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Spectroscopic study of a water-soluble 2,2′-bipyridyl-based europium complex and its interaction with human serum albumin

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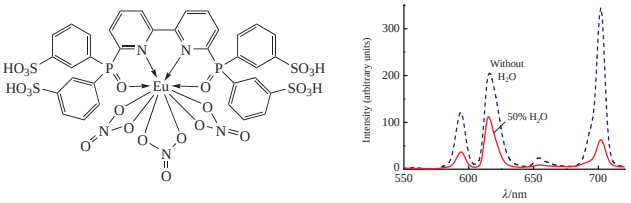
The water-soluble complex of europium with 6,6′-bis[di(3-sulfophenyl)phosphinoyl]-2,2′-bipyridine was synthesized and spectroscopically characterized, and its interaction with human serum albumin was studied using a luminescence technique.



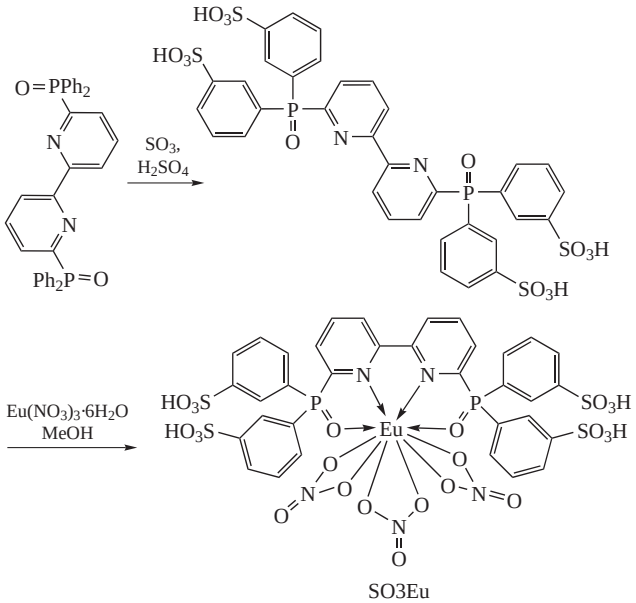
Coordination complexes of rare earth elements (REEs) have found a wide application in biomedical analyses and imaging due to their luminescent properties. They are used as pH,[1] oxygen,[2] hydrogen peroxide[3] and sugar probes[4] along with immunoassays[5] and DNA analysis.[6] The major feature of the complexes for bio-related applications is their water solubility, but the quenching of REE luminescence by water molecules requires the protection of REE ions from water coordination. Thus, the design of ligands capable of reacting with REEs and, at the same time, providing donor centers for metal binding with a high coordination number is a complicated task. Therefore, the design of the water-soluble REE complexes possessing good photophysical properties is of interest in the coordination chemistry of lanthanides.[7] The phosphinoyl complexes of REEs possess bright luminescence in organic solutions,[8] however published information on the water-soluble phosphinoyl complexes is scarce.[9]

The aim of this work was to study the chemical and spectroscopic properties of europium complex SO3Eu (6,6′-bis[di(3-sulfophenyl)phosphinoyl]-2,2′-bipyridine europium trinitrate) and its interaction with human serum albumin (HSA), which is a relevant component of human blood, using a luminescence technique. Thus, we investigated the phosphorescence of the europium complex in the presence of water and the spectral properties of albumin in the presence of this europium complex.

The water-soluble sulfated phosphinoyl ligand and its complex were prepared according to Scheme 1.† The positions of sulfation were found from ^{1}H and 2D COSY NMR spectra.

The interaction of the ligand with europium nitrate gives the complex SO3Eu in a high yield. The IR spectrum exhibited characteristic absorption bands due to bidentate chelate coordinated NO_3 groups at 1637 (ν_5), 1253 (ν_1) and 1035 (ν_2) cm^{-1} in accordance with previously published data.[10] The structure and composition of the complex were confirmed by IR, NMR, absorbance and luminescence spectroscopy and elemental analysis data.

Spectral measurements were performed for the complex dissolved in DMSO at concentrations from 1×10^{-5} to 7×10^{-5} mol dm^{-3} and in an acetate buffer at different pH values.‡ The absorption



