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Protein and ligand enhanced dissolution of BeO at pH 7 ☆

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Dedicated to Robert J. Angelici.

Abstract

Be is a toxic metal used in both aerospace and defense industries. Lung exposure to Be can lead to a specific immune response called chronic beryllium disease (CBD). CBD has the unique characteristics that it can be triggered by very low level exposures, yet the onset of systems can be delayed from one to over 20 years. This variable delay in the onset of systems implies that a change in the local environment leads to dissolution and bio-availability of the particulate Be. We report here on the dissolution of the highly insoluble BeO in the presence of known Be ligands including the iron transport protein, transferrin, and the ubiquitous citric acid. The presence of ligands even at the 100 μM level led to dissolution of Be to levels that have been shown to cause immune response in both the blood and the lung. Dissolution occurred at pH 7 and was significantly enhanced in a 10 mM phosphate buffer.

Keywords: Beryllium; Dissolution; Protein; Citric acid; Transferrin

1. Introduction

The unique properties of beryllium make it desirable for many applications. Beryllium is lightweight, six times stronger than steel, has a high melting point (1278 °C), has a high heat absorption capacity, is nonmagnetic and corrosion resistant, among other desirable qualities [1]. Because of these qualities, beryllium is used in a number of industrial applications, such as missile systems in the defense industry, re-entry vehicles in the space industry, switches and relays in the computer industry, among many others. While industrially important, beryllium and beryllium containing materials are toxic and carcinogenic and processing of Be metal or Be oxide can result in worker exposure. A recent NIOSH report estimates that up to 125 000 workers in the government and private sectors have been potentially exposed to Be [2].

Occupational exposure to beryllium is a known health hazard, causing lesions in the skin, acute pneumonia, sarcoid-like granulomata in the lungs and other organs, as well as pulmonary fibrosis. Chronic beryllium disease (CBD), the most serious of these conditions, can lead to death in some exposed workers. This disease, which results from the cell-mediated immune response to inhaled Be has been studied for decades, however the mechanism for disease development and the factors which influence the progression of the disease are not well understood [3–5]. It is estimated that 1–20% of individuals that have inhaled beryllium develop CBD [5].

Inhalation of BeO and Be metal particles causes them to be deposited in the lungs. In order for the particles to trigger a cell-mediated immune response (CBD or Be sensitization), they must be dissolved to yield a Be species that can interact with the cellular immune system. A Be concentration as low as 1 μM can trigger the immune response-driven cell proliferation of T-cells in the Be-LPT screening for beryllium sensitization [6]. However, in the absence of ligands beryllium is highly insoluble and is known to precipitate as a polymeric hydroxide in water above pH 5.5

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[7]. Under these conditions, BeO would not be dissolved. It is necessary for Be to be complexed with ligands in order to become soluble under biological conditions, yet the interactions of ligands/proteins with particulate BeO remains unexplored. It is well known that very low levels of Be exposure can trigger CBD, and the delay of symptoms can vary from one to over 20 years. A fundamental understanding of how physiological conditions affect particulate dissolution and the kinetics of particle dissolution could help shed light on the initial mechanism of disease and begin to explain the odd combination of a low threshold to trigger disease with a highly variable onset time for symptoms.

It has been shown that ligands containing carboxylates with nearby alcohols or phenols in appropriate positions are able to bind Be under physiological conditions [7–9]. Based on earlier work in our group on Be binding modes with these classes of ligands, we chose to look at the ligand enhanced dissolution in the presence of citric acid (CA), 2,3-dihydroxybenzoic acid (DHBA), and apo transferrin (aTF). CA is abundant in the body and is known to solubilize beryllium across the entire pH range [10]. DHBA has a the highest measured binding constant for BeL complex and aTF, an iron transporting protein, has been shown to bind up to 12 atoms of Be per protein [11,12]. Be binding to these ligands has been well characterized and CA and aTF are physiologically relevant species that could be responsible for Be dissolution in the lungs. Recent reports suggest that the binding in aTF, DHBA and CA have a common factor in that the Be binds by displacing a strong hydrogen bond [12]. Previous biological studies with Be have used soluble stock solutions of $BeSO_4$ followed by pH adjustment. Although a great deal of work with Be solutions has been done in the past, little has been done with the solid form of Be that is the initial source of the immunological response in the lungs. CBD is a lung disease and occurs from a lung burden of solid Be containing particulates. Many of the industrial processes which have led to potential exposure used BeO so we chose to examine the dissolution of BeO. Previous work with particulates has been limited to a few reports on in studies with BeO and cells to demonstrate that cells can uptake the particulates by phagocytosis [13–16].

