Oxidation of Aspartic Acid by Cu(III) Complex in Alkaline Medium: A Kinetic and Mechanistic Study

S.-Y. Huo*, J.-H. Shan, C.-Y. Song, S.-G. Shen and H.-W. Sun
College of Chemistry and Environmental Science, Hebei University, Baoding 071002, China

The kinetics and mechanism of oxidation of aspartic acid by the bis(hydrogen periodato) complex of Cu(III), $[Cu(HIO_6)_2]^{5-}$, is studied in an alkaline medium. The reaction rate is first order with respect to Cu(III) and fractional order with respect to aspartic acid. The value of the observed rate constant is found to decrease with the increase in concentrations of either OH^- or IO_4^-. There is a positive salt effect, and the free radical has been determined. In view of these kinetics phenomena, a plausible mechanism is proposed and the rate equations derived from the mechanism can explain all experimental results. The activation parameters along with the rate constants of the rate-determining step are calculated.

INTRODUCTION

Aspartic acid (Asp) is important as a general acid in enzyme active centers, as well as in maintaining the solubility and ionic character of proteins. Serum proteins are essential to maintaining the pH balance of the body. The charged amino acids are largely involved in the buffering properties of proteins. Physiologically, aspartic acid aids in the removal of ammonia, which is highly toxic to the central nervous system. Recent studies have shown that aspartic acid may increase resistance to fatigue and increase endurance. Structurally, aspartic acid is an alanine molecule with one of the β hydrogens replaced by a carboxylic acid group, the pK_a of which in a polypeptide is about 4.0. Aspartic acid and its α-keto homolog, oxaloacetate, are interconvertible by a simple transamination reaction [1].

---

*Corresponding author. E-mail: huoshuyinger@yahoo.com.cn

In this study, Cu(III) mediates the oxidation of aspartic acid to oxaloacetate [2], which is the typical result of the oxidation of the amino acid [3]. As a result of this kinetics study, a possible reaction mechanism was proposed.

EXPERIMENTAL

Materials

All reagents used were of AR grade. All solutions were prepared with doubly distilled water. Aspartic acid was converted to potassium aspartic acid by the addition of an equivalent amount of KOH solution. The Cu(III) complex, $[Cu(HIO_6)_2]^{5-}$, was prepared by the procedure of Balikungeri *et al.*, standardized by the method of Jaiswal [4], and had a UV spectrum consistent with that reported by Rao [5]. Solutions of Cu(III) and aspartic acid were always freshly prepared before used.

Kinetics, Measurements and Product Analysis

All kinetic measurements were carried out under pseudo-first order conditions [reductant]$_0$ >> [Cu(III)]$_0$. A solution (2 ml) with known concentrations of Cu(III), OH$^-$, and IO$_4^-$, and known ionic strength, and a reductant solution (2 ml) of an appropriate concentration were transferred separately to the upper and lower branch tubes of a λ-type two-cell reactor. After thermal equilibration at the desired temperature in circulating water from a thermostat (BG-chiller E10, Beijing Biotech Inc., Beijing), the two solutions were mixed well and immediately transferred to a 1-cm thick glass cell in a cell holder kept at constant temperature (±0.1 K). The reaction process was monitored automatically by recording the disappearance of Cu(III) over time (t) at 415 nm using a TU-1901 spectrophotometer (Beijing Puxi Inc., China). All other species did not absorb significantly at this wavelength.

A solution with known concentrations of Cu(III), OH$^-$ and IO$_4^-$ was mixed with an excess of reductant. When the Cu(III) color (intense red) completely faded, marking the completion of the reaction, the compound formed was estimated by gravimetric analysis to be the 2,4-dinitrophenyldrazone derivative of oxaloacetate. One mole of reductant was found to consume two moles of Cu(III) by weight. The products of the oxidation were the corresponding aldehyde alcohols, as confirmed by their characteristic spot test result [6].

Acrylamide was added under the protection of nitrogen during the course of the reaction. The appearance of white polyacrylamide was consistent with the presence of free radical intermediates in the reaction. Blank experiments in reaction system gave no polymeric suspension.

