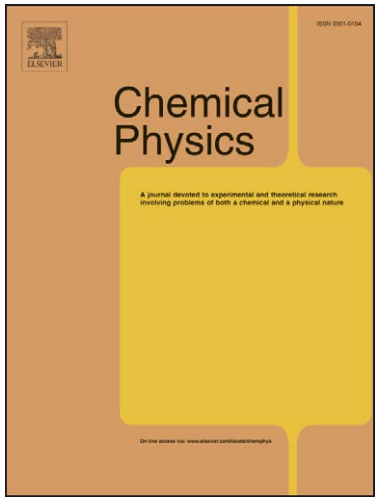

Using Lanthanides as Probes for Polyelectrolyte-Metal Ion Interactions. Hydration Changes on Binding of Trivalent Cations to Nucleotides and Nucleic Acids

Diana Costa, M. Luísa Ramos, Hugh D. Burrows, M. José Tapia, M. da Graça Miguel

PII: S0301-0104(08)00376-5
DOI: 10.1016/j.chemphys.2008.06.014
Reference: CHEMPH 7403

To appear in: *Chemical Physics*

Received Date: 17 September 2007
Revised Date: 14 May 2008
Accepted Date: 25 June 2008

Please cite this article as: D. Costa, M.L. Ramos, H.D. Burrows, M.J. Tapia, M. da Graça Miguel, Using Lanthanides as Probes for Polyelectrolyte-Metal Ion Interactions. Hydration Changes on Binding of Trivalent Cations to Nucleotides and Nucleic Acids, *Chemical Physics* (2008), doi: 10.1016/j.chemphys.2008.06.014

Using Lanthanides as Probes for Polyelectrolyte-Metal Ion Interactions. Hydration Changes on Binding of Trivalent Cations to Nucleotides and Nucleic Acids

Diana Costa[1,*], M. Luísa Ramos[1], Hugh D. Burrows[1], M. José Tapia[2] and M. da Graça Miguel[1]

[1] Department of Chemistry, University of Coimbra, Coimbra, Portugal

[2] Department of Chemistry, University of Burgos, Plaza Misael Bañuelos s/n 09001, Burgos, Spain

Corresponding author:

Diana Rita Barata Costa

Departamento de Química

Universidade de Coimbra

3004-535 Coimbra

Portugal

Fax: +351- 239 82 7703

E-mail address: dcosta@qui.uc.pt

Abstract

The interaction of the trivalent lanthanide ions Ce(III) and Tb(III) with ribonucleic acid (RNA) and guanosine 5`triphosphate (GTP) in aqueous solution has been studied using their luminescence spectra and decays. Complexation is indicated by changes in luminescence intensity. With the system terbium(III)-RNA and terbium(III)-GTP, changes in luminescence with pH are probably related to conformational changes on the structure and also to the different degrees of protonation of phosphate groups and nucleotide bases. The degree of hydration of Tb(III) on binding to RNA and GTP is followed by luminescence lifetime measurements in water and deuterium oxide solutions, and at least one hydration water is lost from the lanthanide ion on binding to RNA or GTP at pH 4.7 and pH 7. Rather different behaviour is observed on binding RNA at pH 9, where six water molecules are lost, possibly due to the lanthanide binding to the bases of the RNA backbone. This is similar to what has previously been seen with DNA, and is supported by ^{31}P NMR spectral measurements, which confirm the possibility of lanthanide binding to both phosphate and bases in the nucleic acids. In the case of GTP at pH 9, two water molecules appear to be lost, probably due to terbium binding both to guanine and a phosphate group. Gd(III) EPR spectral measurements with DNA, RNA and GTP suggest decreased lanthanide ion mobility on binding.

Keywords: dehydration; lifetime measurements; binding;

1. Introduction

Nucleic acids play an important role in biological systems and carry out a broad range of biological functions. Increasing interest is being shown in ribonucleic acids (RNA), both because of their metabolic role and their applications in various types of therapy. Although, both DNA and RNA are nucleic acids involving purine or pyrimidine bases bonded to sugar phosphate ester backbones, relatively small chemical differences between DNA and RNA give rise to great differences in both structural and chemical properties. RNA has the sugar ribose instead of deoxyribose and the base uracil instead of thymine.[1] DNA is normally double-stranded and therefore limited in the number of different structures it can form. RNA is single-stranded but has the possibility to fold back and pair various complementary segments of the same molecule to form different secondary structures such as hairpins, bulges and internal loops with implications for its function.[2-10] The biological function of RNA is often dependent on interactions with different moieties, such as proteins and metal ions,[11-28] and the RNA structure can undergo conformational changes due to ligand binding. Metal ions are important cofactors in RNA structure and function, facilitating RNA folding, tertiary structure stabilization, and catalysis.[27,29,30] Also, the extent of hydration of cations bound to RNA is a key parameter in the energetics of the binding process, as well as a relevant issue in the catalytic roles and structural effects of bound ions. An understanding of the factors involved in metal ion-RNA interactions is, thus, of considerable importance.

Trivalent lanthanide ions form a relatively homogeneous group of 15 elements having attractive, spectroscopic and magnetic properties[31], and have been used as probes of the interactions of metal ions with nucleic acids[32] and other polyelectrolytes.[33]

Given the abundance of negatively charged oxygen donor groups the DNA and RNA molecules easily interact with Ln^{3+} ions, occupy at least some of the inner-sphere

coordination sites of the bound ions, and contribute to the coordination process by completing chelate bridges.[34]Also nucleotides, such as guanosine 5`triphosphate (GTP), a triphosphate group covalently attached to the 5`-hydroxyl group of the corresponding guanine nucleoside (guanosine), show strong ability to interact with lanthanide ions and can mimic some aspects of DNA and RNA behavior in the presence of these ions. The luminescence properties of Tb^{3+}, Eu^{3+} and Ce^{3+} make them quite versatile in their applications to biomolecular structure examination.[35]

The luminescence of the lanthanide ions, except Ce(III), arises from *f*→*f* electron transitions, which can give information on both the coordination environment[36,37] and degree of hydration of these ions.[38,39] The 4f orbitals are shielded, and give rise to narrow emission bands. In addition, there is increasing interest in long-lived luminescent probes[40-42] and lanthanide ions are good candidates for this, particularly as their emissions are not quenched by oxygen. The absorption of Ln(III) ions is extremely weak when compared to organic fluorophores, principally because of the low oscillator strength (~10^{-6}) of their absorption bands,[43,44] which is due to the fact that lanthanide *f*→*f* transitions are generally forbidden by both spin and Laporte selection rules.[45,46] In certain cases, the inherent weakness of Ln(III) ion luminescence may be overcome by an energy transfer process from appropriate organic ligands.[47-51] In contrast, with cerium(III), the lowest energy electronic band in absorption corresponds to the allowed 4*f*→5d transition. Although this results in a much broader band than with the other trivalent lanthanides, it does mean that the transition has a reasonable molar absorption coefficient.[52,53]

Luminescence decay lifetimes of lanthanides provide a direct measure of the number of metal-coordinated water molecules. Replacement of OH oscillators by the OD ones causes the vibronic deexcitation pathway to become exceedingly inefficient, and the

resultant isotope effect on luminescence lifetimes permits the determination of the number of water molecules in the first coordination sphere of metal ion,[54-62] in addition to the changes in hydration on lanthanide ion binding. This technique has been applied to study lanthanide ion dehydration on binding to DNA[32], polyvinylsulfonate[33] and sodium dodecyl sulphate[63] in aqueous solutions as well as to AOT/water/isooctane microemulsions.[64] We extend this study to the behaviour of lanthanide ions on binding to RNA.

