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Multi-Photon Sensitized Excitation of Near Infrared Emitting Lanthanides

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Near infrared (NIR) multi-photon excitation of the NIR-emitting lanthanides neodymium (Nd^{3+}) and ytterbium (Yb^{3+}) sensitized by a fluorescein-linked chelator was demonstrated. Because tissues display minimal absorbance near the excitation wavelength of 800 nm, and because the lanthanides display long decay times, these results suggest the use of Nd^{3+} and Yb^{3+} as luminescent probes in tissues with multi-photon excitation.

KEY WORDS: Lanthanides; fluorescence; multi-photon; near infrared.

INTRODUCTION

The lanthanides terbium (Tb) and europium (Eu) emit in the visible region of the spectrum and, when suitably complexed, display high quantum yields and long luminescent decay times near 2 ms [1–3]. Because of these favorable spectral properties, Tb and Eu are widely used in biomedical assays [4,5]; resonance energy transfer donors [6,7], and occasionally, cellular imaging [8]. There is also growing interest in the use of lanthanides for selective imaging of cancerous regions of tissues [9] and as fluorescently detectable magnetic resonance imaging contrast agents [10]. The use of lanthanides is limited by the need for UV or visible excitation and emission wavelengths that overlap with the autofluorescence of biological tissues.

Recently, growing interest has been expressed in the near infrared (NIR)-emitting lanthanides neodymium (Nd^{3+}), ytterbium (Yb^{3+}), and erbium (Er^{3+}) [11,12]. It has been shown that the NIR emission from Nd^{3+}, Yb^{3+}, and Eu^{3+} can be sensitized by fluorescein and eosin absorbing in the visible wavelength near 480 nm [13–16]. This report demonstrates that the emission from NIR-emitting lanthanides can be sensitized with two-photon excitation of a fluorescein-containing chelator at 800 nm. Because tissues display minimal absorbance near 800 nm, these results suggest the use of multi-photon excitation of NIR-emitting lanthanides to probe biological tissues.

MATERIALS AND METHODS

All chemicals were obtained from Aldrich and used without further purification. The ligand AMF-DTPA was synthesized from 5-aminofluorescein (AMF) and diethylenetriamine pentaacetic acid (DTPA) dianhydride, as described previously [16], yielding the chelator structure AMF-DTPA shown in Fig. 2. The ligand was purified by reverse-phase HPLC on a C_{18} column using a gradient of acetonitrile in water, both containing 0.1% TFA. Multi-photon excitation was accomplished using the 800-nm fundamental output of a mode-locked Ti:sapphire laser with a pulse width near 100 fs. Single-photon excitation was accomplished with the frequency-doubled output of

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[3] ABBREVIATIONS: 2PE, Two-photon excitation; AMF, 5-amino fluorescein; DTPA, diethylenetriamine pentaacetic acid; NIR, near infrared; MPE, Multi-photon excitation.

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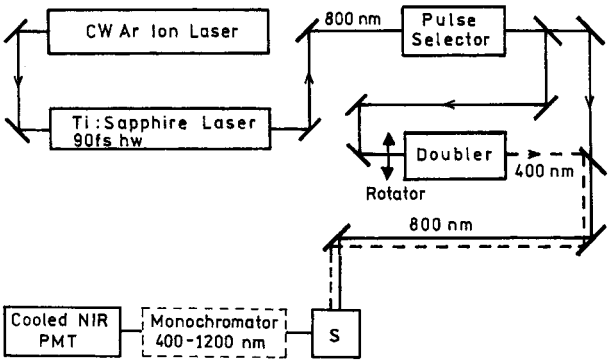

Scheme 1. Ti:Sapphire-based laser system for multi-photon excitation.

this laser at 400 nm. The uncorrected emission spectra were recorded using a 400–1,200 nm monochromator and a cooled R5108 Hamamatsu PMT with a Ag-O-Cs photocathode for NIR sensitivity. All solutions were in D_2O.

