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Energy Transfer from Nucleic Acids to Tb(III): Selective Emission Enhancement by Single DNA Mismatches

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Abstract: Enhanced luminescence resulting from energy transfer (EnT) from nucleic acids to Tb^{3+} has been utilized to investigate the binding of the ions to the bases and nucleotides, as well as in the detection of single mismatches in duplexes. Cytosine enhances the Tb^{3+} emission, but dCMP does not, indicating that the lanthanide bound to the phosphate group is too far away from the base for efficient energy transfer. Conversely, the enhancement of the Tb^{3+} luminescence by dGMP is greater than that of G, where the phosphate appears to aid in the binding of the ion to the base. We propose that the phosphate group in dGMP is able to fold over and permit coordination of the ion to the O6 and N7 atoms of the base while still bound to the anionic phosphate oxygens, thus increasing the binding affinity and promoting efficient EnT. Single-stranded oligonucleotides greatly enhance the Tb^{3+} emission, but duplexes do not. Single mismatches in the sequence of a duplex lead to selective luminescence enhancement in the presence of Tb^{3+}. The largest enhancement was observed for the GG mismatch, followed by CA, GA, and CC, and the smallest emission intensity was measured for TT and TG mismatches. The unexpected role of adenine in the emission enhancement has been explained through preassociation of the Tb^{3+}, thus permitting A to be in the coordination sphere of the ion. It was concluded that A is able to transfer energy to Tb^{3+} when bound to the ion, but in the absence of the supramolecular assembly, it cannot coordinate strongly enough to the lanthanide to effect EnT. The low emission enhancement by the TG mismatch has been explained in terms wobble pair formation. These findings show that the enhanced emission of lanthanides can be successfully utilized to selectively detect single mismatches in duplexes.

Introduction

The sensitive detection of single-stranded regions of DNA, including mutations and mismatches, is critical in nucleic acid hybridization assays with applications that range from the determination of genetic and infectious diseases to providing accurate personal identification.[1−5] Luminescence enhancement of a given probe in the presence of nucleic acids can in principle yield such detection, with marked safety and environmental advantages over radioactive labeling. Owing to the emissive properties of Eu^{3+} and Tb^{3+}, including their luminescence enhance-

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ment through energy transfer,[10−14] and their ability to bind single-stranded regions of DNA,[15,16] these ions are potentially valuable for the selective detection of base mismatches.

The luminescence of aqueous Tb^{3+} and Eu^{3+} is weak owing to low absorption cross sections and nonradiative deactivation through the O−H vibrations of coordinated water molecules; therefore, addition of chelating agents or encapsulation of the lanthanide leads to longer emission lifetimes and quantum yields.[17−22] Significantly greater emission intensities can be obtained upon chelation of the ion by ligands that, when excited with light, can transfer energy to the emissive state of the lanthanide. These systems have been probed extensively for potential applications as optical sensors.[23−26] In addition, the emission from lanthanides has proven useful as a sensitive detection method in biological systems and has facilitated their understanding.[27−30] The changes in the intensity of the Eu^{3+} luminescence upon binding to proteins have been utilized to examine the ligation sphere within the active site,[31] whereas distance and conformational information under physiological conditions has been obtained from energy transfer studies either between two lanthanide ions or from the protein's residues to Eu(III) or Tb(III) bound to the active site.[32−34]

Single-stranded oligonucleotides are known to enhance the emission of Eu^{3+} and Tb^{3+} ions in solution. This feature has been utilized in the detection of distorted DNA regions[35] and to probe DNA− and RNA−drug interactions.[36,37] Lanthanide chelating agents tethered to oligonucleotides have proven important in luminescence energy transfer experiments,[38] as well as in the detection of DNA following complexation of the emissive Tb^{3+} or Eu^{3+} ions.[39−41] In addition, the enhancement of lanthanide emission in the presence of DNA with added ligands has been a subject of intense investigation owing to potential application in nucleic acid hybridization assays.[42−44]

In the present study we explore the enhanced emission of Tb^{3+} as a potential tool in the detection of single base mismatches in DNA duplexes. The enhancement of the lanthanide ion emission upon binding to the four bases, their respective 5′-deoxynucleotides, as well as single- and double-stranded oligonucleotides has been explored. Although previous studies have reported enhancement by various single-stranded sequences, it has been believed that the trivalent ion interacts mostly with the phosphate groups and that energy transfer occurs only from guanine to the ion over long distances. This work leads to the conclusion that direct coordination between the energy donor base and Tb^{3+} is necessary for efficient energy transfer to take place. In addition, selective enhancement by mismatched base pairs in purified duplexes was observed.

