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Research Article

Behavior of Ascorbic Acid and Biological Systems, a Study of Interactions in Cu(II) Binary and Ternary Complexes in Aqueous Solution

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ABSTRACT

The acidity and stability constants of M(Asc)[1] M: Cu^{2+}, $Cu(Bpy^{2})^{2+}$, and $Cu(Phen^{3})^{2+}$ complexes, were determined by potentiometric pH titration. It is shown that the stability of the binary Cu(L), (L: Asc) complex is determined by the basicity of the enediol group. It is demonstrated that the equilibrium, $Cu(Har^{4})^{2+} + Cu(L) \rightleftharpoons Cu(Har)(L) + Cu^{2+}$, is displacement due to the well-known experience that mixed ligand complexes formed by a divalent 3d ion, a heteroaromatic N base and an O donor ligand possess no increased stability. The stability constants of the 1:1 complexes formed between Cu^{2+}, $Cu(Bpy)^{2+}$ or $Cu(Phen)^{2+}$ and L^{2-}, were determined by potentiometric pH titration in aqueous solution (I = 0.1 M, $NaNO_3$, 25°C). The order of the stability constants was reported. A comparative investigation between ternary complexes of Asc is made. The comparison of stability constants of these ternary complexes show that Cu(Har)(Asc) is found near 100% in ternary form. The difference between stability constants show that mixed ligand complexes formed by a divalent 3d ion, a heteroaromatic N base and an O donor ligand possess no increased stability. This means that there is no interaction between $Cu(Har)^{2+}$ and Asc^{2-} and no increased stability were observed in complex building of ternary systems.

KEYWORDS: Ascorbic acid, divalent metal ions, potentiometric titration, acidity and stability constants.

[1]Asc: Ascorbic acid
[2]Bpy: 2,2'-Bipyridyl
[3]Phen: 1,10-phenanthroline
[4]Har: Heteroaromatic ligand such as Bpy or Phen

1. INTRODUCTION

Ascorbate may also act as an antioxidant against oxidative stress [1-4]. However, the fact that the enantiomer D-ascorbate (not found in nature) has identical antioxidant activity to L-ascorbate, yet far less vitamin activity, [5] underscores the fact that most of the function of L-ascorbate as a vitamin relies not on its antioxidant properties, but upon enzymic reactions that are stereospecific.

"Ascorbate" without the letter for the enantiomeric form is always presumed to be the chemical L-ascorbate. Relatively large doses of ascorbic acid may cause indigestion, particularly when taken on an empty stomach. Although ascorbic acid does not have the "classic" acidic functional carboxylic, sulfonic or phosphonic acid, it is considerably acidic. With a pK_a of 4.03 (this work), 4.25 [1,6] it is more acidic than acetic acid with pK_a = 4.8. This is lower than physiological conditions as ascorbate anion Asch. This is partly due to the enediol structure. Enols are already significantly more acidic than alcohols. In addition, the acidity is enhanced by ascorbic acid in the second enolic hydroxyl group and by the adjacent carbonyl group (Fig.1).

Now it is interesting to investigate the complex building of ternary systems with Asc. We would like to determine the thermodynamic constants of ternary complexes such as Cu(Har)(L). This kind of structure of L complex can show new aspect of L's properties in biological systems.

2. EXPERIMENTAL

2.1. Materials

Chemicals were purchased from Merck. Copper(II) nitrate trihydrated, sodium nitrate, potassium hydrogen phthalate, L-ascorbic acid and standard solutions of sodium hydroxide (titrasol), 2,2'-bipyridyl, 1,10-phenanthroline, nitric acid, EDTA and of the buffer solutions of pH 4.0, 7.0 and 9.0 were from Merck. All the starting materials were pro analysis and used without further purification. Water was purified by Mili-Q water purification system, deionized and distillated.

2.2. pH titrations

Reagents: Carbonate-free sodium hydroxide 0.03 M was preparated and standardized against sodium hydrogen phthalate and a standard solution of nitric acid 0.5 mM. Copper (II) nitrate solution (0.03 M) was prepared by dissolving the above substance in water and was standardized with standard solution of EDTA 0.1 M (triplex).

2.3. Apparatus

All pH titrations was performed using a Metrohm 794 basic automatic titrator (Titrino), coupled with a Hero thermostating bath at 25°C (±0.1°C) and a Metrohm combined glass electrode (Ag/AgCl). The pH meter was calibrated with Merck standard buffer solutions (4.0, 7.0 and 9.0).

2.4. Procedure

For the determination of acid dissociation constants of the ligand Asc an aqueous solution (0.3 mM) of the protonated ligand was titrated with 0.03 M NaOH at 25°C under nitrogen atmosphere and ionic strength of 0.1 M, $NaNO_3$. For the determination of binary (one ligand and Cu^{2+}) and ternary systems (Cu^{2+}, one of the other L ligand (Har) and Asc), the ratios used were 1:1, 1:2 Cu(II) : ligand and 1:1:1, Cu(II) : Asc : Har, 0.3 mM. This solution was titrated with 0.03 M NaOH under the same conditions mentioned above. Each titration was repeated seven times in order to check the reproducibility of the data.

