acid, dihydrolipoic acid and cysteines [28–30]. However, this method relies on the exchange of the surfactant coating ligands with the substitutional molecule of which one end carrying a functional group that is reactive toward the nanocrystal surface and the other end carrying a hydrophilic group. Therefore, it is difficult to have a universal substitutional molecule for all kinds of nanoparticles in ligand-exchange strategy. For this reason, up to date, although the water-soluble UCNPs have been obtained via the methods of oxidizing ligands or encapsulation, no feasible surface–ligand-exchange approach has been developed, owing to the difficulties in obtaining compatible and small substitutional ligands [20]. It is thus desirable to find proper substitutional ligand molecules and to develop a feasible surface–ligand-exchange method for getting the biocompatible water-soluble UCNPs.

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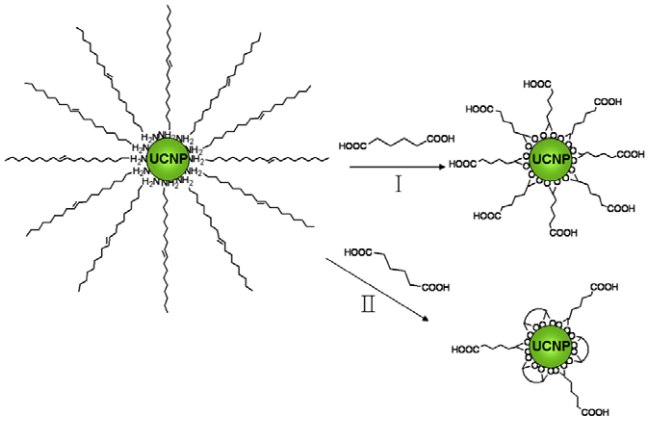
In this paper, the hexanedioic acid (HDA) molecules are selected as substitutional ligand, based on the following two reasons. First, the HDA molecule has two —COOH groups which make it in principle possible to coordinate one —COOH group to the UCNPs surface due to strong coordination of —COOH group with lanthanide ions (Ln^{3+}), and to leave the other end —COOH group free for water compatibility. Second, the HDA molecule, owing medial six carbons, proper in length since too short-chain alkanedioic acid will chelate easily to the surface of nanoparticles whereas too long-chain alkanedioic acid will be less favorable in dissolving in water. The ligand-exchange process is performed at relatively high temperature (~240 °C, which is close to DEG solvent boiling point ~245 °C) to enhance the efficiency of the ligand-exchange reaction. Besides, in the reaction system, high concentration (~0.4 mmol/mL) of the HDA molecules is kept to reduce the possibility of chelating of the HDA on the particle surface. The results show that the UCNPs are successfully transferred from hydrophobic to hydrophilic. It is anticipated that this method might be applied to the ligand exchange for other kinds of nanoparticles, which have strong ability of coordination with —COOH group, such as Fe_3O_4, TiO_2 and Au, etc. [31–33]. Scheme 1 schematically illustrates the general principle of the ligand-exchange approach.

The efficient ligand-exchange process is affirmed by various characterizations. The resulting HDA coated UCNPs maintain strong upconversion emission, small size and long term stability in aqueous solution. Moreover, the specific molecular recognition capacity of avidin-modified hydrophilic UCNPs is studied with fluorescence microscopy, indicating that the HDA modified UCNPs are suitable for biological labeling.

2. Materials and methods

2.1. Materials

All the rare-earth trifluoroacetates were prepared by dissolving the respective rare-earth oxides in trifluoroacetic acid (CF_3COOH; 99%, Aldrich). Trifluoroacetic acid sodium salt (CF_3COONa; 97%, Acros), oleylamine (OM; >80%, Acros), hexanedioic acid (HDA, 99%, Tianjin Chemical Co.), diethylene glycol (DEG; 98%, Tianjin Chemical Co.) ethyl-3-dimethyl amino propyl carbodiimide (EDAC, 98%, Aldrich), N-hydroxysulfosuccinimide sodium salt (Sulfo-NHS, Aldrich), bovine serum albumin (BSA), 2-(N-morpholino)ethanesulfonic acid (MES) hydrate, phosphate buffered saline (PBS) buffer, Tween 20, human IgG and bioinylated goat anti-human IgG were purchased from Beijing Dingguo Biotechnology Corporation. All chemical materials were used without further purification.



