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Europium Luminescence of EF-Hand Helix–Turn–Helix Chimeras: Impact of pH and DNA-Binding on Europium Coordination

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A series of Eu(III) metallopeptides, designed on the basis of the structural similarity of the helix–turn–helix and EF-hand motifs, have been studied by Eu(III) $^7F_0 \rightarrow {}^5D_0$ excitation spectroscopy. The impact of EF-hand ligand set differences on the hydration number and Eu(III) coordination environment are compared among the peptides. The conditional binding affinities were determined by Eu titration (P3, log $K_a = 6.0 \pm 0.4$; P3W, log $K_a = 5.9 \pm 0.2$; P5b, log $K_a = 5.3 \pm 0.1$). Two similar coordination environments occur in each case, consistent with structural flexibility about the metal site. The coordination environments are consistent with 8- or 9-coordinate Eu(III), including six peptide-based ligands and two to three water molecules (P3, $q = 1.9 \pm 0.2$; P3W, $q = 2.3 \pm 0.2$; P4a, $q = 1.9 \pm 0.3$; P5b, $q = 2.6 \pm 0.2$). The Eu(III) $^7F_0 \rightarrow {}^5D_0$ excitation spectra are pH-dependent, as reported for several EF-hand proteins (oncomodulin, parvalbumin). A higher energy transition occurs at pH > 6, and has been assigned to deprotonation of coordinated water. The pK_a leading to this new transition is dependent on Eu(III) Lewis acidity, which varies with the inner and outer sphere ligand set. The noncoordinating ninth position of the Eu-binding loop, which is poised to make second-sphere contacts to the coordinated water, stabilizes the deprotonated form of the coordinated solvent more effectively when it is Thr (P5b) than Asp (P3W). Upon DNA-binding by the metallopeptides, the pK_a of the pH-dependent peak increases, but no new DNA-dependent transitions are observed. This indicates no DNA-based Eu(III) ligands are introduced, such as phosphate oxygen atoms of the DNA backbone. The hydration number decreases in the presence of DNA (P3W + DNA, $q = 1.9 \pm 0.2$; P5b + DNA, $q = 1.7 \pm 0.2$), indicating that DNA-binding by the metallopeptides organizes rather than compromises the Eu-binding site within the peptide.

Introduction

Lanthanide ions have interested bioinorganic chemists for decades because of their versatility in probing calcium functional sites in vivo, as well as their application and promise as pharmaceutical agents. These ions have unique spectroscopic characteristics such as sharp luminescence emission and strong paramagnetic shift properties, and display a preference for coordination environments that parallel that of Ca^{2+} ions. Thus, lanthanides have been used extensively to study EF-hands, a common helix−loop−helix Ca-binding motif in signaling proteins.[1−3] However, unlike Ca-loaded EF-hands, we have found in earlier studies that peptides comprising lanthanide EF-hands are hydrolytically active toward phosphate cleavage near neutral pH,[4,5] and thus, we have utilized the similarity of the helix−turn−helix (HTH) DNA-binding motif and the EF-hand structure to redesign an EF-hand to be a monomeric, DNA-binding metallopeptide nuclease.[4−9]

The redesigned EF-hand motif presents the metal-binding site in a unique monomeric context,[9] which allows us to

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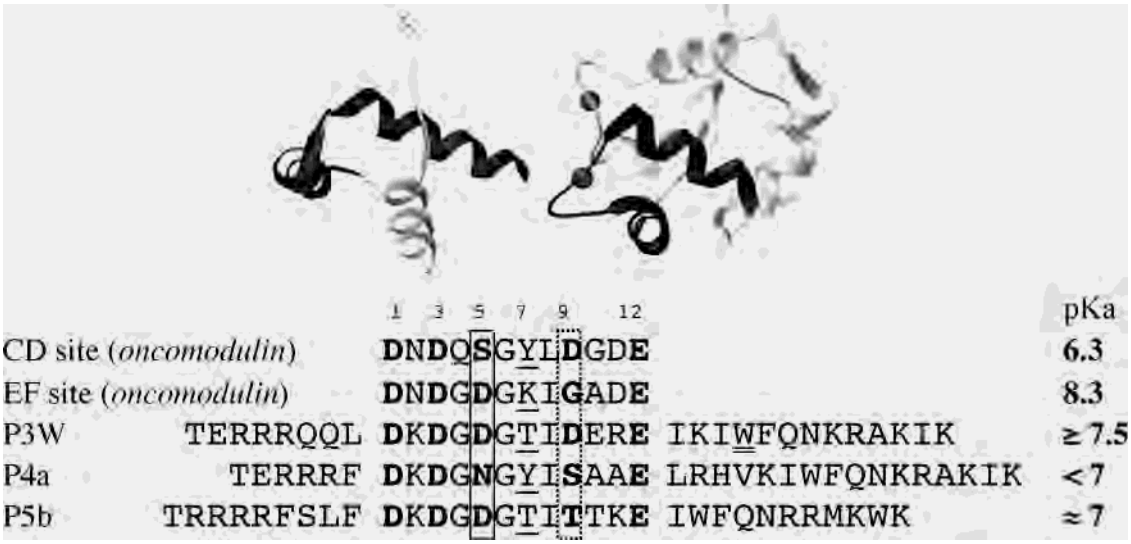