RESULTS AND DISCUSSION

Absorption spectra of Nd^{3+} and Yb^{3+} in D_2O are shown in Fig. 1. Nd^{3+} shows multiple absorption bands from 450 to 850 mn. Yb^{3+} shows a single absorption peak near 960 nm. In both cases the extinction coefficient is small, as can be judged by the 100-mM concentration used to record the spectra.

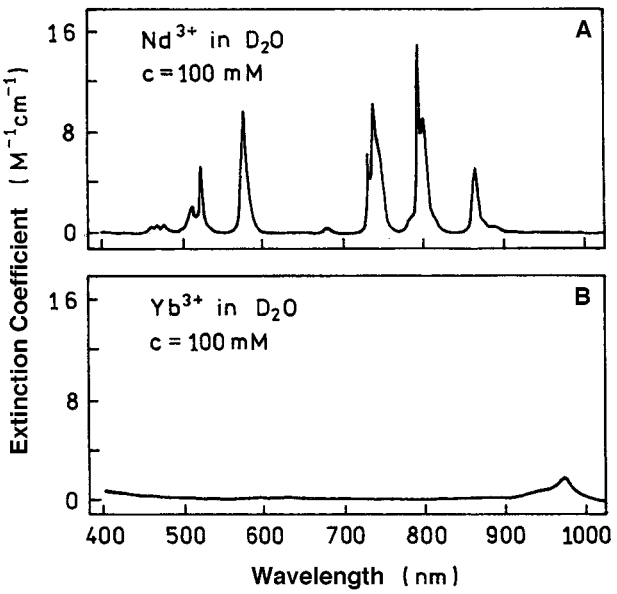

Fig. 1. Absorption spectra of Nd^{3+} (A) and Yb^{3+} (B).

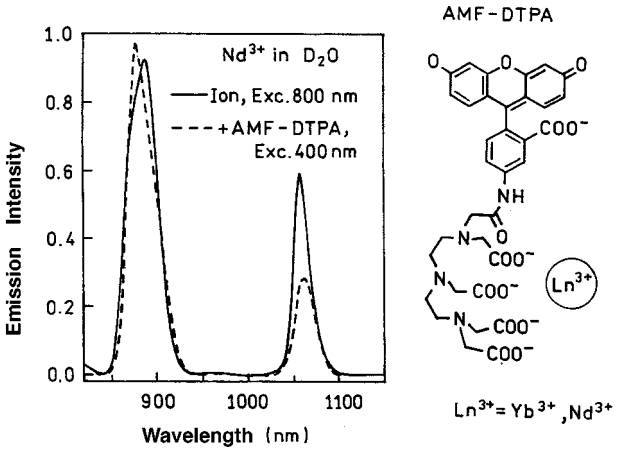

Fig. 2. Emission spectra of Nd^{3+} with direct excitation into the absorption band at 800 nm (—) and Nd-AMF-DTPA with excitation at 400 nm (- - -).

Figure 2 compares the emission spectra of uncomplexed Nd^{3+} in D_2O and when complexed with AMF-DTPA, also in D_2O. In the case of uncomplexed Nd^{3+}, excitation was into the 800-nm absorption band, whereas for Nd-AMF-DTPA excitation was at 400 nm. Essentially identical emission spectra were observed for free and complexed Nd^{3+}.

Figure 3 shows the emission spectra of Nd^{3+} and Nd-AMF-DTPA in D_2O for excitation at 840 nm. With