RESULTS AND DISCUSSION

Under the conditions of [reductant]$_0$ >> [Cu(III)]$_0$, the concentration of Cu(III) was varied from 3.14×10^{-3} to 9.43×10^{-3} M, at fixed [Asp], [OH$^-$], [IO$_4^-$] and ionic strength. The pseudo-first-order constant, k_{obs}, was insensitive to the variations in [Cu(III)]$_0$ (Table 1), and the linear plots of ln(A_t - A_∞) *vs.* time indicate that the reaction is first order with respect

Table 1. Dependence of k_{obs} on [Cu(III)] Concentration[a]

10^3[Cu(III)] (M)	3.14	4.72	6.29	9.43
$10^2 k_{obs}$ (s^{-1})	1.66	1.68	1.65	1.72

[a]Conditions: [IO$_4^-$] = 3.50×10^{-4} M,　[OH$^-$] = 7.00×10^{-2} M, [Asp] = 7.00×10^{-2} M, μ = 0.281 M, Temperature = 203.2 K.

Table 2. Dependence of $10^3 k_{obs}$ (s^{-1}) on Asp Concentration at Different Temperatures[a]

Temp (K)	[Asp] (M)				
	0.02	0.04	0.06	0.08	0.10
298.2	3.25	5.89	7.99	10.06	12.06
303.2	5.04	8.98	13.77	18.06	21.17
308.2	7.25	13.92	20.63	26.05	32.83
313.2	11.50	20.98	35.93	42.50	53.09

[a]Conditions: [Cu(III)] = 7.911×10^{-5} M, [IO$_4^-$] = 5.00×10^{-4} M, [OH$^-$] = 8.00×10^{-2} M, μ = 0.441 M.

to [Cu(III)], where A_t and A_∞ are the absorbance at time t and at infinite time, respectively. The pseudo-first order rate constants, k_{obs}, were evaluated by the method of least squares (r = 0.999). To calculate k_{obs}, generally we used 8-10 A_t values within three times the half-life, and averaged k_{obs} values of at least three independent experiments, with a reproducibility within ±5%.

At fixed concentrations of Cu(III), OH$^-$, and IO$_4^-$, ionic strength and temperature, increases in [Asp] resulted in increased k_{obs} values (Table 2), giving linear plots of $1/k_{obs}$ *vs.* 1/[Asp], with a fractional order with respect to [Asp].

At fixed [Cu(III)], [Asp], [IO$_4^-$], ionic strength and temperature, k_{obs} values decreased with an increase in the concentration of OH$^-$ (Table 3). The plot of $1/k_{obs}$ *vs.* f([OH$^-$])([OH$^-$]) was linear.

At fixed [Cu(III)], [OH$^-$], [Asp], ionic strength and temperature, k_{obs} values decreased with an increase in　[IO$_4^-$]

Table 3. Dependence of k_{obs} on OH^- Concentration[a]

$[OH^-]$ (M)	0.02	0.04	0.06	0.08	0.10
$10^3 k_{obs}$ (s^{-1})	11.35	10.25	9.36	8.36	7.69

[a]Conditions: $[Cu(III)] = 7.911 \times 10^{-5}$ M, $[IO_4^-] = 4.50 \times 10^{-4}$ M, $[Asp] = 7.00 \times 10^{-2}$ M, $\mu = 0.315$ M, Temperature = 298.2 K.

Table 5. Dependence of k_{obs} on Ionic Strength

μ (M)	0.21	0.31	0.39	0.59	0.71
$10^2 k_{obs}$ (s^{-1})	1.36	1.50	1.84	1.96	2.11

[a]Conditions: $[Cu(III)] = 7.911 \times 10^{-5}$ M, $[IO_4^-] = 3.00 \times 10^{-4}$ M, $[OH^-] = 3.00 \times 10^{-2}$ M, $[Asp] = 6.00 \times 10^{-2}$ M, Temperature = 303.2.