In previous studies with DNA,[32] we have shown that the binding of lanthanides is strongly dependent upon pH, and that at high pH both phosphate groups and bases may be involved. Such two-site binding has previously been suggested from both luminescence[65] and ^{1}H NMR spectroscopy.[66] Phosphorous-31 NMR spectroscopy has proved to be a powerful technique here for studying the conformation, dynamics and binding of nucleic acids in aqueous solutions.[67-69] We have therefore used this technique for characterizing the interactions between nucleic acids and lanthanides in these systems.

Further, electron paramagnetic resonance (EPR) is a selective and sensitive tool for studying systems with unpaired electrons, e.g. radicals and paramagnetic transition metals. With EPR spectroscopy, detailed information about the nature, location and the electronic structure and dynamics of the centers with an unpaired spin can be obtained and thereby provide complementary information for understanding of the mechanisms of interaction of lanthanides with the nucleic acids. Gd(III) is the only trivalent lanthanide whose EPR spectrum can be observed routinely at room temperature because of its relatively long electron relaxation times, 10^{-9} to 10^{-10} s. These are slower than those of other lanthanides (around 10^{-13} s), where band broadening is observed, [70,71] but

of a similar order of magnitude to lifetimes of exchange of water molecules on the lanthanide ions.[31,72]

We have therefore carried out a detailed study of the association of trivalent lanthanide ions Ce^{3+} and Tb^{3+} with RNA and GTP using their luminescence spectroscopy and lifetimes, complemented by ^{31}P NMR measurements and EPR studies on solutions of single stranded DNA, RNA and GTP using Gd^{3+}.

2. Materials and Methods

Cerium (III), terbium (III) and gadolinium (III) perchlorates from Aldrich were of the purest grade available and were used as received. Guanosine 5´triphosphate (GTP), sodium salt, and yeast tRNA from Sigma were used as supplied and were of the best grade available. Water purified by a Milli-Q system (Milipore Corporation, Bedford, MA) was used for all solutions. An appropriate buffer (20 mM Tris-HCL, pH 7.5) was used for RNA dilution. RNA concentration was measured by its absorbance at 260 nm, $\varepsilon_{260} = 5x10^5\ M^{-1}\ cm^{-1}$. Deoxyribonucleic acid sodium (DNA) from salmon testes (Sigma) was used as supplied. Nucleic Acids molarities are expressed in moles of nucleotide base. Its molecular weight is around 2000 base pairs (bp). DNA was thermally denatured to produce ss-DNA by heating at 90ºC for 10 min and then cooling rapidly by injecting the sample into a beaker that is cooled by immersion into a mixture of cold ice and ethanol. Following this treatment, the DNA in aqueous solution for the EPR experiments was shown to be single stranded by differential scanning calorimetry (DSC)[32]. DNA concentration was measured by its absorbance at 260 nm, $\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$. A_{260}/A_{280} ratio of RNA and DNA solutions was about 1.8-1.9 indicating the absence of protein contamination.[73]

Deuterium oxide (99.9 at. %) from Aldrich was used in preparing solutions for determining the number of water molecules in the phosphorescence decay measurements. The normal pH of the solutions was 4.7. The solution pH was modified from pH 4.7 to pH 7 and 9 by the addition of small amounts of NaOH (or NaOD).

Absorption spectral measurements were made in 1 cm quartz cuvettes on a Shimadzu UV-2100 spectrophotometer. For luminescence spectral measurements, a Spex Fluorolog 111 was used in 90° configuration, with emission and excitation spectra recorded using appropriate excitation or emission wavelengths. In experiments with the system RNA-terbium(III) an appropriate filter was introduced in front of the emission monochromator to eliminate higher order bands. Terbium(III) luminescence lifetimes were measured using the Spex 1934D phosphorimeter accessory with the Fluorolog 3-22 instrument, and decays were analyzed by using the program Origin 6.0 (Microcal).

The ^{31}P spectra were obtained on a Varian Unity-500 NMR spectrometer (202.326 MHz), using H_3PO_4 (85%) as external reference, sw = 25,000 Hz, at = 1.0 s and d_1 = 5.0 s.

The solutions were prepared starting from ss-DNA stock solution and terbium(III) was added to the solutions in adequate amounts. The pH was adjusted by addition of DCl and NaOD; the pH values quoted are the direct pH-meter readings (room temperature) after standardization with aqueous buffers.

Electron paramagnetic resonance (EPR) measurements were carried out in aqueous solutions using samples in the sealed capillary part of Pasteur pipettes. Spectra were recorded on a Bruker EMX10/12 spectrometer, equipped with Bruker N_2 temperature controller device BVT3000, operating at X band and calibrated with DPPH (α,α´-diphenyl-β-picrylhydrazyl).

3. Results and Discussion

Luminescence studies. Initial observations were made on the phase behaviour of aqueous solutions of RNA and GTP in the presence of lanthanide ions. Upon addition of cerium(III), terbium(III) to an aqueous solution of RNA (10^{-4} M) and GTP (10^{-4} M) at room temperature, formation of a precipitate was observed at lanthanide concentrations above $5x10^{-3}$ M. The concentration of lanthanide ions, on adding RNA and GTP, was then decreased until the concentration limit detected to avoid precipitation and clear isotropic solutions were observed. Information was obtained at a molecular level on aqueous solutions of cerium(III) perchlorate in the presence of RNA and GTP by studying the emission spectrum. In the presence of RNA (10^{-4}M) and GTP (10^{-4}M), increases in Ce(III) luminescence intensity were observed, with the effect being more marked in the presence of RNA, as shown in Figure 1. Emission spectra were also obtained for the system RNA-cerium(III) at different cerium concentrations keeping the RNA concentration constant. Small blue shifts in the emission maxima and a marked increase in the luminescence intensity were observed, as shown in Figure 2, due to a selective binding of the lanthanide to the RNA, probably with cerium lying close to the phosphate groups. Similar behavior was previously observed for DNA-cerium(III) at different cerium concentrations keeping the DNA concentration constant.[32] As shown in Figure 3, the increase of Ce^{3+} emission intensity is linear up to about 5 x 10^{-4} M for both systems (cerium in the presence of RNA and GTP) but tends to a plateau at higher cerium concentration.

