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Notes

Binding Characteristics and Dissociation Kinetics for Iron(II) Complexes with Seawater Extractable Organic Matter and Humic Substances in a Compost

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A steel-slag/compost fertilizer can be useful in supplying complex Fe(II) species to barren coastal regions. Seawater extractable organic matter (SWEOM) was examined for use as a novel chelator of Fe(II) in the compost. The dissociation kinetics for Fe(II)-SWEOM were evaluated, based on the rate of ligand-exchange with *ortho*-phenanthroline. The $\Delta H^{\ddagger}$ for the Fe(II)-SWEOM (19 kJ mol^{-1}) was significantly smaller than the corresponding values for Fe(II) complexes with humic substances (27 kJ mol^{-1}), suggesting that the Fe(II)-SWEOM is kinetically less stable.

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Introduction

Areas with a lack of dissolved Fe species of in coastal seawater are referred to as barren ground, a situation in which coastal areas are no longer able to maintain the growth of seaweed.[1] Thus, the fertilization of barren coastal ground with soluble Fe is an important technique, in terms of restoring seaweed-beds. In coastal seawater in the presence of oxygen, Fe species are converted into insoluble Fe(III)-hydroxides, which result in the Fe no longer being available as a nutrient for the growth of seaweed. Therefore, soluble Fe species in seawater are present in the form of complexes with dissolved organic matter (DOM), such as humic substances that contain humic (HA) and fulvic (FA) acids. Based on these issues, a steel-slag/compost fertilizer was developed to supply dissolved Fe complexed with DOM to barren coastal areas, which resulted in the restoration of seaweed-beds.[2] In this technique, it is assumed that Fe species are eluted from the steel-slag *via* complexation with HA and/or FA.[2,3] However, it has been reported that HA and FA are flocculated at higher ionic strengths, like seawater.[4] This suggests that HA and FA in the compost cannot serve as carriers of Fe from the fertilizer. Thus, a seawater extractable organic matter (SWEOM) fraction should be the major DOM fraction from the compost. However, information related to the complexation of Fe with SWEOM from the compost does not appear to be readily available.

On the other hand, Fe(III)-oxides, such as hematite and magnetite, are found on the surface of steel slag.[5] Such Fe(III)-oxides can be reduced to soluble species in the presence of DOM, such as humic substances.[6] DOM is capable of reducing Fe(III), and all of the reduced Fe(II) becomes complexed with DOM.[7] Thus, in the case of fertilization using a steel slag/compost mixture, Fe(II)-DOM complex species would be expected to be the major Fe component. In terms of the stability of Fe(II)-DOM complexes, their dissociation kinetics as well as their well-known complexing abilities are important because of their impact on the lifetime of such species in the environment. The dissociation kinetics of metal complexes with HA and FA have been evaluated, based on the alteration of complexation equilibria: *e.g.*, pH changes,[8] the addition of competing cations,[9] and the removal of free metal species by chelation using resins or anodic stripping voltammetry.[10,11] *Ortho*-phenanthroline (OP) is a well-known colorimetric reagent that is used in the analysis of Fe(II).[12] Because OP produces a color change only when complexing with free species of Fe(II), this has been used in speciation analysis and evaluating the binding abilities of HAs to Fe(II).[13] If free Fe(II) would bind to OP in the presence of DOM, the Fe(II)-DOM complexes would undergo dissociation, and the formation of Fe(II)-OP complexes would be increased *via* ligand-exchange reactions. In the present study, Fe(II)-binding abilities and dissociation kinetics for Fe(II)-DOM complexes were evaluated by a colorimetric method using OP, and parameters for SWEOM were compared with the corresponding values for HA and FA fractions that were extracted from the same compost sample.

Experimental

Materials

A matured bark compost (Mori Industry Co., Ltd., Hokkaido, Japan) was freeze-dried, and particles smaller than 2 mm diameter were used in the tests. Artificial seawater was prepared by dissolving the following salts (g) in 1 kg of ultrapure water: NaCl, 28.5; $MgSO_4 \cdot 7H_2O$, 6.82; $MgCl_2 \cdot 6H_2O$, 5.16; $CaCl_2$, 1.11; KCl, 0.725; $SrCl_2 \cdot 6H_2O$, 0.024; NaBr, 0.084; H_3BO_3, 0.024. Prior to use in the test, the pH of the artificial seawater was adjusted to 8.1 using dilute aqueous NaOH.

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SWEOM

To separate the SWEOM from a seawater matrix, ultrafiltration and dialysis were employed. The equilibration period for the extraction with seawater was preliminary checked by monitoring the UV-vis absorption spectra and TOC, and 3 days of shaking were found to be sufficient. A mixture of dry compost and artificial seawater at pH 8.1 (solid/liquid = 1/10, w/w) was shaken under a N_2 atmosphere for 3 days. The suspension was then centrifuged at 10000 rpm for 15 min, and the supernatant was filtered through 5A filter paper (ADVANTEC). The filtrate was then deionized by ultrafiltration with an ultrafiltration membrane filter (molecular weight cut-off of 0.5 kDa, regenerated cellulose, Millipore). The retained fraction was washed with ultrapure water several times. The obtained fraction was then dialyzed against ultrapure water using a Spectra/Por® dialysis tube (molecular weight cut-off of 0.5 kDa). After dialysis, the fraction in the dialysis tube was freeze-dried, and a powdered sample of SWEOM was obtained.