Table 4. Dependence of k_{obs} on Different IO_4^- Concentration[a]

$[IO_4^-]$ (M)	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.10
$10^3 k_{obs}$ (s^{-1})	10.43	9.67	9.13	8.26	7.84	7.37	7.09	6.81	6.57

[a]Conditions: $[Cu(III)] = 7.911 \times 10^{-5}$ M, $[OH^-] = 7.00 \times 10^{-2}$ M, $[Asp] = 7.00 \times 10^{-2}$ M, $\mu = 0.283$ M, Temperature = 298.2.

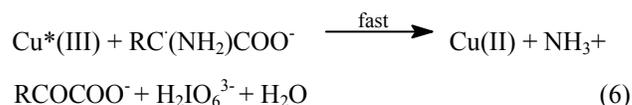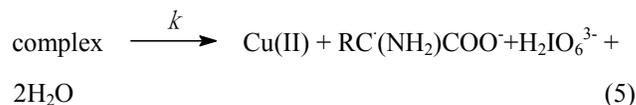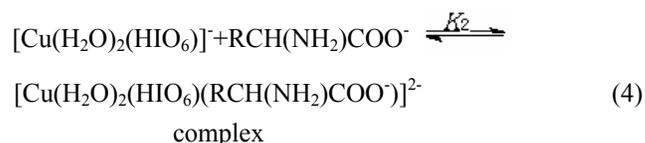(Table 4), which, when plotted as $1/k_{obs}$ vs. $[IO_4^-]$, shows a linear relationship. Additionally, the values of k_{obs} increased with an increase in μ (Table 5), indicating a positive salt effect.

Based on our previous work [7], the main species of periodate were $H_2IO_6^{3-}$ and $H_3IO_6^{2-}$, so $[IO_4^-]_{ex} \approx [H_3IO_6^{2-}] + [H_2IO_6^{3-}]$, and Eqs. (1) and (2) can be obtained as follows:

$$[H_2IO_6^{3-}] = \frac{\beta_3[OH^-]}{\beta_2 + \beta_3[OH^-]} \cdot [IO_4^-]_{ex} = f([OH^-]) \cdot [IO_4^-]_{ex}$$

$$(1)$$

$$[H_3IO_6^{2-}] = \frac{\beta_2}{\beta_2 + \beta_3[OH^-]} \cdot [IO_4^-]_{ex} = \phi([OH^-]) \cdot [IO_4^-]_{ex}$$

$$(2)$$

Under these experimental conditions, the main form of IO_4^- is $H_2IO_6^{3-}$, and the Cu(III) complex may be represented by $[Cu(HIO_6)_2]^{5-}$ as a solid [4]. From the kinetics phenomena in which the rate decreases with the decrease in $[OH^-]$ and $[IO_4^-]$, the active form of Cu(III) in the reaction is deduced to be $[Cu(H_2O)_2(HIO_6)]^-$. The reaction mechanism is proposed as follows:

$$[Cu(HIO_6)_2]^{5-} + 3H_2O \xrightarrow{K_1} [Cu(H_2O)_2(HIO_6)]^- + H_2IO_6^{3-} + OH^- \quad (3)$$

$$[Cu(H_2O)_2(HIO_6)]^- + RCH(NH_2)COO^- \xrightarrow{K_2}$$
$$[Cu(H_2O)_2(HIO_6)(RCH(NH_2)COO^-)]^{2-} \quad (4)$$
$$\text{complex}$$

$$\text{complex} \xrightarrow{k} Cu(II) + RC^\cdot(NH_2)COO^- + H_2IO_6^{3-} + 2H_2O \quad (5)$$

$$Cu^*(III) + RC^\cdot(NH_2)COO^- \xrightarrow{fast} Cu(II) + NH_3 + RCOCOO^- + H_2IO_6^{3-} + H_2O \quad (6)$$

where Cu*(III) stands for the solution in which Cu(III) exists in equilibrium. If subscripts T and e stand for the total and equilibrium concentrations, respectively, then $[Cu(III)]_T = [Cu(HIO_6)_2]^{5-}_e + [Cu(H_2O)_2(HIO_6)]^-_e + [complex]_e$. Eq. (5) represents the rate-determining step.

