TERBIUM BINDING IN HIGHLY LUMINESCENT POLYMER COMPLEXES

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Received February 9, 2006
Accepted May 3, 2006

Polymer ligands containing a covalently bonded quinolinone fluorophore and COOH (**L1**) or COONa (**L2**) binding sites were synthesized by the reaction of high-molecular-weight poly[styrene-*alt*-(maleic anhydride)] with 7-amino-4-methylquinolin-2(1*H*)-one and methanol and subsequent neutralization. The ligand-to-metal energy transfer and ligand binding properties in a series of [Tb(III)-ligand] complexes were investigated by steady-state and time-resolved luminescence spectroscopy in methanol or deuterated methanol. The intensity of the long-lived terbium(III) ion emission at 490, 545, 585 and 620 nm was greatly enhanced upon addition of **L1** or **L2**. Based on the differences in luminescence data obtained for [Tb(III)-**L1**] and [Tb(III)-**L2**] complexes, a qualitative model for the interaction of terbium(III) ion with **L1** and **L2** is put forward. The experimental luminescence decay curves were double-exponential (τ_1, τ_2) with predominating longer component (rel B_1 > 85%) for both [Tb(III)-**L1**] and [Tb(III)-**L2**] complexes. About 2.5 or 2 methanol molecules were coordinated to Tb^{3+} in [Tb(III)-**L1**] or [Tb(III)-**L2**] complexes, respectively, whereas ca. 6.5 methanol molecules were coordinated to Tb^{3+} in methanol.

Keywords: Terbium luminescence; Resonance energy transfer; Quinolinone fluorophore; Functionalized polymers; Time-resolved fluorescence spectroscopy; Fluorescent materials; Lanthanides.

The study of lanthanide(III) ions binding to synthetic polymers is of both theoretical and practical importance. Stable, highly luminescent lanthanide(III) complexes with polymer ligands can be used as alternatives to low-molecular-weight complexes in a wide range of attractive applications, including emitters in electroluminescent devices[1,2], plastic fibre lasers and optical amplifiers[3], or photophysical labels in fluoroimmunoassay and analytical chemistry[4,5]. Moreover, lanthanide(III) ions mimic the behavior of various biologically important ions (including Ca^{2+} or Mg^{2+}) and their luminescence has been extensively utilized in studies of interactions between metal ions and biomacromolecules[6], such as DNA [7], RNA [8] or proteins[9].

Collect. Czech. Chem. Commun. 2006, Vol. 71, No. 9, pp. 1333–1349

doi:10.1135/cccc20061333

The importance of the luminescence of lanthanide(III) ions stems from its peculiar characteristics. Terbium(III) ion (Tb^{3+}) exhibits a long-lived excited state $^5D_4$ and its emission spectrum consists of several sharp bands. This is due to the fact that the $^5D_4$ emitting excited state and all ^7F_J (J = 0, 1, ..., 6) terms of the ground state multiplet have the same $4f^8$ electronic configuration and that the 4f electrons are well shielded from external charge by outer $5s^2$ and $5p^6$ shells. Electric dipolar transitions (absorption, emission) which involve only redistribution of electrons within the 4f orbitals are strictly parity-forbidden by the Laporte rule. In addition, a number of the discussed transitions are also forbidden by the spin cross-over rule. For the same reasons, the Tb^{3+} has an extremely low absorption coefficient (of the order of 10^0) and direct population of its $^5D_4$ excited state is inefficient. Nevertheless, strongly luminescent lanthanide(III) complexes based on encapsulating supramolecular structures (cryptands[5], chelates[10], podands[11], calixarenes[12], crown ethers[13], and macrocycles[14]) have been designed. These "light conversion molecular devices"[15] make use of energy transfer (Fig. 1) from strongly absorbing receptor molecule to the bound lanthanide(III) ion by a process called the "antenna effect"[16]. In addition, the encapsulated lanthanide(III) ion is shielded from environmental vibronic quenching. The idea of using macromolecular structures instead of the synthetically rather complicated supramolecular ligands seems to be well-founded. We have assumed[17–20] that a polymer coil forms a steric "cage" for lanthanide(III) ions.

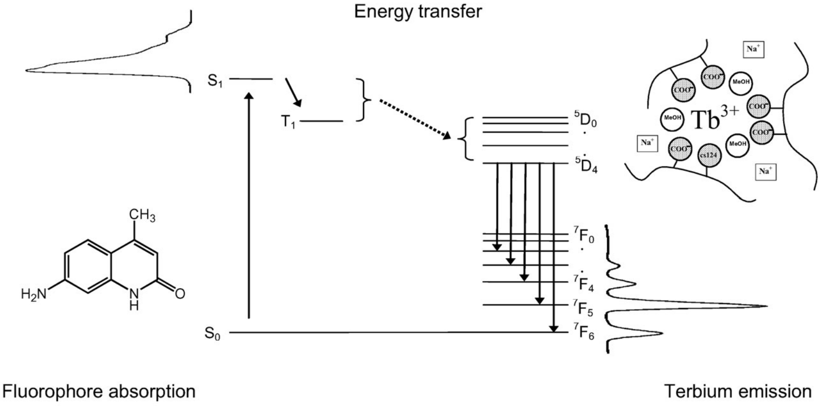

FIG. 1
Schematic energy level diagram for fluorescence resonance energy transfer in the [Tb(III)– ligand] complex

Recently[21] we have synthesized statistical and alternating copolymers of 9-vinylcarbazole with diethyl fumarate, diethyl maleate, methacrylic acid, and maleic acid. Steady-state and time-resolved luminescence of a series of their terbium(III) complexes revealed that the complexing properties of copolymers containing carboxy groups were better than of those containing carboxylic ester groups.