Figure 1. (Top) Ribbon representation of the engrailed homeodomain (left, from PDB coordinates 1ENG)[18] and oncomodulin (right, from PDB coordinates 1RRO).[19] The residues comprising the helix−turn−helix of the engrailed homeodomain and the EF Ca-binding site of oncomodulin are highlighted in black, to illustrate the similarities of the supersecondary turns utilized to design the metallopeptides. (Bottom) Sequences of the metal-binding loops of oncomodulin and three chimeric helix−turn−helix/EF-hand peptides. Positions 1, 3, 5, and 12 (bold) of the Ca-binding site make inner sphere contacts via side chains, position 7 (underlined) makes a carbonyl backbone contact, and position 9 (bold, dashed box) is involved in outer sphere coordination to coordinated solvent. The solid box indicates the inner sphere ligand varied in this study. P3 differs from P3W only in the C-terminal helix, with the underlined tryptophan replaced by histidine. The pK_a or pK_a estimate of the pH-dependent band in the Eu luminescence spectra is indicated.

investigate the impact of loop sequence variations and target-binding (DNA) on the metal coordination and nuclease activity. We find that these peptides not only bind trivalent lanthanide ions with affinities typical of classic EF-hand peptides, but also bind to and cleave DNA with modest sequence selectivity.[7] The NMR solution structure of one (LaP3W) shows that the metallopeptide comprises a well-organized central loop folding about the metal like its EF-hand parent.[9] The hydrolytic activity, as is typical for Lewis acid catalysts, is somewhat pH dependent, presumably due to the deprotonation of a water or hydroxyl nucleophile (pK_a of 8.2 for one such 20-mer EF-hand peptide[4]).

It has been observed that EF-hand proteins such as oncomodulin and parvalbumin exhibit differences in europium binding affinity, as well as pH-dependent Eu coordination behavior, as a function of ligand set.[10−12] This pH dependence has been assigned to the deprotonation of a hydroxyl ligand (serine or threonine), with a p$K_a \leq 8$. However, reports on a number of small-molecule Eu nucleases have shown that the deprotonation of an inner sphere water molecule may also occur in this pH range, and this deprotonation is often directly correlated to an increase in nuclease activity.[4,13,14] Therefore, understanding the coordination environment of the europium site, its pH dependence, and the influence of DNA-target-binding on these chimeras may shed light on coordination changes in native EF-hand proteins, as well as elucidate effects of the peptide ligand environment and pH on hydrolytic activity.

Europium(III) $^7F_0 \rightarrow {}^5D_0$ excitation spectroscopy is a powerful technique that allows the direct interrogation of the metal site in the context of large protein or nucleic acid biomolecules.[3,15] In addition to determining the number of binding sites in a given system, this method allows the characterization of the ligand set through a correlation of excitation energy with ligand nephelauxetic parameters.[16] Moreover, the difference in the observed excited-state lifetimes in H_2O and D_2O is linearly proportional to q, the number of coordinated inner sphere water molecules.[17]

An important premise of our chimeric design is that the replacement of the turn of the DNA-binding HTH does not appreciably alter the HTH geometry, and thus presents a well-organized structure to bind DNA. If these metallopeptides are predisposed to bind to the major groove like the parental HTH structure, the thermodynamics and coordination set of the metal ion may be affected by its interaction with nucleic acids. The present work confirms the metal ion binding affinity, establishes the ligand set, and determines the number of associated inner sphere water molecules for a series of HTH/EF-hand Eu chimeras. Additionally, the influence of the EF-hand ligand set (in particular, the noncoordinating ninth position of the consensus loop; Figure 1) on the Eu coordination environment was studied as a function of DNA-binding and pH. The effect of DNA-binding on the europium chemical environment and hydration number is indicative of whether the Eu(III)-binding site is

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maintained or compromised upon interaction with DNA. We find that the chimeric peptides bind Eu with affinities and coordination environment typical of EF-hands, and DNA-binding does not disrupt the metal-binding site.