Various attempts have been made to model the binding of high valent ions by polyelectrolytes. This is not simple due to the fact that the Poisson-Boltzmann distribution fails for these systems. To measure the extent of cerium binding to both RNA and GTP, a multiple equilibrium model was used. The multiple equilibrium model

has the same mathematical form as that of the Langmuir isotherm, the expression for which in the linear form is[74]

$$\frac{c}{w} = \frac{1}{w_{\max} K} + \frac{c}{w_{\max}} \qquad (1)$$

where c is the total Ce(III) concentration, w is the bound cerium $[Ce]^{ads}$, $w_{\max}$ is the maximum of $[Ce]^{ads}$, and K is a the binding constant. We assumed that the experimental emission intensity is proportional to the concentration of cerium bound to RNA and GTP ($[Ce]^{ads}$).

$$I^{\exp} = \alpha [Ce]^{ads} \qquad (2)$$

To normalize the data, we calculate α in all cases from the emission intensity for the most dilute Ce(III) solution (5×10^{-5} M). With this constant value, α, and Ce(III) concentrations, the sample emission intensity can be calculated, I^{norm}.

$$I^{norm} = \alpha [Ce(III)] \qquad (3)$$

With these assumptions, the ratio c/w (total Ce(III) concentration $/[Ce]^{ads}$) can be assumed to be proportional to the ratio $I^{norm}/I^{\exp}$

A plot of $I^{norm}/I^{\exp}$ versus Ce(III) concentration for a 10^{-4} M RNA and 10^{-4} M GTP solutions and Ce(III) concentrations ranging from 5×10^{-5} M to 5×10^{-3} M is linear (correlation coefficients 0.989 and 0.978 for RNA and GTP, respectively), with slopes

19 125 M^{-1} and 18 800 M^{-1}, for RNA and GTP, respectively. The slope ($1/w_{max}$) gives a measure of the maximum binding of cerium to RNA and GTP. The higher slope for the cerium-RNA system provides evidence of a stronger binding to RNA than to GTP.

In agreement with this observation, the biggest effect on the emission intensity was observed in the presence of RNA. As shown in Figure 3, GTP is a weak enhancer of cerium emission intensity when compared to RNA. This is probably due to the smaller number of binding sites available in GTP, with a maximum of three phosphate groups and one nitrogenous base (guanine), against the much greater number of phosphate groups and purine and pyrimidine bases available in RNA.

Emission spectra were studied of aqueous solution of Tb(III) in the presence of RNA and GTP (Figure 4). The emission spectrum of an aqueous solution of terbium (III) is also shown for comparison. In the presence of RNA and GTP the most sensitive band is in the region 530 to 550 nm; the increase in intensity in this region is more significant than with the other bands. As was observed with cerium, RNA is a more efficient emission enhancer than GTP. The emission intensity of Tb(III) in the presence of GTP is only twice that Tb(III) alone, which, as mentioned above, is due to the existence of fewer binding sites with this nucleotide than with the nucleic acids. These results also suggest that at these concentrations a maximum of one GTP molecule is bound to each lanthanide ion. Increases in the intensity of the emission bands, particularly in the presence of DNA and RNA, has been attributed to energy transfer.[66] However, as we have previously discussed[32] we believe that this is not the only factor involved, and that changes in hydration of the cation may also be responsible. The interaction of lanthanide ions with these polyelectrolytes is likely to change the local coordination sphere, probably leading to substitution of coordinated water molecules of lanthanide ion by phosphate groups or bases.

The effect of the pH. The behaviour of nucleic acids in solution shows a significant dependence upon pH due to the protonation equilibria of phosphate groups and bases, and of the consequent effect of these on chain conformations. Nucleotides undergo protonation in acid solutions, and deprotonation in the presence of base. At neutrality, there is no charge on any of the bases. The bases adenine, cytosine and guanine undergo protonation as the pH is lowered.[75] The pKa values of the purine and pyrimidine bases and phosphate groups are presented in Table 1.

Information on the effect of pH on metal binding was obtained by studying the emission spectrum of aqueous solution of terbium (III) in the presence of RNA and GTP at pH values of 4.7, 7 and 9. The results for the system RNA-terbium are presented in Figure 5. Differences in the luminescence intensities were observed, with the highest intensity at pH 9. The spectra at pH 4.7 and 7 are quite similar suggesting that the same mechanism of binding is involved in this pH region. At neutral pH, RNA is highly charged and the phosphate groups are essentially deprotonated: the phosphomonoester is partially mono-protonated at pH 7, while all the phosphodiester linkages in RNA are entirely deprotonated at pH 7. At pH 4.7 and 7 we believe that, as for DNA-terbium system,[32] terbium binding to RNA mainly involves the phosphate groups. Changes in the RNA structure to a more open form may occur at pH 9, and it is likely that terbium also binds to the nitrogen bases. We have found that, at pH 9, terbium leads to the disruption of the DNA double helix and induces formation of the single stranded conformation.[32] There is evidence from circular dichroism and electrooptical measurements that changes in DNA conformation may occur at even lower pH values, but that this does not lead to strand breaking. Transfer RNA has a secondary structure in which the single stranded RNA folds back on itself to form a double stranded helix.

Terbium can induce some perturbations in the stability of this double helix, as it does with DNA.[32] As with single stranded DNA[32] and poly(vinylsulfonate)[33] it can act as a chelating ligand. Similar, but less pronounced, behaviour was observed for the system GTP-terbium at the same pH values, as shown in the inset of Figure 5. The higher luminescence intensity observed at pH 9 than at pH 4.7 or 7 (whose spectra almost overlap) strongly supports the idea that two possible mechanisms of binding are involved. As with guanosine-5`-phosphate[75], the secondary phosphate of GTP has a pK_a around 6 while guanine has pK_a values 2.4 and 9.2. According with this, the interaction of terbium with GTP probably involves binding to the phosphate at pH 4.7 and 7, and to both phosphate and guanine at pH 9.

We show below that these two different ways of binding are in agreement with the different number of water molecules which are released from the terbium ion coordination sphere at different pH values and with differences in the ^{31}P NMR spectra. We will also see that EPR results confirm the existence of strong interaction and differences on binding depending on the pH.

Hydration studies via lifetimes in H_2O and D_2O solutions. Additional information on terbium-RNA and terbium-GTP interaction was obtained by studying the decay of the terbium(III) ion luminescence. As indicated in the introduction, the terbium lifetimes are very sensitive to the detailed nature of the ligand environment, due to deactivation of excited states by coupling to ligand vibrational modes. Then, the study of Tb(III) luminescence decay in H_2O and D_2O solutions provides a valuable method for measuring the number of coordinated water molecules.[38,44] Water (H_2O) molecules (and OH^- ions) are generally much more effective nonradiative relaxers of lanthanide excited states than are other ligands or ligand donor groups, while D_2O molecules (and OD^-

