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Phosphorylation State-Responsive Lanthanide Peptide Conjugates: A Luminescence Switch Based on Reversible Complex Reorganization

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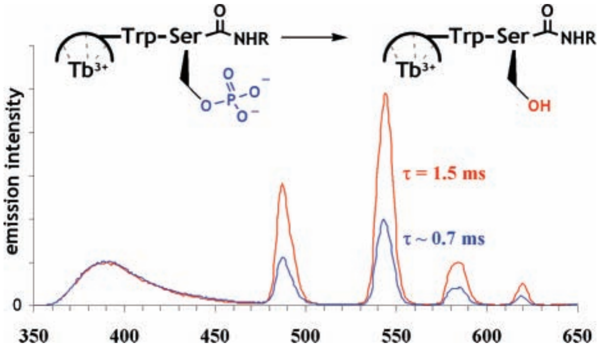
ABSTRACT



A luminogenic probe for peptide dephosphorylation has been developed. It consists of a serine-/tyrosine-containing peptide modified on the N-terminus with a tryptophan residue and a DTPA chelate capable of binding Tb^{3+}. We propose a mechanistic model for the luminescence enhancement based on the interconversion of monomeric and dimeric lanthanide species, which is affected by the phosphorylation state of the serine or tyrosine residue. The optical switch reports effectively on phosphatase-catalyzed dephosphorylation in vitro.

Probe molecules that relay information about chemical transformations via an optical response enable monitoring and imaging of these processes in complex systems, such as live cells.[1] A significant challenge in probing biological systems is the presence of native chromophores that emit UV/visible light on the nanosecond time scale ("cellular autofluorescence"). This is typically overcome by using fluorescent probes that emit at longer wavelengths than the native chromophores.[1] However, an additional degree of resolution can be achieved by transposing the signal output to the millisecond time domain using sensitized lanthanide luminescence.[2] In this context, lanthanide luminescence has been used for studying the structure[3] and metal-binding affinity of proteins[4] as well as for sensing biologically relevant analytes[5] and redox poise.[6]

Phosphorylation of proteins and peptides stands out among posttranslational modifications as the predominant mechanism of cellular signaling.[7] Consequently, these processes have been targets of optical probing strategies. Although significant advances have recently been reported,[8] robust

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[1] Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 10th ed.; Molecular Probes: Eugene, OR, 2005.

[2] Parker, D.; Dickins, R. S.; Puschmann, H.; Crossland, C.; Howard, J. A. K. *Chem. Rev.* **2002**, *102*, 1977−2010.

[3] (a) Nitz, M.; Sherawat, M.; Franz, K. J.; Peisach, E.; Allen, K. N.; Imperiali, B. *Angew. Chem., Int. Ed.* **2004**, *43*, 3682−3685. (b) Horrocks, W. D., Jr.; Sudnick, D. R. *Acc. Chem. Res.* **1981**, *14*, 384−392.

[4] (a) Franz, K. J.; Liu, L. L. *J. Am. Chem. Soc.* **2005**, *127*, 9662−9663. (b) MacManus, J. P.; Hogue, C. W.; Marsden, B. J.; Sikorska, M.; Szabo, A. G. *J. Biol. Chem.* **1990**, *265*, 10358−10366.

[5] Gunnlaugsson, T.; Leonard, J. P. *Chem. Commun.* **2005**, 3114−3131.

[6] Lee, K.; Dzubeck, V.; Latshaw, L.; Schneider, J. P. *J. Am. Chem. Soc.* **2004**, *126*, 13616−13617.

[7] Ahn, N. *Chem. Rev.* **2001**, *101*, 2207−2208.

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optical switches for phosphorylated/dephosphorylated peptide pairs have been difficult to design owing to their relatively subtle structural differences.[9]

As part of a broad program aimed at sensing chemical/enzymatic transformations via optical probes,[10] we became interested in developing an optical switch based on the phosphorylated state of a peptide that would employ lanthanide luminescence and that would potentially be able to report on phosphorylation/dephosphorylation processes. We synthesized ligands **1** and ***p*1**, which contain a diethylenetriaminepentaacetic acid (DTPA) ligand, a tryptophan residue capable of sensitizing Tb^{3+} luminescence,[4b] and serine and phosphoserine residues, respectively (Figure 1a). We investigated their optical properties and found that in the presence of Tb^{3+} there was a significant difference in luminescence intensity (Figure 1b). We were surprised to see that ***p*1** was less intense than **1** because the initial design was based on the premise that the coordination of the phosphoserine of ***p*1** to Tb^{3+} should provide enhanced luminescence. We further investigated this behavior by titrating **1** and ***p*1** with Tb^{3+} (Figure 1c). Interestingly, these experiments showed that for both **1** and ***p*1** luminescence maximized after the addition of ~0.5 equiv of Tb^{3+}, suggesting the formation of a ternary (or higher order) adduct rather than the expected 1:1 Tb/DTPA−peptide complex.[11] The decrease in luminescence intensity after this maximum was more pronounced for ***p*1** than for **1**, such that in the presence of 1−2 equiv of Tb^{3+} the two compounds were easily differentiated.