Experimental Section

Peptide Design and Synthesis. The details of the design and synthesis of 33-mer peptides P3, P3W, and P4a have been described elsewhere.[6,9] P5b was designed in an analogous manner by structural overlays of protein coordinates, and all peptides were obtained by solid-phase peptide synthesis (New England Peptide or Caltech Peptide Synthesis facility, >95% by HPLC). Briefly, crystal coordinates were obtained from the Protein Data Bank, and the HTH and EF-hand motifs manually overlayed using the freeware program Swiss PDBviewer. The coordinates for calmodulin (1OSA)[20] were aligned with those of the engrailed homeodomain (1ENH)[18] for P3, P3W, and P4a, and antennapedia homeodomain (1AHD; NMR structure)[21] for P5b.

Materials and Methods. Europium stock solutions were made fresh by weight from $EuCl_3 \cdot 6H_2O$ (99.8%, Aldrich), and standardized using an Arsenazo III chelometric technique.[22] Peptide concentrations were determined by UV spectroscopy, monitoring the absorbance at 280 nm (P3W, $\epsilon = 7289\ M^{-1}\ cm^{-1}$; P3, $\epsilon = 254\ M^{-1}\ cm^{-1}$; P4a, $\epsilon = 7739\ M^{-1}\ cm^{-1}$; P5b, $\epsilon = 12700\ M^{-1}\ cm^{-1}$). Samples were buffered in 50 mM HEPES and adjusted to the desired pH (or uncorrected pD in D_2O) with concentrated NaOH or HCl. Excitation spectra were collected as 1:1 metallopeptide adducts at 60 μM for P3W and P5b and 33 μM for P4a and P3. For DNA-binding experiments, a 14-mer self-complementary oligonucleotide (5′-GAGCTAATTAGCTC-3′) was added from concentrated stock to form a 1:1:1 (Eu/peptide/DNA duplex) ternary complex. Under these conditions, the 14-mer is double-stranded as determined by thermal denaturation studies, and CD titrations indicate that the similar metallopeptide GdP3W binds 1 equiv of this duplex DNA sequence (Supporting Information). Solutions were allowed to equilibrate 5 min prior to data collection.

Eu(III) excitation spectra and excitation lifetimes were obtained with a Continuum YG-581 C pulsed Nd:YAG laser-pumped tunable TDL 50 dye laser as an excitation source. The $^7F_0 \rightarrow {}^5D_0$ transition was accessed by a mixture of Rhodamine 590 and 610 dyes while the $^7F_0 \rightarrow {}^5D_2$ emission at 612 nm was monitored. The $^7F_0 \rightarrow {}^5D_0$ transition of the Eu(III) ion was excited with laser light at 578−581 nm (pulse width 7 ns; resolution 0.01 nm). The laser operates at 10 Hz, with a power level of approximately 40 mJ/pulse. A further description of the laser setup can be found elsewhere.[23]

The program PeakFit (Jandel Scientific) was used for spectral peak fitting and luminescence decay analysis. PeakFit uses a Marquardt algorithm based nonlinear regression method to analyze data. Excitation spectra were deconvoluted into peaks described by a Lorenztian−Gaussian product function, as detailed previously.[24] Decays were resolved into their component exponentials, providing both amplitudes as well as lifetimes. For lifetime measurements, decays were collected for 4.0 min at a single wavelength (λ_{max}), for equivalent samples in H_2O and D_2O. Binding isotherms were obtained by plotting the total luminescence intensity measured at a particular wavelength from the excitation spectra as a function of Eu(III) concentration, and fitting with a nonlinear least-squares algorithm to a 1:1 association model using a nonlinear least-squares fit.

Results and Discussion

Europium Coordination Environment. A series of chimeric peptides comprising helices from DNA-binding homeodomain proteins (engrailed, antennapedia) have been synthesized and characterized, as described previously (Figure 1).[5,6,9] These peptides are based on the structural similarity of the HTH and EF-hand motifs, resulting in a chimeric metallopeptide that binds both metals (calcium and lanthanides) and DNA.[6,7] Each 33- or 34-mer peptide has a single EF-hand Ca-binding site (Figure 1; center, numbered), flanked by sequences derived from DNA-binding proteins (P3, P3W, and P4a from the engrailed homeodomain; P5b from antennapedia). These chimeric peptides were studied by Eu luminescence spectroscopy to determine the Eu(III) binding affinity and coordination environment, including waters, of the Eu(III) ion within the designed peptides.