Experimental Section

Materials. $TbCl_3$ was purchased from Aldrich; the nucleobases, nucleotides, NaCl, and Trizma base were purchased from Sigma and were utilized without further purification. The various 10-mer oligonucleotide sequences were purchased from the Midland Reagent Co. and consisted of 5′-CGCAXYTGCG-3′, with XY = AT, GT, CT, TT, AA, AG, AC.

Methods. The annealing of the 10-mers to form duplexes was performed by placing a solution containing 1−10 mM bases in 50 mM NaCl, 20 mM Tris (pH = 7.0) at 90 °C for 7−9 min, then cooling slowly in the heat block to room temperature (~3 h). The duplexes were separated from leftover single-stranded oligonucleotides utilizing fast protein liquid chromatography (FPLC) with a Mono-Q 5/5 (Pharmacia) strong anion exchange column, were eluted using a NaCl gradient (from 0 to 1 M NaCl in 60 min) in 20 mM Tris buffer, pH =

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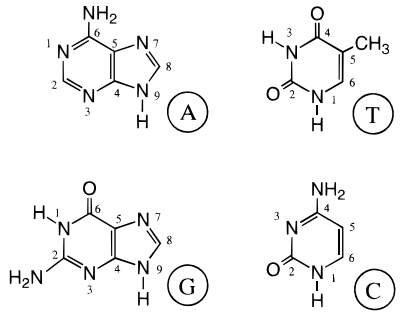

Figure 1. Structures of the four DNA bases.

7.5 (detected using absorption at 254 nm), and were then lyophilized. All solutions were prepared, handled, and stored in plastic Eppendorf tubes and tips. The samples for the emission experiments were allowed to mix for ~30 min at room temperature prior to measurement in either a 0.2 × 1.0 cm (~300-mL sample volume) or a 1 × 1 cm quartz cuvette (Wilmad). Unless otherwise stated the emission experiments were performed in air with $\lambda_{ex} = 260$ nm and $\lambda_{em} = 545$ nm and a 530-nm long-pass filter (CVI Laser Corp.) placed at the entrance slit of the emission monochromator. The error in the emission measurements determined from reproducibility was ±5%.

Instrumentation. Absorption measurements were performed in a Hewlett-Packard diode array spectrometer (HP 8453) with HP8453Win System software installed in an HP Vectra XM 5/120 desktop computer. The melting temperature measurements were carried out utilizing a Peltier temperature control system (HP 89090A) coupled to the absorption instrument and driven by its software. Emission spectra were collected on a SPEX FluoroMax-2 spectrometer equipped with a 150-W xenon source, a red-sensitive R928P photomultiplier tube, and DataMax-Std software on a Pentium microprocessor.

Results

Nucleobases and Nucleotides. A solution containing 25 μM Tb^{3+} is weakly emissive upon 260-nm excitation, owing to the low molar extinction coefficient of the lanthanide ion at that wavelength. Since nucleic acids exhibit large absorption cross sections in the 250−280-nm range, energy transfer (EnT) from the excited base to the emissive $^5D_4$ state of bound Tb^{3+} is possible. Such EnT would result in enhanced Tb^{3+} luminescence in the presence of each nucleic acid (structures shown in Figure 1). The relative emission intensity of 25 μM Tb^{3+} with increasing concentration of each nucleobase is shown in Figure 2a (50 mM NaCl, 2 mM Tris buffer, pH ~ 7.5). Only a decrease in the overall luminescence was observed as the concentration of A (adenine) and T (thymine) were increased, and a lesser decrease was detected upon addition of similar concentrations of G (guanine). In contrast, an enhancement of the Tb^{3+}emission intensity at 543 nm was observed upon addition of C (cytosine), where a plateau with an intensity of 3.5 relative to that measured in the absence of nucleobase is reached at [C] ≥ 40 μM (Figure 2a).

