Tagging of avidin immobilized beads with biotinylated YAG:Ce\(^{3+}\) nanocrystal phosphor

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Abstract YAG:Ce\(^{3+}\) nanoparticles 9.5±1.2 nm in diameter have been synthesized from aluminium isopropoxide and acetates of yttrium and cerium in 1,4-butanediol (1,4-BD) by autoclave treatment at 300 °C for 2 h. After replacing 1,4-BD by ultrapure water, NH\(_2\) groups were introduced on the surface of YAG:Ce\(^{3+}\) nanoparticles by addition of 3-aminopropyltrimethoxysilane then biotinylation with sulfo-NHS-LC-biotin. We demonstrated that avidin immobilized beads are tagged by biotinylated YAG:Ce\(^{3+}\) nanoparticles by the selective avidin-biotin interaction, furnishing a green fluorescent image on excitation with blue light. This result indicates that YAG:Ce\(^{3+}\) nanoparticle phosphors have much potential in biological applications.

Keywords YAG:Ce\(^{3+}\) · Nanoparticle · 3-aminopropyltrimethoxysilane · Avidin-biotin interaction · Fluorescent image · Biological application

Abbreviations QDs quantum dots YAG:Ce\(^{3+}\) yttrium aluminium garnet doped with Ce\(^{3+}\) 1,4-BD 1,4-butanediol APTMS 3-aminopropyltrimethoxysilane Sulfo-NHS sulfosuccinimidyl-6(biotinamido) LC-biotin hexanoate biotinylating agent

Introduction

Since the development of highly sensitive CCD cameras fluorescent organic dyes have been widely used for biological imaging and quantification of biomolecules. The fluorescent intensity is, however, affected by dissolved oxygen, which causes rapid degradation [1]. Oxidation inhibitors, for example p-phenylenediamine and 1,4-diazobicyclo[2.2.2]octane are therefore needed for observation over long periods. Stokes shifts of most organic dyes are, furthermore, small, e.g. ∼30 nm for fluorescein isothiocyanate and ∼15 nm for cyanine. Spectral overlap between excitation and the emission reduces the S/N ratio and also makes it difficult to excite several dyes simultaneously using a light of a single wavelength.

Several efforts have been made to solve such problems. In the first attempt a dye was embedded in a matrix to prevent photochemical oxidation. The photostability of cyanine was improved by embedding in silica nanoparticles [2]. In the second attempt metal complexes were used for bio-labeling instead of organic dyes. Some complexes of rare-earth elements, for example europium and terbium, emit by exciting a ligand and then by energy transfer from a ligand to a central metal ion. Their Stokes shifts are larger

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than those of organic dyes. The fluorescence lifetime of a metal complex is several hundred microseconds, at least three orders of magnitude longer than that of an organic dye. In time-resolved fluoroimmunoassay, emission of the former can be distinguished from autofluorescence of tissues enabling more sensitive detection of a target molecule \([3, 4]\). By taking advantage of the same methods as for organic dyes, metal complexes are embedded in silica nanoparticles to improve sensitivity and photostability \([5–7]\).

The third attempt is biological application of semiconductor nanocrystals. CdSe/ZnS core/shell QDs are very photostable and have high quantum efficiency (ca. 40%) and tunable emission \([8–12]\). Several QDs of different sizes can also be simultaneously excited by light of a single wavelength. These advantages enabled us to perform simultaneous multiplexed toxin analysis \([13]\) and lineage-tracing experiments in embryogenesis \([14]\). The cytotoxicity of cadmium in CdSe/ZnS QDs has been discussed \([15, 16]\); surface modification with silica solved this problem \([17]\). QDs containing cadmium must, however, be treated as toxic substances and careful attention is needed for disposal. Radiation of UV light of less than 400 nm for the excitation of CdSe/ZnS QDs results in DNA lesions \([18]\).