HA and FA

The mixture of dry compost and 0.05 M aqueous NaOH (solid/liquid = 1:10, wt/wt) was shaken under a nitrogen atmosphere for 24 h. The HA and FA fractions in the extract were separated and purified, as described in previous reports.[14] The characteristics of SWEOM, HA and FA are summarized in Tables S1 and S2 of Supporting Information.

Complexometric titration

Stock solutions of DOM for SWEOM, HA and FA (1000 mg L^{-1}) were prepared by dissolving powdered samples in 0.05 M aqueous NaOH. A stock solution of Fe^{2+} (0.5 mM) was prepared by dissolving $FeSO_4(NH_4)_2SO_4{\cdot}6H_2O$ in a 0.02 M aqueous HCl solution. Buffer solutions (pH 3.6) were prepared by mixing 0.2 M aqueous acetic acid and sodium acetate. A 0 – 100 µL aliquot of the Fe^{2+} stock solution was mixed with the buffer solution (4.7 – 4.8 mL) including the DOM stock solution (200 µL) in a 15-mL glass tube, and this was incubated at 25°C for 24 h in the dark. After adding a 100-µL aliquot of aqueous OP (5 mM) and allowing the solution to stand for 30 min, the absorbance at 510 nm was measured using a V-630 type UV-vis spectrometer (Japan Spectroscopic Co., Ltd.).

Dissociation kinetics

A 4.8-mL aliquot of buffer solution including DOM (20 mg L^{-1}) was placed to a 10-mL glass tube, and a 100-µL of aliquot of the Fe^{2+} stock solution was then added. This mixture was incubated for 24 h at 10, 20, 30 and 40°C. After adding a 100-µL of OP (5 mM) and allowing the solution to stand for 30 min, the absorbance at 510 nm was measured. The initial concentration of Fe(II)-DOM complex was determined from the total and free concentrations of Fe(II) in that time. After 2-, 4-, 6- or 8-h incubation periods at 10, 20, 30 or 40°C, the absorbance values were measured at 510 nm, and the concentrations of the Fe(II)-DOM complexes calculated for each incubation period.

Results and Discussion

In the presence of Fe^{2+} and DOM, such as HA, FA and SWEOM, the OP binds to free Fe(II), but not to Fe(II)-DOM complexes.[13] Such properties of OP can be applied to complexometric titrations to evaluate the Fe(II)-binding abilities of DOM.[13] The colored species of Fe(II)-OP is a complex between Fe^{2+} and OP in a 1:3 molar ratio. Under the optimized conditions, the 1:3 complexes are the dominant species.[12] Thus, Fe(II)-OP in this study denotes 1:3 complexes between Fe(II) and OP. Assuming a 1:1 molar ratio for the complexation between Fe^{2+} and an arbitrary binding site in DOM (DOM_i), the conditional binding constant (K') can be defined as

$$K' = \frac{[\text{Fe(II)-DOM}_i]}{[\text{Fe}^{2+}][\text{DOM}_i]}. \quad (1)$$

For complexometric titration, the total Fe^{2+} concentration was varied from 0 to 10 µM at a constant concentration of DOM (20 mg L^{-1}), and [Fe^{2+}] was colorimetrically determined using OP. Thus, the total concentration of complex species ($\sum$[Fe(II)-DOM_i]) can be calculated by subtracting [Fe^{2+}] from the total concentration of Fe^{2+}. The total concentration of the binding sites to Fe(II) in DOM (C_{DOM}) can be expressed as: $C_{DOM} = \sum[DOM_i] + \sum[\text{Fe(II)-DOM}_i]$. Combining this with Eq. (1), the relationship between [Fe^{2+}] and $\sum$[Fe(II)-DOM_i] can be derived as follow:

$$\sum[\text{Fe(II)-DOM}_i] = \frac{C_{DOM}K'[\text{Fe}^{2+}]}{1+K'[\text{Fe}^{2+}]}. \quad (2)$$

A non-linear least-squares curve-curve fitting of the experimental data set for [Fe^{2+}] and $\sum$[Fe(II)-DOM_i] to Eq. (3) resulted in estimated K' and C_{DOM} values. The binding capacity of DOM to Fe(II) (N) can be calculated as:[3]

$$N = \frac{C_{DOM}}{[\text{DOM(g L}^{-1})]\times\%\text{C}}\times 100. \quad (3)$$

The estimated log K' and N values for the DOM are summarized in Table 1. In the present study, the acetate buffer was used to adjust the pH, which may have affected the complexation equilibrium between Fe(II) and DOM. However, the stability constants for Fe(II)-acetate (K = 0.54 – 1.40)[15] were much smaller than those for Fe(II)-DOM complexes (K = 100000 – 430000), indicating that the influences of Fe(II)-acetate formation would be negligible. The log K' values for SWEOM and FA were significantly larger than that for HA, suggesting that the stability of the complex produced between Fe(II) and SWEOM is comparable to that with FA. However, the N value for SWEOM was significantly smaller than those for HA and FA, which was consistent with the low value for the cation exchange capacity in SWEOM (Table S1).