Scheme 1. Schematic illustration of the general principle of ligand-exchange approach using hexanedioic acid as substitutional ligand. I is the ideal model, II is close to actual situation.

2.2. Synthesis of $NaYF_4$:20%Yb, 2% Er (or Tm) NCs

OM coated $NaYF_4$:20% Yb, 2% Er (or Tm, Ho) nanoparticles were synthesized following the routes described previously [12]. Briefly, the mixture of CF_3COONa (2 mmol), $(CF_3COO)_3Y$ (0.78 mmol), $(CF_3COO)_3Yb$ (0.2 mmol), and $(CF_3COO)_3Er$ (or Tm) (0.02 mmol) was dissolved in oleylamine (10 mL), the mixture was then heated to 120 °C to remove water and oxygen, with vigorous magnetic stirring under argon flow for 1 h, followed by 2 h heating at 330 °C in the presence of argon protection. The resulting transparent yellowish reaction mixture was cooled down to 80 °C before ethanol (20 mL) was added. After purification using the standard precipitation–dissolution procedure, the as-synthesized nanoparticles were dissolved in chloroform before further treatment.

2.3. Surface–ligand exchange of UCNPs by HDA

The DEG solution (8.0 mL) containing HDA (500 mg, 3.4 mmol) was heated up to 110 °C with vigorous stirring under argon flow. A chloroform solution of UCNPs (~20 mg) was injected into the hot solution which became turbid immediately. The system was heated to 240 °C and kept at this temperature for about 1.5 h until the solution became clear. After the solution was cooled to room temperature, excess aqueous solution was added, and the NCs were isolated by centrifugation and decantation. Finally the sample was washed three times with pure water and redispersed in deionized water for analysis.

2.4. Bioconjugation of nanoparticles and fluorescence microscope imaging

The experimental processes are as follows: 2.0 mg nanoparticles were suspended in 1.0 mL of 0.02 M MES buffer (pH 6.0), containing 5 mg EDAC and 15 mg sulfo-NHS, and then stirred for 4 h at room temperature. After centrifugation resulting particles were redispersed in MES buffer, and 1 mg avidin was added. The mixture was stirred for 48 h at 4 °C. Finally, the avidin-coated nanoparticles were centrifuged, washed and suspended into 5 mM borate buffer (PH 8.5), containing 1% BSA, 0.05% Tween 20.

Human IgG with concentration of 0.1 mg/mL was spotted manually on an aldehyde silicon surface, allowed to incubate for 2 h at 37 °C. After washing with PBST (PBS containing 0.05% Tween 20) buffer three times, 2% BSA solution was added to block the active sites on the slice for 1 h. Then the silicon slice was put into biotinylated goat anti-human IgG or goat anti-human IgG with concentration of 0.1 mg/mL solution and incubated at 37 °C for 2 h. After washed with PBST and deionized water, the silicon wafers subjected to fluorescent microscopy. Slices were examined under a Motic AE30 microscope equipped with a Canon A630 camera. The excited light comes from an adscititious semiconductor diode laser with 980 nm.

2.5. Characterization

Morphology of the UCNPs was characterized at 200 kV using a Hitachi H-8100 IV transmission electron microscopy (TEM). The mean hydrodynamic size and size distribution were determined by dynamic light scattering (DLS) using Zetasizer 3000HSA. The ligand exchange was identified by Fourier transform infrared spectra (FT-IR, Perkin-Elmer spectrophotometer) and thermogravimetric analysis (TGA, Perkin-Elmer TGA-7). The upconversion emission spectra were recorded by a Jobin-Yvon LabRam Raman spectrometer system equipped with a Peltier air-cooled CCD detector excited by 980 nm laser. The fluorescence microscope imaging was taken under a Motic AE30 microscope equipped with a Canon A630 camera excited by a semiconductor diode laser