Scheme 1

† *6,6′-Bis[di(3-sulfophenyl)phosphinoyl]-2,2′-bipyridyl*. 6,6′-Bis(diphenylphosphinoyl)-2,2′-bipyridyl (182 mg, 0.33 mmol) was added with violent stirring to a mixture of conc. H_2SO_4 (0.10 ml, 1.8 g cm^{-3}) and oleum [0.23 ml, $\omega(SO_3)$ = 60–65%] heated to 50–55 °C under an inert atmosphere. After stirring for 24 h, the reaction mixture was quenched by dropwise addition to crushed ice (50 ml) and neutralized by $NaHCO_3$ to pH ≈ 5–6. After neutralization, 30 ml of ethanol was added, and the white precipitate of $Na_2SO_4\cdot10H_2O$ was filtered off. The resulting colorless clear solution was concentrated *in vacuo* to obtain 221 mg (77%) of white powder. ^{1}H NMR (400 MHz, methanol-d_4) δ: 7.62 (td, 2H, *J* 7.76, 3.06 Hz), 7.97 (dd, 2H, *J* 11.74, 7.83 Hz), 8.05 (d, 2H, *J* 8.07 Hz), 8.22 (dq, 1H, *J* 7.70, 7.54 Hz), 8.15–8.27 (m, 1H), 8.51 (d, 2H, *J* 11.98 Hz), 8.62 (dt, 1H, *J* 7.64, 1.68 Hz). ^{31}P NMR, δ: 21.79. IR (KBr, ν/cm^{-1}): 3448, 3062, 2956, 2923, 2854, 1637, 1569, 1550, 1465, 1436, 1427, 1384, 1139, 1137, 1108, 1037, 995, 798, 742, 692, 620, 539, 487. Found (%): C, 46.79; H, 3.27; N, 3.51. Calc. for $C_{34}H_{26}N_2O_{14}P_2S_4$ (%): C, 46.58; H, 2.99; N, 3.20.

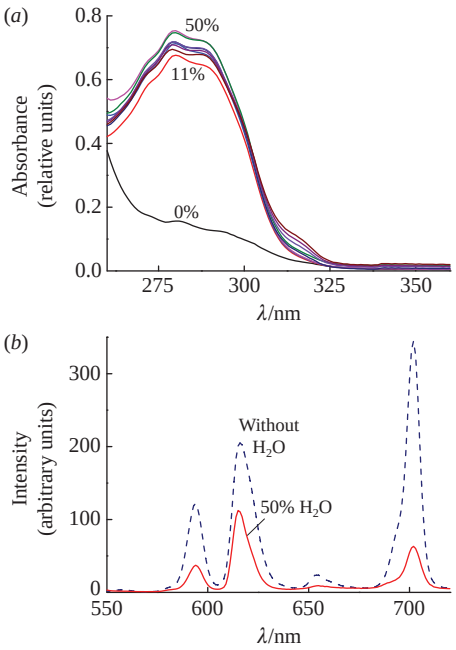


Figure 1 (*a*) Absorption and (*b*) emission (λ_{ex} = 270 nm) spectra of complex SO3Eu in DMSO upon the addition of water.

spectrum of the complex SO_3Eu dissolved in DMSO had no peaks in a spectral range of 250–350 nm, and the absorbance decreased with the wavelength. After the addition of a small amount of water, the absorption band of the complex immediately changed, and further its spectral shape and position of individual peaks did not change with raising the amount of water (Figure 1). This can be due to changes in complex configuration on coordination with water molecules.

In a study of the phosphorescence of complex SO3Eu in solution, we detected emission spectra with the excitation wavelength λ_{ex} = 270 nm. Europium phosphorescence has characteristic peaks at 618 (very strong hypersensitive band) and 594 nm due to the $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_1$ transitions, respectively.[11] The phosphorescence line at 700 nm is prevailing in the emission spectrum of SO3Eu in DMSO (Figure 1). A band at 700 nm corresponds to the $^5D_0 \rightarrow {}^7F_4$ transition. Phosphorescence of complex SO3Eu in DMSO is very high; its absolute value is > 68% (Table 1). After the addition of water, the phosphorescence of europium sharply decreased by a factor of 5.2, especially the line at 700 nm ($^5D_0 \rightarrow {}^7F_4$ transitions). The average luminescence quantum yield after the addition of water is 13±2%.

Europium trinitrato 6,6′-bis[di(3-sulfophenyl)phosphinoyl]-2,2′-bipyridine (SO3Eu). Dry methanol (25 ml) was added to 6,6′-bis[di(3-sulfophenyl)phosphinoyl]-2,2′-bipyridyl (57 mg) and europium trinitrate hexahydrate (29 mg). The mixture was stirred for 30 min and heated under reflux, then leaved overnight. The precipitate formed was collected by filtration, washed with cold methanol and air dried. Yield, 100 mg (85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.62 (br. s, 1 H), 7.93 (dd, 1H, *J* 8.07, 7.34 Hz), 8.03 (d, 1H, *J* 5.14 Hz), 8.27 (br. s, 1H), 8.56 (d, 1H, *J* 9.29 Hz), 8.63 (d, 1H, *J* 6.85 Hz). ^{31}P NMR, δ: 14.10. IR (KBr, ν/cm^{-1}): 3390, 3072, 2927, 2859, 1637, 1589, 1567, 1469, 1444, 1402, 1384, 1253, 1168, 1105, 1035, 860, 796, 688, 617, 545, 457. Found (%): C, 33.23; H, 1.70, N. 5.60. Calc. for $EuC_{34}H_{26}N_5O_{23}P_2S_4$ (%): C, 33.62; H, 2.16; N, 5.77.

‡ Absorption spectra of europium complex and HSA were recorded on a Hitachi U-1900 spectrophotometer. Luminescence emission and excitation spectra were measured on a Hitachi F-7000 spectrometer. Absolute external luminescence quantum yields for the solutions of europium complexes in DMSO and water–DMSO mixtures were calculated from absorbance values and integrated over spectrum luminescence intensities using a classical reference material approach[15] and a solution of Rhodamine 6G as a standard as described previously.[16]

Table 1 Luminescence quantum yields and phosphorescence lifetimes (λ_{em} = 618 nm) for complex SO3Eu in DMSO–water mixtures (λ_{ex} = 270 nm).