Calculation

The acid dissociation constants, $K^{H}_{H_2(Asc)}$ and $K^{H}_{H(Asc)}$ for H_2(Asc) were calculated by an algebraic method. The equilibria involved in the formation of 1:1 complex of Asc and a divalent metal ion may be expressed as equations (3) & (4).

3. RESULTS AND DISCUSSION

In this section we would discuss the resulted data.

3.1. Acidity constants

Ascorbate (Asc^{2-}) can accept one proton on enediol group, for which the following deprotonation equilibria hold:

$$H_2(Asc) \rightleftharpoons H^+ + H(Asc)^- \quad (1a)$$

$$K^{H}_{H_2(Asc)} = [H(Asc)^-][H^+]/[H_2(Asc)] \quad (1b)$$

Asc can release one other proton from amine group according following deprotonation equilibria:

$$H(Asc)^- \rightleftharpoons H^+ + Asc^{2-} \quad (2a)$$

$$K^{H}_{H(Asc)} = [Asc^{2-}][H^+]/[H(Asc)^-] \quad (2b)$$

The two protons in H_2(Asc) are released from $C_6H_8O_6$ according to equilibrium (1) & (2). It is

also closed to the de-protonation of enediol which occurs at the terminal groups of related ascorbic acids [7,8]. Asc can release the first proton from the enediol group. Hence, here due addition to equilibrium (1) should be considered, which takes place near a pH≈ 4 (tab. 1). The second proton can release from the same enediol group (equilibrium (2)).

3.2. Stability of binary and ternary complexes

If we abbreviate for simplicity Cu^{2+}, $Cu(Bpy)^{2+}$, and $Cu(Phen)^{2+}$ with M^{2+}, one may write the following two equilibria (3) & (4):

$$M^{2+} + H(Asc)^{-} \rightleftharpoons M(H;Asc)^{+} \quad (3a)$$

$$K^{M}_{M(H;Asc)} = [M(H;Asc)^{+}]/[M^{2+}][H(Asc)^{-}] \quad (3b)$$

$$M^{2+} + (Asc)^{2-} \rightleftharpoons M(Asc) \quad (4a)$$

$$K^{M}_{M(Asc)} = [M(Asc)]/[M^{2+}][Asc^{2-}] \quad (4b)$$

The experimental data of the potentiometric pH titrations may be completely by considering the above mentioned equilibria (1) through (4), if the evaluation is not carried into the pH range where hyrdoxo complex formation occurs.

The stability of ternary complexes may be evaluated by the following equilibrium:

$$mA + nB + qM + rH \rightleftharpoons A_mB_nM_qH_r \quad (5a)$$

where M is the metal ion, H is the proton, A and B are the ligand. The global stability constant for the ternary complexes may be represented as following:

$$\log\beta_{pqrs} = [A_mB_nM_qH_r]/[A]^m[B]^n[M]^q[H]^r \quad (5b)$$

It is possible to define the stability constants for ternary complexes in relation to their binary ones [9], represented by the equilibrium (6) & (7).

$$M+L_1 \rightleftharpoons ML_1 \quad (6a)$$

$$K^{M}_{M(L_1)} = [ML_1]/[M^{2+}][L_1] \quad (6b)$$

$$ML_1 + L_2 \rightleftharpoons ML_1L_2 \quad (7a)$$

$$K^{M}_{M(L_1L_2)} = [ML_1L_2]/[ML_1][L_2] \quad (7b)$$

Differences between the stability constants of the ternary and binary complexes show the tendency of the formation of ternary species [10]. This could be expected by Eq. (8):

$$\Delta\log K = \log K^{ML_1}_{M(L_1L_2)} - \log K^{M}_{M(L_2)} \quad (8)$$

$$= \log K^{ML_2}_{M(L_1L_2)} - \log K^{M}_{M(L_1)}$$

The difference between the constant refined from experimental data and those calculated statistically using Eq. (8) indicates the possibility of ligand-ligand interaction.

3.3. Potentiometric analyses

The enediol structure due to the reducing (antioxidant) properties of ascorbic acid as enediols can be easily oxidized to diketones. Enediols with adjacent carbonyl group are called, also reductones. Equilibrium reactions of ascorbic acid H_2(Asc). This can gradually give up electrons and protons, and finally to the oxidized form (Asc), dehydroascorbic acid (DHA), reach. This is in aqueous solutions as a monohydrate. The other enolic hydroxyl group has only weak acidic properties pK_a= 10.95 (this work), (pK_a= 11.79 [1]), since the anion less resonance structures can be formed to stabilize. Following the submission of both protons arising from ascorbic acid dianion (Asc^{2-}). The intermediate form, which is caused by loss of an electron and a proton H(Asc.), Is a very strong acid (pK_a = –0.45) [1]. The acid radical-ion of ascorbic acid is called ascorbate [11]. It is produced by transfer of a hydrogen ion (H + proton) to a protonated solvent such as water. The reaction is an equilibrium reaction: Ascorbic acid is a strong reducing agent in aqueous solutions. This may be oxidized to dehydroascorbic via intermediates (DHA).

