Complex Formation between Aspartic Acid and Molybdenum(VI)

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Equilibria of the reaction of molybdenum(VI) with aspartic acid have been studied in aqueous solution of pH 5−6.5 using spectrophotometric and optical rotation methods at constant ionic strength (0.15 mol dm^{-3} sodium perchlorate) and (25 ± 0.1) °C. Our studies have shown that aspartic acid forms a mononuclear complex of 1:1 with Mo(VI) at pH 5.8, and the stability constant of this complexation has been determined.

Introduction

It is recognized that molybdenum found in enzymes in microorganisms, plants, and animals plays an important biochemical role (Kendrick et al., 1992; Ochiai, 1987).

The equilibria of Mo(VI) in aqueous solution are complex, and various polynuclear species form in acidic solution (pH < 4.5) (Johnson and Jons, 1981). In basic solution, little or no complex forms due to the exceedingly high stability of MoO_4^{2-} (Cotton and Wilkinson, 1972).

The present paper describes the complexation of Mo(VI) with L-aspartic acid determined by spectrophotometric and polarimetric methods at (25 ± 0.1) °C and an ionic strength of 0.15 mol dm^{-3} (sodium perchlorate) in aqueous solution of pH ranging from 5 to 6.5.

Experimental Section

Reagents. Sodium perchlorate, perchloric acid, sodium hydroxide, sodium molybdate, and L-aspartic acid were obtained from Merck as analytical reagent grade materials, and were used without further purification. Dilute perchloric acid solution was standardized against $KHCO_3$. A 50 mass % sodium hydroxide solution free from carbonate was prepared from analytical grade material filtered through a G4 Jena glass filter and stored in a polyethylene bottle; dilute solutions were prepared from double-distilled water with conductivity equal to (1.3 ± 0.1) $\mu\Omega^{-1}$, and this stock solution was standardized against $HClO_4$.

Measurement. All measurements were carried out at (25 ± 0.1) °C. The ionic strength was maintained at 0.15 mol dm^{-3} with sodium perchlorate. An Eyela pH meter, PHM 2000, was used for pH measurements. The hydrogen ion concentration was measured with an Ingold UO 3234 glass electrode and an Ingold UO 3236 calomel electrode. A 1.00 × 10^{-2} mol dm^{-3} perchloric acid solution containing 0.14 mol dm^{-3} sodium perchlorate was employed as a standard solution of hydrogen ion concentration (Gharib et al., 1995; Zare et al., 1979). Spectrophotometric measurements were performed on a UV−vis Shimadzu 2100 spectrophotometer with a GDU 20 C computer and using thermostated matched 10 mm quartz cells. The measurement cell was a flow type. A Masterflex pump allowed circulation of the solution under study from the potentiometric cell to the spectrophotometric cell so the absorbance and pH of the solutions could be measured simultaneously. Polarimetric measurements were made at (25 ± 0.1) °C with an Atago Model Polax-D polarimeter, equipped with a sodium lamp. A water-jacketed cell of 200 mm length and 20 cm^3 total volume was used.

For each experiment (for both spectrophotometric and polarimetric methods) two solutions of Mo(VI) + L-aspartic acid had been prepared with the same concentration, but the ionic strength of the first was maintained with sodium perchlorate, and the second with sodium hydroxide. The first solution was then titrated with the second one to reach the pH of interest.

Results and Discussion

Polarimetric Studies. Assuming that the optical activity of aspartic acid will change upon complexation, polarimetric measurements were made to determine if Mo(VI) is complexed by aspartic acid. Thus, we prepared different solutions of Mo(VI) and L-aspartic acid and L-aspartic acid alone with the same concentration but different pH. The effect of pH on the optical rotation of such solutions is shown in Figure 1. When the pH is <7, the optical rotation of the Mo + aspartic acid solution becomes increasingly different from that of aspartic acid, indicating complexation by Mo. The difference reaches a maximum between pH 5 and pH 6 and decreases as the pH is decreased below 5, indicating that the largest amount of complex forms in the pH range 5−6. At higher pH, the complex dissociates due to the competitive formation of MoO_4^{2-}, while at low pH isopolymolybdates form. In order to determine the composition of the complex, a continuous variations study, according to the method of Job, Beck, and Nagypal (1990), was made at pH 5.8. The results are given in Table 1. A rather sharp maximum at a mole fraction of Mo(VI) of 0.5 was obtained, indicating a 1:1 complex.