More recently[22] we have prepared a polymer ligand containing the quinolinone fluorophore and carboxyl binding sites. An efficient ligand-to-Tb^{3+} resonance energy transfer and excellent binding properties were found out by luminescence spectroscopy in spite of the fact that the ligand was actually an oligomer with a low degree of polymerization (P ~ 8).

In this paper we report on the synthesis and characterization of high-molecular-weight polymer ligands (P ~ 1730) containing an efficient 7-amino-4-methylquinolin-2(1*H*)-one fluorophore and COOH (ligand **L1**) or COONa (ligand **L2**) groups as binding sites (Chart 1). Quinolinone derivatives are strong donors in terbium(III) luminescence[23] and the preference of lanthanide(III) ions for ionic binding to “hard” ligands is also notorious[6].

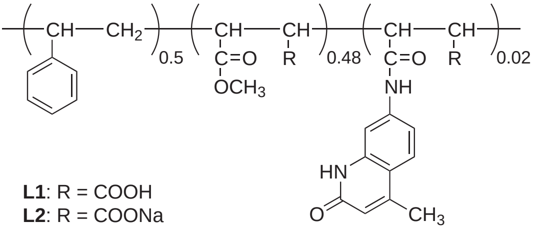

CHART 1

EXPERIMENTAL

Materials

7-Amino-4-methylquinolin-2(1*H*)-one (fluorophore, donor) was synthesized by a Knorr-type condensation reaction of 1,3-phenylenediamine with ethyl acetoacetate[24,25]. The detailed synthesis and characterization as well as the synthesis of model compound, *N*-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)succinamic acid (Chart 2), were described in our previous paper[22]. Methanol-*d* (MeOD), 99.5 mole % D and anhydrous terbium(III) chloride (Aldrich), methanol for UV spectroscopy (MeOH, Lachema Brno, Czech Republic) and other chemicals (Fluka or Aldrich) were used as received.

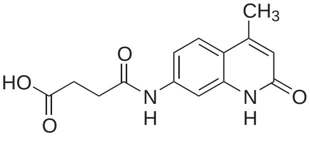

CHART 2

Synthesis of Polymer Ligand (**L1**)

Poly[styrene-*alt*-(maleic anhydride)] copolymer, M_w = 350 000, 50 mole % of styrene, containing 10–15% of monomethyl ester was purchased from Scientific Polymer Products, Inc. (Ontario (NY), U.S.A.). The copolymer (10 g, 0.05 mol of anhydride groups) was dissolved in 70 ml of dry acetone and 7-amino-4-methylquinolin-2(1*H*)-one (0.44 g, 0.0025 mol, i.e. 5 mole % relative to anhydride) in dry dimethylformamide (20 ml) was added dropwise under argon. The reaction mixture was refluxed under argon for 12 h, and dry methanol (50 ml) was added. Then the reaction mixture was refluxed for another 5 h and stirred at laboratory temperature over weekend. The modified polymer was precipitated into a large amount of ethyl acetate, filtered off, and dried. The crude material was twice reprecipitated from methanol into diethyl ether, yielding 6.5 g of white polymer ligand **L1**. Elemental analysis calculated (after Chart 1): 66.88% C, 5.98% H, 0.47% N; found: 66.95% C, 5.72% H, 0.50% N. IR (film on KBr): 3447 (O–H stretching); 3029, 2926, 2854 (C–H stretching); 1718 (C=O stretching); 1653 (amide I band); 1496 (C–C ring stretching); 1455 (CH_2 scissoring); 1400 (C–O–H bending); 1200, 1171 (C–O–C antisym. stretching); 763, 702, 548 (C–H out-of-plane, ring bending) cm^{-1}.

Synthesis of Sodium Salt of Polymer Ligand (**L2**)

The modified polymer **L1** (0.5 g, i.e. 0.002 mol COOH) was dissolved in a solution of NaOH (0.1 g, 0.0025 mol) in 20 ml of methanol and 100 ml of water. The solution was dialysed (Sigma seamless cellulose tubing, molecular weight cut-off 12 000) against water for a week and then freeze-dried, yielding 0.45 g of neutralized polymer ligand **L2**. Elemental analysis calculated (after Chart 2): 61.26% C, 5.10% H, 0.43% N, 8.78% Na; found: 61.73% C, 5.74% H, 0.43% N, 8.60% Na. IR (film on KBr): 3029, 2926, 2854 (C–H stretching); 1718 (C=O stretching); 1653 (amide I band); 1570 (COO^- antisym. stretching); 1495 (C–C ring stretching); 1455 (CH_2 scissoring); 1405 (COO^- sym. stretching); 1200, 1171 (C–O–C antisym. stretching); 764, 702, 548 (C–H out-of-plane, ring bending) cm^{-1}.