ions) are less efficient. This difference in the excited lanthanide ions lifetimes in H_2O versus D_2O solutions can be exploited in determining the number of water molecules coordinated to Tb(III). The decay of Tb(III) luminescence was studied alone ([Tb(III)]=10^{-4}M) and in the presence of RNA ([Tb(III)] = 10^{-4}M, [RNA] = 10^{-4}M) and GTP ([Tb(III)] = 10^{-4}M, [GTP] = 10^{-4}M) in H_2O and D_2O solutions. Good single exponential decays were observed. Although more than one Tb(III) species is present in these systems, such that in principle multiexponential decays may be expected, terbium(III) complexes are know to be labile,[76] such that the rate of ligand exchange with this cation is likely to be faster than its excited state decay, and only a single exponential is seen in each case. From these, the number of bound water molecules was determined (Table 2). The values for aqueous solution in the absence of RNA and GTP are in good agreement with literature data[44] and are consistent with Tb(III) being coordinated to 9 water molecules for pH values 4.7, 7 and 9, as been shown by neutron scattering.[77] However, in the presence of RNA and GTP, the number of bound water molecules appears to decrease to approximately 8 at pH 4.7 and 7, and to approximately 3 and 7 water molecules at pH 9, respectively. This loss of one, six and two water molecules is probably related to the different way terbium binds to RNA and GTP at different pH values. At low pH values, the behaviour is similar to that observed in our previous study with double stranded DNA. It has been suggested for lanthanide ion binding to polynucleotides that this involves mainly charged phosphate goups.[78] In this region, substitution by this group on the nucleic acids or nucleotide is likely to replace one water molecules. For GTP at pH 9, as discussed in the previous section, since this is a nucleotide with one nitrogenous base, binding the lanthanide may involve one phosphate group and the guanine base, leading to release of two water molecules from the terbium ion. The biggest effect is seen with RNA at pH 9, where six water

molecules are lost. This is similar to what has been seen on binding of lanthanide ions to single stranded DNA[32] or to poly(vinyl sulfonate)[33], and is probably due to chelation of the lanthanide ion by the polyelectrolyte leading to loss of so many water molecules. A similar chelation effect has been identified with samarium (III) in a high-resolution crystal structure determination of a metal substituted manganese peroxidase.[79] The loss of six water molecules is fully consistent with the marked increase in luminescence intensity seen with RNA at this pH, and, as discussed in the previous section, we believe that both changes in the secondary structure of RNA and involvement of phosphate groups and bases contribute to the binding. The results with GTP strongly support binding by both phosphate and bases with RNA.

We believe that the confirmation of lanthanide ion dehydration in these systems is important and, although it is dangerous to extrapolate to the behaviour of other metal ions and polyelectrolytes, feel that the entropy change caused by cation dehydration may be one of the important thermodynamic parameters in the binding in these systems. There is increasing evidence for the importance of counter ion dehydration on binding to polymer and surfactant systems, [32,33,64,80] possibly involving the dehydration entropy.[81]

^{31}P Nuclear Magnetic Resonance Studies

Phosphorous-31 NMR spectroscopy is likely to provide valuable information on the extent of metal ion binding to phosphate groups in nucleic acids. Due to experimental reasons, it was not possible to obtain ^{31}P NMR spectral data with RNA. However, studies were made using single strand DNA (ss-DNA), and the results are presented in Table 3. The ^{31}P chemical shifts of ss-DNA show four broad signals spanning from around -28 to around 30 ppm. One of the most important factors that affect ^{31}P chemical

shifts is the degree of imposed conformational constraint. Generally, the ^{31}P chemical shifts of the phosphates move upfield as the position of the phosphate moves toward the centre of the helix.[68] In the presence of Tb(III), the ^{31}P NMR spectra of ss-DNA, for pH values 4.7 and 7, shows the presence of inorganic phosphate (Pi) complexed with Tb(III), together with two other signals, with positive chemical shifts, which can be assigned to phosphate moieties of DNA complexed with Tb(III), supporting the model of binding at this pH obtained from luminescence studies. The loss of two of the ^{31}P signals of the DNA and the presence of a new signal around 40 ppm may be associated with both paramagnetic broadening and lanthanide induced chemical shift, but may also indicate changes in the DNA conformation on binding. The presence of inorganic phosphate suggests that Tb(III) is inducing hydrolysis, in agreement with previous reports.[82] This is not seen in the luminescence studies, which suggests that at the level of the first coordination sphere of the lanthanide ion there is little difference between coordination by inorganic phosphate and phosphate groups on the DNA.

However, at pH 9, no signals could be observed in the ^{31}P NMR spectra of ss-DNA in the presence of the same concentration of Tb(III), due to severe broadening, suggesting that the interaction between metal ions and ss-DNA is stronger, probably, as previously suggested [83], involving both phosphate groups and the N-donor groups of the bases.

Similar experiments were carried out with GTP (guanosine 5' triphosphate) and Tb(III). The results are also presented in Table 3. ^{31}P NMR signals of GTP have previously been assigned.[84] In the presence of Tb(III) new signals appear, which suggest that the interaction of metal ions with GTP also gives rise to hydrolysis to inorganic phosphate at all the pH values studied (4.7-9). Both the oxygen atoms of the sugar phosphate moieties and the nitrogen atoms of the base residues are available as potential binding

sites in GTP. Previous studies on the complexation of Ru(II) with ATP and GTP have provided evidence of the possibility of macrochelate formation through coordination of a metal centre by both the guanine base and phosphate residues, with coordination by the N-donor atoms of the base occurring in alkaline solutions.[85] Macrochelates are only formed with 5'-di and -tri-phosphates, facilitating a slow metal-assisted cleavage. We note that recent density functional calculations on the binding of the related hydrated aluminium(III) ion to nucleic acid bases suggest that binding involves the nitrogen atom of the five ring of guanine.[86] The ^{31}P NMR results all support the view that in neutral or acid solution, complexation of the lanthanide involves phosphate groups. The differences observed with the ^{31}P NMR spectra for the systems Tb(III)/ss-DNA and Tb(III)/GTP are probably related with differences in the lanthanide ion binding. With GTP, macrochelation is possible, and may involve more than one phosphate group. This cannot occur with the single strand nucleic acid. However, it is possible that the lanthanide may bind more than one phosphate group at different parts of the polymer chain.

Electron Paramagnetic Resonance Studies (EPR). Additional experimental evidence showing lanthanide ions interacting with nucleic acids and GTP comes from Gd(III) X band EPR spectra.

The Gd(III) EPR spectra was studied in pure water and in solutions of ds-DNA, ss-DNA, GTP and RNA at different pH values (pH 4.7, 7 and 9) and different gadolinium concentrations. In the case of ds-DNA for the three pH values the samples precipitate and the only signal detected was due to free Gd(III) (data not shown). For RNA, the signal at pH 9 seems to be more affected by precipitation than at the other two pH

values, what would be in agreement with the loss of a higher number of coordinated water molecules when lanthanides interact with RNA at pH 9.

The first evidence of the interaction of Gd(III) with GTP and nucleic acids comes from the effect of pH on the Gd(III) signal. In aqueous solution, the Gd(III) signal is pratically unaffected by pH. However, in the presence of nucleic acids (ss-DNA and RNA) or GTP, the intensity of the signal increase markedly with pH, as it is shown in Figure 6 for ss-DNA with a Gd(III) concentration of $5x10^{-3}$ M. With this technique differences between pH 4.7, 7 and 9 become evident and in this sense, EPR spectroscopy studies using different pH values are more sensitive than luminescence.

The second evidence which probes the interaction of Gd(III) with nucleic acids and GTP comes from the slight broadening of the Gd(III) signal with respect to the signal in aqueous solution, as it is shown in Figure 7.

