BBA - General Subjects 1862 (2018) 1760–1769

Contents lists available at ScienceDirect

BBA - General Subjects

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

Investigating the effect of ascorbate on the Fe(II)-catalyzed transformation of the poorly crystalline iron mineral ferrihydrite

Wei Xiao, Adele M. Jones, Richard N. Collins, T. David Waite*

UNSW Water Research Centre, School of Civil and Environmental Engineering, UNSW Australia, Sydney, NSW 2052, Australia

ARTICLE INFO

Keywords:
Ferritin
Pathological ferritin
Iron oxides
Iron
Fe(II)-catalyzed transformation
Ascorbic acid

ABSTRACT

The inorganic core of the iron storage protein, ferritin, is recognized as being analogous to the poorly crystalline iron mineral, ferrihydrite (Fh). Fh is also abundant in soils where it is central to the redox cycling of particular soil contaminants and trace elements. In geochemical circles, it is recognized that Fh can undergo Fe(II)-catalyzed transformation to form more crystalline iron minerals, vastly altering the reactivity of the iron oxide and, in some cases, the redox poise of the system. Of relevance to both geochemical and biological systems, we investigate here if the naturally occurring reducing agent, ascorbate, can effect such an Fe(II)-catalyzed transformation of Fh at 25 °C and circumneutral pH. The transformation of ferrihydrite to possible secondary Fe(III) mineralization products was quantified using Fourier transform infrared (FTIR) spectroscopy, with supporting data obtained using X-ray absorbance spectroscopy (XAS) and X-ray diffraction (XRD). Whilst the amount of Fe (II) formed in the presence of ascorbate has resulted in Fh transformation in previous studies, no transformation of Fh to more crystalline Fe(III) (oxyhydr)oxides was observed in this study. Further experiments indicated this was due to the ability of ascorbate to inhibit the formation of goethite, lepidocrocite and magnetite. The manner in which ascorbate associated with Fh was investigated using FTIR and total organic carbon (TOC) analysis. The majority of ascorbate was found to adsorb to the Fh surface under anoxic conditions but, under oxic conditions, ascorbate was initially adsorbed then became incorporated within the Fe(III) (oxyhydr)oxide structure (i.e., co-precipitated) over time.

1. Introduction

Ascorbate (or Vitamin C) is a vital antioxidant in the human body and is particularly abundant in the human brain [1]. The body is unable to synthesize ascorbate with humans mostly reliant on plant-derived ascorbate to meet metabolic requirements [1,2]. In addition to producing ascorbate, plants can exude ascorbate into the soil where it has been associated with the uptake of iron by plants due to its ability to induce the reductive dissolution of solid Fe(III) minerals to soluble Fe (II) species [3]. Even at circumneutral pH, ascorbate is able to reduce Fe (III) (oxyhydr)oxides (herein termed Fe(III) oxides) to aqueous Fe(II), despite the lower extent of ascorbate sorption to mineral surfaces relative to that at more acidic pHs [4,5]. Ascorbate has also been used as a proxy for other naturally occurring reductants that are recognized to induce the dissolution of Fe(III)-bearing minerals [6]. In the absence of inhibitors of Fe(III) oxide transformation [7,8], the presence of Fe(II) is recognized to catalyze the transformation of the poorly crystalline Fe (III) oxide, ferrihydrite (Fh), to more crystalline oxides such as goethite and lepidocrocite within hours if sufficient Fe(II) is available [8,9]. As such, there has been speculation that ascorbate may stimulate the transformation of Fh into more crystalline Fe(III) oxides at circumentral pH due to its ability to partially reduce Fh and form Fe(II) [10], but there has been no definitive study of this possibility. This is the focus of the study described herein.