The $^7F_0 \rightarrow {}^5D_0$ excitation spectrum of each Eu peptide at pH 7.0 is presented in Figure 2. In all four cases, the metallopeptides have spectra that include two peaks centered at approximately 579.2−579.4 nm (a) and 579.6−579.8 nm (b). Additionally, a higher energy, pH-dependent transition is observed at approximately 578.7−579.0 nm (c) for P5b and P4a, and is discussed in detail in later sections (*vide infra*). The relatively small difference in energy between the two lower energy transitions observed for all four peptides (a, b) is consistent with a pair of isomeric metal environments with an equivalent set of ligands, organized in a slightly different symmetry about the metal. The excited-state lifetimes for these peaks are nearly identical, indicating similar coordination environments, or an averaged value between them (Supporting Information table). The amplitudes of these two peaks occur in different ratios for each metallopeptide, but the energies are typical for a traditional EF-hand ligand set (six oxygen donor atoms from the EF-hand loop, plus water molecules), on the basis of the correlation between transition energy and nephelauxetic parameters for the ligating atoms.[16] As such, it is likely that the energy barrier between these structural "isomers" is low, perhaps representing a pseudorotation between two similar environments within a flexible site.

It might be expected that only a single transition be observed, deriving from a unique europium environment in the peptide. However, the observation of two closely spaced transitions for a given binding site is quite common for EF-hand proteins as well as small-molecule Eu complexes. Henzl and co-workers have previously reported that Eu-loaded oncomodulin exhibits two excitation peaks each for both the EF and CD binding sites.[10−12] Moreover, pairs of isomeric peaks have been reported for various small-molecule com-

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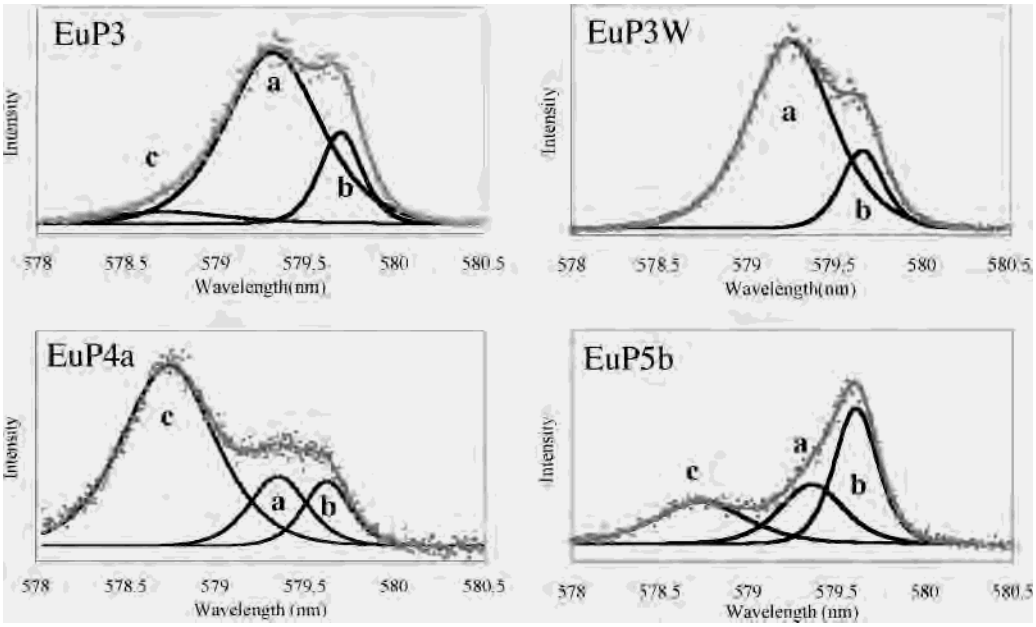

Figure 2. Eu $^7F_0 \rightarrow {}^5D_0$ excitation spectra ($\lambda_{em} = 612$ nm) of Eu HTH/EF-hand chimeras at pH 7.0 in 50 mM HEPES buffer. The raw data and fit (gray) overlay the deconvoluted peaks (a−c).

plexes of DOTA and DTPA derivatives, for which the number of coordinated water molecules and nature of the donor set are well-established.[25−27]