Although the factors responsible for the linewidth of Gd(III) EPR spectra in aqueous solutions have been extensively studied and discussed in detail,[87-91] the situation is complex, involving both inner- and outer-sphere exchange processes, together with dipole-dipole interactions, and effects due to transient distortions of complexes, factors which depend on both the symmetry and the number of coordinated water molecules. However, while the actual mechanism responsible for the slight increase in band width in the present case is not clear, the results are consistent with changes in the coordination sphere of the cation on binding to single stranded DNA or GTP. Also the fact that signals seem slightly wider at higher pH is in agreement with a stronger interaction between the lanthanide and ss-DNA, RNA or GTP under these conditions, as suggested by the luminescence results.

Conclusions

Luminescence studies have been used to study the interaction between the trivalent ions cerium (III) and terbium (IIII), and RNA and GTP. The luminescence spectra in the presence of RNA and GTP show significant differences which suggest complexation. With cerium this binding is evident from the dependence, both for RNA and GTP, of emission intensity on concentration, where a plateau regime has been found. In the case of terbium-RNA and terbium-GTP systems, interesting dependences on pH were observed; this is related to the protonation equilibria of the phosphate groups and nucleotide bases, which also will affect the helical structure of RNA. Two possible mechanisms of binding seem to be present. At pH 4.7 -7 binding is suggested to involve just the phosphate groups. However, at pH 9 the mechanism may involve both phosphate groups and the bases in the case of RNA, or the guanine base in the case of GTP. This is consistent with the analysis of the Tb(III) lifetimes in H_2O and D_2O at different pH, where it was found that the lanthanide ion loses one water molecule on binding the RNA and GTP at pH 4.7 and 7, and six and two water molecules at pH 9, respectively. Further information comes from EPR spectroscopy, whose strongly supports the existence of interaction. The Gd(III) EPR spectra in the presence of ss-DNA, GTP and RNA show slight broadening, supporting changes in the lanthanide ion coordination sphere. In agreement with the results obtained with the other spectroscopic techniques used in this work, the increase of Gd(III) spectrum intensity with pH also shows that lanthanide interaction with nucleic acids and GTP is stronger at higher pH.

Acknowledgements

We are grateful to POCTI (POCTI/QUI/35415/2000, /39593/2001, /45344/2002 and 58291/2004), FCT (SFRH/BD/16736/2004), and FEDER for financial support. We also thank *Conselho de Reitores das Universidades Portuguesas/Ministério de Ciencia y Tecnologia (Acção Integrada Luso-Espanhol* E-4/05) for further support for the collaboration between Coimbra and Burgos.

Figure Captions

Figure 1. Emission spectra (λexc = 280 nm) for: cerium (III) ($[Ce^{3+}] = 10^{-4}$M); cerium (III)-GTP ($[Ce^{3+}] = 10^{-4}$M, [GTP] = 10^{-4}M) and cerium (III)-RNA ($[Ce^{3+}] = 10^{-4}$M, [RNA] = 10^{-4}M). Temperature 25°C.

Figure 2. Emission spectra (λexc = 280 nm) for the complex Ce(III)-RNA with different cerium concentrations ($5x10^{-5}$M, 10^{-4}M, 10^{-3}M and $5x10^{-3}$M). RNA concentration is constant ([RNA] = 10^{-4} M).Temperature 25°C.

Figure 3. Corrected Ce(III) emission intensity versus Ce(III) molar concentration in GTP and RNA aqueous solutions ([GTP] = 10^{-5} M) and [RNA] = 10^{-5} M). Temperature 25°C.

Figure 4. Emission spectra (λexc = 280 nm) for: terbium (III) ($[Tb^{3+}] = 10^{-4}$M); terbium(III)-GTP ($[Tb^{3+}] = 10^{-4}$M, [GTP] = 10^{-4}M) and terbium (III)-RNA ($[Tb^{3+}] = 10^{-4}$M, [RNA] = 10^{-4}M). Temperature 25°C.

Figure 5. Emission spectra (λexc =280) for the complex Tb(III)-RNA ([Tb(III)] =10^{-4} M, [RNA] =10^{-4} M) with different pH values (4.7, 7 and 9) and in the inset for the complex Tb(III)-GTP ([Tb(III)] =10^{-4} M, [GTP] =10^{-4} M) with different pH values (4.7, 7 and 9).Temperature 25 °C.

Figure 6. The EPR Gd(III) ([Gd(III) = $5x10^{-3}$ M]) spectra in the presence of ss-DNA ([ss-DNA]) = 10^{-4} M]) in aqueous solution at different pH (4.7, 7 and 9). Temperature 25ºC.

Figure 7. The normalized EPR Gd(III) ([Gd(III) = $5x10^{-3}$ M]) spectra in aqueous solution (dotted line) and in the presence of RNA ([RNA]) = 5 $x10^{-3}$ M], straight line) at pH 4.7. Temperature 25ºC.

References

(1) L. Stryer, Biochemistry (W. H. Freeman and Company, San Francisco, 1994)**.**

(2) C. R. Woese, S. Winker and R. Gutell, *Proc. Natl. Acad. Sci. US* **A87**, (1990) p.8467.

(3) V. P. Antao, S. Y. Lai and I. Jr. Tinoco, *Nucleic Acids Res.* **20**, (1991) p.819.

(4) A. Gluck, Y. Endo and I. G. Wool, *J. Mol. Biol.* **115**, (1992) p.91.

(5) T. Hjalt and E. G. Wagner, *Nucleic Acids Res.* **20**, (1992), p.6723.

(6) P. Blomberg, H. M. Engdahl, C. Malmgren, P. Romby, and E. G. Wagner, *Mol. Microbiol.* **12**, (1994) p.49.

(7) H. W. Pley, K. M. Flaherty and D. B. Mckay, *Nature* **372**, (1994), p.68.

(8) E. V. Puglisi and J. D. Puglisi, *Nat. Struct. Biol.* **5**, (1998), p.1033.

(9) S. S. Ashraf, G. Ansari, R. Guenther, E. Sochacka, A. Malkiewicz and P. F. Agris, *RNA* **5**, (1999), p.503.

(10) F. Kolb, H. M. Engdahl, J. G. Slagter-Jäger, B. Ehresmann, C. Ehresmann, E. Westhof, E. G. Wagner and P. Romby, P. *EMBO J.* **19**, (2000), p.5905.

(11) R. A. Lerner, S. J. Benkovic and P. G. Schultz, *Science* **252**, (1991) p.659.

(12) J. Greenblatt, J. R. Nodwell and S. W. Mason, *Nature* **364**, (1993), p.401.

(13) R. R. Samaha, B. O`Brien, T. W. O`Brien and H. F. Noller, *Proc. Natl. Acad. Sci. USA* **91**, (1994), p.7884.

(14) V. Ramakrishnan, C. Davies, S. E. Gerchman, B. L. Golden, D. W. Hoffmann, T. N. Jaishree, J. H. Kycia, S. Porter and S. W. White, *Biochem. Cell. Biol.* **73**, (1995), p.979.

(15) A. Liljas and M. Garber, *Curr. Opin. Struct. Biol.* **5**, (1995), p.721.

(16) D. I. Friedman and D. L. Court, *Mol. Microbiol.* **18**, (1995), p.191.

