

Energy transfer study of europium–tetracycline complexes

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Available online 24 March 2006

Abstract

The aim of this work was to study the energy transfer mechanisms from the ligand to the central lanthanide ion of tetracycline–europium (Tc–Eu) complex. The absorption spectra of a $\text{EuCl}_3 \cdot \text{H}_2\text{O}$ solution was measured and absorption cross-section of Eu^{3+} was estimated. The observed overlap predicts a resonant energy transfer from Tetracycline to Eu^{3+} . The microscopic parameter of energy transfer was obtained using the overlap integral calculation considering a dipole–dipole energy transfer mechanism and results were compared considering an energy transfer by exchange.

In this work, we report also the optical properties of Tc–Eu complex when urea hydrogen peroxide is added to the aqueous solution.
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Keywords: Lanthanides; Optical sensor; Glucose; Urea; Tetracycline; Energy transfer

1. Introduction

The emission from lanthanides ions has been utilized extensively in biological systems [1]. Although trivalent lanthanides ions (Ln^{3+}) have small absorption coefficients, it is possible to produce highly luminescent lanthanides complexes chelating these ions in strongly absorbing ligand as the tetracycline molecule. Tetracycline–Europium (Tc–Eu) complex is the first lanthanide-based probe that can be excited by 405 nm diode laser.

Sensitized 4f luminescence in lanthanides complexes is efficiently used as alternative to organic fluorophores, particularly where there are problems of background auto-fluorescence due to their spectral characteristics. These characteristics include long lifetime (μs to ms), sharply spiked emission spectrum (under 10 nm bandwidth), large Stokes shift, no self-quenching, high quantum yield for lanthanides luminescence and excellent solubility [2]. Upon excitation of the sensitizer, the energy is transferred to the lanthanide ion. For efficient energy transfer the sensitizer needs to be in close proximity of the ion and the energy levels of sensitizer and lanthanide ion should match.

Sensitizers are being used to excite the lanthanide ions via an energy transfer from the sensitizer (or antenna) to the lanthanide ion [3]. Upon absorption of light the sensitizer is excited to the singlet excited state. This state can transfer its energy to the lanthanide ion (singlet energy transfer), can convert to the triplet state (intersystem crossing), or can decay to the ground state (by fluorescence or non-radiatively). From the triplet state, energy is transferred to the lanthanide ion or the triplet state decays to the ground state. In general, energy is transferred via the triplet state because the intersystem crossing is enhanced by the nearby, paramagnetic lanthanide ions, and because energy transfer via the singlet state is not fast enough to compete with the fluorescence or the intersystem crossing.

In this work, we report the optical properties of Tc–Eu complex, the energy transfer mechanisms were studied and an enhancement on europium emission band when urea hydrogen peroxide is added to the Tc–Eu aqueous solution was observed for the first time.

2. Materials and Methods

The absorption spectra of all samples were measured at room temperature in the range 200–2500 nm using a Varian Spectrometer Cary 17 D. The emission spectra were

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obtained by exciting the samples (1 mm thickness) with a 150 W xenon lamp. The emissions of the samples were analyzed with a 0.5 m monochromator (Spex) and a PMT detector. The signal was amplified with an EG&G 7220 lock-in and processed by a computer. The relative errors in the emission measurements are estimated to be <5%.

All inorganic salts used have analytical purity and were obtained from Sigma Aldrich and Molecular Probe. All solutions were prepared in 10 mmol l⁻¹ 3-(*N*-Morpholino) propanesulfonic acid (Mops from Carl Roth, Germany) buffer. Tetracycline–HCl used was a secondary pattern gently provided by Bunker Indústria Farmacêutica Ltda. Urea peroxide 98% used in this work is from Sigma.

Solution I: 2.3 g of Mops salt (*N*-morpholino propanesulfonic acid from Carl Roth, Germany) in 100 ml of distilled water with NaOH to adjust the pH to 6.9.

Solution II: 115.3 mg of EuCl₃·6H₂O in 10 ml of Mops buffer solution.

Solution III: 50.5 mg of tetracycline–HCl in 10 ml of Mops buffer solution.

Solution IV: Tc–Eu solution. Mix 10 ml of solution II and 10 ml of solution III.

Solution V: Tc–Eu–urea hydrogen peroxide solution. Mix 10 ml of Solution IV on different concentrations of urea hydrogen peroxide.

3. Results

Optical absorption of Tc–Eu solution was measured and the results are shown in Fig. 1. In this figure we can observe that the tetracycline absorption band shifts to red when perturbed by europium ions. Tetracyclines have several proton-donating groups which offer different possibilities of complexation with lanthanide ions depending on the pH. For pH around 7.0, lanthanides are probably bound to oxygen atoms. Chelation sites include the β-diketone system and the enol and carboxamide groups of the A ring [4,5].

