Coordination Chemistry Reviews 273–274 (2014) 30–46

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr

Review

Designing reactivity-based responsive lanthanide probes for multicolor detection in biological systems

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Contents

ARTICLE INFO

Article history:
Received 31 August 2013
Accepted 15 October 2013
Available online 26 October 2013

Keywords:
Lanthanide
Luminescence spectroscopy
Fluorescent probe
Cyclen
Hydroporphyrin
Cu-catalyzed azide-alkyne cycloaddition
Near infrared

ABSTRACT

Strategies for the design and synthesis of responsive luminescent probes based on lanthanide complexes are surveyed. The sensitive detection of analytes ranging from ions to small molecules to large biomolecules such as enzymes in complex biological milieu drives the quest for even more selective probes with improved photophysical properties and broad availability for non-specialists. Here, reactivity-based probes, *i.e.* those in which the sensing event is accompanied by the breaking of a covalent bond are reviewed, with an emphasis on the strategies that could be generalized to the detection of additional analytes. Syntheses providing advanced cyclen-based ligands with minimum effort, as well as those that enable post-complexation modification of lanthanide-bound structures are presented.

1. Introduction

The complexity of biological systems presents a formidable challenge to those engaged in their molecular-level study. Fluorescence technologies are well-suited to this task due to their non-invasiveness, their high sensitivity, and their high spatiotemporal resolution. However, the interconnectedness of biological processes means that the investigation of only one species or transformation at a time gives only a severely limited and often distorted picture of the system. Thus, chemical tools that enable the monitoring of multiple species/processes simultaneously are in great demand. Responsive fluorescent probes

Abbreviations: Asc, ascorbate; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl; CuAAC, Cu-catalyzed azide-alkyne cycloaddition; DIPEA, diisopropylethylamine; DO3A, 1,4,7-tris(carboxymethylaza)cyclododecane-10-azaacetylamid; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; hROS, highly reactive oxygen species; Ln, lanthanide; NIR, near infrared; PeT, photoinduced electron transfer; ROS, reactive oxygen species; TBTA, tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine; TMEDA, tetramethylethylenediamine; TMPi, 2,2,6,6-tetramethylpiperidine.

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http://dx.doi.org/10.1016/j.ccr.2013.10.012

based on organic emitters typically have short emission lifetimes, small Stokes shifts, and broad (tens to hundreds of nm) absorption and emission spectra. In contrast, lanthanide emitters have spiked, metal-specific, and mostly non-overlapping bands, which means that they are readily detected independently of each other [1–4].

Before cocktails of responsive lanthanide-based probes can be used to simultaneously monitor biological processes several obstacles need to be overcome. The most obvious of these is the dearth of generally applicable strategies for designing responsive probes [5–7]. Most examples in the literature concern hand-crafted, fine-tuned probes based on supramolecular analyte recognition [8,9]. The recognition of a different analyte usually requires the creation of a significantly altered system, with changes in antenna, recognition principles, and even lanthanide-binding site. Reactivity-based probes overcome this limitation by enabling the grafting of various detection sites ('cages') onto a common platform. An additional advantage of reactivity-based probes is the possibility to accumulate recognition events over an extended period of time, thus amplifying very weak signals arising from the detection of short-lived species present in very low concentrations. A further possible improvement in detection limit is through catalytic signal amplification, a thus far underutilized approach. The drawback of reactivity-based probes is that with a few exceptions, reversible processes are not readily monitored, something which supramolecular probes are able to do.

Here we present a systematic overview of strategies to construct reactivity-based responsive lanthanide probes. Some general solutions to the problem of detection are discussed, followed by the problem of access to lanthanide complexes. This aspect of probe design is often overlooked. However, complex synthesis represents a significant part of the research in this area, and unless readily available, lanthanide-based probes are unlikely to become widespread in biochemistry and chemical biology.

