Redox reactions of nitrite ions on the surface of colloidal magnetite particles coated with chondroitin sulfate

Abstract Colloidal magnetite particles coated with chondroitin sulfate (abbreviated as Fe/CS) were prepared under conditions of varying Fe(II) fractions at a fixed Fe concentration and a given concentration of CS. The average size of the magnetite core region was estimated as 7 nm from transmittance electron microscopy measurements, while the size of the Fe/CS particles ranged 155–175 nm, as estimated using Rayleigh scattering measurements by reference to a control size derived from the dynamic light scattering. The reaction of various Fe/CS with NO$_3^-$ in aqueous solutions was determined by fluorometry using 2,3-diaminonaphthalene as a probe and by gas chromatography–mass spectrometry. The concentrations of NO$_3^-$ in the reaction mixtures decreased in the presence of Fe/CS to a greater extent under Ar compared with aerobic conditions. The reactivity of Fe/CS toward NO$_2^-$ under aerobic conditions increased with decreasing the size of Fe/CS particles or with increasing content of Fe(III) in the Fe/CS solutions, but was independent of the Fe(II) fraction in the preparation process. While CS molecules had no influence on the NO$_3^-$ decomposition, those coated with the magnetite core may prevent the diffusion of NO$_3^-$ to be adsorbed on the core surfaces. NO$_3^-$ was concluded to undergo redox reactions with Fe(II) and Fe(III) located on the core surface of magnetite crystalline structures of Fe/CS.

Key words Colloidal magnetite particles · Chondroitin sulfate · Nitrite ions · Redox reactions · Core surface

Introduction

The redox reaction of nitrogen oxides (NO$_x$) has been extensively studied in relation not only to physiological action to cells, but also to air pollution produced by automobiles and factories, photochemical ozone formation and degradation [1], global nitrogen cycle by organisms involving a series of reductions as in (Eq. 1), etc.

$$\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2 \quad (1)$$

Particularly nitric oxide (NO) is well known to play important roles in vivo as a vasodilator, i.e., the endothelium-derived relaxation factor [2], neurotransmitter [3], inhibition of blood coagulation [4]. NO is oxidized in vivo to nitrites, which are harmful particularly to new-born babies, because their livers function insufficiently [5]. Nitrites are further oxidized to stable forms of nitrates in the liver. A large amount of NO production, however, is harmful and results in inflammation and infectious diseases, which cause cell death, apoptosis [6]. Moreover, NO and nitrites are associated with diseases such as sepsis [7, 8], Kawasaki disease to infants [9] and methemoglobinemia [10].

NO has been reported to bind with trivalent iron, (Fe(III), in nitrite reductase [11], methemoglobin [12],...
cytochrome P450s [13] and chelaters [14]. Recently, we prepared colloidal magnetite (Fe₃O₄) particles, which were coated with chondroitin sulfates [15]. Since magnetite in the crystal structure contains Fe(III) in the oxidized form, the reactivity of the colloidal magnetite particles toward NO is an interesting subject for investigation.

In this work, we studied the reaction with nitrite ions (NO₂⁻) instead of an unstable compound, NO. For characterizing the redox properties of colloidal magnetite particles coated with chondroitin sulfate (CS). It is noted that CS is one of the naturally occurring mucopolysaccharides and may therefore be less toxic toward living matter. In view of a potential application of the particles to contrast enhancers for magnetic resonance imaging, it is important to study the reaction between nitrites and colloidal magnetite particles.

**Experimental**

Preparation of colloidal magnetite particles coated with CS

Magnetite was produced by a coprecipitation method from aqueous solutions in various compositions of iron(II) chloride tetrahydrate [Fe(II)Cl₂·4H₂O, Wako Chemicals] and iron(III) chloride hexahydrate [Fe(III)Cl₃·6H₂O, Wako Chemicals] [16]. Both Fe(II) and Fe(III) ions in aqueous solution were fed at a fixed total concentration of 14 mM with varying mole fractions of Fe(II) from 11.4 to 57.5%. The oxidation of Fe(II) was prevented by bubbling Ar throughout the aging process of the colloidal magnetite particles.

