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# Bacterial Spore Detection by [$Tb^{3+}$(macrocycle)(dipicolinate)] Luminescence

Morgan L. Cable,[§,#] James P. Kirby,*[,#] Karn Sorasaenee,[§] Harry B. Gray,*[,§] and Adrian Ponce*[,#]

*Beckman Institute, California Institute of Technology, Pasadena, California 91125, and In Situ Instruments Section, Jet Propulsion Laboratory, 4800 Oak Grove Drive, Pasadena, California 91109*

Received March 27, 2006; Revised Manuscript Received December 5, 2006 ; E-mail: james.p.kirby@jpl.nasa.gov; hbgray@caltech.edu; ponce@caltech.edu

Bacterial spores are the most resistant microbial structures toward environmental extremes.[1] Consequently, bacterial spores find extensive use as microbial indicators for evaluating the efficiency of sterilization regimens,[2] and in the most ominous application, *Bacillus anthracis* spores have been employed as delivery vehicles in anthrax attacks.[3] We[4] and others[5] have investigated methods for the rapid detection of bacterial spores based on dipicolinic acid (DPA) detection. DPA—a unique biomarker and major constituent of bacterial spores ($>10^8$ molecules of DPA per spore)—can be released into bulk solution by physical lysis, chemical lysis, or germination.[6] High affinity binding of DPA to $Tb^{3+}$ triggers intense green luminescence under UV excitation, enabling a very sensitive assay for bacterial spore detection.[5a−e,7]

When $TbCl_3$ is employed as the sensor reagent, nonselective binding of aromatic ligands[8] to $Tb^{3+}$(aq) can give rise to false positives, and binding of anions such as phosphate may inhibit DPA binding, which can give rise to false negatives.[9] Moreover, nonradiative quenching from coordinated water in [Tb(DPA)-$(H_2O)_6]^+$ reduces the overall quantum yield and thus the sensitivity of the assay. Our plan is to tether $Tb^{3+}$-macrocycle complexes capable of selective DPA binding from polymers to build solid-state devices such as fiber optic and waveguide sensor components. In a first step, we are employing macrocyclic ligands to construct $Tb^{3+}$-receptor platforms that are specific for DPA, reminiscent of previous work on bioactive anions binding to lanthanide complexes in aqueous solution.[10]

The macrocycle DO2A (1,4,7,10-tetraazacyclododecane-1,7-diacetate) meets our basic requirements for a receptor ligand in that (1) the DO2A fraction bound at micromolar concentrations is near unity, (2) DO2A binding keeps three adjacent coordination sites open and does not inhibit DPA binding, (3) DO2A can be chemically modified with various covalent pendent groups to test receptor site constructs and enable polymer incorporation, and (4) the $[Tb(DO2A)(DPA)]^-$ complex eliminates water quenching. Here we report the first structurally characterized ternary [$Tb^{3+}$(macrocycle)(dipicolinate)] complex, $[Tb(DO2A)(DPA)]^-$.

The DO2A ligand was prepared by hydrolysis of DO2A-*tert*-butyl ester.[11] The principal molecular ion $[Tb(DO2A)(DPA)]^-$ (Figure 1) was crystallized via slow solvent evaporation in the monoclinic space group $P2_1/c$ in a distorted capped-square antiprism geometry. Although there are four possible stereoisomers of the lanthanide−cyclen compound,[12] only $\Delta(\lambda\lambda\lambda\lambda)$ or $\Lambda(\delta\delta\delta\delta)$ is observed in the asymmetric unit. The molecular structure of $[Tb(DO2A)(DPA)]^-$ suggests that there are two hydrogen interactions between the DO2A and DPA ligands (N3H···O3, 2.76 Å, and N5H···O1, 2.75 Å).