Methods and Procedures

UV-VIS spectra were taken on a Perkin–Elmer Lambda 20 spectrometer in methanol using quartz cuvettes (0.1 or 1 cm). ^{1}H NMR spectra were taken on a Bruker ACF-300 spectrometer at 300.1 MHz in deuterated dimethyl sulfoxide using hexamethyldisiloxane as an internal standard. FT IR spectra were measured using a Perkin–Elmer Paragon 1000 PC Fourier transform infrared spectrometer in KBr pellets or as film on a KBr pellet. Steady-state fluorescence spectra were measured in methanol at 30 or 50 °C with a Hitachi Perkin–Elmer MPF-2A spectrometer in L-format arrangement using a quartz cuvette (1 × 1 × 4 cm). The luminescence lifetimes (τ_{MeOH}, τ_{MeOD}) of $TbCl_3$ and [Tb(III)-ligand] complexes were measured in methanol (MeOH) or deuterated methanol (MeOD) with a time-resolved fluorimeter FL900CDT (Edinburgh Analytical Instruments, U.K.) using the time-correlated single-photon counting (TCSPC) method[26]. Measurements were performed on a millisecond time scale using a computer-controlled μF900 xenon flashlamp for excitation. Both [Tb(III)-**L1**] and [Tb(III)-**L2**] complexes were excited at 345 nm and their emission was recorded at 545 nm. The luminescence decay, $F(t)$, was analyzed using the FL900CDT software with experimental function

$$F(t) = C + \sum_t B_i \exp(-t/\tau_i)\,, \tag{1}$$

where B_i is a pre-exponential factor representing the fractional contribution to the time-resolved decay of the component with a life-time τ_i [rel $B_i = (B_i\tau_i/\sum_j B_i\tau_i)$, C is background and t is time. Least-square analysis of the observed time-resolved data, $R(t)$, was used for calculation of the impulse response function $F(t)$. The goodness-of-fit parameter, χ^2, was calculated from

$$\chi^2 = \sum_t \frac{[R(t) - R_{\text{calc}}(t)]^2}{R(t)} = \sum_t \left[\frac{R(t) - R_{\text{calc}}(t)]}{\sigma(t)}\right]^2 = \sum_t u^2(t)\,, \tag{2}$$

where $R_{\text{calc}}(t)$ is the calculated decay of luminescence, $\sigma(t)$ the standard deviation of each data point and $u(t)$ are the weighted residuals. A good fit should give the value $\chi^2 < 1.7$ and a residual curve that contains only random noise distributed around zero.

RESULTS AND DISCUSSION

Polymer Design, Synthesis and Characterization

The design of the polymer ligand was based on the general knowledge that it should (i) contain a suitable strongly absorbing (organic) fluorophore for an efficient energy transfer to the otherwise weakly absorbing Tb^{3+} and (ii) be able to tightly bind the ions. As it is assumed, the macromolecular nature of the ligand then ensures shielding of the bound ions and prevents deactivation of their excited states by the solvent environment. According to Pearson's theory[27], lanthanide(III) ions are hard acids. Much of their coordination chemistry involves anionic oxygen donors and it is well established that carboxylates coordinate the lanthanide(III) ions very well. Hence, the COOH or COO^- groups are good candidates for efficient binding sites of the ligand. Using the methods of polymer chemistry, these requirements were matched by the described one-pot modification reaction of parent high-molecular-weight poly[styrene-*alt*-(maleic anhydride)] with 7-amino-4-methylquinolin-2(1*H*)-one and methanol yielding ligand **L1** and, after neutralization, ligand **L2** (Chart 1). It is of importance to know the fluorophore content in the synthesized ligands. As this content is low (5 mole % relative to anhydride in the feed), UV-VIS spectrometry in methanol was used for its evaluation. Absorption spectra of the prepared ligands **L1** and **L2** are shown in Fig. 2 together with those of free quinolinone fluorophore and a model compound. It turned out that the characteristic absorption band of the free fluorophore with a maximum at 347 nm was not retained in the spectrum of the polymers with covalently bonded fluorophore. Both **L1** and **L2** show two absorption bands at 330 and 345 nm. Obviously, the amide bond formation causes the splitting and a blue shift of the absorption spectrum. Similar results were reported when the amino group of the same fluorophore was replaced by the dimethylamino group[28] or when absorption spectra of various N^7-acylated derivatives of 7-amino-4-methylquinolin-2(1*H*)-one were investigated[29], both experimentally and theoretically. Based on this knowledge, which can be accounted for by a larger inductive effect of the substituted amino group, a model compound, *N*-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)succinamic acid (Chart 2), was synthesized. It shows two absorption bands at 330 and 345 nm with ε_{330} = 17 000 and ε_{345} = 16 000 l mol^{-1} cm^{-1}, respectively (Fig. 2). Assuming that the molar absorption coefficient of the quinolinone moiety in the polymer is the same as that of the model compound, the quinolinone content in polymer ligands (**L1** or **L2**) was determined the same, 2.0 mole % (see Chart 1). This result was in good agreement with that determined by elemental analysis (ex N). It should be pointed out that the low fluorophore content is desirable because the formation of terbium complex with two quinolinone units is less probable. It is assumed that in such complexes the quinolinone units interact to form a new triplet state which lies slightly below the terbium $^5D_4$ level. The new state presents an efficient pathway for nonradiative deactivation[30].