Because YAG is a chemically and thermally stable material, YAG:Ce\(^{3+}\) is used as the white solid-state light-emitting device. We have previously reported wet chemical synthesis of YAG:Ce\(^{3+}\) nanocrystal phosphors of \(\sim 10\) nm diameter by a glycothermal method \([19, 20]\). We believe YAG:Ce\(^{3+}\) nanocrystal phosphor has much potential in biological applications for several reasons:

1. YAG:Ce\(^{3+}\) can be excited by blue light, which causes less damage to living cells;
2. YAG:Ce\(^{3+}\) nanocrystal phosphor has a luminescence quantum efficiency as high as 20–40%, because of the allowed 4f\(\rightarrow\)5d transition of Ce\(^{3+}\) \([21]\); and
3. YAG:Ce\(^{3+}\) is not regarded as a toxic material.
Here we report the preparation of biotinylated YAG:Ce$^{3+}$ nanoparticles and the labeling of avidin immobilized beads with these by use of the avidin–biotin interaction.

**Experimental**

**Chemicals and materials**

Yttrium acetate tetrahydrate (99.99%), cerium acetate hydrate (99.99%), aluminium isopropoxide (99.9%), and 1,4-butanediol (97.0%) were purchased from Kanto Chemical. 3-Aminopropyltrimethoxysilane and sulfo-NHS-LC-biotin were purchased from Chisso Chemical Division and Pierce, respectively. Ultrapure water was prepared by use of a Milli-Q system (Millipore). Avidin immobilized agarose gel beads and avidin immobilized polystyrene-copolymer beads (Micromer) were purchased from Sigma and Micromod Partikeltechnologie, respectively.

**Synthesis of YAG:Ce$^{3+}$ nanoparticles by glycothermal method**

Yttrium acetate tetrahydrate (2.5014 g), cerium acetate hydrate (0.0251 g), aluminium isopropoxide (2.553 g), and 1,4-BD (63.6 mL) were poured into a 120-mL autoclave (Taiatsu Techno TVS-120-N2). The sealed autoclave was heated to 300 °C at 3.1° min$^{-1}$ with stirring at 300 rpm, kept at the same temperature for 2 h, and cooled to room temperature to furnish the YAG:Ce$^{3+}$ colloidal 1,4-BD solution. This solution was left for 1 week to induce sedimentation of aggregated particles. The supernatant colloidal 1,4-BD solution was used for the conjugation with biotin.

**Fig. 3** Particle-size distribution measured by DLS for YAG:Ce$^{3+}$ nanoparticles diluted in ultra pure water (solid line) and APTMS-modified YAG:Ce$^{3+}$ nanoparticles diluted in ultra pure water (broken line)

**Fig. 4** PL and PLE spectra of the as-prepared YAG:Ce$^{3+}$ nanoparticles dispersed in 1,4-BD. PL and PLE spectra were measured by monitoring the optimum wavelengths, i.e. 450 and 530 nm, respectively

**Fig. 5** FTIR spectra: (a) YAG:Ce$^{3+}$ nanoparticles, (b) APTMS-modified YAG:Ce$^{3+}$ nanoparticles
Conjugation with biotin

Figure 1 shows the process of the conjugation between YAG:Ce$^{3+}$ nanoparticles and biotin. APTMS (150 μL) was poured into the mixture of YAG:Ce$^{3+}$ colloidal 1,4-BD solution (5 mL) and ultrapure water (10 mL), and was stirred for 2 h. After removal of free APTMS by centrifugation, sulfo-NHS-LC-biotin-PBS solution (1 mL) was poured into the colloidal solution and stirred for 2 h. Free biotinylation agents were removed to furnish the biotinylated YAG:Ce$^{3+}$ colloidal aqueous solution.

Tagging of avidin beads with biotinylated YAG:Ce$^{3+}$ nanoparticles

Biotinylated YAG:Ce$^{3+}$ colloidal aqueous solution (100 μL) and avidin immobilized agarose gel beads (100 μL) or avidin immobilized polystyrene-copolymer beads (50 μL) were poured into ultrapure water (5 mL) and stirred for 2 h. This mixture was centrifuged in the ultrafiltration microtube (pore size 5.0 μm) to remove free biotinylated YAG:Ce$^{3+}$ nanoparticles. YAG:Ce$^{3+}$ nanoparticles without biotin was also used in the above-mention procedure as the control.

