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Beryllium binding at neutral pH: The importance of the Be—O—Be motif

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Abstract

Beryllium speciation at physiological conditions is critical to understanding chronic beryllium disease (CBD). The MHC-class II receptor alleles that have been linked to CBD have more than six carboxylates in a short 20 amino acid segment of the binding pocket and it has been suggested that beryllium may bind within the MHC-class II receptor via the carboxylates. Previous reports also show that citric acid binds beryllium significantly stronger than similar carboxylate ligands such as tartaric acid and is one of the few ligands that can compete with hydrolysis to solubilize beryllium across the entire pH range at molar concentrations. We have characterized the binding of Be to citric acid and shown using a combination of NMR, mass spectrometry and ligand competition studies that Be_2L and Be_4L_2 species dominate. A Be—O—Be linkage with the bridging oxygen coming from the aliphatic alcohol is critical to the stability of the complex. We show through competition experiments that the most stable Be—O—Be arrangement has one Be in a five-member ring and the other Be in a six-member ring. The unusual deprotonation of an aliphatic alcohol ($pK_a = 18$) at neutral pH has significant ramifications on the potential interactions of Be with biological ligands such as carbohydrates and Ser and Thr residues.

Keywords: Beryllium; Citric acid; Binding; Binuclear

1. Introduction

Despite the high toxicity of beryllium, it is widely used due to its unique properties. Beryllium has a high melting point (1285 °C), is lightweight, non-magnetic and six times stiffer than steel [1]. The use of beryllium in industry and government has increased over time and shows no signs of decline. 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 to inhaled beryllium in 6–20% of exposed individuals. The onset of CBD can be delayed for 10–40 years after exposure, and no strong correlation is known between dose and development of CBD. The nature and effects of CBD have been well studied [2] but the role of beryllium to trigger CBD is not well understood [3,4]. Research efforts tend to focus on areas of biological and environmental effects of beryllium with far less effort devoted to the speciation and interactions of beryllium under physiological conditions.

CBD is unique compared to asbestosis or silicosis in that it involves an immune response of proliferating CD4+ T cells [5]. The CD4+ T cell proliferation has been linked to a set of MHC-class II receptors that are only present in a certain portion of the population. The set of MHC-class II receptors linked to CBD have a larger concentration of carboxylate residues. Over

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six carboxylate residues exist in a short 20 amino acid sequence that forms part of the binding pocket of the MHC-class II receptor. The binding pocket is designed to bind antigens, such as foreign polypeptides from infectious agents, and present them on the outside of the cell. One theory for the triggering of CBD involves Be binding to the pocket either on its own or as a peptide complex. The potential binding of Be is strongly dependent on the 3D structure of the protein and the exact position of the carboxylates. Modeling studies suggest that Be binding sites are available on the MHC-class II receptor, but there has been no experimental verification [6]. We have undertaken an investigation into the speciation of beryllium with multi-carboxylate ligands in aqueous media as a foundation to understand what type of Be species may be involved in CBD. Previous work with potentiometric titrations has shown that citric acid, with three available carboxylates, has a considerably higher affinity for beryllium than other aliphatic carboxylate ligands and can solubilize beryllium across the entire pH range [7,8].

Beryllium speciation in water in the absence of ligands other than H_2O and OH has been well documented [7]. One of the interesting results is the tendency of Be to form clusters, such as the $Be_3(OH)_3^{3+}$ which exists as a cyclic six member ring structure and is the predominate species in solution from approximately pH 4.5 to 5.5 (at 2 mM Be) [7]. Above pH 5.5, Be precipitates as a polymeric hydroxide. Despite the strong evidence for cluster formation in water, most aqueous research has focused on monomeric BeL or BeL_2 complexes at low pH. A recent report has shown that a Be—O—Be motif is highly stable and selective for Be with the bridging O coming from a phenolate [9]. Also another recent report showing a Al/Be motif (Al—O—Be) utilizing CA [10]. Theoretical studies also indicate the importance of the Be—O—Be unit [11]. Herein, we experimentally demonstrate the importance of the Be—O—Be structural unit in beryllium binding to citric acid and demonstrate the ability of Be to deprotonate aliphatic alcohols at neutral pH. We report on the dominant ligand to metal ratio for beryllium binding to citric acid and its derivatives. Key functional features of the ligands are identified that impact both ligand design in the future and potential interactions with proteins and carbohydrates. Our results are discussed in respect to other hydroxy-acids, such as tartaric acid [12–14], malic acid [15] and glycolic acid [12,15] that have been examined previously.