The emission intensity of 25 μM Tb^{3+} was measured as the concentration of the 5′-deoxymonophosphate of each nucleobase, dCMP, dGMP, dAMP, and dTMP, was increased (Figure 2b). As previously reported,[45] only dGMP showed enhancement of the Tb^{3+} emission with relative intensity of ~12 at [dGMP]/[Tb^{3+}] > 1. As shown in Figure 2b, addition of similar concentrations of dCMP, dAMP, and dTMP to the Tb^{3+} solutions does not appear to enhance the luminescence of the lanthanide ion. The emission quantum yield of 25 μM Tb^{3+} with 30 μM dGMP ($\lambda_{exc} = 260$ nm) was measured to be 0.041(4), relative to a standard solution of quinine bisulfate (1 N H_2SO_4).[46]

Fits of the emission enhancement as a function of nucleobase or nucleotide concentration to a 1:1 Tb^{3+}:B (B = C, dGMP) binding model are shown in parts a and b of Figures 2 for C and dGMP, respectively. Since the 1:1 model resulted in good fits of the data, other binding stoichiometries were not explored. The binding constants obtained for the binding of Tb^{3+} to C and dGMP were 5.3×10^5 and 2.8×10^6 M^{-1}, respectively.

The excitation spectra of 25 μM Tb^{3+} in the absence and presence of 50 μM dGMP are shown in Figure 3. The excitation spectrum collected for Tb^{3+} is consistent with its weak absorption in the 250−320-nm region, where strong emission is only observed at wavelengths below 240 nm. In contrast, in the presence of dGMP. strong lanthanide emission is observed in the 240−300-nm region, consistent with the absorption spectrum of the nucleotide. Similar results were observed in the presence of 50 μM cytosine.

Single- and Double-Stranded Oligonucleotides. The emission of 25 μM Tb^{3+} was enhanced by a factor of ~11 in the presence of single-stranded oligonucleotides, where the data points for the addition of the single-stranded 10-mer with sequence 5′-CGCAATTGCG-3′ to 25 μM Tb^{3+} (50 mM NaCl, 2 mM Tris buffer, pH ~ 7.5) are shown in Figure 4. Similar enhancement was observed for other sequence-related 10-mers, such as 5′-CGCAXTTGCG-3′ (X = C, T, and G). When the experiment is conducted with annealed duplex (following the removal of any remaining single strand) of the palindromic sequence 5′-CGCAATTGCG-3′, no Tb^{3+} emission enhancement is observed (Figure 4).

The presence of a single mismatched base pair in the sequence of the 10-mer duplex leads to emission enhancement for certain pairs of nucleotides. The enhancement observed for a duplex containing a GG mismatch (sequence shown in Table 1) is greater than that measured for CA and GA mismatches, followed by CC; the smallest emission enhancement was measured for TT and TG mismatches (Figure 5). As seen in Figure 5 for duplexes that enhance the lanthanide ion emission, the luminescence intensity of 25 μM Tb^{3+} increases with increasing mismatched duplex up to [bases] ~ 50 μM, at which point the overall emission begins to decrease with further increase in the oligonucleotide concentration.

The duplex sequences and mismatches investigated are listed in Table 1, along with the emission intensity of 25 μM Tb^{3+} in the presence of 50 μM bases of each duplex (relative to that observed for the duplex with no mismatches at the same base concentration). The GG mismatch produces the largest relative Tb^{3+} emission enhancement (9.6), followed by GA and CA (7.0 and 6.7, respectively), whereas duplexes containing TT and GT mismatches exhibit the lowest enhancement (3.6 and 3.4, respectively). The melting temperatures, T_m, measured for all the duplexes are also listed in Table 1.

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(46) The emission spectrum of Tb^{3+} spectrum was collected from 450 to 650 nm ($\lambda_{exc} = 260$ nm; 435-nm emission long-pass filter) and integrated after conversion to energy. The sample and standard were optically matched at the excitation wavelength, and both samples were bubbled with N_2 prior to each measurement. The quantum yield of the sample, Φ_{sam}, was determined relative to that of the quinine bisulfate standard ($\Phi_{st} = 0.546$ in 1 N H_2SO_4) and calculated using $\Phi_{sam} = \Phi_{st}(A_{st}/A_{sam})(I_{sam}/I_{st})(\eta^2_{sam}/\eta^2_{st})$, where A_{st} and A_{sam} represent the absorbance of the standard and sample at the excitation wavelength and I_{st} and I_{sam} are the integrated emission intensities, respectively. The refractive index of the sample, η_{sam}, and standard, η_{st}, were assumed to be equal (Scaiano, J. C., Ed. *CRC Handbook of Organic Photochemistry*; CRC Press: Boca Raton, FL, 1989; pp 231−237).