Butcher et al. (1976) have shown by vibrational spectroscopy that a 1:1 Mo(VI) + aspartic acid complex isolated from neutral solution has the MoO_3 core, from which we conclude that the composition of the complex indicated by the optical rotation measurements is MoO_3L^{2-}, where L represents the fully dissociated amino acid anion. The formation of the complex is described by (Funahashi et al., 1981)

$$MoO_4^{2-} + 2H^+ + L^{2-} \rightleftharpoons MoO_3L^{2-} + H_2O \qquad (1)$$

Aspartic acid exists in solution in one or more of four

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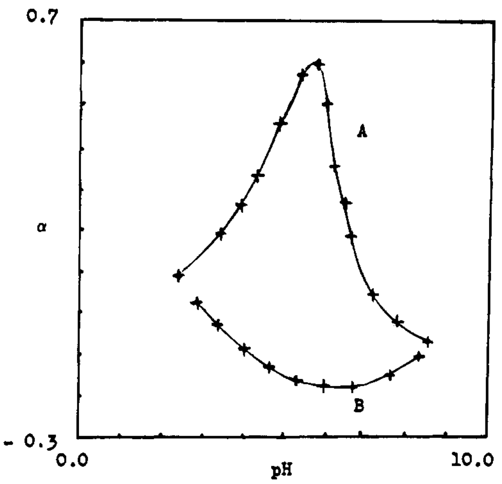

Figure 1. Effect of pH on optical rotation, α, of (A) Mo(VI) (10^{-2} M) + L-aspartic acid (10^{-2} M) and (B) L-aspartic acid (10^{-2} M).

Table 1. Continuous Variation Data for the Mo(VI) + Aspartic Acid System at pH 5.8, (25 ± 0.1) °C, and Ionic Strength 0.15 M ($NaClO_4$)

x^a	α(Mo + Asp)^b	α(Asp)^c	Δα^d
0.0	−0.215	−0.215	0.000
0.1	−0.110	−0.200	0.090
0.2	−0.025	−0.195	0.170
0.3	0.085	−0.160	0.245
0.4	0.155	−0.140	0.295
0.5	0.250	−0.125	0.375
0.6	0.255	−0.115	0.372
0.7	0.250	−0.065	0.315
0.8	0.230	−0.055	0.285
0.9	0.090	−0.025	0.115
1.0	0.000	0.000	0.000

[a] Mole fraction of Mo(VI). [b] Optical rotation of [Mo(VI)] + [aspartic acid] = 3.5 × 10^{-2} M. [c] Optical rotation of aspartic acid at the same concentration as in the Mo + aspartic acid solution. [d] α(Mo + aspartic acid) − α(aspartic acid).

forms, depending on the solution pH (Chang, 1977):

$$LH_3^+ \underset{pK_1 = 2.40}{\rightleftharpoons} LH_2 \underset{pK_2 = 3.80}{\rightleftharpoons} LH^- \underset{pK_3 = 9.20}{\rightleftharpoons} L^{2-} \qquad (2)$$

L^{2-}, LH^-, LH_2, and LH_3^+ refer to the different forms of aspartic acid existing at different pH values. pK_1 and pK_2 refer to the first and second dissociation constants of the first and second carboxyl groups, respectively, and pK_3 denotes the deprotonation of the amino group. The above pK values were determined by potentiometric titration under the same condition of temperature and ionic strength as described before (Gharib et al., 1993).

We have previously described (Gharib et al., 1995) that, at pH 5.8, H_2L and HL^- are the major species. Since the complex apparently involves the amino group, however, L^{2-} must be the species which complexes.

