Designer Ligands for Beryllium

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Despite the high toxicity of beryllium, it is widely used due to its unique properties. Beryllium is toxic both as a carcinogen and as the agent that initiates chronic beryllium disease (CBD). CBD, a granulomatous lung disease, is a cell-mediated immune response of inhaled beryllium in 6−20% of exposed individuals. The nature and effects of CBD have been well-studied,[1] but the role of beryllium in triggering CBD is not well understood.[2,3] The onset of CBD can be delayed for 10−40 years after exposure, and there is no clear dose response correlation. Research efforts tend to focus on areas of biological[4] and environmental[5] effects of beryllium with far less effort devoted to the speciation and interactions of beryllium under physiological conditions. Currently, beryllium detection is accomplished by ICP-AE (inductively coupled plasma-atomic emission) or ICP-MS, requiring a large capital investment.[5] A selective fluorescent sensor would offer several advantages, including: low-cost instrumentation, quick analysis, field portability, a small sample size (100 μM) for analysis, and potential for imaging in a biological system.

Previous research has focused on the binding of beryllium by ligands to make BeL and BeL$_2$ species with chelating ligands such as chromotropic acid.[6−8] This strategy has resulted in ligands with fairly high binding constants (chromotropic acid $K = 16.2$) but poor selectivity.[6] Ligands such as chromotropic acid that show fluorescence changes upon binding beryllium are very intriguing for their potential as fluorescent indicators, but the poor selectivity requires the addition of ethylenediamine tetraacetic acid (EDTA) as a masking agent and limits their utility as a fluorescent sensor.[9] Although most literature has focused on BeL and BeL$_2$ species, it is well-known that the aqueous speciation of beryllium involves polynuclear complexes such as Be$_3$(OH)$_3$.[6] Recent reports have shown that polynuclear species are also dominant in the speciation of Be complexes of citric acid (CA) that feature a central Be−O−Be unit with the O coming from the alkoxide in CA.[10] The preferred geometry for the Be in the Be−O−Be unit in CA analogues consists of one five-member ring and one six-member ring. The Be−O−Be motif with a bridging RO has been previously observed in crystal structures for the Be glycolate system[11] and with ^{t}butoxide[12] or ethoxide[13] as the bridging RO species, but in these cases the binding constants for the ligands are very weak (log K_1 Be-glycolate = 1.49).[6] Herein, we report on beryllium binding to ligands that are intentionally designed to bind polynuclear species of Be based on the Be−O−Be motif. Two ligands, depicted in Figure 1, have been evaluated: 2-hydroxyisophthalic acid (HIPA) and 2,3-dihydroxy-benzoic acid (DHBA). Both ligands have a high affinity for beryllium over a wide range of concentrations/pH and exhibit luminescent changes upon binding the beryllium.

HIPA and DHBA were chosen to support the Be−O−Be motif in two different manners; HIPA can form two six-member rings, and DHBA can form one five-member ring and one six-member
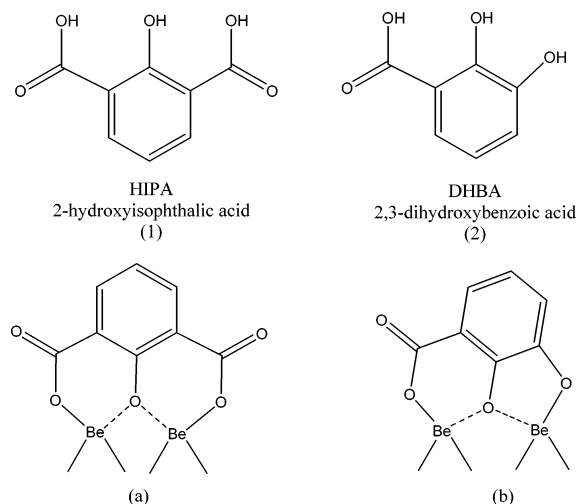
Figure 1. (Top) Structures of 2-hydroxyisophthalic acid (**1**) and 2,3-dihydroxybenzoic acid (**2**). (Bottom) Two different binding pockets for Be with HIPA (a) and DHBA (b).