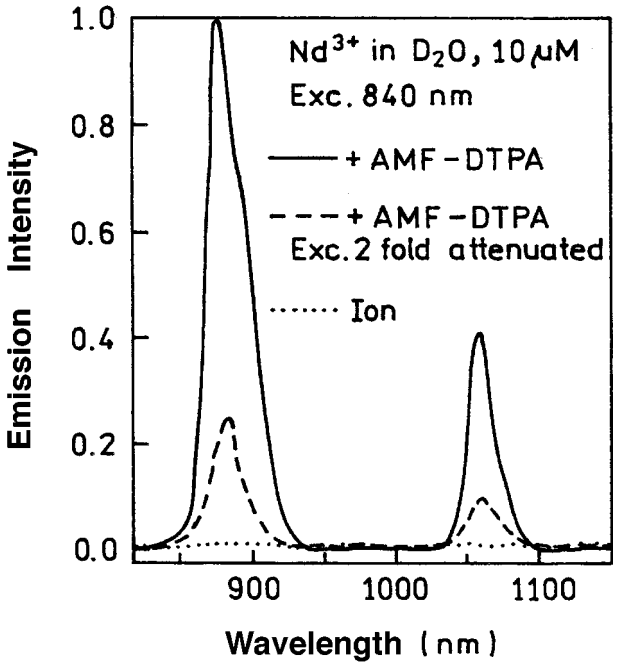

Fig. 3. Emission spectra of ND^{3+} for 840 nm excitation. In absence of AMF-DTPR, there is no emission observed (••••).

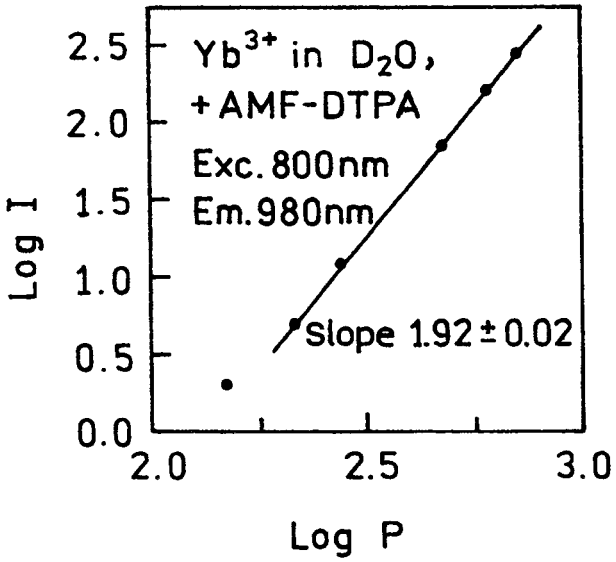

Fig. 4. Dependence of Yb-AMF-DTPR emission on excitation power. The incident power is in mW.

the same illumination intensities the emission of uncomplexed Nd^{3+} is manyfold less than that of Nd-AMF-DTPA. The ligand AMF-DTPA does not absorb at 840 nm; thus the emission is probably due to multi-photon absorption by the fluorescein chromophore. A two-photon absorption process was demonstrated by the four fold decrease in the emission of Nd-AMF-DTPA when the excitation at 840 nm is attenuated two fold.

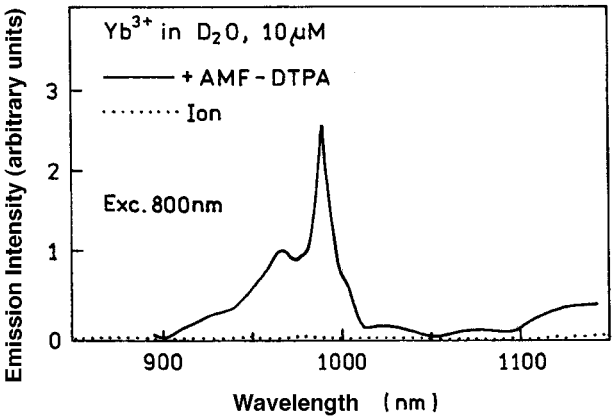

Fig. 5. Two-photon induced emission spectrum of Yb-AMF-DTPR.

Finally, Yb^{3+} in D_2O uncomplexed and complexed with AMF-DTPA was examined. The square dependence of the emission intensity on the incident power is shown in Fig. 4. With 800-nm excitation there was no detectable emission from the uncomplexed ion. In contrast, the Yb^{3+} emission was easily detectable from a 10-μm solution of Yb-AMF-DTPA (Fig. 5). Because the fluorescein chromophore does not absorb by a single-photon process at 800 nm, the Yb^{3+} emission is sensitized by multi-photon absorption of AMF-DTPA.

In summary, the results demonstrate multi-photon–sensitized excitation of Nd^{3+} or Yb^{3+} and suggests the use of such complex probes in tissues.

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