As the rate of the disappearance of Cu(III) was monitored,

the rate of the reaction can be derived as:

$$-\frac{d[Cu(III)]_T}{dt} = \frac{2kK_1K_2[Asp]}{K_1 + [H_2IO_6^{3-}][OH^-] + K_1K_2[Asp]}[Cu(III)]_T$$
$$= k_{obs}[Cu(III)]_T$$

(7)

$$k_{obs} = \frac{2kK_1K_2[Asp]}{K_1 + [H_2IO_6^{3-}][OH^-] + K_1K_2[Asp]}$$

(8)

$$\frac{1}{k_{obs}} = \frac{1}{2k} + \frac{K_1 + [H_2IO_6^{3-}][OH^-]}{2k_1K_1K_2}\frac{1}{[Asp]}$$

(9)

$$\frac{1}{k_{obs}} = \frac{1}{2k} + \frac{K_1 + f([OH^-])[OH^-][IO_4^-]_{ex}}{2k_1K_1K_2}\frac{1}{[Asp]}$$

(10)

Equation (9) suggests that the plots of $1/k_{obs}$ *vs.* $1/[Asp]$ should be linear. From their intercepts, the rate-determining step constants (k) at different temperatures were evaluated. Equation (10) shows that the plots of $1/k_{obs}$ *vs.* $[IO_4^-]_{ex}$ should also be linear. The activation parameter data of the reaction, obtained by the previously published method [8], are listed in Table 6. Note that the activation energy and $\Delta H^{\ddagger}$ value of this reaction are very large. Because aspartic acid has a long c-chain, it has a large steric hindrance when the transition state complex, $[Cu(H_2O)_2(HIO_6)(RCH(NH_2)COO^-)]^{2-}$, is formed. The value of the entropy of the rate-determining step, $\Delta S^{\ddagger}$, is alterable when the reaction occurs in aqueous solution. In this reaction, we think the structure of the transition state is very loose, resulting in a positive $\Delta S^{\ddagger}$ value.

ACKNOWLEDGEMENTS

Table 6. Rate Constants at Different Temperatures and Activation Parameters of the Rate-determining Step.

298.2 K	$10^2 k = 1.69$ s^{-1}
303.2 K	$10^2 k = 4.97$ s^{-1}
308.2 K	$10^2 k = 11.6$ s^{-1}
313.2 K	$10^2 k = 19.5$ s^{-1}
E_a	127 kJ mol^{-1}
$\Delta H^{\ddagger}$	125 kJ mol^{-1}
$\Delta S^{\ddagger}$	141 J mol^{-1} K^{-1}

REFERENCES

[1] H.J. Bilbert, Basic Concepts in Biochemistry, Beijing, Peking University Medical Press, 2002, p.188.

[2] H.J. Shan, H.Y. Wei, L. Wang, S.G. Shen, J. Hebei Acad. Sci. 18 (2001) 93.

[3] H.Y. Zhang, H.Q. Wan, Biochemstry, Beijing, Chemical Industry Press, 2001, p.227.

[4] a) A. Balikungeri, M. Pelletier, D. Monnier, Inorg. Chim. Acta 22 (1977) 7; b) P.K. Jaiswal, K.L. Yadava, Indian J. Chem. 11 (1973) 837.

[5] K.B. Reddy, B. Sethuram, T.N. Rao, Indian J. Chem. 20A (1981) 395.

[6] F. Feigl, Spot Tests in Organic Analysis, Elsevier Publishing Co., New York, 1956, p. 208.

[7] J.H. Shan, S.Y. Huo, S.G. Shen, H.W. Sun, Chem. J. Chin. Univ. 26 (2005) 706.

[8] J.H. Shan, T.Y. Liu, Acta Chim. Sinica 52 (1994) 1140.