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A Sensitized Europium Complex Generated by Micromolar Concentrations of Copper(I): Toward the Detection of Copper(I) in Biology

Romain F. H. Viguier and Alison N. Hulme*

School of Chemistry, The University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, United Kingdom

Received June 15, 2006; E-mail: alison.hulme@ed.ac.uk

Copper is required as a cofactor in nearly 20 enzymes and is an essential micronutrient for all known life forms. Conversely, copper(I) catalyzes the production of highly reactive oxygen species from hydrogen peroxide via Fenton chemistry, and the accumulation of solvent-exposed copper(I) in amyloid beta fibrils is implicated in the progression of Alzheimer's disease.[1] The detection of solvent-exposed copper(I) in biology is an unexplored field, and the development of a copper(I) sensor could lead to new diagnoses for a range of conditions, including Menkes syndrome and Wilson's disease. The principal challenge in detecting copper(I) in vivo is that it is almost exclusively found coordinated to copper-binding proteins (e.g., superoxide dismutase, metallothionein) or ligands (e.g., glutathione) and not as free ions.[2] Thus metal sensors designed around ion chelates which detect the presence of free (or labile) copper(I) ions have severe limitations.[3]

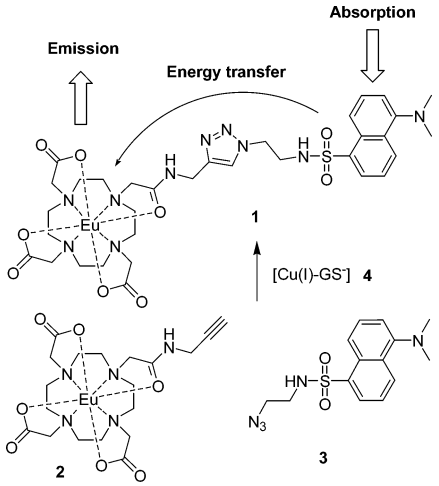
In a new approach to copper(I) detection, we have designed a lanthanide-based luminescent sensor **1** (Scheme 1). Our catalysis-based approach to metal detection[4] makes use of the highly versatile copper(I)-catalyzed variant of the Huisgen 1,3-dipolar cycloaddition reaction recently developed by Sharpless[5] to couple alkynyl Eu(III) complex **2** with dansyl azide **3**.[6] We expect this methodology to find widespread application, allowing the attachment of lanthanide complexes to a diverse range of organic or inorganic substrates. The "click" reaction offers many advantages for the development of copper(I) sensors for use in vivo: it is specifically catalyzed by the presence of copper(I) complexes (even under aqueous conditions);[7] it is bio-orthogonal and has been successfully performed in biological media.[8]

Luminescent lanthanide chelates themselves offer considerable advantages over the use of standard fluorescent dyes for detection in vivo, especially when there is significant autofluorescence. The lanthanide f−f electronic transitions are Laporte-forbidden, and lanthanide ions are generally considered to be photophysically inert.[9] However, in the presence of a proximal fluorophore, indirect excitation can be achieved through energy transfer.[10] Such sensitized lanthanide complexes show highly desirable spectral characteristics, including millisecond lifetimes, spiked emission (<10 nm full width at half-maximum), large Stokes shifts (<150 nm), and potentially high quantum yields (~1).[11]

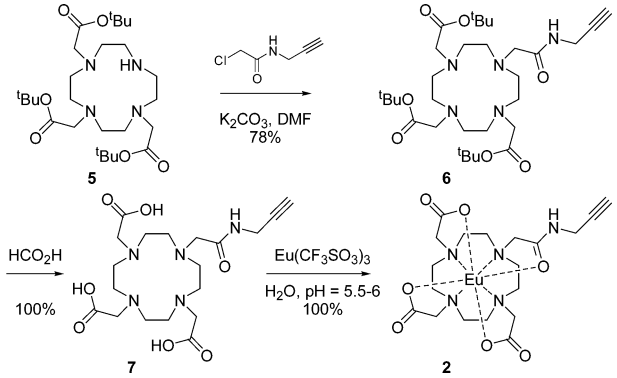
Lanthanide complex **2**, required as one of the sensor components, was synthesized by alkylation of commercially available DO3^{t}Bu **5** with *N*-(2-propynyl)chloroacetamide to give compound **6** in 78% yield (Scheme 2). Cleavage of the *tert*-butyl esters was achieved in quantitative yield using formic acid, revealing the octadentate ligand **7**. This ligand was then readily converted to alkynyl Eu(III) complex **2** on reaction with europium(III) trifluoromethane sulfonate.