(17) G. Simos, G. Tekotte, H. Grosjean, A. Segref, K. Sharma, D. Tollervey and E. Hurt, *EMBO J.* **15**, (1996), p.2270.

(18) M. Markus, A. Hink, S. Huang, D. Draper and D. A. Torchia, *Nature Struct. Biol.* **4**, (1997), p.70.

(19) K. M. Weeks, *Curr. Opin. Struct. Biol.* **7**, (1997), p.336.

(20) D. Crothers, *Proc. Natl. Acad. Sci. USA* **95**, (1998), p.15163.

(21) R. A. Weisberg and M. E. Gottesman, *J. Bacteriol.* **181**, (1999), p.359.

(22) M. Menger, F. Eckstein and D. Porschke, *Biochemistry* **39**, (2000), p.4500.

(23) J. Barrick, T. Takahashi, J. Ren, T. Xia and R. Roberts, *Proc. Natl. Acad. Sci. USA* **98**, (2001), p.12374.

(24) D. C. Clary and A. J. H. M. Meijer, *J. Chem. Phys.* **116**, (2002), p.9829.

(25) R. Hanna and J. A. Doudna, *Curr. Opin. Struct. Biol.* **4**, (2000), p.166.

(26) A. M.Pyle, *J. Biol. Inorg. Chem.* **7**, (2002), p.679.

(27) V. DeRose, *Chem. Biol.* **9**, (2002), p.961.

(28) G. Bokinski, D. Rueda, V. Misra, M. M. Rhodes, A. Gordus, H. P. Babcock, et al. *Proc. Natl. Acad. Sci. USA* **100**, (2003), p.9302.

(29) A. M. Pyle and J. B. Green, *Curr. Opin. Struct. Biol.* **5**, (1995), p.303.

(30) M. J. Fedor, *Curr. Opin. Struct. Biol.* **12**, (2002), p.289.

(31) J.-C. Bünzli, *Acc. Chem. Res.* **39**, (2006), p.53.

(32) D. Costa, H. Burrows and M. Miguel, *Langmuir* **21**, (2005), p.10492.

(33) M. Tapia and H. Burrows, *Langmuir* **18**, (2002), p.1872.

(34) L. G. Marzilli, *Prog. Inorg. Chem.* **23**, (1977), p.255.

(35) F. S. Richardson, *Chem. Rev.* **82**, (1982), p.541.

(36) W. DeW. Horrocks Jr and M. Albin, *Prog. Inorg. Chem.* **31**, (1984), p.1.

(37) G. R. Chopin and Z. M. Wang, *Inorg. Chem.* **36**, (1997), p.249.

(38) W. DeW. Horrocks Jr and D. R. Sudnick, *Acc. Chem. Res.* **14**, (1981), p.384.

(39) R. D. Shannon, *Acta Crystallogr.* **A32,** (1976), p.751.

(40) M. Almgren, *Adv. Colloid Interface Sci.* **41**, (1992), p.9.

(41) M. Miguel, H. Burrows, S. Formosinho, B. Lindman, *J. Mol. Struct.* **563**, (2001), p.89.

(42) H. Burrows and M. Miguel, *Adv. Colloid. Interface Sci.* **89**, (2001), p.485.

(43) G. Stein and E. Würzberg, *J. Chem. Phys.* **62**, (1975), p.208.

(44) W. DeW. Horrocks Jr and D. R. Sudnick, *J. Am. Chem. Soc.* **101**, (1979), p.334.

(45) W. T. Carnall, P. R. Fields and K. Rajnak, *J. Chem. Phys.* **49**, (1968), p.4447.

(46) H. N. Halladay and M. Petersheim, *Biochemistry* **27**, (1998), p.2120.

(47) D. Parker and J. A.G. Williams, *J. Chem. Soc. Dalton Trans.* (1996), p.3613.

(48) M. P. O.Wolbers, F. C. J. M. van Veggel, F. G. A. Peters, E. S. E. van Beelen, J. W. Hofstraat, F. A. J. Geurts and D. N. Reinhoudt, *Chem. Eur. J.* **4**, (1998), p.772.

(49) G. R. Choppin and D. R. Peterman, *Coord. Chem. Rev.* **174**, (1998), p.283.

(50) D. Parker, *Coord. Chem. Rev.* **205**, (2000), p.109.

(51) S. Lis, M. Elbanowski, B. Makowska and Z. Hnatejko, *J. Photochem. Photobiol. B* **150**, (2002), p.233.

(52) G. Blasse, W. Schipper and J. Hamelink, *J. Inorg. Chim. Acta* **189**, (1991), p.77.

(53) K. Okada, Y. Kaizu, H. Kobayashi, K. Tanaka and F. Marumo, *Mol. Phys.* **54**, (1985), p.1293.

(54) J. Kropp and M. W. Windsor, *J. Phys. Chem.* **71**, (1967), p.477.

(55) J. L. Kropp and M. W. Windsor, *J. Chem. Phys.* **39**, (1963), p.2769.

(56) J. L. Kropp and M. W. Windsor, *J. Chem. Phys.* **45**, (1966), p.761.

(57) A. Heller, *J. Am. Chem. Soc.* **88**, (1966), p.2058.

(58) Y. Haas and G. Stein, *J. Phys. Chem.* **75**, (1971), p.3668.

(59) Y. Haas and G. Stein, *J. Phys. Chem.* **75**, (1971), p.3677.

(60) Y. Haas and G. Stein, *J. Phys. Chem.* **76**, (1972), 1093.

(61) S. T. Frey and W. DeW. Horrocks Jr, *Inorg. Chem.* **30**, (1991), p.1073.

(62) A. Beeby, L. M. Bushby, D. Maffeo and J. A. Williams, *JCS Perkin Trans.* **2**, (1999), p.493.

(63) M. Tapia, H. Burrows, M. E. D. G. Azenha, M. Miguel, A. A. C. Pais and J. Sarraguça, *J. Phys. Chem B* **106**, (2002), p.6966.

(64) H. Burrows and M. Tapia, *Langmuir* **18**, (2002), p.6706.

(65) D. S. Gross and H. Simpkins, *J. Biol. Chem.* **256**, (1981), p.9593.

(66) D. S. Gross, H. Simpkins, E. Bubienko and P. Borer, Arch. *Biochem. Biophys.* **219**, (1982), p.401.

(67) D. Gorensetin, *J. Am. Chem. Soc.* **97**, (1975), p.898.

(68) D. Gorensetin, *Chem.Rev.* **94**, (1994), p.1315.

(69) P. Moore, *Acc. Chem. Res.* **28**, (1995), p.251.

(70) E. Stephens and C. Grisham, *Biochemistry* **18**, (1979), p.4678.

(71) T. Hyde and T. Sarna, *J. Chem. Phys.* **68**, (1978), p.4439.

(72) C. Cossy and A. E. Merbach, *Pure Appl. Chem.* **60**, (1988), p.1785.

(73) C. Cantor and P. Schimmel, (*Biophysical Chemistry,* San Francisco, CA 1980)

(74) B. Jönsson, B. Lindman, K. Holmberg and B. Kronberg, (*Surfactants and polymers in aqueous solution*, West Sussex, 2nd Edition, 2003)

(75) G. Zubay, (*Biochemistry*, Dubuque, IA, 4th edn., 1998).