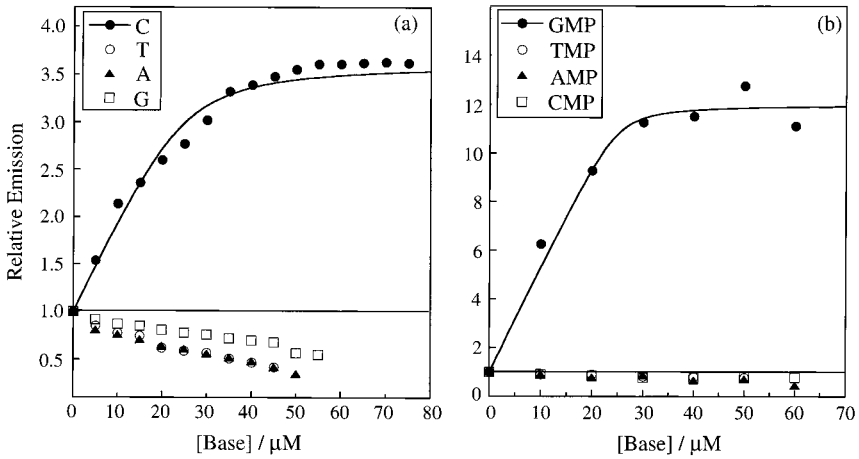

Figure 2. Relative emission of 25 μM Tb^{3+} (λ_{exc} = 260 nm, λ_{em} = 545 nm) in 50 mM NaCl, 2 mM Tris buffer, pH ~ 7.5, as a function of added (a) bases and (b) deoxymonophosphates of each nucleic acid (±5% error in reproducibility).

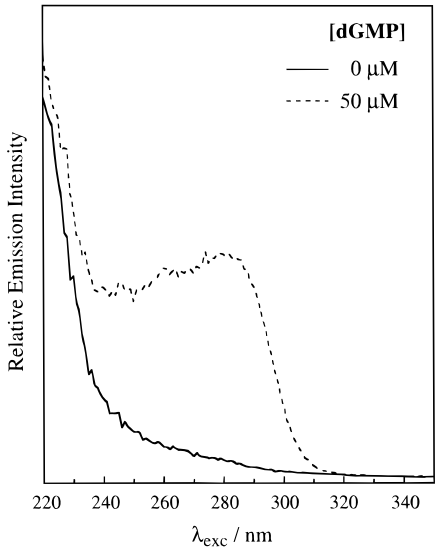

Figure 3. Excitation spectra (λ_{det} = 545 nm) of 25 μM Tb^{3+} in the absence and presence of 50 μM dGMP (50 mM NaCl, 2 mM Tris buffer, pH ~ 7.5).

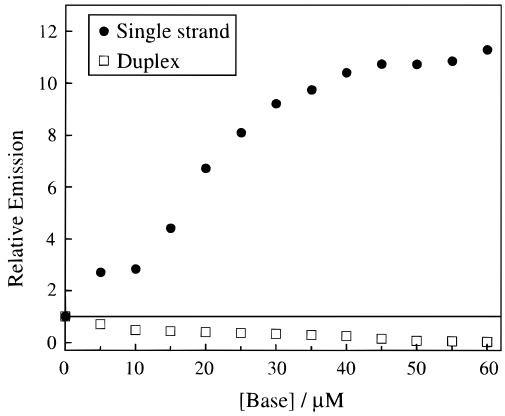

Figure 4. Relative emission of 25 μM Tb^{3+} (λ_{exc} = 260 nm, λ_{em} = 545 nm) in 50 mM NaCl, 2 mM Tris buffer, pH ~ 7.5, as a function of added single strand and duplex (±5% error in reproducibility).