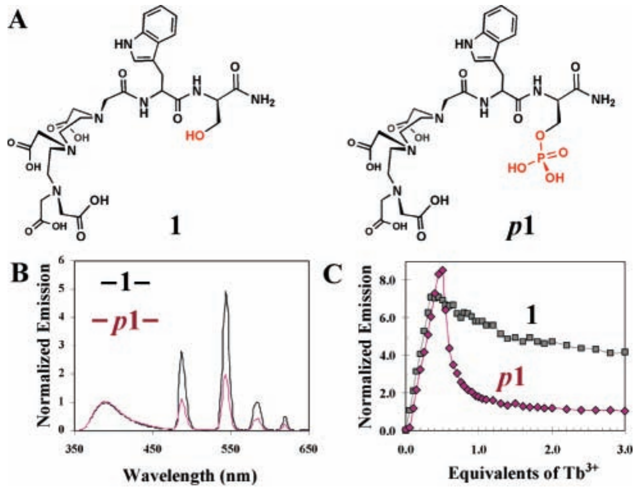

Figure 1. (a) Structures of **1** and ***p*1**. (b) Steady-state luminescence spectra of **1** and ***p*1** in the presence of 1 equiv of $TbCl_3$. (c) Titrations of **1** (gray squares) and ***p*1** (purple diamonds) with $TbCl_3$ (emission monitored at 545 nm). Spectra are normalized to emission at 390 nm, which remains constant after ~1 equiv.

Time-resolved emission experiments were carried out on selected titration points (Figure 2). Luminescence decay curves of ***p*1** in the presence of 0.3 or 0.5 equiv of Tb^{3+} were monoexponential, indicating the presence of a single luminescent species with a lifetime of about 1.7 ms. At higher Tb^{3+} concentrations (0.8, 1.0, 3.0 equiv), the luminescence is markedly shorter lived, and the decay curves are no longer monoexponential, indicating the presence of multiple luminescent species. By contrast, the luminescence decay curves of **1** were observed to be monoexponential at all Tb^{3+} concentrations, although the lifetimes decreased with increasing Tb^{3+} concentration.[16]

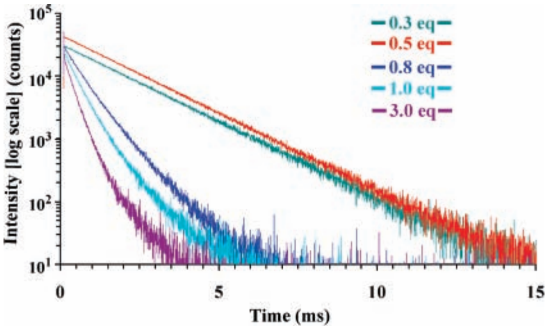

Figure 2. Luminescence decay curves for ***p*1** in H_2O (100 μM) with 0.3 (teal), 0.5 (red), 0.8 (blue), 1.0 (cyan), and 3.0 (purple) equiv of $TbCl_3$.

Guided by both the steady-state and the time-resolved emission measurements, we propose the following model for the observed luminescence switch **1**/***p*1**. Each peptide probe (**1** and ***p*1**) exists in solution containing Tb^{3+} (≥1 equiv) as an equilibrium of two species, the expected monomeric and the dimeric complex (Figure 3).[12] The dimeric species (for both **1** and ***p*1**) is more luminescent than the corresponding monomer because it is likely that fewer quenching water molecules are bound to the metal center. The position of the equilibrium between these two species is affected by the phosphate group. Specifically, in the presence of the phosphate moiety, the equilibrium is shifted in favor of the monomeric ***p*1/Tb** complex, presumably due to intramo-

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(8) (a) Chen, C.-A.; Yeh, R.-H.; Lawrence, D. S. *J. Am. Chem. Soc.* **2002**, *124*, 3840−3841. (b) Chen, C.-A.; Yeh, R.-H.; Yan, X.; Lawrence, D. S. *Biochim. Biophys. Acta* **2004**, *1697*, 39−51. (c) Shults, M. D.; Imperiali, B. *J. Am. Chem. Soc.* **2003**, *125*, 14248−14249. (d) Rothman, D. M.; Shults, M. D.; Imperiali, B. *TRENDS Cell Biol.* **2005**, *15*, 502−509.