The negative ion mode ESI mass spectrum of $[Tb(DO2A)(DPA)]^-$ in $CH_3OH$ was observed at $m/z = 610.0$ (calcd 610.4 g/mol for $Tb_1C_{19}H_{25}N_5O_8$). The UV absorption spectrum of $[Tb(DO2A)(DPA)]^-$ revealed peaks at 271 and 278 nm, attributable to the $\pi \rightarrow \pi^*$ transitions of bound DPA. Excitation and emission spectra (Figure S1) are consistent with a DPA $\rightarrow$ $Tb^{3+}$ energy transfer mechanism, where the most intense emission occurs at 545 nm ($^5D_4 \rightarrow {}^7F_5$, 278-nm excitation).[13]

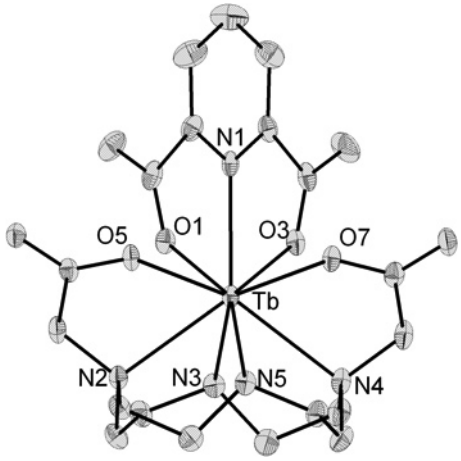


***Figure 1.*** Thermal ellipsoid plot of $[Tb(DO2A)(DPA)]^-$ complex with 50% probability. Hydrogen atoms are omitted for clarity.

The optimal stoichiometry for complex formation with $[Tb(DO2A)]^+$ and DPA was determined by Job's method of continuous variations. The luminescence intensity peaks at a mole fraction of 0.5, indicating the formation of the ternary complex $[Tb(DO2A)(DPA)]^-$ (Figure 2). Lifetime measurements in $H_2O$ and $D_2O$ strongly support ternary complex formation with concomitant exclusion of water from the $Tb^{3+}$ coordination sphere (Table 1).[14]

The exclusion of water from the inner sphere of the ternary $[Tb(DO2A)(DPA)]^-$ complex minimizes the nonradiative quenching of the $Tb^{3+}$ emission resulting in a quantum yield ($\Phi_{em} \approx 0.1$) that is higher than that of $[Tb(DPA)]^+$ ($\Phi_{em} \approx 0.06$). The increased quantum yield affords a corresponding improvement in the bacterial spore detection limit.

The $^5D_4 \rightarrow {}^7F_4$ transition can be employed as a spectroscopic handle, because the band shapes for $[Tb(DO2A)(DPA)]^-$, $[Tb(DPA)(H_2O)_6]^+$, and $[Tb(DPA)_3]^{3-}$ are remarkably distinct (Figure S4), presumably because of ligand field effects amplified by the highly allowed $\pi \rightarrow \pi^*$ transition of the bound DPA. Competitive binding experiments show that the affinity of $[Tb(DO2A)]^+$ for $DPA^{2-}$ ($K_A = 10^{10.7}$ $M^{-1}$) is much greater than that of $Tb^{3+}$(aq) ($K_A = 10^{8.7}$ $M^{-1}$).[16] In these experiments, the characteristic band shape of $[Tb(DO2A)(DPA)]^-$ remains unchanged down to 6.0 nanomolar, corresponding to $2.1 \times 10^4$ spores per mL[4c] (Figure 3).

We conclude that the remarkable ternary $[Tb(DO2A)(DPA)]^-$ complex stability is due to additional binding, such as the interligand

[§] California Institute of Technology.
[#] Jet Propulsion Laboratory.


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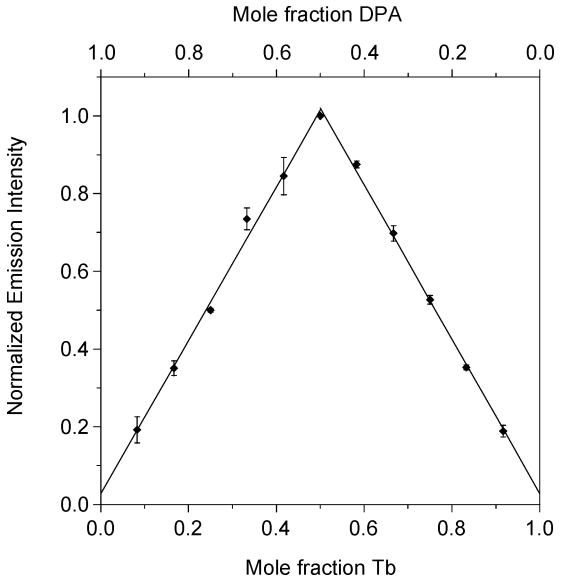