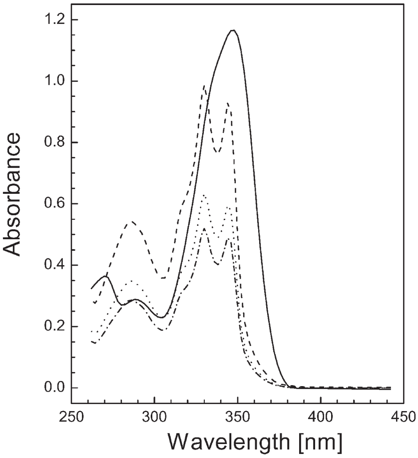

FIG. 2
Absorption spectra of 7-amino-4-methylquinolin-2(1*H*)-one (solid, 6.34 × 10^{-5} mol l^{-1}), model compound *N*-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)succinamic acid (dashed, 5.76 × 10^{-5} mol l^{-1}), ligand **L1** (dotted, 0.226 g l^{-1}) and ligand **L2** (dashed-and-dotted, 0.200 g l^{-1}) in methanol (1 cm)

*Steady-State Luminescence of [Tb(III)-**L1**] and [Tb(III)-**L2**] Complexes*

The aim of the study was to evaluate and compare luminescence properties of [Tb(III)-**L1**] and [Tb(III)-**L2**] complexes in methanol solution. When excited at 345 nm, both ligands show a short-lived broad-band emission spectrum with maxima at 372 and 395 nm, which are due to the bonded fluorophore (Fig. 3, dashed line). When Tb^{3+} was added to ligands and a complex was formed, the ligand emission intensity decreased. This was accompanied by an increase in the typical long-lived terbium(III) ion emission bands at 490, 545, 585 and 620 nm, corresponding to the $^5D_4 \rightarrow {}^7F_6$, $^5D_4 \rightarrow {}^7F_5$, $^5D_4 \rightarrow {}^7F_4$ and $^5D_4 \rightarrow {}^7F_3$ transitions, respectively. Emission spectrum of the [Tb(III)-**L1**] complex is shown in Fig. 3 (solid line). The $^5D_4 \rightarrow {}^7F_5$ transition centred at 545 nm is the most intense one. It should be emphasized that we were almost unable to detect the luminescence of $TbCl_3$ in methanol at the same fluorimeter sensitivity, because of its low absorption coefficient and methanol quenching (Fig. 3, dotted line). The ligand emission spectrum overlaps the absorption spectrum[31] of Tb^{3+}. Evidently, the resonance energy transfer[26] (RET) from the donor (7-amino-4-methylquinolin-2(1*H*)-one) to the acceptor (Tb^{3+}) is responsible for the increase in terbium(III) emission intensity (Fig. 1). Whereas the energy transfer between lan-

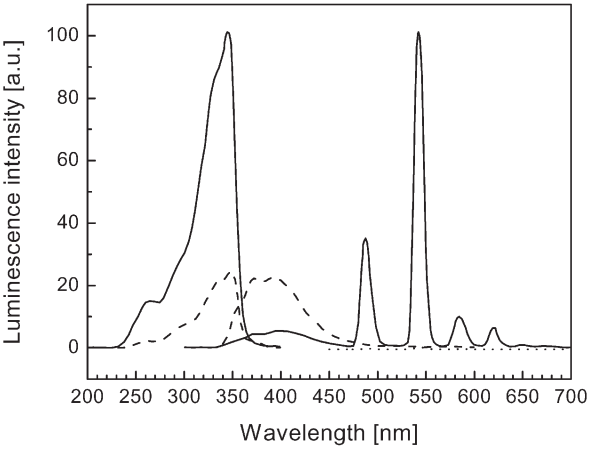

FIG. 3

Luminescence excitation (λ_{exc}) and emission (λ_{em}) spectra of ligand **L1** (dash, λ_{exc} = 345 nm, λ_{em} = 395 nm), the [Tb(III)-**L1**] complex, (solid, λ_{exc} = 345 nm, λ_{em} = 545 nm) and $TbCl_3$ (dotted, λ_{exc} = 345 nm) in methanol; $c_{\mathbf{L1}}$ = 0.24 g l^{-1}, ~[COOH] = 0.001 mol l^{-1}, $[Tb^{3+}]$ = 0.001 mol l^{-1}