2. Strategies for responsive probe design

Compared to purely organic reporters, lanthanide-based probes provide several unique features which make them attractive for use as bio-probes. These include long excited state lifetimes, large Stokes shifts, narrow emission wavelengths and high water solubility. For an efficient Ln-based probe several aspects need to be considered: (1) A strongly coordinating ligand is required in order to minimize non-radiative deactivation of the excited Ln^{III} state and to avoid dissociation of the toxic lanthanide. While a number of multidentate ligands exist for *in vitro* use, the strict requirements for *in vivo* experiments (pLn > 20) limit the possibilities. Cyclen derivatives and cryptates provide the greatest stability in this respect [1]. (2) Since Ln-ions are poor absorbers of light, a strongly absorbing Ln-sensitizing antenna with a suitable triplet excited state is needed for maximum brightness. (3) In order to avoid back energy transfer the antenna triplet state should ideally be >2000 cm^{-1} above the Ln emitting state. For Eu and Tb this limits the choice of sensitizer to those which absorb below 430 and 400 nm, respectively. (4) The sensing mechanism must be selective, and respond with reasonable kinetics to the analyte in question. Several ways exist to place the lanthanide emission under analyte control (for an excellent review see [7]). In this section, the focus will be on reactivity based probes, which for the purpose of this review can be defined as those that undergo a bond breaking/making event in response to an analyte.

3. Reactivity based responsive Ln probes

With the above-mentioned antenna requirements in mind, designing a reaction based lanthanide probe can be governed by the same considerations as when designing a fluorescent reporter [10,11]. That is, processes which serve to deactivate the antenna excited state will generally translate to a decrease in the coupled lanthanide emission. Under this framework of mechanism-based probe design, a number of luminescent lanthanide probes for the detection of small-molecule analytes, metals, and enzymatic activity have been reported. These are presented in the following sections. Some of these probes belong to several categories, and were sorted based on their most striking characteristic.

3.1. Probes based on phenol and aniline releasing reactions

Given the wealth of reactions which release phenols and anilines it should come as no surprise that many probes rely on such transformations. In terms of antenna structure, the simplest reaction-based Ln-probes to depend on such a reaction have utilized an amide/aniline switch to alter the photophysical properties of the antenna. The first reported example of this type of probe was developed by Kikuchi et al. to detect protease activity (Scheme 1a) [12]. The amide pre-antenna is caged by either a leucine or a succinyl-leucine-tyrosine, cleavable by leucine aminopeptidase or calpain, respectively. Enzymatic cleavage of the cages resulted in a sizeable increase in the Tb-emission when the probe was excited at 250 nm. Relying on the same aniline antenna, Chang et al. have developed a H_2O_2 responsive Tb-based probe (Scheme 1b) [13]. The probe incorporates a boronate cage linked to the aniline through a self immolative carbamate. H_2O_2-induced oxidation of the boronate reveals an unstable *p*-hydroxybenzyl carbamate which spontaneously fragments to reveal the aniline. By exciting the probe at 280 nm a 6-fold increase of Tb-emission was observed upon reaction with H_2O_2. Despite the rather low excitation wavelength of 280 nm, by employing time resolved emission spectroscopy endogenous H_2O_2 production in live stimulated RAW 264.7 macrophages could be detected. The boronate-to-phenol oxidation has been widely employed as the recognition event for detecting H_2O_2 [14,15], and more recently $ONOO^-$ [16–19]. A potential pitfall of such heptadentate core structures is the presence of labile inner-sphere water molecules on the Ln. The replacement of these upon binding to *e.g.* phosphate or serum proteins can significantly alter the luminescent behavior of the complexes even in the absence of a recognition event [20].

Due to the basicity of amines and phenolates, probes relying on their formation generally respond not only to their intended analyte but also to pH changes, as the protonated species often has photophysical properties similar to the caged compound. However, by designing the probe so that the amine or phenol formation is part of a tandem process, such pH sensitivity can be avoided. Our lab has relied on a phenol-to-lactone tandem reaction to generate a Tb- and Eu-sensitizing coumarin antenna [21]. The caged phenol is positioned *ortho* to a malonate. Upon analyte-triggered uncaging the phenolate initially formed will undergo a subsequent lactonization to form a coumarin (Scheme 2) [22,23]. Because the coumarin formation relies on the unmasking of a phenol, in principle any species which mediates such a reaction can be detected based on this method.

To demonstrate this approach we synthesized Pd(0), H_2O_2, β-galactosidase and F^- responsive Eu and Tb-based probes (relying on an allyl- [24], boronate- [14], β-galactose- [25] and TIPS-cage [22], respectively, Scheme 3). In order to preserve the integrity of the cages, a divergent synthesis was chosen where the caged coumarins were coupled to a Ln-DO3A chelate in the final step by CuAAC. The probes were able to detect H_2O_2 and F^- in the low μM range (1