2. Experimental

Safety note. CAUTION! Beryllium and its derivatives are extremely toxic and should be handled with care.

2.1. Materials and methods

All aqueous solutions were prepared with water that was purified using a MilliQ® analytical deionization water treatment system. Stock solutions 1 M of $BeSO_4$ in water were prepared in a high efficiency particulate air (HEPA) filtered box from $BeSO_4$ (Acros). Tartronic acid (TTA) (Alfa Aesar), citric acid (CA), 3-hydroxy-3-methylglutaric acid (HMGA), tricarballylic acid (TCA), malic acid (MA), citramalic acid (CMA), potassium hydroxide and ammonium hydroxide were purchased from Acros and used as received. Dimethylcitric acid (DMCA) was prepared from trimethylcitrate (Aldrich) by a previously reported method [16]. NMR data (^1H, ^9Be, ^{13}C) were obtained on Bunker 400 MHz instruments. T_1 experiments followed literature precedent [17]. The longest T_1 value for the ligand's methylene carbons was calculated at 0.93 s (MA) and 4.29 s (HMGA) for the α-carbon to the hydroxide. Chemical shifts are reported relative to DSS for ^1H and ^{13}C, $Be(H_2O)_4^{2+}$ for ^9Be and are in ppm. 10% D_2O was added to the NMR solution to provide of lock signal. Raman data were recorded on a Thermo-Nicolet FT-RAMAN module instrument. Electrospray ionization (ESI) (positive) Mass spectra (MS) were recorded on a Perkin–Elmer/Mariner. pH values were measured using a Metrohm AG 9101 Herisau probe.

2.2. Solution studies

Citric acid. Solutions (0.1–1 M) of citric acid:Be were prepared in ratios (ligand:Be) of 2:1 (**1**), 1:1 (**2**), 1:2 (**3**), and 1:3 (**4**). The pH was adjusted with KOH in the range from 4 to 11. All Be compounds were soluble except **4** which precipitated as $(Be(OH)_2)$ as the pH was raised to pH 5.8. Raman data for these compounds were similar to that of free citric acid.

(**1**) ^9Be NMR (pH 9.0): δ 1.92, 3.42; (pH 7): δ 1.45, 2.01, 3.53; ^{13}C NMR (pH 9.0): δ 46.0–46.7 (CH_2, CA:Be), 48.8 (CH_2, CA), 49.8 (CH_2, CA:Be), 78.1 (CO^-, CA), 79.1–80.2 (C, CA:Be), 180.4–181.9 (CH_2COO^-, CA:Be), 182.1 (CH_2COO^-, CA), 184.6 (COO^-, CA), 186.5–187.1 (COO^-, CA:Be).

(**2**) ^9Be NMR (pH 9.7): δ 1.89, 3.56; (pH 7): δ 1.45, 1.91, 3.42; ^{13}C NMR (pH 9.7): δ 46.4–46.9 (CH_2, CA:Be), 48.8 (CH_2, CA), 49.8–50.1 (CH_2, CA:Be), 78.1 (CO^-, CA), 79.1–80.2 (C, CA:Be), 180.7–182.0 (CH_2COO^-, CA:Be), 182.2 (CH_2COO^-, CA), 184.6 (COO^-, CA), 186.5–187.1 (COO^-, CA:Be); ^{13}C NMR (pH 3.0): δ 41.8–42.7 (CH_2, CA:Be), 43.8 (CH_2, CA), 76.7–78.0 (C, CA:Be), 73.9 (C, CA), 173.6–174.0 (CH_2COO^-, CA:Be), 175.0 (CH_2COO^-, CA), 176.9–178.3 (CH_2COO^-, CA:Be), 178.9 (COO^-, CA), 181.3–182.9 (COO^-, CA:Be).

(**3**) ^9Be NMR (pH 9.3): δ 1.90, 3.52; (pH 7.2): δ 1.53, 1.91, 3.14; ^{13}C NMR (pH 9.3): δ 46.1–46.8 (CH_2,