(9) (a) Bowman, B. F.; Peterson, J. A.; Stull, J. T. *J. Biol. Chem.* **1992**, *267*, 5346−5354. (b) Post, P. L.; Trybus, K. M.; Taylor, D. L. *J. Biol. Chem.* **1994**, *269*, 12880−12887. (c) Higashi, H.; Sato, K.; Ohtake, A.; Omori, A.; Yoshida, S.; Kudo, Y. *FEBS Lett.* **1997**, *414*, 55−60. (d) Ting, A. Y.; Kain, K. H.; Klemke, R. L.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 15003−15008.

(10) (a) Yee, D. Y.; Balsanek, V.; Sames, D. *J. Am. Chem. Soc.* **2004**, *126*, 2282−2283. (b) Chen, G.; Yee, D. Y.; Gubernator, N. G.; Sames, D. *J. Am. Chem. Soc.* **2005**, *127*, 4544−4545. (c) Tremblay, M. S.; Sames, D. *Org. Lett.* **2005**, *7*, 2417−2420. (d) Froemming, M. K.; Sames, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 637−642.

(11) (a) Martell, A. D.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1989. (b) Anderegg, G.; Arnaud-Neu, F.; Delgado, R.; Felcman, J.; Popov, K. *Pure Appl. Chem.* **2005**, *77*, 1445−1495.

(12) Although higher-order adducts may be present in solution, the simplest ligand/metal stoichiometry that is consistent with the titration data is 2:1. Mass spectrometry in various ionization modes was unsuccessful in confirming the identity of the oligomeric species, although the monomeric ***p*1** was readily observed; see Supporting Information for additional details.

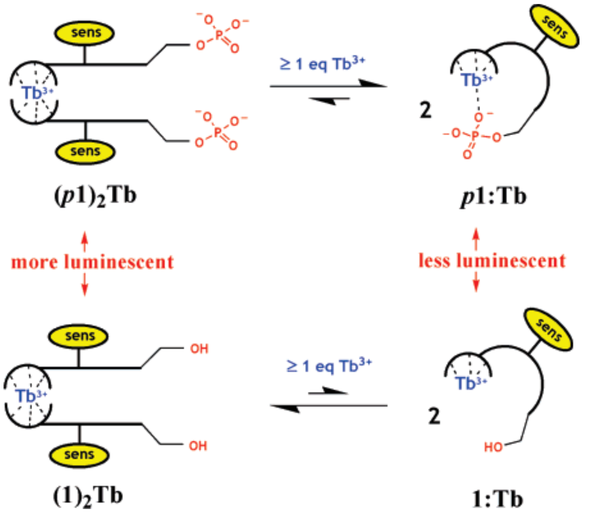

Figure 3. Proposed model for the observed luminescence switch **1**/***p*1** based on reversible complex reorganization. The presence of phosphate shifts the equilibrium toward the less-luminescent monomeric ***p*1**:**Tb** species, presumably because of intramolecular interaction between the phosphate and the metal.

lecular interaction between the phosphate and the metal.[13] It should be noted that it is likely that this behavior is the observed sum of a large number of interactions resulting from a variety of microenvironments fostered by the presence of the phosphate group. In contrast, in the absence of the phosphate, the equilibrium favors the dimeric complex.

Thus, the luminescence increase produced by dephosphorylation is a result of converting one equilibrium, which favors the monomer, to another equilibrium, which favors the more luminescent dimer. Although interconversion of mono- and bis-ligated lanthanide species is known to occur with tripodal aminomethylene tris(aryl) ligands,[14] there are no reports of such behavior with polyaminocarboxylates such as DTPA.[15]

Further support for the dimerization of these peptide probes was provided by monitoring the Tb^{3+} titration of ***p*1** by ^{31}P NMR.[16] The size of the peak at 0.5 ppm corresponding to the phosphoserine residue decreases linearly as Tb^{3+} is added and is completely consumed after the addition of 0.5−0.6 equiv. The disappearance of the peak is consistent with the incorporation of the phosphopeptide into a Tb^{3+} complex, which is known to cause extreme peak broadening.[4a] The same trend was observed for a phosphotyrosine analogue of ***p*1**. Unfortunately, no new phosphorus signal was observed after the disappearance of the free ligand peak (>0.5 equiv of Tb^{3+}), which prevented us from obtaining direct evidence for the proposed phosphoserine coordination to Tb^{3+} in the monomeric species.[17]