ring upon binding. The aryl ring provides the potential for fluorescence, serves to add rigidity to the ligand's binding site, and increases the acidity of the ROH that serves as the bridging RO unit in the Be−O−Be moiety. Binding of Be to HIPA and DHBA ligands was characterized by a combination of ^{9}Be and ^{13}C NMR, UV, and MS. By monitoring both ^{13}C NMR to observe free/bound ligand and ^{9}Be NMR to observe free/bound Be as the Be:ligand ratio was changed from 1:2 to 2:1, the binding ratio of beryllium to the ligand was found to be 2:1. The ^{9}Be NMRs of the 2:1 complex are consistent with the structures in Figure 1, where the HIPA:Be complex has only one peak at 3.40 ppm for two identical Be sites in a six-member ring and the DHBA:Be complex has two peaks at 4.52 and 2.65 ppm corresponding to one Be in a five-member ring and one Be in a six-member ring, respectively. The UV/vis spectrum for Be$_2$DHBA has a peak at 325 nm in agreement with the fully deprotonated ligand in concentrated NaOH. The MS for HIPA with Be shows a base ion at 429 m/z [Be$_4$(HIPA)$_2$(H$_2$O)-(OH)$^+$] with a daughter peak at 215 [Be$_4$(HIPA)$_2$(H$_2$O)$_2^{2+}$, $z = 2$], suggesting that the species can readily dimerize as previously seen for citric acid.[10]

The HIPA and DHBA ligands exhibit a red shift in the absorbance of 10 and 14 nm, respectively, when Be is added in a 1:2 ratio of ligand to Be (Figure 2). The stability constants were determined by spectrophotometric titrations (absorbance vs pH), utilizing the data analysis program pHAB.[14] The stability constants for the Be/ligand complexes are summarized in Table 1. The simple binding constants for BeL formation are slightly higher than that of chromotropic acid (one of the highest reported binding constants in the literature). The stability constants for the 1:2 species are extremely high and demonstrate the importance of the Be−O−Be motif. Speciation plots based on the stability constants show that
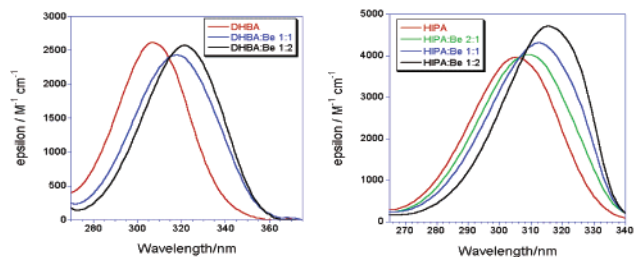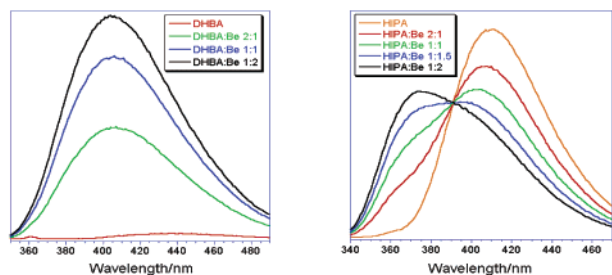
Figure 2. (Left) Absorption spectrum of DHBA:Be (pH = 7.5). (Right) Absorption spectrum of HIPA:Be (pH = 8.5). All solutions: [ligand] = 1 x 10^{-4} M in 0.1 M KCl.

Table 1. Stability Constants for Ligands **1** and **2** with Beryllium[a]

| quotient	HIPA (**1**) log β	DHBA (**2**) log β
[LH]/[L][H]	13.7	13.3
[LH2]/[LH][H]	19.1	23.5
[LH3]/[LH2][H]	22.3	26.2
[LBe]/[L][Be]	17.5	18.4
[LBe2]/[LBe][Be]	27.0	28.5

[a] All constants are determined at 298 K, 0.1 M KCl.