Fe(II)-DOM complexes can be dissociated in the presence of a strong chelator, such as OP. The dissociation of the Fe(II)-DOM complex may proceed *via* the formation of a ternary complex (OP-Fe(II)-DOM), analogous to that reported for metal-OP complexes,[16]

Table 1 Binding characteristics and activation parameters[a] for Fe(II) complexes with HA, FA and SWEOM

Sample	Binding abilitiy		Activation parameter		
	log K'	N/ µmol g^{-1} C	$\Delta H^{\ddagger}$/ kJ mol^{-1}	$\Delta S^{\ddagger}$/ J K^{-1} mol^{-1}	$\Delta G^{\ddagger b}$/ kJ mol^{-1}
SWEOM	5.63 ± 0.15	80.0 ± 7.9	18.8 ± 1.1	–267 ± 4	94.3 ± 2.2
HA	5.01 ± 0.26	235 ± 62	26.6 ± 3.4	–243 ± 11	95.5 ± 6.6
FA	5.39 ± 0.10	112 ± 10	27.0 ± 1.6	–236 ± 5	93.8 ± 3.1

a. Each analysis was conducted 5 – 10 times. "±" represents standard deviation.
b. $\Delta G^{\ddagger}$ values were evaluated at 283 K.

$$\text{Fe(II)-DOM} + \text{OP} \rightleftharpoons [\text{OP-Fe(II)-DOM}]^{\ddagger} \rightleftharpoons \text{DOM} + \text{Fe(II)-OP} \quad (4)$$

The dissociation kinetics of the Fe(II)-DOM complexes were monitored by an increase in the Fe(II)-OP complex content during reaction (4). Therefore, the pseudo-first-order rate constant (k_{obs}) was evaluated based on a decrease of $\sum$[Fe(II)-DOM$_i$], as described in by

$$\ln \frac{\sum[\text{Fe(II)-DOM}_i]_{t=t}}{\sum[\text{Fe(II)-DOM}_i]_{t=0}} = k_{obs} \times t, \quad (5)$$

where t represents the incubation time after adding OP. In reaction (4), the tentative species, [OP-Fe(II)-DOM]‡, can be regarded as an activated complex that is produced during the dissociation of Fe(II)-DOM to form Fe(II)-OP *via* a ligand-exchange. To evaluate the activation parameters for reaction (4), the temperature dependence on k_{obs} was investigated in the range of 10 – 40°C. The free energy of activation, $\Delta G^{\ddagger}$, can be expressed as[7]

$$k_{obs} = \frac{k_B T}{h} \exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right), \quad (6)$$

where h, k_B, T and R are Planck's constant, the Boltzmann constant, the absolute temperature and the universal gas constant, respectively. From Eq. (6), $\Delta G^{\ddagger}$ can be evaluated using k_{obs} at an arbitrary temperature. Since $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$, Eq. (6) can be rearranged by using the activation enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$),

$$\ln \frac{k_{obs}}{T} = \ln \frac{k_B}{h} + \frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{R} \times \frac{1}{T}. \quad (7)$$

From the linear relationships between ln k_{obs}/T and $1/T$ for each Fe(II)-DOM complex (Eyring plots), $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ were evaluated. The evaluated $\Delta G^{\ddagger}$ (283 K), $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are summarized in Table 1. In general, $\Delta S^{\ddagger}$ provides information on the stability of activated complex species.[17] The levels of $\Delta G^{\ddagger}$ and $\Delta S^{\ddagger}$ were similar for Fe(II) complexes with HA, FA and SWEOM. These results suggest that the reaction intermediate for the dissociation of Fe(II)-DOM complex *via* a ligand-exchange with OP is the ternary complex, OP-Fe(II)-DOM, and its stability is similar for the HA, FA and SWEOM ligands. However, the $\Delta H^{\ddagger}$ value for the Fe(II)-SWEOM complex was significantly smaller than those for Fe(II) complexes with HA and FA. $\Delta H^{\ddagger}$ can be regarded as the energy for dissociation of the activated complex, in which the smaller $\Delta H^{\ddagger}$, the more dissociable is the complex. Therefore, the smaller $\Delta H^{\ddagger}$ for SWEOM indicates that the Fe(II)-SWEOM complex is more dissociable and exchangeable than Fe(II) complexes with HA and FA.

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Supporting Information

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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