Obtaining a better understanding of the interaction of ascorbate with Fh has potential relevance to biogeochemical redox processes in soils and the impact of these processes on contaminant dynamics. For example, as Fh transforms to more crystalline Fe(III) oxides, the reduction potential of these Fe(II)-Fe(III) oxide systems can decrease [11] thereby facilitating the reduction of a wider range of reducible contaminants and, potentially, increasing the reduction rate of readily reducible species [9,12,13]. Understanding the interaction of ascorbate with ferrihydrite also has relevance to possible transformation pathways of iron in the human body as recent studies indicate that the iron storage protein ferritin is structurally analogous to the inorganic Fe(III) oxide ferrihydrite that is ubiquitous in soils [14,15]. This is of particular interest as the transformation pathways of iron in the body are not well understood, although there is recent data and evidence to suggest that

* Corresponding author.
E-mail address: d.waite@unsw.edu.au (T.D. Waite).

https://doi.org/10.1016/j.bbagen.2018.05.005
Received 13 March 2018; Received in revised form 7 May 2018; Accepted 7 May 2018
Available online 09 May 2018

the Fe(III) oxides goethite, magnetite and maghemite form in the human brain [16,17]. There is also an increased interest in the relationship between pathological ferritin, increased dietary iron intake and accumulations in the brain, and a variety of neurodegenerative disorders associated with the formation of reactive oxygen species due to the formation and oxidation of poorly-chelated Fe(II) [18–26]. Transformation of ferrihydrite to other Fe mineral forms is one such avenue under investigation, as this may dramatically alter the redox properties of stored Fe in the body, and therefore its ability to produce neurotoxic reactive oxygen species [16,27,28], in a similar manner to that which occurs in the presence of Fe(III)-dopamine complexes when there is a deficiency of adenosine triphosphate (ATP) [29]. Indeed, previous studies have already found that β-amyloid proteins can reduce Fh and induce the formation of magnetite and maghemite in Alzheimer's disease (AD) afflicted plaques in the human brain [26,30].

To obtain further insight into the possible role of ascorbate in the transformation of Fh, mineralogical changes in Fh suspensions initiated by the partial reductive dissolution of Fh by a range of ascorbate concentrations under anoxic and oxic conditions were monitored in this study using Fourier transform infra-red spectroscopy (FTIR), X-ray absorption spectroscopy (XAS) and X-ray diffraction (XRD). The adsorption of ascorbate to Fh and the possible incorporation of ascorbate within Fh have also been investigated using total organic carbon (TOC) and FTIR measurements.

Previous studies of the interaction of ascorbate with ferrihydrite have largely focused on the reactivity of the iron oxide, with kinetic equations used to describe the reductive dissolution process [6,31]. Results of these studies demonstrate the distinctly higher reactivity of Fh towards reductive dissolution relative to more crystalline Fe(III) oxides such as goethite and lepidocrocite [6] with this higher reactivity largely due to the lower crystallinity and small grain size of Fh relative to other Fe(III) oxides. The study by Tan et al. [32] is the only one to date to investigate the ability of ascorbate to catalytically transform ferrihydrite (Fh) to another Fe(III) oxide (in this case, hematite (Hm)) with this study performed at 100 °C over pH 4 to 9 and for a reaction period of 10 h. Tan et al. [32] found that the concentration of ascorbate affects the shape of the Hm particles formed by differential surface adsorption onto particular crystal planes. The optimum pH for transformation was found to be pH 7 with the mechanism of action related to the formation of Fe(II) with this reduced iron species shown to catalyze the transformation of Fh to Hm at 100 °C in the absence of ascorbate [33,34]. In this study the nature of the Fe(III) oxides formed following reaction of ascorbate with Fh at room temperature (25 °C) at neutral pH over a 28-day period is examined.

2. Materials and methods

2.1. Chemicals and synthesis of Ferrihydrite

Ammonium hydroxide (NH_4OH, puriss. approx. 25% NH_3), ferric nitrate nonahydrate ($Fe(NO_3)_3.9H_2O$, ≥98%), ferrous chloride tetrahydrate ($FeCl_2.4H_20$, ≥99%), L-ascorbic acid (≥99%), potassium hydroxide (KOH, ≥90%), N,N,N′,N′-tetraethylethylenediamine (TEED, 98%), sodium hydroxide (≥98%), 1,10-phenanthroline (≥99%) and sodium fluoride (≥99%) were all purchased from Sigma-Aldrich (St Louis, MO, USA). Hydrochloric acid (37%) and nitric acid (65%), employed for solution preparation and/or glass- and plastic-ware cleaning, were obtained from Merck (Ajax Finechem Pty Ltd., MA USA). All working solutions and Fe(III) oxide suspensions were prepared using Milli-Q (Millipore Corp., MA, USA) water (resistivity > 18 MΩ·cm). Synthetic 2-line ferrihydrite (Fh) was prepared using the methodology outlined by Jones et al. [8]. A portion was freeze-dried for use as a reference with the remainder employed, undried, for transformation experiments within one week of preparation. As each new stock solution was found to completely dissolve in 0.1 M, pH 3 ascorbic acid over a period of 1 h when prepared to a final Fe(III) concentration of 10 mM Fe(III), the reactivity of the various stock solutions employed were considered comparable. The Fe(III) oxides chloride-green rust (Cl-GR), goethite (Gt), hematite (Hm), lepidocrocite (Lep) and maghemite (Mgh) were also prepared for use as reference standards using established synthetic methods [35]. Magnetite (Mt), for use as a reference standard, was purchased from Sigma-Aldrich (St Louis, MO, USA) as 50–100 nm Fe(II)Fe(III) oxide (≥97%). Gt, Lep and Mt were also synthesized in the presence or absence of ascorbic acid, with the details of the methods employed provided in Sections 2.4–2.6.

