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Stabilities of Metal Complexes of Mugineic Acids and Their Specific Affinities for Iron(III)

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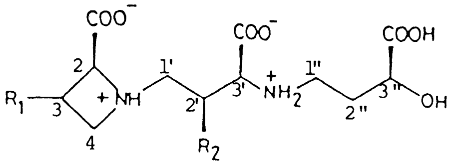
Equilibria between several metal ions and mugineic acids, phytosiderophores in gramineous plants, were potentiometrically investigated in aqueous solutions and the stabilities of the complex species formed were determined. Only iron(III) formed very stable species with the quadrivalent anions of mugineic acids, and their stability constants were estimated to be $10^{32.5}$~$10^{33.3}$.



epi-HMA $R_1=R_2=OH$
MA $R_1=H$, $R_2=OH$
DMA $R_1=R_2=H$

Iron is one of the essential metals for living organisms. Low molecular-weight iron-chelators, termed "phytosiderophore", occur in gramineous plants, and are involved in the acquisition of this essential metal from soils. Typical phytosiderophores are mugineic acid (MA), 2(S),2'(S),3'(S),3"(S)-N-[3-carboxy-(3-carboxy-3-hydroxypropylamino)-2-hydroxypropyl]-azetidine-2-carboxylic acid, and its analogs, 3-epi-hydroxymugineic acid(epi-HMA) and 2'-deoxymugineic acid(DMA), which have been isolated from root-washings of barley and wheat cultivars. These mugineic acids are a poly-functionalized sort of amino acid with amino, carboxyl, and hydroxyl groups.

The structural, physicochemical, and biochemical properties of mugineic acids have been reported and reviewed.[1,2] It has been suggested that, as compared with microbial siderophores, MA has a much smaller affinity for iron(III) ion, while having very high efficiency in iron-extraction from high pH soils.[3] So far reported little is known about the properties of epi-HMA and DMA as a chelator, that is, about the influence of the 3-OH introduction into MA or the 2'-OH elimination from MA on the chelating ability. We have potentiometrically studied the equilibria between the mugineic acids and metal ions including iron(III) and several bivalent metal ions in aqueous solutions, and determined the stability constants of the complex species formed.

Mugineic acids used, epi-HMA, MA, and DMA, were isolated from root-washings of iron-deficient water-cultured barley cultivars, Tochigi-goldenmelon, Minorimugi, and Rikuzenmugi, respectively, and purified by means of column chromatographies.[4)] The potentiometrical titrations were carried out for the solutions(1~ 2x10^{-3} mol/dm^3) with ligand(mugineic acids) only and with both of metal ion and ligand in a 1:1 molar ratio at 25.0±0.1 °C and I=0.1 mol/dm^3(KNO_3) under moistened N_2. The apparent ion product of water(pKw') and apparent proton activity(y_H) were determined to be 14.028 and 0.87, respectively. The metal ion concentrations were standardized by means of atomic absorption spectrometry(Mn^{2+}, Al^{3+}) and chelatometry with edta(Fe^{3+}, Fe^{2+}, Ca^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}).

The proton-dissociation constants for these mugineic acids were evaluated with a least-squares process of the titration data(Table 1). The value of Ka_1, corresponding to the dissociation in 3"-COOH, is almost the same among epi-HMA, MA, and DMA, which indicates that both of 3- and 2'-OH have no influence on this dissociation, probably due to their remote positions from 3"-COOH. However, these OH groups affect the second and third proton-dissociations in the amino groups: in particular, the tertiary ammonium group in the azetidine ring of epi-HMA is made much acidic by the electron-withdrawing property of the OH groups.

Table 1. Proton-dissociation constants[a)]

	pKa_1	pKa_2	pKa_3
epi-HMA	3.226(5)	7.10(1)	9.62(1)
MA	3.231(3)	7.94(1)	9.85(2)
DMA	3.193(4)	8.25(2)	10.00(1)

a)Values in parentheses denote errors in the last figures.

The equilibrium constants, β_{pqr}, defined by Eq. 1 were determined with the computational process of the titration data for the 1:1 systems using SUPERQUAD,[5)] a non-linear least-squares program(charges are omitted for simplicity, but it should be noted that L represents the tervalent anions of the mugineic acids):

$$pM + qL + rH = M_pL_qH_r, \qquad \beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r \qquad (1)$$

where p, q, and r are the numbers of metal ion, mugineic acid, and proton, respectively, in the complex, $M_pL_qH_r$.