Figure 3. (Left) Emission spectra of DHBA with increasing [Be]. [Ligand] = 10^{-5} M in HEPES buffer (0.05 M), pH = 7, λ_{exc} = 320 nm. (Right) Emission spectra of HIPA with increasing [Be]. [Ligand] = 10^{-5} M in acetate buffer (0.05 M), pH = 5, λ_{exc} = 320 nm.

the Be$_2$L species dominates even at concentrations as low as 1 μM.[12] The Be$_2$L species at low concentration (10 μM) is supported by the presence of the Be$_4$(HIPA)$_2$(H$_2$O)(OH)$^+$ peak in the MS data.

As shown in Figure 3, both ligands demonstrate a strong luminescence in the presence of Be when excited at 320 nm. The HIPA ligand emits at 411 nm and shows a blue shift to 375 nm upon addition of beryllium. The DHBA ligand has a nominal emission spectrum, but in the presence of Be a strong emission is observed at 402 nm. The fluorescence changes coupled with the high binding constants of these ligands enable both ligands to be used as fluorescent indicators for Be at very low levels. A 10 μM solution of DHBA can be used to determine 50 nM of Be (4.5 pg/mL) as depicted in the emission spectrum in Figure 4. Other fluorescent indicators have been reported for Be with similar detection limits based on simple BeL binding, but all have severe interferences from other metals such as aluminum and iron.[5] Aluminum interferes strongly in most systems, because of the similarity in the charge-to-size ratio and the fact that in the case of simple metal−ligand binding with chelating ligands, the O−O distance between the two nearest chelating oxygens in a tetrahedrally bound Be and an octahedrally bound Al are identical to within 0.02 Å. By designing a ligand to bind with the Be−O−Be motif, we have imposed a tremendous selectivity for Be. We have demonstrated this selectivity by looking at fluorescence as a function of Be concentration in the presence of other metals. In a solution with 10 μM of DHBA and a cocktail of metal ions including Al, Fe, Cr, Cu, Zn, Cd, and Pb, all at 10 μM each, the background
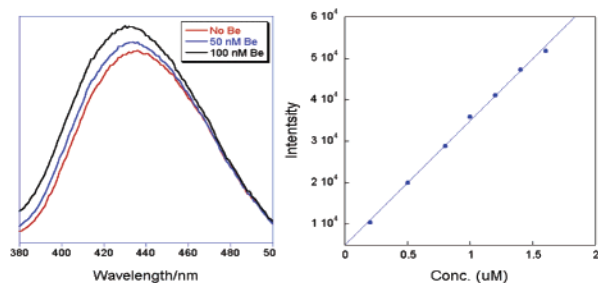
Figure 4. (Left) Emission spectrum of DHBA with Be at low concentration. [Ligand] = 10^{-5} M in HEPES buffer (0.05 M), pH = 7, λ_{exc} = 320 nm. (Right) Fluorescence intensity as a function of [Be]. [Ligand] = 10^{-5} M in HEPES buffer (0.05 M), pH = 7, λ_{exc} = 320 nm.

fluorescence remains minimal. The fluorescence intensity as a function of [Be] (see Figure 4) gives a linear plot with no interference from the presence of all seven metals down to 200 nM Be (1.8 ng/mL). This dramatic result demonstrates for the first time that ligands can be rationally designed to selectively bind beryllium based on binding polynuclear species. The DHBA ligand detects Be at pH 7 with no interference, making it ideal for biological imaging of Be.

Although the importance of polynuclear species is known in simple aqueous beryllium species with no ligands present, no previous work has purposely exploited the potential of binding polynuclear species for the detection of Be. The results with HIPA and DHBA demonstrate that ligands can be designed to bind Be strongly and selectively based on binding polynuclear structures. The high binding constants observed for both ligands suggest that binding polynuclear species may be a viable strategy for developing therapeutic agents for CBD, and the stability of the bridging RO group indicates that amino acids such as tyrosine, threonine, or serine may play a role in biological interactions with Be clusters. Future work will explore if such polynuclear speciation can offer insight into physiological interactions and why Be leads to CBD while there is no analogous chronic aluminum disease.

Supporting Information Available: Experimentals and a speciation figure of the beryllium complexes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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