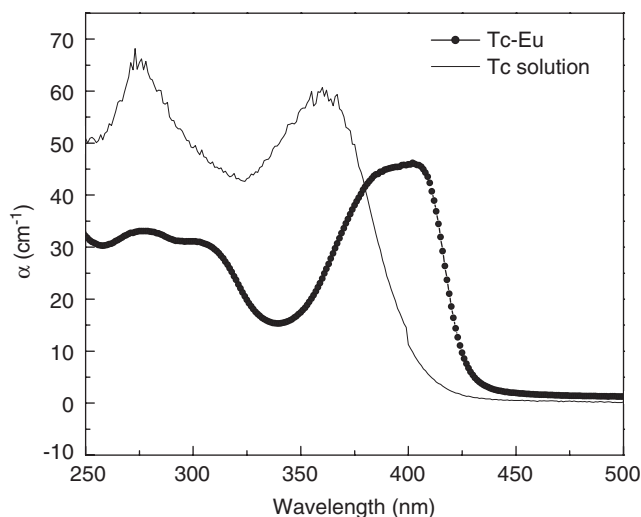


Fig. 1. Optical absorption of Tc–Eu complexes.

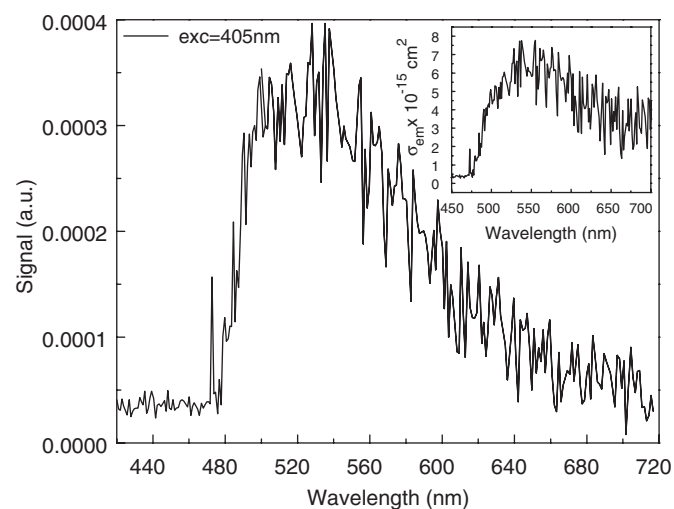


Fig. 2. Emission spectra of tetracycline excited at 405 nm. Inside figure shows the emission cross-section calculated considering radiative lifetime ~ 25 ps.

The tetracycline emission band occurs around 520 nm (Fig. 2) and this emission must be transferred to europium ions that emit light. Tc–Eu complex showed a strong red emission, at 619 nm and its intensity depends on the pH of the solution. The best pH range is 6.8–70.

The intense europium luminescence originates from the intramolecular energy transfer through the excited state of the tetracycline to the emitting level of Eu³⁺. Energy transfer can proceed via dipolar or multipolar interactions between sensitizer and acceptor (lanthanide ion), a Förster [6] mechanism of transfer, or via an exchange mechanism, the Dexter mechanism of transfer. The main difference between these two types of energy transfer is that in the Dexter mechanism orbital overlap is needed, whereas in the Förster mechanism the transfer is through space and is strongly dependent on the spectral overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor. In the Dexter mechanism, the spectral overlap is independent of the oscillator strength of the transitions (emission and absorption spectra are normalized such that the areas of the spectra are unity). Energy transfer by the Dexter mechanism is only efficient at very small distances (<10 Å), whereas Förster energy transfer has been reported to occur over much longer distances.

Using the resonant energy transfer theory, a non-radiative quantum mechanical process that does not require collisions and as consequence production of heat. For Tc–Eu complex, we obtained several important parameters, the microparameter (C_{DA}) involved in the energy transfer from the excited states of donor (Tc) to the acceptor (Eu³⁺) can be calculated by

$$C_{DA} = \frac{R_c^6(D-A)}{\tau_D}, \quad (1)$$

where τ_D is the total lifetime of the donor state without the presence of the acceptor. The critical radius R_c is calculated