A mixture of Fe(II) and Fe(III) was added to 5% CS C (Maruhana) with stirring at 80 °C under Ar. The mixture of iron and CS C was adjusted promptly to pH 11 by adding 5 N NaOH, subsequently to pH 8.0 by adding 5 N HCl, and further refluxed for 1 h under Ar for aging of the magnetite particles. The formation of magnetite is represented by

\[
\text{Fe}^{2+} + 2\text{Fe}^{3+} + 8\text{OH}^- \rightarrow \text{Fe₃O₄} + 4\text{H₂O}
\]  

After the aging process, precipitates of the reaction mixture were removed by centrifuging at 3000 g for 30 min. Supernatants filled in a cellulose membrane (Viskase, fractionation Mₜ, 12,000–14,000) were dialyzed for longer than 3 days against deionized water, which was replaced several times with fresh deionized water. The resulting CS coated magnetite (abbreviated as Fe/CS) particles were concentrated under reduced pressure. The color of the Fe/CS particles varied from brown to dark brown, depending on the composition of Fe(II) and Fe(III) in the preparation. The particles were stable in the colloidal state for longer than 2 years.

Measurement of the particle size of Fe/CS

The contents of Fe(II) in Fe/CS were determined by atomic absorption spectrometry (Shimadzu, MPS-2000). The particle size of Fe/CS (57.5%) containing 57.5% Fe(II) in the feed was estimated using a dynamic light scattering apparatus (Otsuka Electronics, DLS-7000). In addition, attempts were made to measure the sizes of the Fe/CS particles with a Coulter counter (Coulter Electronics, ZM). The core region within the Fe/CS, which appeared to consist of magnetite, was observed by transmission electron microscopy (TEM) (Hitachi, H-500) without staining.

Fluorometric assay of NO₂⁻ concentrations

The concentrations of NO₂⁻ were measured by fluorometry using a Hitachi F-2000 fluorescence spectrophotometer, using the reaction with 2,3-diaminonaphthalene (DAN) [17–19]. Treatment of a mixture (1.0 ml) of nitrite (1.0 μM) and Fe/CS (200 μM) in 10 μM tris(hydroxymethyl)aminomethane HCl at pH 7.6 with DAN (0.05 mg, 7.6 μM, 25 μl) in 6.2 N HCl formed 1-naphthotriazole as a fluorescent product. Aliquots of NaOH were added to the solution after the reaction for 10 min. The concentrations of 1-naphthotriazole thus formed were determined by fluorometry at 450 nm upon excitation at 365 nm, where unreacted DAN had no influence on the fluorescence intensity. The calibration curve of the fluorescence intensity was linear in the range 0.1–1.0 μM NO₂⁻.

The effect of Fe/CS on the decomposition of NO₂⁻ was compared with the effect of FeSO₄ (Wako Chemicals), Fe₃(SO₄)₂ (Wako Chemicals), Fe₃O₄ (Nakarai Tesq) and Fesin (Yoshitomi Medical, [Fe(OH)₃]ₐ[C₃H₂O₁₅]₂). CS sodium salt (Nakarai Tesq) was dissolved through a cellulose membrane (Nakarai Tesq, fractionation Mₜ, 8000) against deionized water, which was replaced three times every 48 h. The deionized water of 15 Ml/cm used throughout the measurements was obtained from a mega-pure-water purification system (Corning, MP-190).

Gas chromatography–mass spectrometry

The gas-phase composition of the reaction system of Fe/CS and NO₂⁻ under an Ar atmosphere was analyzed by gas-chromatography–mass spectrometry (GC-MS) (Shimadzu, GC-14A). Aliquots (2.0 ml) were collected with a syringe from the gas phase in contact with a reaction mixture of 10 mM Fe/CS (20.2%) and 0.1 mM NO₂⁻ incubated for 10 min and was analyzed by an electron impact method (GC-EIMS).

UV–vis absorption spectra

Commercially available Fe₃O₄ was ground sufficiently and suspended in 2 M H₂SO₄. The Fe₃O₄ suspension was filtered with a filter (Gelman Sciences, E031, 3 mm in diameter and 0.45 μm pore size) and measured with a UV–vis spectrophotometer (Shimadzu, MPS-2000).

In the measurement of 0.2 mM Fe/CS (20.2%) in 2 M H₂SO₄, a sheet of Teflon of 0.3-mm thickness was fixed on the side of the transmitted light of two quartz cuvettes, which led to scattering of both transmitted light and scattered light. Such an opal-glass method [20] resulted in more sensitive spectra against each wavelength despite reduced light transmittance. UV–vis absorption spectra of various Fe/CS particles in deionized water were measured in a similar manner.

**Results and discussion**

Reaction of Fe/CS with NO₂⁻

NO₂⁻ (1 μM) reacted with various Fe species (100 μM) in solution, colloid and suspension to decrease the concentration of NO₂⁻ in the reaction mixtures, as shown in Table 1. Both Fe(II) and Fe(III) appear to be responsible for the reaction with NO₂⁻ by both reduction and oxidation mechanisms. The reactivity was higher to some extent under anaerobic conditions than under aerobic conditions. Oxygen may regenerate NO₂⁻ in the suppression. Under anaerobic conditions of