An alternative explanation for the ∼579.3 and ∼579.7 nm peak pair (a, b) is a monomer−dimer equilibrium, not uncommon for isolated EF-hand peptides in solution.[28−31] However, the intensity change of the transitions as a function of metal shows a clear break at 1:1 stoichiometry, and both isomer peaks increase equivalently as a function of metal. This is incompatible with a dimerization equilibrium, unless the binding affinity of the metal site is equivalent in the monomer and dimer, or in rapid exchange, which remains a possibility. However, it is notable that the NOE-restraint-based NMR solution structure of LaP3W, as well as static light scattering experiments,[9] suggests that the Ln(III)P3W metallopeptides are monomeric species in solution. Previous fluorescence and circular dichroism studies show no evidence for more than 1:1 Ln/peptide association at these concentrations for the other sequences as well, although P3 appears to dimerize at concentrations above 200 μM.[6] These data suggest that the chimeras are monomeric species under the conditions used for these luminescence studies.

The binding affinity of P3, P3W, and P5b for Eu(III) was determined by titrating peptide (33−60 μM) and $EuCl_3$ and following the increase in luminescence intensity at λ_{max}. As shown in Table 1, these three peptides have conditional metal binding affinities less than 10 μM, in good agreement with previously measured affinities of lanthanide P3W complexes.[6−8] Thus, although P5b is not structurally well-organized in the putative helical regions, P5b binds Eu(III) with affinity typical of isolated EF-hand peptides and other HTH/EF-hand chimeras.

Table 1. Lifetime Values, Hydration Number, and Binding Affinities for Eu Peptide Chimeras (Peaks a and b) in the Presence and Absence of DNA (pH 7.0, 50 mM HEPES)

peptide	τ_{H_2O} (ms)	τ_{D_2O} (ms)	$q \pm 0.2$	log K_a
P3	0.41	2.40	1.9	6.0 ± 0.4
P4a	0.38	1.65[a]	1.9[a]	*b*
P3W	0.35	2.11	2.3	5.9 ± 0.2
P5b	0.32	2.04	2.6	5.3 ± 0.1
P3W + DNA[c]	0.41	2.49	1.9	*b*
P5b + DNA[d]	0.41	1.64	1.7	*b*

[a] Corrected for 85% D_2O solvent; $q \pm 0.3$. All other samples measured in >99% D_2O. [b] Not measured. [c] pH 8.0. [d] pH 5.0.

The number of coordinated inner sphere water molecules was determined by comparing luminescence emission lifetimes in H_2O and D_2O (Table 1).[17] Using the previously developed relationship $q = 1.11[\tau^{-1}_{H2O} - \tau^{-1}_{D2O} - 0.31]$, the hydration numbers of the complexes were determined for the chimeric peptides (Table 1).[32] A hydration number of 2.0−2.3 agrees quite well with the predicted 8-coordinate EF-hand geometry of our design for the reasonably well-folded EuP3 and EuP3W peptides. EuP5b, in contrast, has little secondary structure except in the presence of trifluoroethanol, as determined by circular dichroism (unpublished data). The fact that EuP5b is less well-folded than EuP3 or EuP3W likely correlates with increased dynamic motion and solvent accessibility at the europium center, which is in turn