Amount of water added (wt%)	Luminescence quantum yield (%)	Phosphorescence lifetime/ms
0	68.4	1.09
11	11.5	0.75
20	11.4	0.75
27	11.6	0.79
33	11.0	0.97
38	12.9	0.81
42	15.1	0.65
46	14.7	0.81
50	16.3	0.65

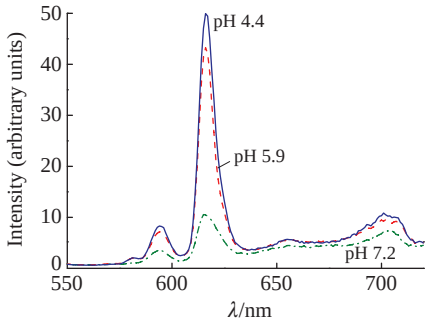


Figure 2 Luminescence emission spectra of complex SO3Eu in DMSO at different pH (λ_{ex} = 270 nm).

While the luminescence quantum yield decreased by a factor of 5, the spectral shape of bands in absorption and phosphorescence excitation spectra after the addition of water to DMSO was not changed. Therefore, the complex SO3Eu was not destroyed upon the addition of water.

The stability of complex SO3Eu was tested at various pH values. In the phosphate buffer with pH 7.2, the phosphorescence of the complex was very weak, whereas in the acetate buffer (pH 4.4), it decreased a little (Figure 2). We observed that the complex behavior is pH-dependent; thus, it can be used as a marker at low pH values.

Since albumin fluorescence in the presence of DMSO collapses, it is impossible to use it as a solvent without dilution. Thus, albumin fluorescence measurements were carried out by the addition of europium complex diluted with an acetate buffer. The spectra in Figure 3 clearly demonstrate that the albumin emission band remains constant in shape and its intensity remains undisturbed except for a small decrease in the intensity upon excitation at λ_{ex} = 215 and 225 nm.

After the addition of complex SO3Eu to a solution of albumin in acetate buffer, we found that albumin did not change its fluorescence emission, while fluorescence excitation at 227 nm disappeared and greatly decreased at 284 nm (Figure 4).[12] The measured luminescence quantum yield of europium was 1.3%.

Only three amino acids (tyrosine, phenylalanine and tryptophan) contribute to UV protein fluorescence. There are six major binding sites of HSA: centers I and II for the binding of small organic molecules, centers III and IV for long-chain fatty acids, center V for ligands with free SH groups and center VI for the binding of metal ions. Some binding reactions are provided by electrostatic interaction, and others are covalent in nature causing a chemical modification of side chains of amino acid residues. In the first binding site the HSA has tyrosine due to which one can expect a decrease in the intensity of the fluorescence excitation spectrum. Using a standard luminescence technique, we detected changes in tryptophan fluorescence, which was also studied by other researchers;[13] a disturbance of photoexcitation energy transfer

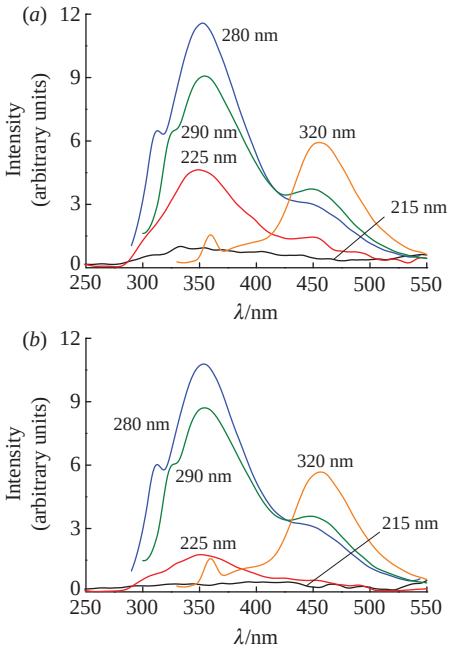


Figure 3 Fluorescence spectra of (*a*) HSA and (*b*) HSA + SO3Eu in aqueous solution upon excitation with different wavelengths.

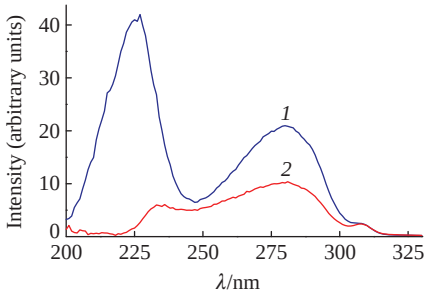


Figure 4 Fluorescence excitation spectra of (*1*) HSA and (*2*) complex SO3Eu in aqueous solution (detected at 345 nm).

from tyrosine residues to tryptophan is expected.[14] We may suppose that complex SO3Eu probably enters the first binding site of albumin. The obtained result demonstrates the usefulness of the photophysical parameters of tyrosine for the probing of peptide binding sites.

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