(76) L. Helm and A.E. Merbach, *Chem. Rev.* **105** (2005), p.1923.

(77) G. W. Neilson and J. E. Enderby, *Adv. Inorg. Chem.* **34**, (1989), p.195.

(78) R. M. Izatt, J. J. Christensen, J. H. Rytting, *Chem. Rev.* **71**, (1971), p.439.

(79) M. Sundaramoorthy, H. L. Youngs, M. H. Gold, T. L. Poulos, *Biochemistry* **44**, (2005), p.6463.

(80) L. Romsted, *Langmuir* **23**, (2007), p.414.

(81) C. G. Sinn, R. Dimora, C. Huin, O. Sel and N. Antonietti, *Macromolecules* **39**, (2006), p.6310.

(82) M. Komiyama, N. Takeda and H. Shigekawa, *Chem.Comm.* **12**, (1999), p.1443.

(83) D. S. Gross, H. Simpkins, E. Bubienko and P. N. Borer, *Arch. Biochem. Biophys*. **219**, (1982), p.401.

(84) P. Lee, A. Gorrell, H. J. Fromm and R. F. Colman, *Arch. Biochem. Biophys.* **372**, (1999), p.205 *and references therein.*

(85) S. Korn and W. S. Sheldric, *J. Chem. Soc. Dalton Trans.* (1997), p.2191.

(86) D. Mazzuca, N. Russo, M. Toscano and A. Grand, *J. Phys. Chem. B* **110**, (2006), p.8815.

(87) J. Reuben, *J. Phys. Chem.* **75**, (1971), p.3164.

(88) R. V. Southwood-Jones, W. L. Earl, K. E. Newman and A. E. Merbach, *J. Chem. Phys.* **73**, (1980), p.5909.

(89) D. H. Powell, A. E. Merbach, G. González, E. Brücher, K. Micskei, M. F. Ottaviani, K. Köhler, A. von Zelewsky, O. Ya. Grinberg and Y. S. Lebedev, *Helv. Chim. Acta* **76**, (1993), p.2129.

(90) S. K. Sur and R. G. Bryant, *J. Magn. Reson. Chem. B* **111**, (1996), p.105.

(91) S. Rast, P. H. Fries, E. Belorizky, A. Borel, L. Helm and A. E. Merbach, *J. Chem. Phys*. **115**, (2001), p.7554.

(92) E. M. Moody, T. S. Brown and P. C. Bevilacqua, *J. Am. Chem. Soc.* **126**, (2004), p.10200.

Figures

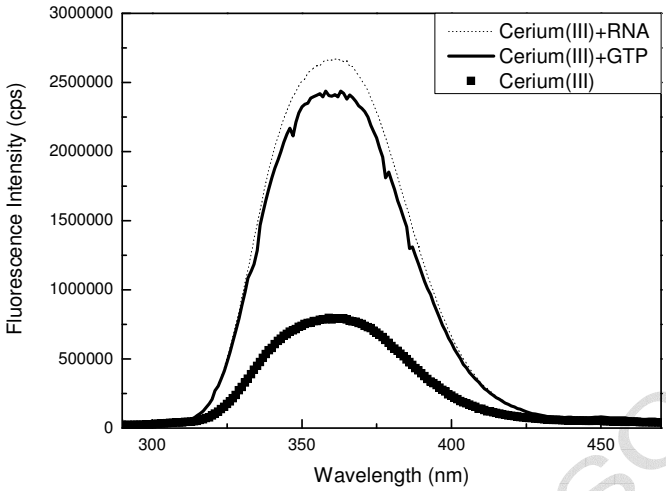

Figure 1.

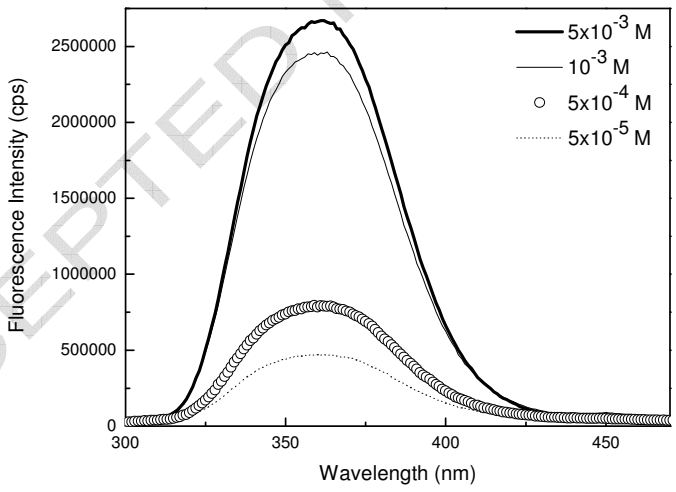

Figure 2.

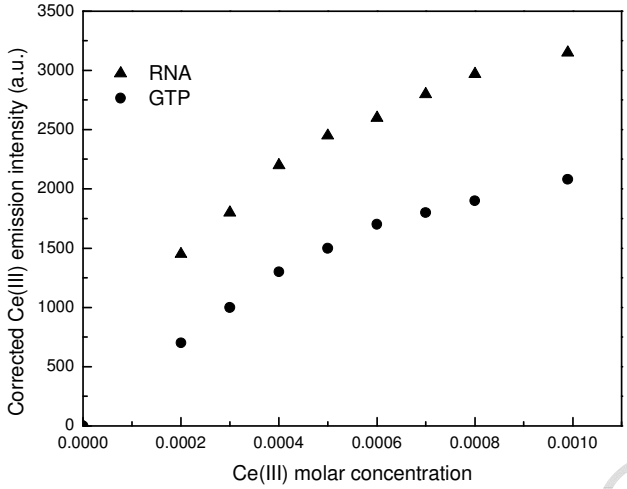

Figure 3.

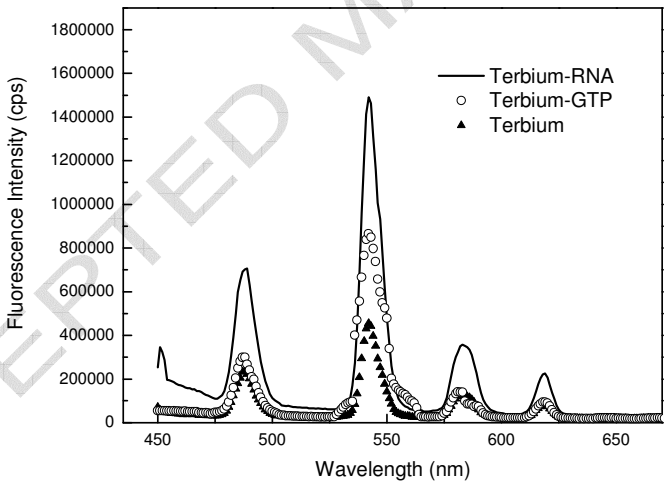

Figure 4.