thanide(III) ion pairs has been widely studied[32], less is known about the precise mechanisms and selection rules involved in the process of energy transfer between an organic donor and lanthanide(III) ion in coordination compounds. However, most of the recent works tend to suggest that the donor T_1 state is involved and the Förster dipole–dipole mechanism is more probable for the energy transfer to lanthanide(III) ion. The efficiency of this type of energy transfer depends[33] upon the spectral overlap of the emission spectrum of donor with the absorption spectrum of acceptor, the quantum yield of donor in the absence of acceptor, the relative orientation of donor and acceptor transition dipoles, and the distance between the donor and acceptor molecules. In our case we have a fixed spectral overlap and donor quantum yield ($\phi_D = 0.68$)[28]. Thus, the efficiency of RET depends mainly on the donor–acceptor distance and orientation, i.e. on structural properties of the ligand. The donor–acceptor distance has not a fixed value as is the case in structurally more rigid low-molecular-weight complexes. There is a distribution of quinolinone-Tb^{3+} distances in polymer ligand complexes. For that reason, luminescence intensities (λ_{exc} = 345 nm, λ_{em} = 545 nm, ${}^5D_4 \rightarrow {}^7F_5$), rather than energy transfer efficiencies were used for comparison of [Tb(III)-**L1**] and [Tb(III)-**L2**] luminescence. Here we assume that only sensitized luminescence from terbium(III) ions bound to the polymer chain is observed under our experimental conditions and that it carries averaged information on the ion microenvironment – mainly the mean donor–acceptor distance, which is crucial for energy transfer.

The starting experimental concentrations of both ligands ($c_{\mathbf{L1}}$ = 0.24 g l^{-1}, [COOH] = 0.001 mol l^{-1}, $A_{\mathbf{L1}}$ = 0.68; $c_{\mathbf{L2}}$ = 0.26 g l^{-1}, [COONa] = 0.001 mol l^{-1}, $A_{\mathbf{L2}}$ = 0.68) were chosen on the basis of fluorimeter sensitivity and absorption of the ligands in the L-format arrangement (inner filter effect). The absorbance (A) of solution did not change when Tb^{3+} was added, because its absorption coefficient is several orders of magnitude lower than that of the ligand. Thus, maintaining the same ligand concentration, it is possible to compare luminescence properties of the complexes with various Tb^{3+} concentrations without a need to compensate different sample absorbances. The increase in terbium(III) emission intensity for the [Tb(III)-**L1**] complex (Fig. 4, squares) is not linear with increasing terbium concentration but tends to a plateau when the binding ability of the polymer coil is saturated ([Tb^{3+}] > 1/3 [COOH]). This strongly resembles adsorption behavior. As a matter of fact, the Langmuir adsorption isotherm was frequently used to fit experimental data of complex formation from small cations and polymers[34,35]. In our case, there is an evident deviation from the Langmuir-like behavior in the terbium(III) emission intensity dependence, especially at lower Tb^{3+} concentrations (it can be best seen when the linear form of the isotherm is used for data-fit). As was pointed out above, the observed terbium luminescence intensity depends not only on the equilibrium concentration of the ions bound to the polymer chain, but also on the mean donor–acceptor distance in the complex. Hence, with the fixed fluorophore content in the coil ("effective fluorophore concentration inside the coil") and a random distribution of ions inside the coil, the mean donor–acceptor distance rapidly increases for lower Tb^{3+} concentrations and the measured terbium(III) emission intensity shows a negative deviation from the expected ideal adsorption behavior. The importance of the "effective fluorophore concentration inside the coil" and its influence on the energy transfer efficiency was further confirmed by measuring the [Tb(III)-**L1**] luminescence at a higher temperature. We observed a 15% decrease in terbium(III) luminescence intensity in the whole concentration range when measured at 50 °C and compared with the 30 °C data (the curve shape was the same). Evidently, the binding ability of the coil is the same (the COOH content unchanged, the same curve shape), but at higher temperature the polymer coil expands and the discussed "effective fluorophore concentration inside the coil" was somewhat smaller.

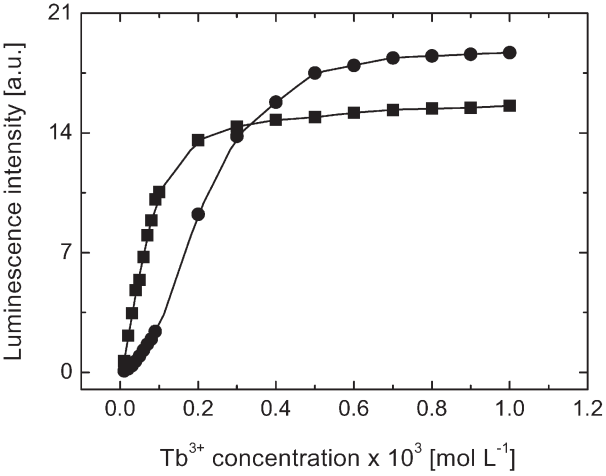

FIG. 4
Luminescence emission intensity (λ_{exc} = 345 nm, λ_{em} = 545 nm) of [Tb(III)-**L1**] (■) and [Tb(III)-**L2**] (●) complexes as a function of Tb^{3+} concentration in methanol; $c_{\mathbf{L1}}$ = 0.24 g l^{-1}, ~[COOH] = 0.001 mol l^{-1}, $c_{\mathbf{L2}}$ = 0.26 g l^{-1}, ~[COONa] = 0.001 mol l^{-1}