Discussion

Nucleobases and Nucleotides. From the relative emission of Tb^{3+} as the concentration each base G, A, T, and C is increased (Figure 2a), it is apparent that A and T do not enhance the emission of the lanthanide. The decrease in the overall emission can be explained by an inner filter effect, since the bases absorb the excitation wavelength but do not transfer energy to Tb^{3+}. Correction for the number of photons absorbed by A and T utilizing the absorption of each sample at the excitation wavelength results in no overall enhancement of the emission.[47] Although the overall Tb^{3+} luminescence decreases as G is added,

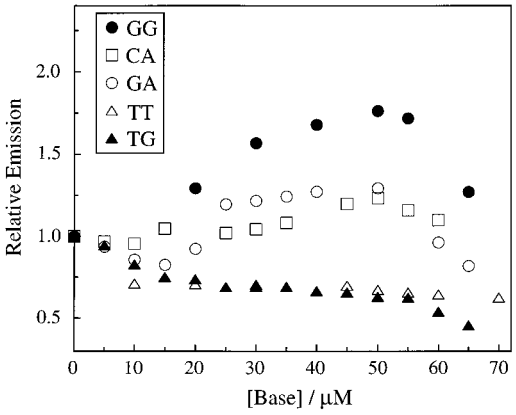

Figure 5. Relative emission of 25 μM Tb^{3+} (λ_{exc} = 260 nm, λ_{em} = 545 nm) in 50 mM NaCl, 2 mM Tris buffer, pH ~ 7.5, as a function of added duplexes possessing a single GG, CA, GA, TT, and TG mismatch (±5% error in reproducibility).

Table 1. Duplex Sequences with Appropriate Mismatch, Measured Melting Temperatures, T_m, and Emission Intensities of 25 μM Tb^{3+} with 50 μM Bases Relative to No-Mismatch Duplex

5′−CGCA**X**TTGCG−3′
3′−GCGT**Y**AACGC−5′

X	Y	mismatch	T_m, °C[a]	relative intensity
A	T	None	72	1.0
G	G	GG	66	9.6
A	G	GA	64	7.0
A	C	CA	61	6.7
C	C	CC	64	6.3
C	T	TC	58	4.7
A	A	AA	59	4.5
T	T	TT	60	3.6
G	T	GT	66	3.4

[a] ±1 °C.

(47) The absorption by the sample containing 50 μM A or T ($A \sim 0.67$ in 1-cm pathlength) at the emission excitation wavelength was used to calculate the relative number of photons absorbed by the bases, thus not available for absorption by the emissive Tb^{3+}. For the 90° geometry of the instrument, the assumption was made that the emission was collected after the incident light travelled 0.3 cm up to 0.7 cm. This leads to a relative $I/I_o \sim 0.48$.

it can be seen in Figure 2a that the intensities are greater than those observed for similar concentrations of A and T. Indeed, relative to the emission intensity of A and T, an enhancement factor of ~1.6 is calculated for [G] ≥ 40 mM. As previously reported by various authors, dGMP leads to enhancement of the Tb^{3+} emission whereas the other nucleotides do not.[45,48,49] Although C enhances the lanthanide ion emission, dCMP does not appear to enhance the luminescence to a greater extent than dAMP or dTMP.

Upon ligand excitation in the presence of Tb^{3+}, two mechanisms for the enhancement of the lanthanide emission are possible in water. Energy transfer from the excited ligand to Tb^{3+} is expected to provide the largest enhancement, although a small increase in the emission intensity can arise from the replacement water molecules from the first coordination sphere of the ion by other ligands, resulting in a decrease of the excited-state deactivation through the O−H vibrational modes of coordinated water molecules. Whereas the former is dependent on excitation wavelength (where the ligand absorbs), the latter is not. Experiments conducted with 25 mM Tb^{3+} and λ_{exc} = 488 nm, where C and G do not absorb light, showed no enhancement of the emission upon addition of up to 70 μM C or dGMP. Therefore, it may be concluded that energy transfer is the mechanism of Tb^{3+} emission enhancement in the presence of C and dGMP. This result is not surprising, since it is known that the emissive Tb^{3+} excited state is not as sensitive to water vibronic deactivation compared to that of Eu^{3+}.[50] The excitation spectra shown in Figure 3 provide conclusive evidence of energy transfer in the observed Tb^{3+} emission enhancement in the presence of dGMP. The difference in the Tb^{3+} excitation spectrum (λ_{det} = 545 nm) in the absence and presence of dGMP corresponds to the absorption of the nucleotide in the 250−300-nm range, indicative of absorption by dGMP followed by energy transfer to the emissive excited state of the lanthanide ion. Similar excitation spectra were collected in the presence of C and single-stranded 10-mer.