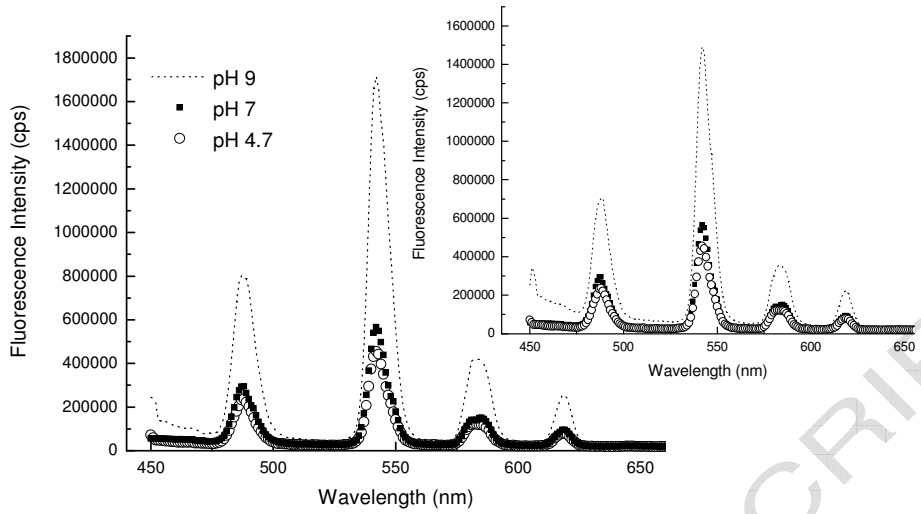

Figure 5.

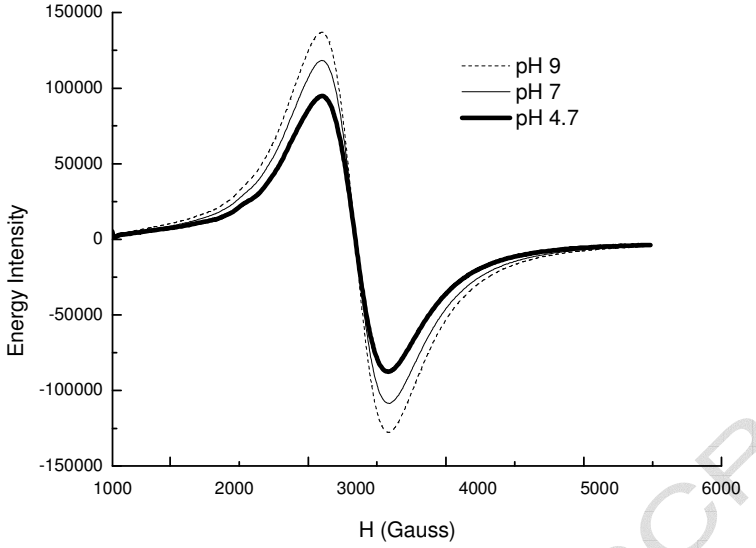

Figure 6.

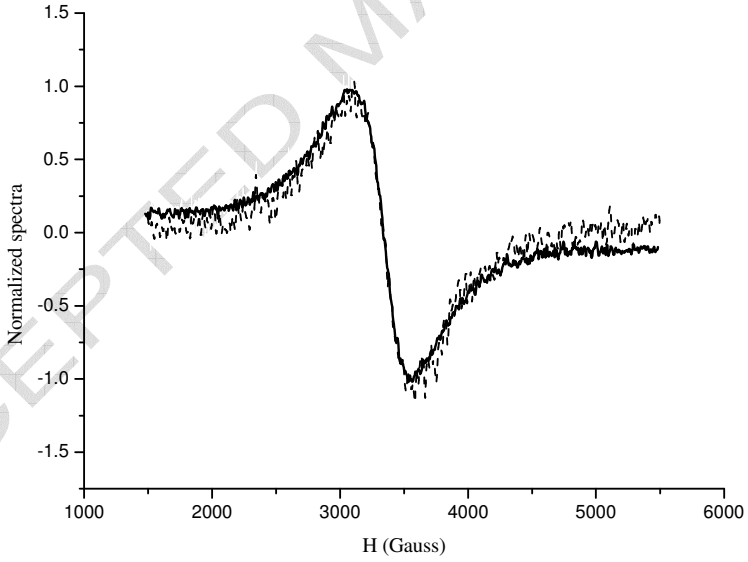

Figure 7.

Table 1: pKa values of pyrimidine and purine bases and phosphate groups.[78,92]

Base	**Atom**	**pKa**
Adenine	N-1	3.5
Cytosine	N-3	4.2
Guanine	N-7	3.6
Guanine	N-1	9.2
Thymine	N-3	9.7
Uracil	N-3	10.1
RNA (Phosphates)		between 3 and 4

Table 2: Lifetime, τ, and the Number of Coordinated Water Molecules, n, of Tb(III) in water and D_2O, and in RNA and GTP Aqueous and D_2O solutions at different pH values.

Samples	$\tau_{H2O\ (ms)}$	$\tau_{D2O\ (ms)}$	n[a]
Tb pH 4.7	0.46	4.43	9
Tb-RNA pH 4.7	0.52	3.17	8
Tb-GTP pH 4.7	0.43	2.02	8
Tb pH 7	0.43	4.26	9
Tb-RNA pH 7	0.42	2.03	8
Tb-GTP pH 7	0.43	2.03	8
Tb pH 9	0.49	4.35	9
Tb-RNA pH 9	0.57	0.65	3
Tb-GTP pH 9	0.41	3.05	7

a. Estimated error ± 0.5 water molecules.

Table 3: ^{31}P NMR parameters[a] for ss-DNA, ss-DNA/Tb(III), GTP and GTP/Tb(III) solutions

ss-DNA [b]				
pH* 4.7	28.73	11.70	-13.40	-25.68
pH* 7.0	30.27	11.75	-14.60	-28.00
pH* 9.0	30.10	11.85	-15.35	-28.46

ss-DNA/Tb(III) [c]			
	Pi	remaining peaks	
pH* 4.7	5.64	40.71	26.75
pH* 7.0	5.64	40.83	28.78
pH 9.0	- [d]	- [d]	- [d]

GTP [e]			
	α-GTP	β-GTP	γ-GTP
pH* 4.7	-7.93 (J_{P-P} 19.08)	-19.49 (J_{P-P} 19.08; 19.07)	-6.63 (J_{P-P} 19.07)
pH* 7.0	-7.97 (J_{P-P} 19.08)	-19.62 (J_{P-P} 19.08; 19.07)	-6.72 (broad)
pH* 9.0	-7.99 (J_{P-P} 19.08)	-19.58 (J_{P-P} 19.08; 19.07)	-6.41 (broad)

GTP/Tb(III) [f]		
	Pi	α-GTP+β-GTP
pH* 4.7	3.31	-6.42 (broad)
pH* 7.0	3.29	-6.41 (broad)
pH 9.0	3.29	-6.39 (broad)

a δ Values, in ppm, relative to H_3PO_4 (85%) as external reference, *J* values in Hz.
b 10 mmol dm^{-3} ss-DNA solution.
c 10:1 mmol dm^{-3} DNA:Tb(III) solution.
d not observed.
e 10 mmol dm^{-3} GTP solution.
f 10:1 mmol dm^{-3} GTP:Tb(III) solution.