Figure 1 shows the titration curves for the M^{n+}-MA systems, and each curve is almost identical with the corresponding one for the epi-HMA and DMA systems. The proton-release from the ligand almost completes at a=3 in the presence of a bivalent metal ion, while, in the cases of Fe^{3+} and Al^{3+}, another proton is released. In particular, the exceptional lowering of pH in the presence of Fe^{3+} indicates the formation of the very stable species, $[Fe^{III}LH_{-1}]^-$, where LH_{-1} represents the quadrivalent anions of mugineic acids.

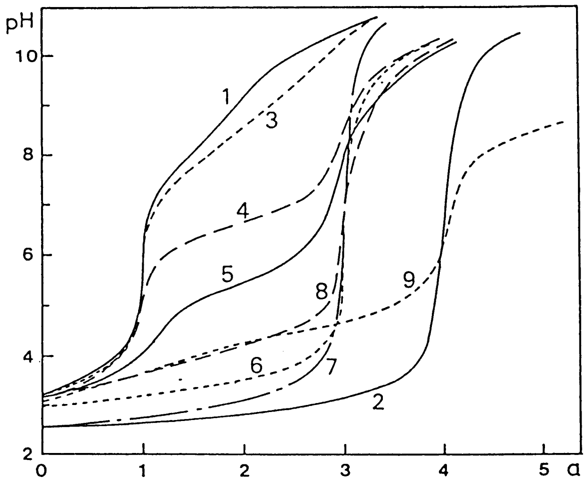


Fig. 1. Titration curves of the M^{n+}-MA systems.
1:MA only, 2:Fe^{3+}, 3:Ca^{2+}, 4:Mn^{2+}, 5:Fe^{2+}, 6:Ni^{2+}, 7:Cu^{2+}, 8:Zn^{2+}, 9:Al^{3+}. a is moles of NaOH added per mole of ligand.

As Table 2 shows, for the Fe^{3+} systems with MA and DMA, the two constants, β_{110} and β_{11-1}, represented by Eqs. 2 and 3, respectively, were determined(charges are omitted):

$$Fe + L = FeL, \qquad \beta_{110} = [FeL]/[Fe][L] \qquad (2)$$

$$Fe + L + H_{-1} = FeLH_{-1}, \qquad \beta_{11-1} = [FeLH_{-1}]/[Fe][L][H]^{-1} \qquad (3)$$

where β_{110} is equal to K_{FeL}, the stability constant of the $[Fe^{III}L]$ species. For the Fe^{3+}-epi-HMA system, however, only β_{11-1} was left to be determined during the computational refinement of the titration data: in the pH range of the titration only the [11-1] species($[Fe^{III}LH_{-1}]^-$), in which the three numbers express p, q, and r in $M_pL_qH_r$, is formed, but no [110] species($[Fe^{III}L]$) presences as shown in Fig. 2. Similarly in the Fe^{3+}-MA and -DMA systems, the [11-1] species occupies already high ratios before NaOH is added, and its distribution leads to 100% at about pH 4.

Table 2. β_{pqr} values for the Fe^{3+} species$^{a)}$

	$\log\beta_{110}$	$\log\beta_{11-1}$
epi-HMA	-	15.49(2)
MA	17.71(2)	15.39(1)
DMA	18.38(3)	16.25(1)

a)Values in parentheses denote standard deviations in the last figures.

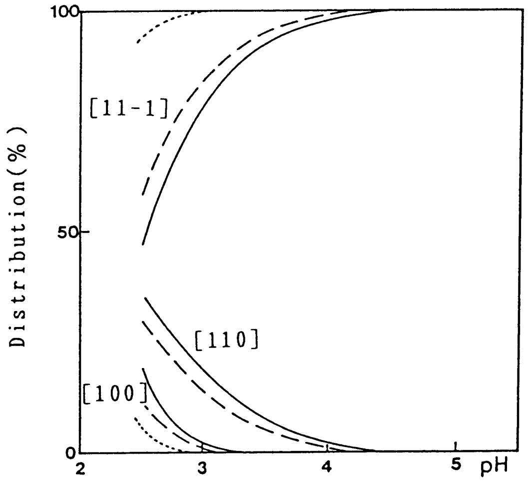


Fig. 2. Species distributins of the Fe^{3+} complexes.
——— MA, -------- epi-HMA, — — — DMA