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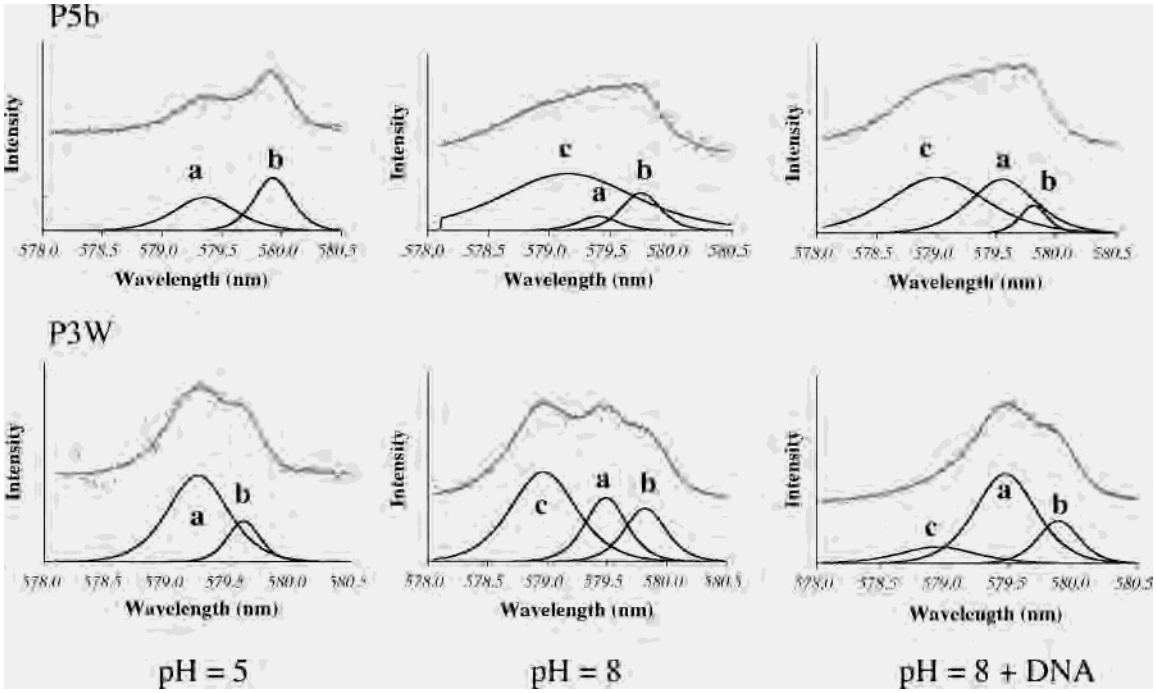

Figure 3. Eu $^7F_0 \rightarrow {}^5D_0$ excitation spectra ($\lambda_{em} = 612$ nm) of EuP3W and EuP5b chimeras at pH 5.0 (left), pH 8.0 (center), and pH 8.0 in the presence of a self-complementary 14-mer DNA duplex (50 mM HEPES buffer) (right). The raw data and fit (gray) overlay the deconvoluted peaks (a−c).

reflected in the average higher number of inner sphere water molecules (between two and three). The increased hydration number may also correspond to a higher total coordination number of nine rather than eight donor atoms.

Impact of DNA-Binding on the Eu(III) Environment. The intriguing question of how the metal-binding site changes upon DNA-binding was addressed for metallopeptides EuP3W (well-folded in the absence of DNA) and for EuP5b (metal-binding, but poorly folded in the adjacent helical regions). These differences in protein folding stability are likely due to choices in the sequences flanking the 12-amino acid EF-hand metal-binding loop. To test the effect of DNA-binding on the metal environment, a 1:1 mixture of peptide (P3W or P5b) and duplex DNA was titrated with $EuCl_3$. The self-complementary 14-mer oligonucleotide 5′-GAGC*TAATTA*GCTC-3′ includes the engrailed binding site (italic), although this site is not necessarily the highest affinity target for these peptides (unpublished data). In agarose gel shift assays with random sequence DNA, we have found that the apopeptides have nonspecific DNA affinity very similar to that of the metallopeptides (for peptide P5b, this affinity is $K_d \approx 8\ \mu$M, and for P3 and P3W, $K_d \approx 15\ \mu$M).[6,7] Circular dichroism studies (EuP5b; to be published) and magnetic resonance relaxivity measurements (GdP3W)[33] confirm that the lanthanide peptides bind this 14-mer oligomer sequence with at least comparable affinity, and can be reversibly "salted off" with NaCl[33] (see also the Supporting Information).

It is notable that no new DNA-dependent transitions occur upon titration with DNA (within the resolution of these spectra). This indicates that there is no change in the Eu coordination sphere, such as direct bonds to phosphate oxygens of the DNA backbone (Figure 3). However, although no new species are formed, some important changes are observed upon DNA-binding. The two lower energy peaks present in both EuP3W and EuP5b spectra (a, b) occur in somewhat different intensity ratios, with DNA-binding tending to favor the higher energy species of the pair (a) at both pH 5 and pH 8. Additionally, DNA-binding has a dramatic effect on the intensity of the pH-dependent peak (c). In both peptides, the population of this species decreases as DNA is added (Figure 3). This is particularly obvious for EuP3W, for which the higher energy species is nearly abrogated upon DNA-binding even at pH 8.0, suggesting a significant increase in pK_a for this deprotonation event in the presence of the DNA polyanion. The changes in luminescence spectra are reversible upon the addition of $\geq$100 mM NaCl, as expected for electrostatic peptide−DNA interactions (Supporting Information).