According to this mechanistic hypothesis, analogues of peptides **1**/***p*1** containing other substituents capable of chelating the oxophilic, cationic metal center should also show a similar luminescence switching. This prediction was confirmed by the following examples (Figure 4). The tyrosine/phosphotyrosine peptide pair exhibits luminescence properties very similar to those for **1**/***p*1**. Also, peptides containing a carboxylate on the residue adjacent to the DTPA-Trp fragment showed less intense Tb^{3+} luminescence than those containing neutral functionalities at this position; for example, DTPA-TrpAspNH$_2$ and DTPA-TrpAlaNH$_2$ constitute an optical switch as well. Furthermore, the phospho-/dephosphopeptide pair DTPA-Trp(*p*)SerPheArg$_4$NH$_2$ exhibited switching behavior nearly identical to the parent pair **1**/***p*1**, despite the presence of four cationic arginine residues, suggesting that interaction between the peptide chains is not a contributing factor (Figure 4).

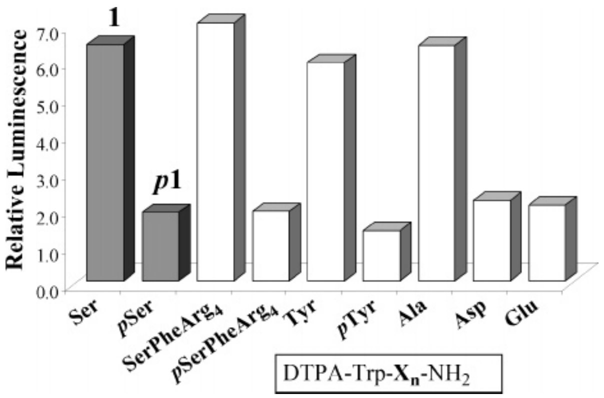

Figure 4. Tb/Trp emission ratios (545/390 nm) for analogous DTPA peptide conjugates (50 μM in 100 μM TbCl$_3$, 10 mM HEPES, 100 mM NaCl, pH 7.4, 25 °C). Peptides with anionic residues in the X$_n$ position show decreased luminescence, supporting the intramolecular interaction rationale.

To assess the ability of this optical switch to serve as a luminogenic probe of an enzymatic transformation, ***p*1** was subjected to the action of alkaline phosphatase, a promiscuous enzyme capable of hydrolyzing phosphopeptides. Indeed, at 37 °C in pH 7.4 HEPES buffer, the expected luminescence increase was observed and confirmed to correlate with conversion of ***p*1** to **1** by mass spectrometry.[16] As a result, these probes allow for continuous (real-time) monitoring of peptide dephosphorylation in vitro. According to the mechanistic rationale discussed above, phosphatase converts one equilibrium to another, increasing the relative concentration of the more luminescent dimer complex (Figure 5).

In summary, we have presented a new format for discriminating between phosphorylated and dephosphorylated

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(13) Titration of ***p*1** at various ionic strengths showed that the sharp maximum could be "salted out", which is consistent with the switching phenomenon involving electrostatics.

(14) (a) Lowe, M. P.; Caravan, P.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1998**, *37*, 1637−1647. (b) Piguet, C.; Bünzli, J.-C. G. *Chem. Soc. Rev.* **1999**, *28*, 347−358.

(15) A Eu/DO3A complex is proposed to dimerize at basic pH because of intermolecular complexation of pendant carboxylate arms: Faulkner, S.; Burton-Pye, B. P. *Chem. Commun.* **2005**, 259−261.

(16) See Supporting Information for additional details.

(17) It is important to note that proximity to Tb^{3+}, not only coordination to it, can cause extreme broadening of NMR signals. For more details, see Supporting Information.

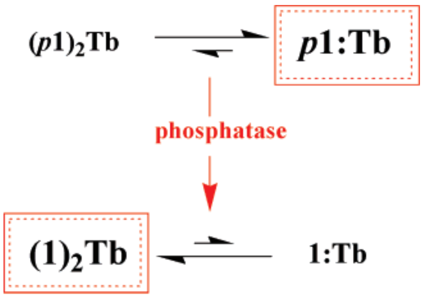

Figure 5. Schematic representation of the phosphatase-mediated conversion of ***p*1** to **1**.

peptides based on a significant change in luminescence properties. A mechanistic model for this behavior invokes the interconversion of mono- and bis-ligated lanthanide species, where the presence of a phosphate group stabilizes the monomeric complex. Although this luminescence switch reports adequately on a dynamic enzymatic process in vitro, its application in vivo may be limited because of the changes in complexation stoichiometry.

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Supporting Information Available: Experimental procedures, steady-state and time-resolved luminescence studies, and MS characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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