Characterization

YAG:Ce$^{3+}$ nanoparticles were observed with a Hitachi H-9000UHR field-emission transmission electron microscope (FE-TEM). The size distribution of YAG:Ce$^{3+}$ nanoparticles was measured by means of a Malvern HPPS dynamic light-scattering (DLS) instrument; the as-prepared colloidal solution was diluted in ultrapure water and the refractive index of bulk YAG, 1.82, was used.

Powdered samples of YAG:Ce$^{3+}$ were obtained from YAG:Ce$^{3+}$ colloidal solutions by washing with ethanol and centrifuging. The molecules adsorbed on the surface of YAG:Ce$^{3+}$ powdered samples were analyzed by Fourier-transform infrared (FTIR) absorption spectrometry (Bio-Rad, FTS-60A) by the diffuse-reflection method. Diffuse reflectance spectra of powdered samples were measured by ultraviolet-visible (UV-vis) optical absorption spectrometry (Jasco, V570), in which the white plate (Spectralon SRS-99) was used as reference standard and calibration was

<table>
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<th>Peak number</th>
<th>APTMS-modified YAG:Ce$^{3+}$</th>
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<th>Assignment</th>
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<tr>
<td>1</td>
<td>~3700</td>
<td>~3700</td>
<td>v(OH)</td>
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<td>v(OH)</td>
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<td></td>
<td>v(CH)</td>
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$\nu$, stretching; $\delta$, bending; $\omega$, wagging; $\nu^s$, asymmetric; $s$, symmetric
performed using the regression line from 550 nm to 700 nm as background.

Photoluminescence (PL) and its excitation (PLE) spectra of colloidal and powdered samples were measured by means of a Jasco FP-6500 spectrophotometer. Avidin immobilized beads tagged with biotinylated YAG:Ce\textsuperscript{3+} nanoparticles were observed by fluorescence microscopy (Nicon, Eclipse, E600) in which the modified FITC filter cube set (excitation wavelength, 430 to 440 nm; dichroic wavelength 505 nm; emission 515 to 555 nm band pass) was used. Avidin immobilized polystyrene-copolymer beads tagged with biotinylated YAG:Ce\textsuperscript{3+} nanoparticles were also observed with a Hitachi S-4700 field-emission scanning electron microscope (FE-SEM).

**Results and discussion**

Properties of YAG:Ce\textsuperscript{3+} colloidal aqueous solution

Figure 2a shows the FE-TEM micrograph of YAG:Ce\textsuperscript{3+} nanoparticles synthesized by the glycothermal method. Fig. 2b shows the particle size distribution estimated by counting particles in the TEM image. The mean particle size is 9.5, standard deviation 1.2 nm.

Figure 3 (solid line) shows the size distribution of YAG:Ce\textsuperscript{3+} nanoparticles measured by DLS. The mean particle size obtained by DLS is 46.8 nm, standard deviation 7.5 nm, larger than that obtained by use of FE-TEM. This indicates that YAG:Ce\textsuperscript{3+} primary particles aggregate in water to form secondary particles.

Figure 4 shows PL and PLE spectra of YAG:Ce\textsuperscript{3+} nanoparticles. The excitation peaks are observed at 450 and 345 nm. The former is assigned to the 4f\textsuperscript{→}5d\textsubscript{1} transition and the latter to the 4f\textsuperscript{→}5d\textsubscript{2} transition for Ce\textsuperscript{3+} incorporated in YAG. The emission consists of overlaid peaks at 530 and \( \sim 570 \) nm. The former is assigned to the 5d\textsuperscript{1}(\textsuperscript{2}A\textsubscript{1g})\textsuperscript{→}4f (\textsuperscript{2}F\textsubscript{5/2}) transition and the latter to the 5d\textsuperscript{1}(\textsuperscript{2}A\textsubscript{1g})\textsuperscript{→}4f (\textsuperscript{2}F\textsubscript{7/2}) transition for Ce\textsuperscript{3+} incorporated in YAG.\textsuperscript{20} The PL and PLE spectra of YAG:Ce\textsuperscript{3+} nanoparticles do not change after replacing 1,4-BD with ultrapure water.