The hydration number of Eu remains constant for EuP3W in the presence and absence of DNA ($q \approx 2$ in both cases), but for the more flexible and poorly folded EuP5b, the hydration number diminishes from $q \approx 3$ to $q \approx 2$ upon DNA addition, with an accompanying increase in the signal-to-noise ratio.[34] These changes in the coordination sphere result in a EuP5b metal environment more similar to that of the well-folded EuP3W system. Thus, DNA-binding does not compromise the ability of the peptides to bind metals, and in fact apparently serves to organize the Eu-binding site into a structure more like the classic EF-hand of LnP3W, presumably also enhancing the overall integrity of the supersecondary fold.

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pH Dependence of the Europium Environment. The spectra collected at pH 7 allowed us to determine the number of inner sphere water molecules and the binding affinities of the HTH/EF-hand peptides for Eu(III). In addition, a striking difference in the Eu environment among the peptides is observed. In some cases (EuP5b, EuP4a) but not in others (EuP3, EuP3W), a strong third peak is observed at higher energy (c; 578.9 nm), in addition to the two nearly identical peaks typical of Ca-binding proteins (Figure 2).[35]

The intensity of this higher energy peak is pH dependent, as shown in Figure 3 for EuP3W and EuP5b. The high-energy species is not present in either Eu chimera spectrum at pH 5 (Figure 3, left), but as the pH is raised to 7 (Figure 2) and then to 8 (Figure 3, center), this peak occurs earlier in the titration and is more intense for EuP5b than EuP3W. This suggests that the deprotonation event causing the change in the Eu environment occurs with a lower pK_a for EuP5b (p$K_a \approx 7$) than for EuP3W (p$K_a = 7.5-8$).[36]

The behavior of this peak is similar to transitions observed for small-molecule Eu complexes[14] and EF-hand proteins such as oncomodulin and parvalbumin.[10−12,37] Henzl and co-workers have shown that this pH-dependent peak in the Eu luminescence spectrum occurs for some EF-hand Ca-binding sites and not for others, suggesting a difference in pK_a of the deprotonation event. For example, oncomodulin includes two different EF-hand sites (CD and EF) with slightly different sequences, and deprotonation is observed at pH 6.3 for the CD site, but at pH 8.3 for the EF site. As shown in Figure 1, the difference in sequence between the two binding sites is at positions 5 and 9 of the loop. For EF-hand motifs, these residues correspond to an inner sphere and outer sphere ligand, respectively, with the ninth position generally involved in hydrogen bonding to coordinated solvent.[38−41] The observed pK_a decrease between the EF and CD sites correlates with changing a negative carboxylate ligand (Asp) to a neutral alcoholic ligand (Ser) at position 5, along with adding a second-sphere ligand at position 9 (no side chain is available to hydrogen-bond to solvent in the oncomodulin EF site, as the ninth position is Gly). Additionally, point mutation studies exchanging Asp and Ser at position 5 were able to interconvert the pH dependence of the Eu spectra of these two sites.

In the case of the chimeras, the apparent pK_a is correlated with the second-shell ligands at position 9, as the ligand sets of P3W/P3 and P5b differ only at that noncoordinating position. This suggests that the deprotonation we observe is of the coordinated water, rather than a peptide side chain ligand as implicated in the oncomodulin study. It is unlikely that the deprotonation of the Thr alcohol side chain in the second coordination shell would itself cause such a dramatic change at the metal center. This dependence of the pK_a of an inner sphere water molecule on the hydrogen-bond donor and acceptor groups adjacent to the site is well-known in other systems, such as carbonic anhydrase.[42] Changes that increase negative charge in the second shell of this enzyme's active site contribute significantly to increases in $Zn-OH_2$ pK_a (up to 1 pK_a unit). The pK_a of H_2O bound to the zinc active site of carbonic anhydrase is 6.8 when the native Thr is adjacent to the site, but increases to 8.3 when this residue is replaced by Ala, which has no hydrogen-bonding capability.

Further support for the assignment of the Eu chimera pK_a to the deprotonation of coordinated water comes from the observation that EuP4a has a pK_a apparently lower than 7, with both an alcoholic second-shell ligand (position 9) and a neutral inner sphere ligand (position 5). Thus, the loss of a negatively charged direct ligand, which increases the Lewis acidity, and the stabilization of the deprotonated water molecule by the alcohol both serve to lower the pK_a of the water. Additionally, EuP3W does exhibit the higher energy transition (c) at pH 8, despite having no direct peptide ligands to deprotonate. As would be predicted from this assignment, EuP4a is the most active nuclease of the Eu chimeras, since the phosphate ester hydrolysis rate is correlated with activated (deprotonated) water.[5]