To improve the ligand binding properties, polymer **L1** was neutralized yielding polymer **L2** with COONa groups instead of COOH (Chart 1). The preference of lanthanide(III) ions for ionic binding to charged hard bases arises from the fact that 4f orbitals of these ions are effectively shielded from interaction with ligand orbitals by outer 5s and 5p orbitals and complex formation is mostly electrostatic in character. When the luminescence of [Tb(III)-**L2**] complexes was studied under the same conditions as in the case of the [Tb(III)-**L1**] complexes ([COONa] = [COOH], $A_{\mathbf{L2}} = A_{\mathbf{L1}}$, 30 °C), a different terbium(III) emission intensity dependence on the Tb^{3+} concentration was found. If the Tb^{3+} concentration is high ($[Tb^{3+}] > 1/3\ [COO^{-}]$), the emission intensity of the [Tb(III)-**L2**] complex was found to be even higher than that of the [Tb(III)-**L1**] complex. Here the excess of terbium(III) ions compensates the negative charge along the polymer chain and the moderate increase in terbium(III) luminescence intensity (ca. 15%) reflects the better binding ability of polymer **L2**. Moreover, polyions exhibit the most folded conformation structures at approximately three-fold excess of added trivalent salt, as Monte Carlo simulations suggest[36]. By comparing the root-mean-square end-to-end distance, the root-mean-square radius of gyration and the shape factor, it was shown that these structures are even more compact than those of the uncharged chain of the same type. So, there is a higher "effective fluorophore concentration inside the coil" and thus a smaller mean donor–acceptor distance in the [Tb(III)-**L2**] complex than in [Tb(III)-**L1**] at high Tb^{3+} concentrations ($[Tb^{3+}] > 1/3\ [COO^{-}]$). When the Tb^{3+} concentration decreases, the measured terbium luminescence intensity of the [Tb(III)-**L2**] complex reaches a point where it is the same as that of the [Tb(III)-**L1**] complex. It can be seen from Fig. 4 that this happens exactly when $[Tb^{3+}] = 1/3\ [COO^{-}]$. At those ligand-to-metal concentrations, the charges are just equal and the stoichiometric 1:3 complex (metal to COO^{-} of the ligand) is formed. A further decrease in Tb^{3+} concentration leads to a rapid decrease in the luminescence intensity of the [Tb(III)-**L2**] complex. Such a behavior can be explained by a polymer coil expansion. In the dissociated state charged groups along the polymer chain repel each other and, as a consequence, the polymer coil expands. So, when the negative charge is not compensated by counter-ions ($[Tb^{3+}] < 1/3\ [COO^{-}]$), the polymer chain stretches and the mean donor–acceptor distance becomes too large for efficient energy transfer. At the lowest Tb^{3+} concentration measured ($[Tb^{3+}] = 10^{-5}$ mol l^{-1}), the luminescence of the [Tb(III)-**L2**] complex was reduced 8 times when compared with that of the [Tb(III)-**L1**] complex with an unperturbed polymer coil structure.

Finally, steady-state measurements were used to prove the importance of steric properties of macromolecular architecture in binding and in the ligand-to-metal energy transfer process. When the model compound was added to a methanol solution of $TbCl_3$, no significant increase in terbium luminescence was detected[22]. The [Tb^{3+}] and fluorophore concentration were 1.0×10^{-4} and 3.0×10^{-5} mol l^{-1}, respectively. This showed that *N*-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)succinamic acid did not form a complex with Tb^{3+} in methanol solution, even though the acid bears the binding sites (COOH or COONa) and fluorophore unit. On the other hand, we estimated that the terbium luminescence intensity in complexes with the synthesized polymer ligands is approximately four orders of magnitude higher that that for $TbCl_3$ itself. The described behavior of macromolecular ligands is attributed to the "polymer cooperative effect", i.e. the high local concentration of binding sites and fluorophores in the polymer domain ("effective concentration inside the coil")[20,37].

*Time-Resolved Luminescence of [Tb(III)-**L1**] and [Tb(III)-**L2**] Complexes*

Additional information about intimate coordination environment of Tb^{3+} in [Tb(III)-**L1**] and [Tb(III)-**L2**] complexes were obtained by luminescence lifetime measurements in methanol or deuterated methanol. This was used for comparison of binding properties of both ligands. The electronic configuration of the terbium(III) ion is $[Xe]4f^8$. The observed characteristic emission of terbium(III) ion originates at the $^5D_4$ excited state level and the luminescence transitions end at all the J levels of the ^7F_J ground state multiplet ($J = 0, 1, \ldots, 6$, whereas $J = 2$, 1 and 0 are the weakest of these transitions – see Fig. 1). This desirable intrinsic luminescence can be quenched by nonradiative dissipation of excitation energy into suitable vibrational modes of the environment, the process becoming more probable when vibrational quanta of the oscillators or their overtones match the energy gap between the $^5D_4$ emissive level and the highest $^7F_0$ level of the ground state multiplet. When solvent molecules containing O–H groups (such as water or methanol) are coordinated to terbium(III) ion, this deactivation pathway becomes very efficient[38]. On the assumption[39] that every O–H oscillator quenches independently, a quantitative method for estimation of the number of solvent molecules in the first coordination sphere of terbium(III) ion was proposed[40]. It is based on a deuterium isotope effect: if the O–H oscillators (3500 cm^{-1}) are replaced by the low-frequency O–D oscillators (2800 cm^{-1}), the vibronic de-excitation pathway becomes much less efficient and the observed luminescence lifetime of terbium increases. For aqueous solutions, the number of coordinated water molecules (w) is then directly proportional to the difference in the observed luminescence lifetimes in H_2O (τ_H) and D_2O (τ_D)