EnT that leads to the enhancement of the Tb^{3+} emission takes place from an excited state of the given base (donor) to the emissive $^5D_4$ state of lanthanide ion (acceptor). The efficiency of the EnT process is dictated by parameters such as the binding of the lanthanide to the base, rate of energy transfer, and quantum yield of formation of the ligand donor excited state. The Coulombic energy transfer mechanism prevails at large donor−acceptor separations, whereas at short range, spin-allowed electronic exchange becomes dominant. Although the EnT mechanism for the systems presented here has not been determined at this time, it is well-known for other chelating energy transfer donors that efficient EnT is expected only for bases that are bound to the metal ion and remain coordinated during the time of the EnT process.[10−13,51]

Although absorption of the excitation light (260 nm) by the nucleic acids results in the population of their singlet excited states, $^1\pi\pi^*$, with lifetimes in the 1−9-ps range,[52] rapid intersystem crossing to the ligand's $^3\pi\pi^*$ state is known to occur with quantum yields (Φ_{isc}) ranging from 1.27×10^{-2} (C) to 1.23×10^{-4} (T) in the absence of lanthanide.[53,54] EnT from the bases to the emissive $^5D_4$ state of Tb^{3+} is expected to take place from the ligand's lowest triplet excited state, and the ligand-centered Φ_{isc} should be larger in the presence of the heavy lanthanide ion coordinated to the base through increased spin−orbit coupling induced by Tb^{3+}.

EnT from the lowest-lying triplet excited state of aromatic ligands to Eu^{3+} and Tb^{3+} was initially advanced by Crosby and is generally accepted,[55] where for strong chelators the energy of the donor triplet state is the most important parameter for efficient EnT to take place.[24a,56−58] Recent experiments involving aromatic ligands coordinated to Tb^{3+} have shown that the quantum yield of energy transfer to the emissive $^5D_4$ state of the lanthanide ion located 20 500 cm^{-1} above the ground state can be correlated directly with the energy of the lowest $^3\pi\pi^*$ state of the ligand.[59] Luminescence quantum yields ranging from 0.01 to 0.6 were measured for those ligands whose $^3\pi\pi^*$ states were at energies between 21 500 and 27 000 cm^{-1}, whereas those with $^3\pi\pi^*$ states located below 21 000 cm^{-1} exhibited quantum yields of ≤0.001.[59] Since the $^3\pi\pi^*$ states of all the nucleic acids are in the 26 300−27 900 cm^{-1} range,[53,60−62] their energies are well suited for efficient energy transfer to the $^5D_4$ state of Tb^{3+}. Therefore, differences in the observed enhanced emission from the lanthanide ion must be due to either the quantum yield of formation of the triplet state of the donor or to differences in binding of Tb^{3+} to each base. Tb^{3+} is considered a hard acid that binds predominantly through electrostatic interactions.[63] In water, typically only oxygen-containing neutral ligands bind the ion, since nitrogen chelators cannot displace the strongly bound water molecules.[63] Strong chelation of Tb^{3+} in water is therefore better accomplished by ligands that possess two or more adjacent electron density rich regions, especially where at least one of them is an oxygen atom.[64,65] Simple inspection of the structures of the nucleic acids (Figure 1) reveals that this is only possible in C (through O2 and N3) and G (through O6 and N7). The other two bases, A and T, do not possess adjacent high electron density regions with oxygen atoms available for bonding. The differences in enhancement between C and G may be due to differences in quantum yield of triplet formation or differences in binding stability and kinetics.

Since the excited-state kinetics of the nucleotides are known to be very similar to those of the corresponding bases,[53,62] the

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observed differences in energy transfer to the lanthanide ion between dCMP and dGMP can be explained by variations in binding of the trivalent ion in the presence and absence of the anionic phosphate group. Since the energy transfer from dCMP to Tb^{3+} is significantly decreased compared to that observed in C, it is likely that competitive binding between the electron-donating groups of the base itself and the phosphate is taking place, where the equilibrium lies toward the binding to the anionic phosphate group rather than coordination to the neutral base. It appears that in dCMP the Tb^{3+} bound to the phosphate group may be too far away from the base for effective energy transfer to take place, where direct coordination to O2 and N3 of cytosine is necessary for emission enhancement. Similar results were obtained for amino carboxylates, such as EDTA, substituted with aromatic groups, where the distance between the aromatic donor and the lanthanide were too large for effective energy transfer to occur.[66]