Glutathione (GSH) is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) that reduces Cu(II) to Cu(I) and binds strongly to soft metals, such as copper(I), forming very stable complexes even in the presence of oxygen.[13] Glutathione is the most abundant

Scheme 1. Sensitized Eu(III) Chelate **1** Generated by the Presence of Micromolar Concentrations of a Copper(I) Glutathione Complex



Scheme 2. Synthesis of Alkynyl Eu(III) Complex **2**



intracellular nonprotein thiol and is present in all living cells in concentrations varying from 1 to 6 mM. Complexation of Cu(I) to the carboxylate anion of glutathione (GS^-), to give the GS^-−Cu(I) complex **4**, may provide a pooling mechanism for Cu(I) in living cells. Molecular modeling predicts that the glutathione anion binds the copper tightly in a tetracoordinate fashion, in which one oxygen (from the carbonyl of the glycine), two nitrogens (from the main chain of the cysteine and the γ-glutamyl residue), and the sulfhydryl group of cysteine coordinate the copper ion.[14] Given the natural abundance of glutathione, we were particularly interested to determine whether the GS^-−Cu(I) complex **4** might act as a catalyst for the formation of complex **1**. When equimolar concentrations of alkynyl Eu(III) complex **2** and dansyl azide **3** are mixed in the presence of 10 μM concentrations of GS^-−Cu(I) complex **4**, sensor **1** is indeed formed. The formation of **1** was monitored by its europium luminescence emission, which shows a maximum 10-fold enhancement due to the antenna effect after 1 h (Figure 1).

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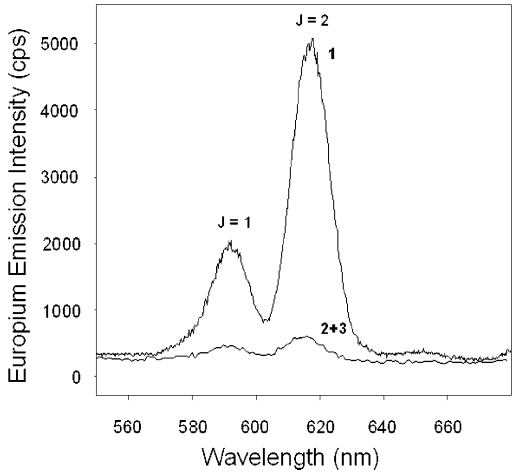

Figure 1. (a) Europium emission spectrum of the sensitized europium complex **1** formed in situ from a 1:1 mixture of **2** and **3** (10 μM) in the presence of $CuSO_4$ (1 μM) and GSH (10 μM) in water. Emission bands arise from the ^5D_o to ^7F_J transitions (λ_{ex} = 350 nm, time delay = 0.076 ms, sample window = 5 ms). (b) The emission spectrum of the control experiment, a 1:1 mixture of **2** and **3** (10 μM) before addition of the catalyst.

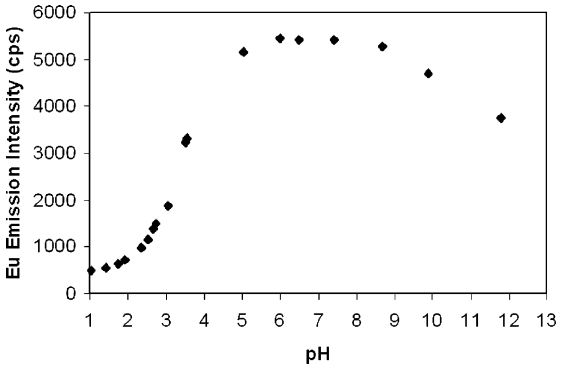

Figure 2. Plot of the europium emission intensity of **1** (10 μM, λ_{ex} = 350 nm) at 615 nm versus pH. The pH was adjusted by addition of concentrated HCl (aq.) or NaOH (aq.) and recorded using a microelectrode (Aldrich CMAW711) connected to a pH meter (Corning 145).

Control experiments using known concentrations of preformed complex **1** indicate that this corresponds to completion of the reaction.

Similar structures to **1**, but lacking a triazole ring, have been reported;[15] in these, coordination of the dansyl sulfonamide to the europium ion above pH 5.7 has been shown to lead to quenching of the lanthanide luminescence.[15c] In contrast, in sensor **1**, the triazole ring acts as a spacer which prevents dansyl coordination of the europium; the effect of this spacer upon the sensor performance is illustrated particularly well by the profile of the europium luminescence emission versus pH. Europium emission intensity shows a maximum plateau from pH 5 to 9 (Figure 2); this profile reflects the known protonation states of the dansyl fluorophore.[15a,16] Thus the absorbance shows a maximum at 330 nm at neutral pH, characteristic of an amine-naphthalene charge-transfer band. On deprotonation of the sulfonamide nitrogen (pH > 10.8), the electron density on the naphthyl ring is increased, moving this charge transfer state to higher energy. The result is a hypsochromic shift with the excitation λ_{max} shifting from 330 to 318 nm. On protonation of the dimethylamino group (pH < 3.8), the dansyl fluorescence diminishes almost to zero, the charge-transfer emissive state having been destroyed. Thus when using an excitation wavelength of 350 nm, these dramatic changes in spectral form are echoed in the luminescence emission spectra of our sensor.

In conclusion, we present a novel approach for detecting Cu(I) based upon a catalytic effect; our methodology relies on the sensitization of a europium complex by a pendant dansyl antenna. Formation of the luminescent device is brought about by the Huisgen 1,3-dipolar cycloaddition reaction catalyzed by the GS^-–Cu(I) complex. This is the first demonstration of catalysis of the Huisgen 1,3-dipolar cycloaddition by a stable biological copper(I) source. On the basis of this design, we are currently optimizing the sensor components for further biological applications, improving characteristics, such as the quantum yield and cell permeability.

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Supporting Information Available: Experimental procedures for the preparation of Eu(III) complex **2** and sensor **1**, spectroscopic data for the compounds **6**, **7**, **2**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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