$$w = q\left(\frac{1}{\tau_H} - \frac{1}{\tau_D}\right), \tag{3}$$

where q is 4.2 ms for Tb^{3+} complexes. On the assumption[41] that methanol behaves like a half of water molecule, the number of coordinated methanol molecules (m) in Tb^{3+} complexes can be determined by carrying out separate experiments in methanol (τ_{MeOH}) and deuterated methanol (τ_{MeOD}) and using a similar formula

$$m = k\left(\frac{1}{\tau_{MeOH}} - \frac{1}{\tau_{MeOD}}\right), \tag{4}$$

where k is 8.4 ms for Tb^{3+} complexes.

Typical experimental decay curves measured in MeOH and MeOD are shown in Fig. 5 and the results are summarized in Table I for [Tb(III)-**L1**] and Table II for [Tb(III)-**L2**] complexes. The experimental decay curves (Eq. (*1*)) were found to be double-exponential (τ_1, τ_2) with predominating

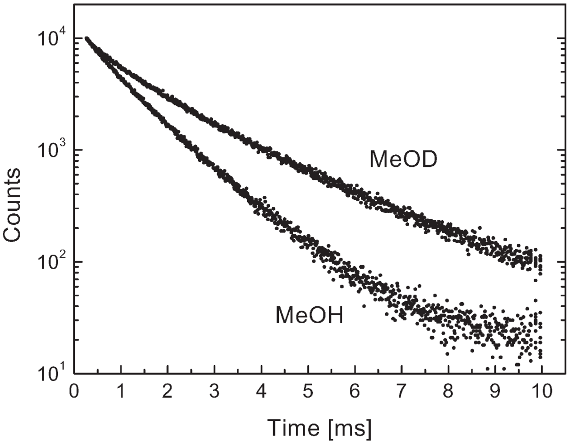

FIG. 5

Typical luminescence decay curves obtained from time-resolved measurements (λ_{exc} = 345 nm, λ_{em} = 545 nm). Data shown for the [Tb(III)-**L1**] complex in methanol (MeOH, τ_1 = 1.197 ms, τ_2 = 0.551 ms, rel B_1 = 84%) and deuterated methanol (MeOD, τ_1 = 1.856 ms, τ_2 = 0.551 ms, rel B_1 = 92%); $c_{\mathbf{L1}}$ = 0.24 g l^{-1}, ~[COOH] = 0.001 mol l^{-1}, [Tb^{3+}] = 0.001 mol l^{-1}

longer component τ_1 (rel B_1 > 85%). The goodness-of-fit parameter, (χ^2, Eq. (*2*)), was around 1.5 and the weighted residuals were randomly distributed around zero. It is known that the physical interpretation of the multiple lifetimes obtained from the mathematical fit of the experimental luminescence decay curve can be difficult. Although the decays fit best to two components, the lifetime of the longer component (τ_1) only was used in calculation of the number of coordinated methanol molecules (*m*). This is a standard approach in luminescence experiments involving terbium(III)

TABLE I

Luminescence lifetimes of [Tb(III)-**L1**] complexes obtained from double-exponential fits (τ_1, τ_2, rel B_1) of decay curves in methanol (MeOH) or deuterated methanol (MeOD) and calculated (Eq. (*4*)) numbers of coordinated methanol molecules (*m*) in the complexes; $c_{\mathbf{L1}}$ = 0.24 g l^{-1}, ~[COOH] = 10^{-3} mol l^{-1}

$[Tb^{3+}]$, mol l^{-1}	Solvent	τ_1, ms	τ_2, ms	rel B_1, %	*m*
10^{-3}	MeOH	1.197	0.551	84	2.5
	MeOD	1.856	0.551	92	
10^{-4}	MeOH	1.172	0.424	90	2.5
	MeOD	1.796	0.579	90	
10^{-5}	MeOH	1.108	0.401	85	2.5
	MeOD	1.650	0.420	91	

TABLE II

Luminescence lifetimes of [Tb(III)-**L2**] complexes obtained from double-exponential fits (τ_1, τ_2, rel B_1) of decay curves in methanol (MeOH) or deuterated methanol (MeOD) and calculated (Eq. (*4*)) numbers of coordinated methanol molecules (*m*) in the complexes; $c_{\mathbf{L2}}$ = 0.26 g l^{-1}, ~[COOH] = 10^{-3} mol l^{-1}

$[Tb^{3+}]$, mol l^{-1}	Solvent	τ_1, ms	τ_2, ms	rel B_1, %	*m*
10^{-3}	MeOH	1.245	0.613	87	2.4
	MeOD	1.942	0.482	97	
10^{-4}	MeOH	1.309	0.580	89	2.1
	MeOD	1.936	0.682	92	
10^{-5}	MeOH	1.296	0.496	93	1.9
	MeOD	1.824	0.637	93	

ion[42]. The short lifetime component (τ_2) indicates that a small population of the terbium(III) ions has a quenched excited-state lifetime as a consequence of the fact that these metal ions are not complexed with **L1** or **L2**.