In the cases of G and dGMP, the phosphate group appears to aid in binding of the donor and acceptor. A comparison of the enhanced luminescence among GMP with its triphosphate and diphosphate analogues, GTP and GDP, respectively, has shown that GMP is a better energy transfer donor in the order GMP > GDP > GTP.[15b] This observation was explained in terms of the distance between the Tb^{3+} bound to the phosphate groups, where the probability of binding further away from the base is greater in GTP than GDP. This explanation is consistent with the behavior observed for dCMP. The difference between dGMP and dCMP may be due to the ability of the phosphate group to fold over and interact with a Tb^{3+} ion coordinated through O6 and N7 in dGMP, whereas an analogous foldover in dCMP may not take place. Molecular models show that the foldover is possible in dGMP (coordinating to O6, N7, and one or two phosphate oxygens), but coordination of the ion to the phosphate as well as the O2 and N3 atoms of dCMP leads to a highly strained molecular geometry. The phosphate foldover has been observed for dGMP in the presence of Mg^{2+}, Cu^{2+}, and Zn^{2+}.[67] The simultaneous coordination of a single transition metal, such as Ru(II), Cu(II), and Cr(III), to both O6 and N7 and phosphate groups of dGMP was also previously reported.[68,69] In contrast, the metals were only observed to interact with the phosphate oxygens of dCMP.[68,69]

Further evidence of the foldover mechanism operative in dGMP arises from sensitization experiments conducted with the structurally related dAMP. It is likely that the two prominent reasons for the lack of Tb^{3+} emission enhancement in the presence of adenine are the inability of A to bind strongly to the lanthanide ion and the base's low quantum yield of intersystem crossing to the energy donor $^3\pi\pi^*$ state (2.3×10^{-3}).[53,54] Sensitization of the bases utilizing a triplet energy donor, such as acetone, can be utilized to obtain higher concentrations of the $^3\pi\pi^*$ excited state of nucleosides and nucleotides.[61] Addition of 5 mM acetone to solutions containing 25 μM Tb^{3+} in water does not lead to significant changes in the luminescence intensity of the lanthanide, since acetone does not coordinate the ion. A decrease in the emission intensity of the Tb^{3+}/acetone solution is observed upon addition of 50 μM A ($I/I_0 = 0.48$), owing to the inner filter effect and the lack of energy transfer. However, when the same experiment is conducted with dAMP, a slight emission enhancement is observed ($I/I_0 = 1.2$), indicating that the Tb^{3+}−dAMP adduct is more strongly bound than its adenine counterpart. Therefore, when both a high quantum yield for production of the adenine $^3\pi\pi^*$ state and stronger binding to the base aided by the phosphate group are operative, energy transfer from adenine to Tb^{3+} can take place. Conversely, no acetone-sensitized emission enhancement was observed for dCMP (25 μM Tb^{3+}, 50 μM dCMP, 5 mM acetone), possibly due to the interaction of Tb^{3+} mostly with the phosphate group rather than coordination to the base itself. The purine moieties in dAMP and dGMP possess the N7 site available for coordination in both nucleotides when the phosphate group folds over. The difference in emission enhancement between dGMP and dAMP can be ascribed to the lack of coordinating oxygen atom in adenine, thus making the binding of Tb^{3+} to dAMP weaker than to dGMP. Since excitation of acetone leads only to the sensitization of the triplet excited states of the nucleotides,[61] the enhanced energy transfer to Tb^{3+} in the presence of acetone shows the participation of the triplet state in the energy transfer process.

Single- and Double-Stranded Oligonucleotides. Single Strand and Duplex. In the presence of single-stranded oligonucleotides, the emission of Tb^{3+} is greatly enhanced (Figure 4), especially with those possessing guanines in the sequence.[70] However, 10-mer duplexes that were purified to remove any remaining single strand did not enhance the Tb^{3+} luminescence (Figure 4).[16] This result indicates that binding to the phosphate backbone, without direct coordination to the base, does not result in efficient energy transfer.[66]

Mismatched Duplexes. Enhancement of the Tb^{3+} luminescence was observed for purified duplexes that possess certain mismatched pairs of bases. As shown in Figure 5 and listed in Table 1, the greatest enhancement was observed for GG, followed by CA and GA mismatches. With the exception of the GT mismatch, the results are consistent with those obtained with nucleobases and nucleotides, where mismatches containing C and G led to the largest emission enhancement. Therefore, a large enhancement for GG and CC mismatches was expected; however, the enhanced Tb^{3+} emission of similar magnitude for CA and GA mismatches was not.