The internal PL quantum efficiency (IQE\textsubscript{sample}) of the sample was calculated by use of the equation:

\[
\frac{IQE_{\text{sample}}}{IQE_{\text{ref}}} = \frac{F_{\text{sample}}}{F_{\text{ref}}} \times \frac{A_{\text{ref}}}{A_{\text{sample}}} \tag{1}
\]
where IQE_{\text{ref}} is the internal PL quantum efficiency (72\%) of the reference, (Y_{2.1}\text{Gd}_{0.9})Al_3O_{12}:Ce^{3+} (Kasei Optonix, P46-Y3), at \lambda_{\text{ex}}=470 nm; F_{\text{sample}}, the integral PL intensity of the sample at \lambda_{\text{ex}}=450 nm; F_{\text{ref}}, the integral PL intensity of the reference at \lambda_{\text{ex}}=470 nm; A_{\text{sample}}, the absorbance of the sample at 450 nm; and A_{\text{ref}}, the absorbance of the reference at 470 nm. The IQE_{\text{sample}} value was 22.0\% for the powder obtained from the YAG:Ce^{3+} colloidal solution.

Surface modification of YAG:Ce^{3+} nanoparticles by APTMS

Figure 5 shows FTIR spectra of YAG:Ce^{3+} nanoparticles with and without APTMS modification. FTIR peak assignments are summarized in Table 1. After APTMS modification new peaks 3 and 8 appear at 3091 and 1643 cm^{-1}, respectively. The former is assigned to the N-H stretching mode and the latter to the H-N-H bending mode. Peaks 13 and 14 at 1176 and 1067 cm^{-1} are assigned to the asymmetric stretching modes of the Si-O-X (X=Al, Y, Ce, and C) bond. These results suggest that the surface of the YAG:Ce^{3+} nanoparticles is coated with APTMS. We note that peak 1 corresponding to the free isolated OH group is observed at 3700 cm^{-1}. This OH group on the surface of YAG:Ce^{3+} nanoparticles possibly reacts with the hydrolyzed APTMS by condensation to introduce the NH_{2} group, as already shown in Fig. 1.

After APTMS modification the mean particle size measured by DLS is 41.3 nm, standard deviation 9.1 nm, as shown by broken line in Fig. 3. Thus, the APTMS modification does not appreciably change the stability of the dispersion.

Fig. 7 Microscopic images of avidin immobilized polystyrene-copolymer beads. (a), (b), (d), (e): after treatment with biotinylated YAG:Ce^{3+} nanoparticles; (c): before treatment with biotinylated YAG:Ce^{3+} nanoparticles; (a): bright field image; (b): fluorescent image; (c)-(e): FE-SEM images
Figure 6 shows fluorescent images of avidin immobilized agarose gel beads. YAG:Ce\(^{3+}\) nanoparticles without biotin are inert toward avidin immobilized agarose gel beads and no image is observed by fluorescence microscopy, as is apparent from Fig. 6c,d. In contrast, for biotinylated YAG:Ce\(^{3+}\) nanoparticles the fluorescence image is the same as the bright field image, as shown in Fig. 6a,b. A similar result was obtained for avidin immobilized polystyrene-copolymer beads, as shown in Fig. 7a,b. FE-SEM observation also reveals that the surface of the beads is uniformly covered with YAG:Ce\(^{3+}\) nanoparticles, as is apparent from Fig. 7d,e and comparison with Fig. 7c. These results indicate that biotinylated YAG:Ce\(^{3+}\) nanoparticles are successfully bound to the surface of avidin immobilized beads by selective avidin-biotin interaction.

Conclusions

YAG:Ce\(^{3+}\) nanoparticles of 9.5±1.2 nm diameter have been prepared by glycothermal method. This phosphor emits green light on excitation with blue light. APTMS modification introduces NH\(_2\) groups on to the surface of the particles to enable conjugation with biotin. Avidin immobilized beads tagged with biotinylated YAG:Ce\(^{3+}\) nanoparticles can be observed by fluorescence microscopy. This demonstrates that conjugation of YAG:Ce\(^{3+}\) nanoparticles with other biomolecules, for example DNA and protein, has promising potential in biological applications.

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