The model of species for this ternary system that was used in superquad program includes all the species of table 1 as well as the hydrolysis of Cu^{2+} [11,12]. The stability constants of the binary complexes were refined separately using the titration data of this system in a 1:1 and 1:2 ligand:Cu^{2+} ratio in the same conditions of

temperature and ionic strength. An important point is that our experimental received results regarding to stability constant of binary complex Cu(Asc) is much higher than in earlier work [10]. They were fixed and, consequently, only ternary species were refined in ternary model of the species. The results are summarized in Table 1. The order of the resulted stability constants are Cu^{2+} = $Cu(Bpy)^{2+}$ = $Cu(Phen)^{2+}$. Figure 2 shows schematic structures of the species with interactions according to equilibrium (4) & (7) for Cu(Phen)(Asc). The results of the acidity constants show good agreement with reported values [13]. The reported stability constant of Cu(Asc) complex is similar to our results (tab. 1).The difference between stability constants according eq. (8) show that mixed ligand complexes [14-17] formed by a divalent 3d ion, a heteroaromatic N base and an O donor ligand possess no increased stability. This means that there is no interaction between $Cu(Har)^{2+}$ and Asc^{2-} and no increased stability were observed in complex building of ternary systems.

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Table 1: Logarithm of the stability constants of binary and ternary complexes of M^{2+} at 25°C, 0.1 M, $NaNO_3$*.

$pK^{H}_{H_2(Asc)}$ = 4.03±0.03			$pK^{H}_{H(Asc)}$ = 10.95±0.04
No.	**Species**	**logK[a]**	**Δlog K[b]**
1	Cu^{2+}	7.45±0.22	–
2	$Cu(Bpy)^{2+}$	7.25±0.20	0.20±0.22
3	$Cu(Phen)^{2+}$	7.26±0.21	0.19±0.22

*The given errors are three times the standard error of the mean value or the sum of the propabable systematic errors. [a]according eq. (4). [b]according eq. (8).

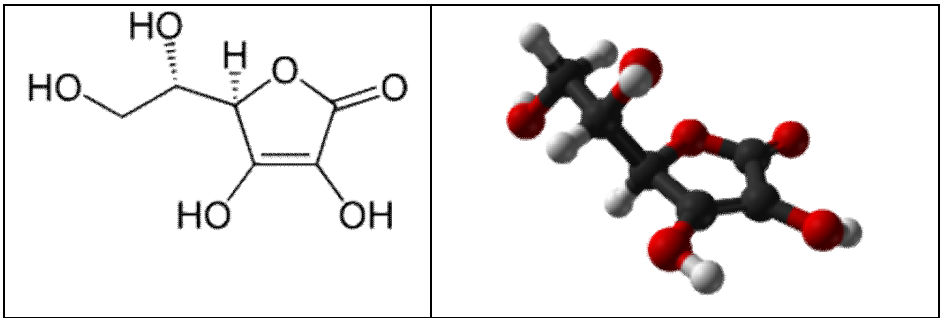

Figure 1: Chemical formula of Ascorbic acid

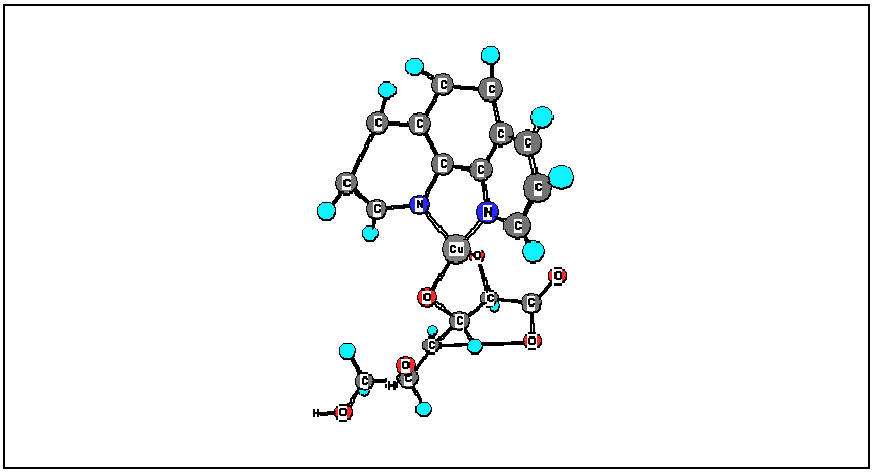

Figure 2: Schematic structures of the species with interactions according to equilibrium (4) & (7) for Cu(Phen)(Asc). The structure in the right part of the figure was drawn with the program CS Chem 3D, version 3.5, from Cambridge Software Corporation.