Photosensitized Near Infrared Luminescence of Ytterbium(III) in Proteins and Complexes Occurs via an Internal Redox Process

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Whereas photosensitization of visible emission from Eu^{3+} and Tb^{3+} in complexes containing chromophoric ligands is a well-established phenomenon with a number of current applications,[1−3] analogous emission from Yb^{3+} in the near-infrared remains relatively unexplored. Early observations of near-infrared emission from the $^2F_{5/2}$ level of Yb^{3+} in diketonate complexes[4,5] were followed much later by three reports involving Yb(III) porphyrins.[6−8] In these systems, the Yb^{3+} is directly coordinated to the chromophoric energy donor. The present work shows that Yb^{3+} luminescence can also be sensitized by a distant chromophore. Using the well-characterized, single tryptophan-containing calcium-binding protein parvalbumin from codfish (pI = 4.75)[9,10] wherein the two bound Ca^{2+} ions have been replaced by Yb^{3+} ions, we observe emission in the near-infrared with a peak at 977 nm when the protein is irradiated with 290 nm light (Figure 1). On the basis of modeling from the known structure of carp parvalbumin,[11,12] the tryptophan (Trp) is approximately equidistant from the two metal ion binding sites with the nearest indole ring atom 8−11 Å from a metal ion site. We propose that sensitized Yb^{3+} luminescence occurs via a long-range electron transfer (ET) process.

Figure 2 shows the energy levels of Eu^{3+}, Tb^{3+}, and Yb^{3+} [13] along with a schematic indication of porphyrin and indole chromophore levels. In Eu^{3+} and Tb^{3+} complexes with directly bonded chromophoric ligands, it is generally thought that energy transfer from ligand triplet levels accounts for the sensitized emission.[14] On the other hand, a through-space Förster mechanism has been established for energy transfer from the singlet excited states of tryptophan[15] or tyrosine[16] to the metal ions bound at Ca^{2+}-binding sites in proteins. The efficiency of energy transfer is directly proportional to the spectral overlap of the donor emission and acceptor absorption. Both Eu^{3+} and Tb^{3+} have many states above their emissive levels for which

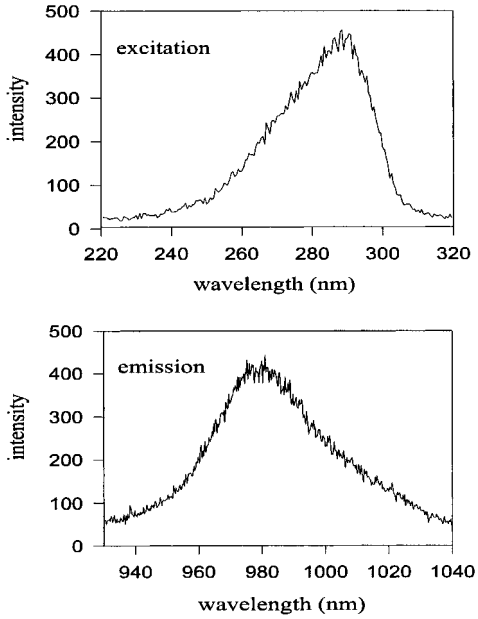

Figure 1. Excitation and emission spectra of sensitized Yb^{3+} luminescence in Yb^{3+}-loaded codfish parvalbumin. The excitation spectrum was taken by monitoring the 977 nm emission, and the emission spectrum was taken while exciting at 288.5 nm. Both are for a 109 μM parvalbumin sample in D_2O buffered to pH 5.8 with piperazine.