In this study, we compare the dissolution of BeO under biological conditions in the presence and absence of CA, DHBA, and aTF. Experiments were performed in HEPES buffer (10 mM, pH 7) and phosphate buffer (10 mM, pH 7) using the high fired BeO source of UOX from Brush Wellman.

2. Materials and methods

HEPES and aTF (Sigma–Aldrich), sodium phosphate (EM Science), CA and DHBA (Acros) were used as received. A beryllium stock solution was prepared by suspending BeO in Milli-Q water generated at 18 Ω. The suspension was accomplished by sonication of the stock solution for 60 min prior to removing aliquots. The concentration of the stock solution was determined to be 1440 ppm Be by the Berylliant fluorometric method [17]. HEPES and phosphate buffers were dissolved in Milli-Q water generated at 18 Ω, and then adjusted to pH 7. The concentration of both HEPES and phosphate buffers was 10 mM.

Four samples were prepared from each buffer (10 mM HEPES and 10 mM phosphate pH 7): one with buffer only, one with a 2:1 Be:CA ratio (1.1 mM Be:0.55 mM CA), one with a 2:1 Be:DHBA (1.1 mM Be:0.55 mM DHBA) ratio, and one with a 16:1 Be/aTF (1.1 mM Be:70 μM aTF) ratio. Initially the samples were prepared by adding the appropriate amount of each ligand to 50 mL of buffer. Then 5 mL were taken from each sample for ICP matrix blanks. Be from the stock solution was added to each sample (45 mL) to achieve 1.1 mM Be. The samples were continually rotated for the duration of the experiment, with the exception of sampling times.

Aliquots for analysis were taken from each sample at given time intervals and analyzed by ICP/AES to determine the amount of Be dissolved in solution. For sampling, the samples were removed from the rotator and centrifuged @3000 rpm for 30 min. A 2.5-mL aliquot of each sample was filtered through a 0.45 μm filter. The filtered sample was analyzed neat on a JY-Ulitma ICP-AES to determine Be concentration in solution in ppb. When no ligand was present in the case of the HEPES buffer, no soluble Be was detected over a 30 week period indicating that the centrifugation and filtering process does adequately remove the particulate BeO.

3. Results and discussion

Fig. 1 shows the increase in BeO dissolution over a period of 30 weeks for samples in HEPES buffer. In the

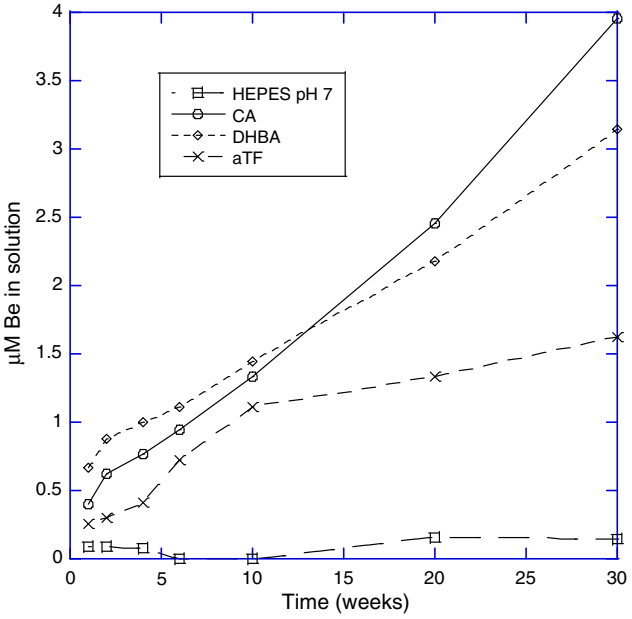

Fig. 1. Dissolution of BeO in pH 7 HEPES.