The stability constant for the 1:1 complex at pH 5.8 was estimated from a continuous variations plot of the results in Table 1. The first problem is based on the fact that the total rotation, α_t, at the maximum (mole fraction of Mo 0.5) is due to the rotation of the complex and the free aspartic acid species, neglecting the rotation of H_3L^+ and L^{2-}, because their concentrations are negligible at this pH:

$$\alpha_t = \alpha_C + \alpha_{H_2L} + \alpha_{HL^-} \qquad (3)$$

Since the rotation of each species in eq 3 is proportional to the concentration of that species, eq 3 may be written as

Table 2. Continuous Variation Data for the Mo(VI) + Aspartic Acid System at pH 5.8, (25 ± 0.1) °C, and Ionic Strength 0.15 M ($NaClO_4$)

	A^b		
x^a	275 nm	270 nm	265 nm
0.06	0.038	0.047	0.065
0.10	0.064	0.079	0.107
0.16	0.105	0.126	0.170
0.20	0.129	0.155	0.211
0.30	0.192	0.232	0.322
0.40	0.255	0.319	0.429
0.50	0.308	0.384	0.515
0.60	0.250	0.320	0.420
0.70	0.182	0.236	0.311
0.80	0.121	0.159	0.203
0.84	0.100	0.122	0.164
0.90	0.061	0.077	0.101
0.94	0.036	0.046	0.058
1.00	0.000	0.000	0.000

[a] Mole fraction of Mo(VI). [b] The corrected absorbance of [Mo(VI)] + [aspartic acid] = 1.75 × 10^{-2} M at different wavelengths.

$$\alpha_t = k_C[C] + k_{H_2L}[H_2L] + k_{HL^-}[HL^-] \qquad (4)$$

where the k's are proprotionality constants and C refer to the concentration of the complex. For the material balance

$$C_L = [C] + [H_2L] + [HL^-] \qquad (5)$$

where C_L is the total concentration of ligand. The values of k_{H_2L} and k_{HL^-} were obtained from the plot of α versus pH for aspartic acid at the respecttive pH values where these forms are maximized and the other species can be neglected (pH 3.10 and 6.50). The value of k_C was obtained from a measurement of α at pH 5.8 for a solution of 0.81 × 10^{-2} M in Mo(VI) and 1.00 × 10^{-3} M in aspartic acid in which the ligand is essentially all complexed. The values obtained were $k_{H_2L} = 2.1$, $k_{HL^-} = -22.5$, and $k_C = 81.5$. Equations 4 and 5 were then solved for [C] and $[HL^-]$. The concentrations of H_2L and L^{2-} were calculated from the proper equations of the dissociation constants. [Mo(VI)] was obtained from

$$C_M = [Mo(VI)] + [C] \qquad (6)$$

where C_M is the total concentration of Mo(VI). The stability constant, K_S, was calculated from

$$K_S = \frac{[MoO_3L^{2-}]}{[MoO_4^{2-}][H^+]^2[L^{2-}]} \qquad (7)$$

This gave the value of log K_S = 18.3 ± 0.1, for the total concentration of 3.5 × 10^{-2} M of Mo(VI) and ligand.

Spectrophotometric Studies. Using the continuous variations method, the absorbances of two different solutions of Mo(VI) and L-aspartic acid of total concentrations 1.75 × 10^{-2} and 1.25 × 10^{-2} M were determined in the UV range 265−280 nm and at a constant pH of 5.8. The observed absorbances were corrected for unreacted Mo(VI), from eq 8, and are summarized in Tables 2 and 3 and

$$A_C = A_{obs} - \epsilon_o[Mo] \qquad (8)$$

plotted in Figure 2. A_C, A_{obs}, and ϵ_o are the absorbance of the complex, the observed absorbance, and the molar absorptivity of Mo(VI), respectively. ϵ_o values, for various

Table 3. Continuous Variation Data for the Mo(VI) + Aspartic Acid System at pH 5.8, (25 ± 0.1) °C, and Ionic Strength 0.15 M ($NaClO_4$)

	A^b		
x^a	280 nm	275 nm	270 nm
0.06	0.019	0.026	0.032
0.10	0.029	0.043	0.052
0.16	0.045	0.067	0.084
0.20	0.069	0.093	0.116
0.30	0.098	0.130	0.177
0.40	0.123	0.164	0.220
0.50	0.148	0.199	0.258
0.60	0.128	0.160	0.218
0.70	0.100	0.126	0.174
0.80	0.071	0.089	0.105
0.84	0.044	0.058	0.077
0.90	0.031	0.039	0.053
0.94	0.019	0.023	0.033
1.00	0.000	0.000	0.000

[a] Mole fraction of Mo(VI). [b] The corrected absorbance of [Mo(VI)] + [aspartic acid] = 1.25 × 10^{-2} M at different wavelengths.