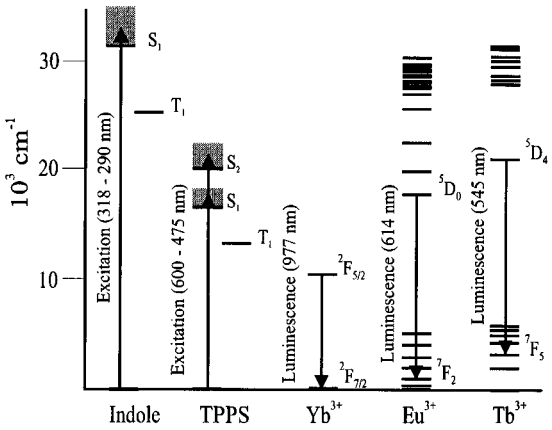

Figure 2. Energy level diagram for Yb^{3+}, Eu^{3+}, and Tb^{3+} with the singlet and triplet levels of tetra-*p*-sulfonatophenylporphrin (TPPS) and indole shown.

an energy match or near match to ligand chromophore excited singlet or triplet states exists. Yb^{3+}, however, has no energy levels above about 10 235 cm^{-1}, thereby eliminating any possible energy match with ligand singlet or triplet levels even in the porphyrin complexes, and of course, the spectral overlap integral of Förster theory is zero. The puzzle is, therefore, how does the energy get from the excited Trp to the Yb^{3+}?

The answer is provided by an observation made in this laboratory a number of years ago: the intensity of Trp fluorescence (singlet emission) in Ln^{3+}-loaded codfish parvalbumin is nearly identical for all Ln^{3+} ions with the exceptions of Eu^{3+} and Yb^{3+} which diminish the fluorescence intensity to 0.24 and 0.46 of the value found for the other ions in the series.[9] At the time, an energy transfer mechanism involving overlap of Trp emission with a ligand to metal charge transfer (LMCT) band of Eu^{3+} or Yb^{3+} was invoked to explain the observed quenching. The requisite LMCT band is neither seen for Yb^{3+}-bound parvalbumin nor found upon reexamination of the Eu^{3+} system. Thus, we are left with an electron transfer mechanism to account for the quenching of parvalbumin Trp fluorescence and for the subsequent Yb^{3+} luminescence emission. These ions

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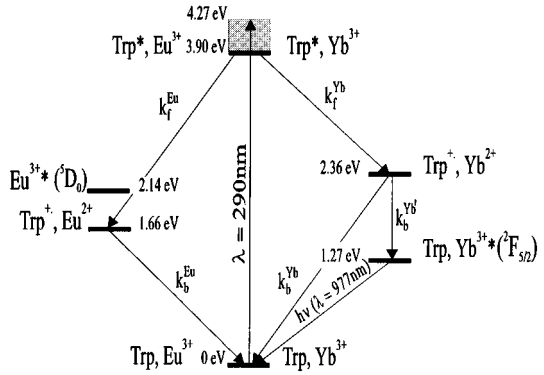

Figure 3. Proposed electron transfer scheme between Trp* and either Eu^{3+} or Yb^{3+} where k_f^{Ln} and k_b^{Ln} are the forward and back ET rate constants where the Ln^{3+} ion is left in its ground state. $k_b^{Yb'}$ is the back ET rate constant for the situation where Yb^{3+} is left in its excited emissive electronic state ($^2F_{5/2}$).

are the first and second most readily reduced ions in the Ln^{3+} series with reduction potentials of -0.35 V (Eu^{3+}) and -1.05 V (Yb^{3+}) vs the NHE.[17] The ground state indole moiety cannot reduce either of these ions, but the excited state can, as indicated in Figure 3. Indeed, an electron transfer mechanism was postulated by Abusaleh and Meares[18] for the quenching of indole and related fluorphores tethered to EDTA chelates of Ln^{3+} ions, with the Eu^{3+} and Yb^{3+} complexes being the most efficient quenchers. Indole quenching by Ln^{3+} ions in chelate complexes has been further investigated recently.[19]