The role of adenine in Tb^{3+} emission enhancement in GA and CA mismatched duplexes is not straightforward. The relative stability of the duplexes containing a single mismatch from their measured melting temperatures, T_m, listed in Table 1 does not appear play a role in the Tb^{3+} emission enhancement, since no correlation between T_m and relative emission intensity is evident. A possible explanation is that adenine itself can transfer energy effectively to Tb^{3+} but cannot coordinate to the lanthanide ion itself, since it does not possess an electron-donating oxygen atom available for binding. Therefore, in the absence of other chromophores that keep the Tb^{3+} and adenine together, the energy transfer does not take place. The observed GA and CA enhancement may be due to the ability of the opposing G and C at the mismatch site or backbone phosphates to aid in the binding of the ion, such that in the preassembled system one of the adenine nitrogens is able to coordinate to Tb^{3+}. Once A is part of the Tb^{3+} coordination sphere, it may be able to transfer energy to the ion when excited with light. Energy transfer from noncoordinating molecules, such as benzene, has been observed in supramolecular systems where the organic molecule and the lanthanide were held in close proximity.[24,71,72] Furthermore, the

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coordination of Tb^{3+} to the electron-rich groups of the nucleoside bases in addition to the binding to the phosphate backbone units is not without precedent, since the two binding sites were reported for the ion in the presence of single-stranded regions of DNA.[73] The singlet excited-state lifetime and triplet state energy of adenine are similar to those of guanine,[52,74] therefore the rate of EnT from A to Tb^{3+} should be similar to that of G if strong binding of both bases to the lanthanide ion is operative. The fact that sensitization of dAMP utilizing acetone leads to Tb^{3+} emission enhancement is consistent with the ability of adenine to transfer energy to the lanthanide ion from its $^3\pi\pi^*$ excited state. Furthermore, the relative Tb^{3+} emission enhancements by CA is significantly greater than that of CT (Table 1), supporting the idea that adenine can transfer energy to Tb^{3+}, whereas thymine cannot.

The lowest emission enhancement relative to duplex was measured for the duplex containing a GT mismatch. Although this result is unexpected if the possibility of energy transfer from G to Tb^{3+} is considered, it can be explained by the known formation of a stable wobble pair between G and T.[75] Such pairing would preclude Tb^{3+} coordination to G in the duplex, thus making the emission enhancement appear much like that by the duplex with no mismatched bases.

In the cases where enhancement was observed, such as that for GG, GA, and CA mismatches shown in Figure 5, the Tb^{3+} emission increases with the concentration of duplex, then a decrease in the luminescence is observed upon further addition of oligonucleotide. This behavior of the raw data can be explained by the initial binding of free Tb^{3+} leading to EnT; however, once all the lanthanide ion is bound, an inner filter effect from absorption by additional nucleotides leads to an apparent decrease in the emission.

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Conclusions

The luminescence enhancement of Tb^{3+} emission was utilized to probe the interactions between nucleic acids and the ion, including the detection of single base pair mismatches in a duplex sequence. It was found that cytosine enhances the Tb^{3+} emission, but dCMP does not, indicating that the lanthanide bound to the phosphate group is too far away from the base for efficient energy transfer. However, the enhancement of the Tb^{3+} luminescence by dGMP is much greater than that of G. It is believed that the phosphate group aids in the binding of the lanthanide ion, which still coordinates to the O6 and N7 atoms of the base for efficient energy transfer to occur.

Although single-stranded oligonucleotides enhance the Tb^{3+} emission, purified duplexes do not. However, certain single mismatches in the sequence of a duplex lead to an increase in the observed Tb^{3+} luminescence intensity. The largest enhancement was observed for the GG mismatch, followed by CA, GA, and CC mismatches. Although it was expected that mismatches containing unpaired C and G bases would enhance the emission, the role of A in the luminescence enhancement was unexpected. It was concluded that A plays a role in energy transfer when Tb^{3+} is preassociated to the duplex, but it cannot itself bind the lanthanide ion. Duplexes containing a single TT or TG mismatch were found to enhance the emission of Tb^{3+} to a very small extent relative to duplex. The low emission enhancement by the TG mismatch has been explained in terms hydrogen-bonded wobble pair formation. These findings show that the enhanced emission of lanthanides can be successfully utilized to selectively detect single mismatches in duplexes.

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