2.2. Experimental conditions and measurement of Fe(II)

All transformation experiments were conducted in 50 mL glass Wheaton serum bottles in a pH 7.0 buffered solution containing 50 mM of the non-complexing buffer N,N,N′,N′-tetraethylethylenediamine (TEED - $pK_a = 6.58$) [36]. The concentration of ferrihydrite was 10 mM of Fe(III) and ascorbate concentrations were between 0.1 and 5 mM. For experiments performed under anoxic conditions, the bottles were capped with a nitrile rubber stopper and all work was conducted in an anaerobic chamber (< 0.1 ppm O_2; UnilabPro MBraun) employing a 99.99% N_2 gas mixture (BOC gases, Australia) with aqueous solutions purged with argon gas for 2 h and stirred overnight in the chamber to < 0.1 $mg{\cdot}L^{-1}$ O_2 (HACH LDO II dissolved oxygen probe) before use. The pH of all buffered solutions was adjusted before and after degassing. Measurement of pH was undertaken using a Thermo Scientific Orion 8172BNWP ROSS glass combination pH probe calibrated with NIST compliant buffer solutions (pH 4.00 and 7.00 at 25 °C). For experiments performed under oxic conditions, the bottles were loosely covered with parafilm to prevent evaporation over time in a 25 °C temperature controlled lab under oxic conditions (i.e. not inside an anaerobic chamber). All suspensions were well-mixed during transformation using an orbital shaker (Thermoline WiseShake) at 180–200 rpm to ensure the particles remained well suspended.

At specific time-points, 1 mL samples of the uniformly suspended mixtures were removed and micro-centrifuged (10,000 rpm for 20 s; Biolab BCMI-505 centrifuge). The Fe(II) concentration in the supernatant was measured using the phenanthroline colorimetric method [37,38]. Following careful removal of all of the supernatant, 1 mL of 10 mM HCl was added to the remaining solid, mixed for 30 s on a vortex mixer and then micro-centrifuged as before. Any Fe(II) adsorbed to the particles was expected to de-sorb into the acid solution, with the Fe(II) concentration then quantified as previously mentioned [37,38]. Complete removal of any sorbed Fe(II) was confirmed as the total concentration of Fe(II) present (as determined from the sum of aqueous Fe (II) and sorbed Fe(II) concentrations) was equal to the total concentration of Fe(II) added to each suspension to within ± 5%. Following careful removal of all of the acidic supernatant, the Fe(III) oxide solid remaining was then rinsed again with de-aerated Milli-Q water and made up to 100 μL with MilliQ water. Quantification of the proportion of the various iron oxides present in this suspension was undertaken using Fourier transform infrared spectroscopy (FTIR). Additional 4 mL aliquots were collected at select time-points, rinsed as described previously and then dried to obtain sufficient solid mass for XAS and XRD quantification.

2.3. Methods to detect secondary Fe(III) mineralization products

FTIR measurements and quantification of particular Fe(III) oxides were undertaken on a PerkinElmer Frontier IR spectrophotometer using the method outlined by Xiao et al. [39], except that normalization of the spectra to an absorbance of 0 was carried out at 920 cm^{-1}. In brief, the 1 mL aliquot of suspension collected at each time point was centrifuged, rinsed multiple times with nanopure water and concentrated 10-fold to 100 uL. Once the background signal was collected, a 3 uL aliquot of this suspension was allowed to dry over a stream of nitrogen on a 3 mm diameter diamond/ZnSe crystal of a universal attenuated