The electron transfer mechanism explains how it is that emission occurs from the $^2F_{5/2}$ state of Yb^{3+} (Figure 3). The driving force, $-\Delta G$ for the forward ET may be estimated with the equation[20] $\Delta G_{Ln} = E(Trp^{\bullet+}/Trp) - E_{Trp^*} - E(Ln^{3+}/Ln^{2+})$ where the reduction potential of the tryptophan radical cation, $E(Trp^{\bullet+}/Trp)$ is 1.13 eV,[21] and E_{Trp^*} (the energy of Trp in its excited singlet state) is 3.9 eV. The reduction potential of the protein-bound metal ion is $E(Ln^{3+}/Ln^{2+})$, estimated as the aqueous reduction potential plus a correction factor of -0.18 to account for the estimate that the binding constant for a Ln^{3+} ion to parvalbumin is 10^3 times that of the Ln^{2+} ion. These values yield a driving force for Yb^{3+} of $-\Delta G^\circ_{Yb} = 1.54$ eV. The initial reduction of Yb^{3+} by tryptophan in its excited singlet state produces the tryptophan radical cation, $Trp^{\bullet+}$, and Yb^{2+}. The former is a strong oxidant and the latter a strong reducing agent, causing the electron to return producing Yb^{3+} and ground state Trp. Since the driving force of the ET back reaction, $-\Delta G = E(Trp^{\bullet+}/Trp) - E(Ln^{3+}/Ln^{2+})$ for the Yb system (2.36 eV) is *greater* than the $^2F_{5/2}$ state energy (1.27 eV), the Yb^{3+} thus formed may be in either the ground or excited state. The fraction of excited Yb^{3+} formed results in near-infrared luminescence.

Moreover, the ET mechanism also explains why very little sensitized emission from the $^5D_0$ excited state of Eu^{3+} is seen. The driving force for the Eu system is $-\Delta G^\circ_{Eu} = 2.24$ eV. It can be seen in Figure 3 that the back ET from Eu^{2+} has a driving force of 1.66 eV which is *less* than the energy of the emissive $^5D_0$ state (2.14 eV). Thus, Eu^{3+}, with a larger driving force than Yb^{3+}, efficiently quenches Trp* fluorescence but is not itself photosensitized by the ET process. The small amount of Eu^{3+} luminescence observed is due to a competing Förster energy transfer process from the Trp excited state.

Having established that long-range ET in a protein is the cause of photosensitized emission from bound Yb^{3+}, it is likely that this mechanism is a general one which will account for Yb^{3+} emission in complexes as well. We have surveyed a variety of Yb^{3+} complexes including $Yb(acac)_3$ in pyridine, Yb(III) tetra-*p*-sulfonatophenylporphrin (TPPS),[22] and Yb^{3+} complexes of quin-2[23] and desferrioxamine,[24] as well as Yb^{3+} bound to the proteins calmodulin, S-100β, and transferrin. All exhibit near-infrared emission. In each case, the emission spectrum is similar to that shown in Figure 1 and the excitation peaks closely correspond to ligand absorption maxima. Future work will further characterize what is essentially an internal chemiluminescent redox reaction. It is expected that the redox properties of the ligands will be particularly important in determining the magnitudes of the fluorescence quenching and sensitized luminescence emission in Yb^{3+} complexes.

Among the ramifications of these finding are the following: (1) Since Yb^{3+} emission occurs in the near-infrared region where biological tissues and fluids (e.g. blood) are relatively transparent, development of Yb^{3+} complexes for various analytical and chemosensor applications[8,25,26] is promising. Our establishment of the ET mechanism of Yb^{3+}-sensitized luminescence provides the basis for the rational design of appropriate systems. (2) For many complexes involving supramolecular and encapsulating aromatic nitrogen donor ligands, the quantum yield of sensitized emission from Eu^{+3} is considerably lower than that for Tb^{+3}.[2] It is likely that an ET mechanism largely accounts for this effect, although the previously postulated energy transfer to LMCT bands may also play a part. Maximizing Eu^{3+} luminescence quantum yields is of obvious importance for immunoassay reagents and our findings may lead to the design of more effective luminophores. (3) Finally, we note that the ET processes discovered here provide the basis for the use of Eu^{3+} and Yb^{3+} as electron acceptor probes for the study of long-range ET in proteins.[27−30] This aspect of our work is developed elsewhere.[31]

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