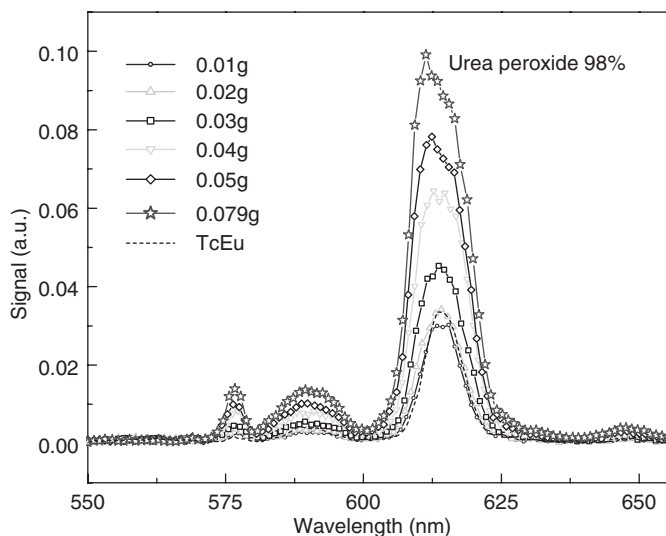


Fig. 3. Urea hydrogen peroxide concentration effect on Tc–Eu–UHP complex emission spectra.

using the overlap integral method based on the calculation of the emission (donor) and the absorption (acceptor) cross-section overlap using the following expression valid for a resonant energy transfer:

$$R_c^6 = \frac{6c\tau_D}{(2\pi)^4 n^2} S(\lambda), \quad (2)$$

where c is the speed of light, n the refractive index of the medium, and $S(\lambda)$ is the overlap integral between the emission and absorption cross-sections of donor and acceptor ($4.88 \times 10^{-32} \text{ cm}^2$), respectively, calculated for D–A transfer. The emission cross-section of Tc was obtained from the emission shown in Fig. 3:

$$\sigma_e(\lambda) = \frac{\lambda^4}{n^2 8\pi c \tau_R} \frac{SE(\lambda)}{\int SE(\lambda) d\lambda}, \quad (3)$$

where n is refractive index of the Tc–Eu complex (measured $n = 1,3385$), $SE(\lambda)$ is the emission spectra, τ_R is radiative lifetime (considered ~ 10 ns dipole–dipole permitted transition) and the τ_D donor emission lifetime of 25 ps [7].

The absorption spectra of a highly concentrated $\text{Eu-Cl}_3 \cdot \text{H}_2\text{O}$ solution was measured and absorption cross-section of Eu^{3+} ($\sim 5 \times 10^{-18} \text{ cm}^2$, $\sigma_{\text{abs}} = \alpha/[\text{Ln}^{3+}]$) was estimated. The observed overlap predicts an efficient resonant energy transfer from Tc to Eu^{3+} . The microscopic parameter of energy transfer obtained using the overlap integral calculation was $C_{\text{DA}} \sim 3.15 \times 10^{-31} \text{ cm}^6/\text{s}$ and $R_c \sim 14.1 \text{ \AA}$.

For these values it was possible to calculate the energy transfer probability ($3.9 \times 10^{10} \text{ s}^{-1}$) given by

$$W_{\text{DA}} = \frac{C_{\text{DA}}}{R_c^6}. \quad (4)$$

For exchange mechanism, with 50% luminescence quantum yields we obtained $R_c = 2.6 \text{ \AA}$. We consider that the electron exchange mechanism of energy transfer must be more effective than dipole–dipole mechanism in Tc–Eu complex.

β -Diketonate complexes form strong 3:1 complexes and have absorption bands, which can be tuned easily by changing substituents on the chelate. In neutral 1:3 complexes, the coordination number on the lanthanide ion is six. This means that the coordination of the lanthanide ions is unsaturated and additional ligands can be incorporated. An increase in europium emission band was observed, for the first time, with addition of urea peroxide in the solution, as we can see in Fig. 3. The shape of the emission band and a shift for the blue region were observed comparing with Tc–Eu and Tc–Eu–urea hydrogen peroxide solutions.

Urea hydrogen peroxide (UHP) is a stable form of hydrogen peroxide (HP) [8,9] and cytotoxic agent. Both UHP and HP have potential deleterious effects on various cells, including those of kidney and heart. It was conceived that this enhancement could be used to determine urea hydrogen peroxide.

4. Conclusions

Tc–Eu complexes were studied and they showed high lanthanide emission. The energy transfer between Tc and Eu was studied and an interaction critical radius of 14.1 \AA for dipole–dipole transfer was obtained. For exchange mechanism the calculated critical radius was 2.6 \AA .

Finally the enhancement of Eu emission with introduction of urea peroxide in Eu–Tc solution suggest a new method for determination of urea hydrogen peroxide in aqueous solutions using the luminescence enhancement of europium tetracycline complex upon binding of urea peroxide.

Acknowledgments

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP, for the support under grants 00/15135-9 and 05/50437-0.

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