Upon DNA-binding, the deprotonation is disfavored, with the pH-dependent peak decreasing in magnitude for EuP5b, and nearly disappearing entirely for EuP3W (pH 8; Figure 3, left). An adjacent polyanion would be expected generally to disfavor the loss of a positive proton, and thus raise the pK_a as the chimera associates with its target. An additional effect of DNA-binding appears to be in organizing and constraining the metal site, as implied by hydration number changes (Table 1), and by CD for both systems (to be published). Therefore, it is possible that DNA-binding forces the second-shell Asp_{17} of P3W (position 9) to be directed "inside" toward the water molecule, rather than flexibly sampling an "outside" position as well. The flexible, outside position was shown by NMR of LaP3W to be the favored structure in solution.[9] Thus, similar to EF-hand proteins where dimerization of EF-hand domains rigidifies the extended loop structure, the negative carboxylate may be more rigidly positioned inside to stabilize the protonated water molecule when the Eu chimera binds to DNA.

The outside position of Asp_{17} in EuP3W provides no stabilization of the inner sphere water molecule, analogous to having no side chain at that position. It is notable that point mutations to the CD site of oncomodulin that convert both sites to the EF site ligand set (Asp at position 5, and Gly at position 9) resulted in a Eu luminescence spectrum that had a small population of the pH-dependent band at pH 8 (although it was attributed to an impurity), which is similar

(35) The 578.9 nm peak is similar in energy to that of the europium aquo complex, but the intensity of the latter is below detection at these concentrations.

(36) A full pH titration study is necessary to determine the explicit pK_a of this deprotonation, and these studies will be reported elsewhere.

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to that of EuP3W.[12] However, point mutations to give Asp in both inner and outer sphere sites (positions 5 and 9, respectively) resulted in no observed higher energy band ($pK_a \geq 8$), analogous to the behavior observed for EuP3W when organized and bound to DNA.

The lifetimes measured at the wavelength of the pH-dependent peak (c) in D_2O and H_2O for EuP3W + DNA (Table 1) correlate with a hydration number of $q = 1.8$, not appreciably lower than 1.9 observed for the a, b peak pair. If the observed pK_a is due to the deprotonation of coordinated solvent, the loss of one OH oscillator would be expected to give a calculated hydration number of 1.5. However, the H^+ exchange rate is almost certainly fast, especially with two inner sphere solvent molecules.[43] The lifetime measurements were made at pH values relatively near the pK_a. Thus, the exchange between protonated and deprotonated solvent, expected to be fast on the time scale of the lifetime measurements, results in an averaged value higher than expected. Additionally, the deprotonation may influence the number of closely diffusing second-sphere molecules (affecting the correction factor in the hydration equations[17]), as described for macrocyclic Eu complexes.[14]

Conclusions

The redesign of a DNA-binding motif by incorporating a structurally equivalent metal-binding turn has been utilized to build a peptide-based artificial nuclease. The success of this modular structural approach to design depends on each of the two functions (DNA-binding and metal-binding) not compromising the other. Therefore, it is important to understand the coordination environment of the metal in detail, and how the lanthanide-binding properties of the chimeras are affected by interaction with duplex DNA.

The interrogation of the Eu center by luminescence spectroscopy allowed us to investigate the nature of the Eu coordination environment in our designed chimeras. We find the Eu peptide HTH chimera behave as "normal" EF-hand sites, despite drastic charge and sequence changes flanking the metal-binding loop. The sites are somewhat flexible, with two similar coordination environments consistent with 8 (or in some cases up to 9) coordinate Eu(III), including six peptide-based ligand atoms and two to three water molecules coordinated in the inner sphere. Significantly, DNA-binding organizes rather than disrupts the Eu-binding site, as evidenced by a hydration number of $q = 2$ upon DNA-binding. This is an important goal of the design. There is no evidence for the introduction of new Eu(III) ligands upon metallopeptide/DNA-binding, such as coordination by DNA phosphate backbone oxygen atoms. The Eu luminescence excitation spectra are pH-dependent, with a new peak observed at higher pH values ($pK_a \geq 6$). This higher energy peak is most consistent with deprotonation of an inner sphere water, rather than an amino acid side chain, and correlates well with the relative nuclease activity of the designed metallopeptides.

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Supporting Information Available: Circular dichroism titration study of GdP3W-binding to DNA, table of excitation peak wavelength maxima and lifetimes, and Eu luminescence spectra of EuP3W + DNA as a function of added NaCl. This material is available free of charge via the Internet at http://pubs.acs.org.

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