It can be seen from data in Table I that only 2.5 methanol molecules, on average, remain in the first coordination sphere of terbium(III) ion in the [Tb(III)-**L1**] complex. Other solvent molecules were replaced by the interaction of ions with ligand binding sites (with COOH and, to a lesser extent[21], with COOMe). Moreover, we found out that the number of methanol molecules is independent of the Tb^{3+} concentration. We believe that this strongly supports the hypothesis of adsorption in the polymer coil inside which there exists an “effective concentration” of both the fluorophore (see discussion on steady-state) and binding sites (COOH). The ion is then surrounded by the same averaged microenvironment.

After neutralization, the binding properties of the polymer improved and, on average, two methanol molecules were coordinated to terbium(III) ion in the [Tb(III)-**L2**] complex. In addition, the number of coordinated methanol molecules varies with Tb^{3+} concentration. This is a consequence of conformational changes of the ligand. Monte Carlo simulations[36] have shown that polyions exert a strong influence on the distribution of counterions in their closest vicinity. For the present discussion regarding the inner coordination sphere of the ion, the most important information from the simulation results is the probability distribution function of the smallest distances between the binding site (COO^- in our case) and the trivalent counter-ion (Tb^{3+} in our case). The main theoretical observation is that the distance of the closest counterion is increased when the salt concentration is increased. Changing the point of view, Tb^{3+} is more tightly bound at lower concentrations and, consequently, the number of coordinated methanol molecules decreases (Table II).

The luminescence lifetimes of $TbCl_3$ in MeOH or MeOD were measured ($[Tb^{3+}] = 1 \times 10^{-4}$ mol l^{-1}, $\lambda_{exc} = 345$ nm, $\lambda_{em} = 545$ nm, ${}^5D_4 \rightarrow {}^7F_5$) to determine the *m* value of Tb^{3+} in methanol. The luminescence decays of free terbium(III) ion follow single-exponential fits with experimental lifetimes $\tau_{MeOH} = 0.985$ ms and $\tau_{MeOD} = 3.578$ ms. Using Eq. (*4*), we estimated that free Tb^{3+} coordinates approximately 6.2 ± 0.5 methanol molecules.

We can compare binding properties (*m*) of the high-molecular-weight ligand **L1** (*P* ~ 1730) with the recently prepared[22] oligomeric ligand (*P* ~ 8) containing the same quinolinone fluorophore and carboxyl binding sites (**LO**). The *m* values in Tb^{3+} complexes determined for **L1** and **LO** ligands were 2.5 and 3.0 methanol molecules, respectively. The large polymer coil aids a better binding (“polymer effect”) but the difference is not as significant as one would expect. On the other hand, the *m* values in Tb^{3+} complexes for neutralized **LO** and **L2** ligands were found to be 1.0 and 2.0 methanol molecules, respectively. We assume that the charged large ligand **L2** expands but because of its size the coil cannot be fully extended, whereas the neutralized oligomer ligand **LO** can expand to a “rigid rod”. Consequently, the [Tb(III)-**L2**] complex is formed by one ligand macromolecule which excludes the volume for another one. On the other hand, the [Tb(III)-**LO**] complex is assumed to be formed by several cooperating rigid rod oligomer molecules around the central Tb^{3+}. As we have already discussed[22], some of them even do not carry the fluorophore unit.

CONCLUSION

Efficient high-molecular-weight polymer ligands for sensitized terbium luminescence based on a quinolinone fluorophore with COOH or COONa groups as binding sites were synthesized and characterized. Steady-state and time-resolved measurements were used to study luminescence properties of terbium complexes with the new ligands. The dependence of the terbium(III) luminescence intensity of [Tb(III)-**L1**] and [Tb(III)-**L2**] on Tb^{3+} concentration was described in terms of the donor–acceptor distance, employing the concept of “effective concentration of fluorophore and binding sites inside the coil”. A qualitative model based on the phenomenon of adsorption (for uncharged ligand) and chain expansion (for charged ligand) was proposed. In time-resolved luminescence, the decay curves were double-exponential (τ_1, τ_2) with predominating longer component (rel $B_1 >$ 85%) for both [Tb(III)-**L1**] and [Tb(III)-**L2**] complexes. About 2.5 or 2 methanol molecules were coordinated to Tb^{3+} in the [Tb(III)-**L1**] or [Tb(III)-**L2**] complex, respectively. The binding properties of high-molecular-weight ligands **L1** and **L2** were compared with those of oligomeric ligand **LO**.

We thank the Grant Agency of the Czech Republic for support of this work (grant No. 203/04/1372) and the Grant Agency of the Academy of Sciences of the Czech Republic (grant No. IAA4050409).

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