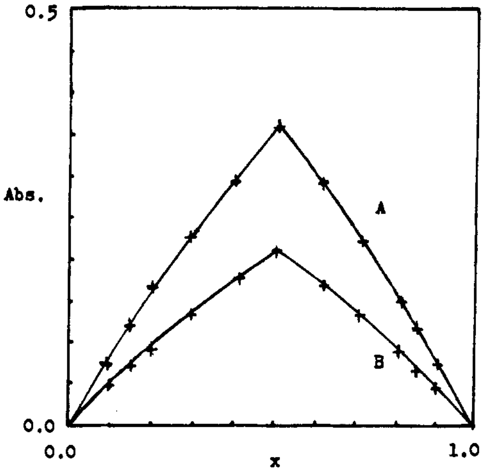

Figure 2. Continuous variation plots of absorbances of MoO_3L^{2-}, Abs., versus mole fraction of Mo(VI), x, for (A) [Mo(VI)] + [aspartic acid] = 1.25 × 10^{-2}, at 265 nm, and (B) [Mo(VI)] + [aspartic acid] = 1.75 × 10^{-2} M, at 280 nm.

Table 4. Values of Molar Absorptivities of Mo(VI), ϵ_0, and MoO_3I^{2-}, ϵ, at pH 5.8, (25 ± 0.1) °C, I = 0.15 M ($NaClO_4$), and Different Wavelengths

	280 nm	275 nm	270 nm	265 nm
ϵ_0	11.6	27.9	66.3	135.2
ϵ_1	24.1	33.9	43.2	58.6

wavelengths, were calculated at the mole fraction of Mo(VI) equal to 1, and are shown in Table 4.

In Figure 2 a rather sharp maximum at a mole fraction of Mo(VI) of 0.5 was obtained, indicating a 1:1 complex. The molar absorptivity of the complex, ϵ, was calculated from the linear part of the curve at low mole fraction of Mo(VI), where essentially all the metal ion was in the form of a complex, and for different wavelengths is shown in Table 4. At the maximum point on the plot, the concentration of the complex is

$$[C] = A_C/\epsilon \tag{9}$$

Equations can be written for the total concentration of Mo(VI), C_M, and the total concentration of ligand, C_L, at this point:

$$C_M = [Mo] + [C] \tag{10}$$

$$C_L = [L] + [C] \tag{11}$$

Combining eqs 9–11 in eq 7, and solving for K_S, gives the stability constant of eq 1. The average of K_S for different concentrations and wavelengths is log K_S = 18.2 ± 0.2.

Since Mo(VI) complexes generally contain the MoO_3 core with a coordination sphere of the metal completed by three donor atoms (Funahashi et al., 1981), it seems unlikely that complexes of 1:2 and 1:3 stoichiometry exist in the pH range of interest (Johnson and Jons, 1981; Funahashi et al., 1981).

Literature Cited

Beck, M. T.; Nagypal, I. *Chemistry of Complex Equilibria*; Ellis Horwood Limited: New York, 1990.

Butcher, R. J.; Powell, H. K. J.; Wilkins, C. J.; Yong, S. H. *J. Chem. Soc., Dalton Trans.* **1976**, 356–359.

Chang, R. *Physical Chemistry with Applications to Biological Systems*; Macmillan Publishing Co.: New York, 1977.

Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 3rd ed.; Interscience: New York, 1972.

Funahashi, S.; Kato, Y.; Nakayama, M.; Tanaka, M. *Inorg. Chem.* **1981**, *20*, 1752–1756.

Gharib, F.; Zare, K.; Khorrami, S. A. *J. Chem. Eng. Data* **1995**, *40*, 186–189.

Gharib, F.; Zare, K.; Khorrami, S. A. *J. Chem. Eng. Data* **1993**, *38*, 602–604.

Gharib, F.; Zare, K.; Khorrami, S. A. *J. Sci. Az-Zahra Univ.* **1995**, in press.

Johnson, J. S.; Jons, O. *Acta Chem. Scand.* **1981**, *35*, 233–237.

Kendrick, M. J.; May, M. T.; Plishka, M. J.; Robinson, K. D. *Metals in Biological Systems*; Ellis Horwood Limited: New York, 1992.

Ochiai, E. I. *General Principles of Biochemistry of the Elements*; Plenum Press: New York, 1987.

Zare, K.; Lagrange, P.; Lagrange, J. *J. Chem. Soc., Dalton Trans.* **1979**, 1372–1376.

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