Thus, for the discussion about the complex formation between iron(III) and mugineic acids, the stability of the $[Fe^{III}LH_{-1}]^-$-type species, not of the $[Fe^{III}L]$-type, is essential. The stability constant of $[Fe^{III}LH_{-1}]^-$ is expressed by Eq. 4:

$$Fe + LH_{-1} = FeLH_{-1}, \qquad K_{FeLH_{-1}} = [FeLH_{-1}]/[Fe][LH_{-1}] \qquad (4)$$

The forth proton to be released in these mugineic acids is assigned to 3"-OH, since it has been confirmed by an X-ray crystallography that, in the cobalt(III) complex of MA, this hydroxyl group(3"-OH) is deprotonated and quadrivalently anionic MA acts as a hexadentate chelator.$^{6)}$

This forth proton-dissociation constant is expressed by Eq. 5, and then $K_{FeLH_{-1}}$ is related to β_{11-1} by Eq. 6.

$$L = LH_{-1} + H, \qquad Ka_4 = [LH_{-1}][H]/[L] \qquad (5)$$

$$K_{FeLH_{-1}} = \beta_{11-1}/Ka_4 \qquad (6)$$

In general, the alcoholic proton is hardly released and in some cases its dissociation constant is too small to be determined experimentally. Although it is the case for the mugineic acids, the Ka_4 value for 3"-OH is estimated to be $10^{-17.1}$ by using the Taft equation, $pKa=15.9-1.42\Sigma\sigma^*$.$^{7,8)}$ Then, we can estimate the stability constant, $K_{FeLH_{-1}}$ (Eq. 4), to be $10^{32.5}$~$10^{33.3}$. This is as large as or larger than those of the iron(III) complexes with hydroxamate-type siderophores such as desferriferrichrome($K=10^{29.1}$) and desferriferrioxamine B($10^{30.6}$).$^{9)}$ Although aluminum(III) is thought to form similar anionic complexes, $[Al^{III}LH_{-1}]^-$, from Fig. 1, their stabilities were estimated to be less than those of the iron(III) complexes by 10^6-10^8.

The main complex species with bivalent metal ions are $[M^{II}L]$, and the species containing the quadrivalent anions of mugineic acids are not formed, or unstable if any, in contrast with the case of Fe^{3+}. Table 3 shows the stability constants for [M^{II}L]$^{-}$, K_{ML}(=β_{110}), most of which are in fair agreement with the values evaluated from the classical Bjerrum's plot of the same titration data, or from the competitive reaction method using tris(2-aminoethyl)amine(tren).

The order of the stability, $Ca^{2+}<Mn^{2+}<Fe^{2+}<Ni^{2+}<Cu^{2+}>Zn^{2+}$, holds for the three mugineic acids, and it is consistent with the so-called "natural order of stability". Except the complexes of Ca^{2+}, the stabilities seem to be increased as the ligand-basicity is increased, that is, epi-HMA<MA<DMA.

It has been revealed from the X-ray crystal structure of the copper(II) complex with MA that bivalently anionic MA coordinates in a hexadentate fashion to form a distorted octahedral geometry, in which the 3'-carboxyl and 3"-hydroxyl groups weakly bind at the pseudo-apical positions of the copper with their protonated forms.10) In aqueous solutions, however, these mugineic acids act probably as quinquedentate chelators for the bivalent metal ions, as a result of the deprotonation of the 3'-carboxyl group.

Table 3. Stability constants of [M^{II}L]$^{-}$ in $\log K_{ML}$(=$\log\beta_{110}$)$^{a,b)}$

	epi-HMA	MA	DMA
Ca^{2+}	4.31(1)	3.81(1)	3.34(2)
Mn^{2+}	8.03(1)	8.30(4)	8.29(2)
Fe^{2+}	10.02(8) [10.15]	10.14(6) [10.23]	10.45(4) [10.49]
Ni^{2+}	14.387(4) [14.41]	14.92(1) [14.72]	14.78(1) [14.60]
Cu^{2+}	17.92(1) [17.70]$^{c)}$	18.101(4) [17.99]$^{c)}$	18.70(1)
Zn^{2+}	12.437(4) [12.43]	12.692(2) [12.65]	12.842(6) [12.82]

a) Values in parentheses denote standard deviations in the last figures.

b) Values in brackets estimated by the Bjerrum's plot.

c) By the competitive reaction using Ca^{2+} and tren.

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