***Figure 2.*** Job's plot method of continuous variations in water at pH 8.5 (adjusted with NaOH). [Tb] and [DPA] are varied inversely from 0 to 12 $\mu$M in 1 $\mu$M increments, with [DO2A] = 100 $\mu$M.

***Table 1.*** Luminescence Lifetime Measurements[a]

| complex[b] | $\tau(H_2O)$/ms | $\tau(D_2O)$/ms | q[c] |
|---|---|---|---|
| $[Tb(H_2O)_9]^{3+}$ | 0.4 | 3.4 | $8.8 \pm 1.1$ |
| $[Tb(DPA)(H_2O)_6]^{+}$ | 0.6[d] | 3.5 | $5.6 \pm 0.7$ |
| $[Tb(DO2A)(H_2O)_3]^{+}$ | 1.1 | 2.6 | $2.4 \pm 0.3$ |
| $[Tb(DO2A)(DPA)]^{-}$ | 1.9 | 2.2 | $0.3 \pm 0.0$ |

[a] Excitation at 266 nm (10 ns, pulsed Nd:YAG laser), emission detected at 544 nm, sample concentrations 1 to 10 $\mu$M, pH 7.5 (adj with NaOH). [b] Waters included assuming that the terbium complex is 9-coordinate. [c] The number of water molecules, $q$, in the $Tb^{3+}$ coordination sphere, where $q = A_{Ln}(\tau(H_2O)^{-1} - \tau(D_2O)^{-1})$, and $A_{Tb} = 4.6$ or $4.2 \pm 0.5$ ms$^{-1}$ per bound water molecule for complexes with and without DO2A, respectively (0.09 ms$^{-1}$ added for each NH amine oscillator in DO2A) (ref 14b). [d] See ref 15.

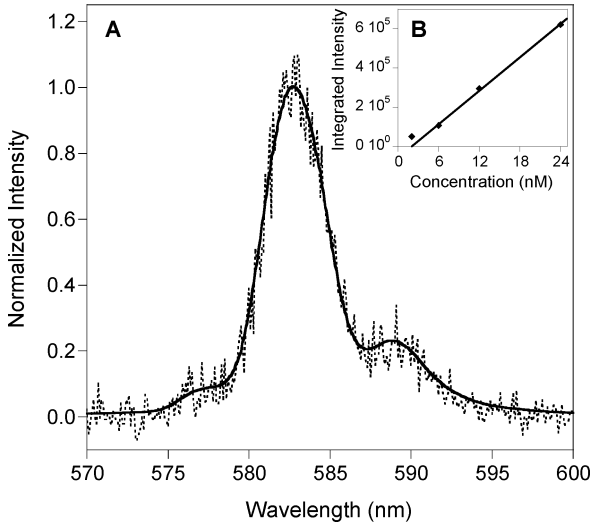


***Figure 3.*** (A) Emission spectra of $[Tb(DO2A)(DPA)]^{-}$ at 6.0 $\mu$M (solid) and 6.0 nM (dashed) at pH 7.0. Excitation wavelength: 278 nm. (B) Concentration dependence of $[Tb(DO2A)(DPA)]^{-}$.

hydrogen interactions between DO2A and DPA indicated in the crystal structure. Our studies have demonstrated that $[Tb(DO2A)]^{+}$ is a viable first generation receptor capable of high affinity DPA binding. We plan to investigate various pendent groups to further optimize the DPA receptor site.

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**Supporting Information Available:** Crystallographic data (CIF), elemental analysis, UV absorption, excitation and emission spectra, pH dependence data, bacterial spore detection data, and experimental preparation of $TBA^{+}[Tb(DO2A)(DPA)]^{-}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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