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Thermodynamics for complex of *L*-histidine with molybdenum (VI) Model Anticancer Drugs

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ABSTRACT

Organometallic complexes offer potential for design as anticancer drugs. A quantitative model that discriminates anticancer compounds from the inactive ones in a training series was obtained.Equilibrium of the reaction of molybdenum (VI) with l-histidine have been studied in aqueous solutions at pH range of 4-8, using spectrophotometry and optical rotation methods at constant ionic strength of 0.15 mol.lit^{-1} of sodium perchlorate at $25 \pm 0.1^0 c$. *L*-histidine has been proposed to be present at the active site of a number of enzymes. In addition, as a part of cytochrome-c, it is apparently involved in the electron transport systems. Also, molybdenum (VI) is an essential component of several enzymes which catalyze reactions. In this project, we have determined the pH in which the complex is formed using spectrophotometry and polarimetry techniques. The molar ratio of metal to ligand in a molecule of complex was 1:1, and the stability constant of this complexation process has been determined by polarimetry and spectrophotometry methods at different wavelengths. The stability constant decreases upon increasing the pH over 6, so the stability constant of complex of Mo (VI) with l-histidine has been determined at pH=5.8. We have used literature to propose a structure for the complex molecules. So the proposed structure is an octahedral with a central core consists of MoO_3 with three binding sites for complexation. Thus, histidine as tridentate ligand can made a chelate with the metal, through the oxygen of carboxylic group, the nitrogen of amides group and the charged nitrogen of imidazole ring donors.

Keywords: Molybdenum (VI); l-histidine; Optical rotation; Spectrophotometry

1. INTRODUCTION

There are a lot of investigation about enzymes containing molybdenum (VI), and it has been proved that molybdenum (VI) exists in structure of some enzymes in live systems and plays an important catalytic role in these systems [1]. So recently catalytic role of Mo (VI) in live creatures has been studied using simple pattern systems which are similar to the natural complex systems (for example enzymes). So thermodynamic and kinetic investigation of complexes of Mo (VI) with amino polycarboxylic acids; has been the issue of several investigations.

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In this research the equilibrium of complex formation of Mo (VI) with amino acid of l-histidine has been investigated at pH range of 4-8 using polarimetry and spectrophotometry methods. Also the stability constant of complex of molybdenum (VI) with l-histidine has been calculated at pH=5.8, 25°C and ionic strength of 0.15 M of sodium perchlorate. Of course the formation constant of this complex has been determined at three pH of 6, 6.3 and 6.8 using; spectrophotometry method and effect of pH on the formation constant has been investigated and finally the obtained results have been analyzed.

2. EXPERIMENTAL

2.1. Materials

Sodium molybdate ($Na_4M_0O_4 2H_2O$) with purity of 99.5%, per chloric acid with weight percentage of 70%, the solution of NaoH(1N)- histidine and the reactant of l-histidine with purity of 99% were obtained from Merck. Sodium perchlorate, with purity of 99.5% was obtained from Ridel-Dehaen. In all experiments double distilled water with specific conduction of $1.28 \pm 0.1 \mu ohm^{-1} cm^{-1}$ was used.

2.2. Apparatuses

All determinations of rotation angle were carried out on an ARAGO PolAX-D polarimeter. The polarimetry had the accuracy of ± 0.003 arc degree. All spectrophotometry determinations were carried out on a Shimadzu UV-2100 double beam spectrophotometer. All pH-metric determinations were done on a pH M-2000 EYELA pH-meter at 25±0.1°C.

2.3. Methods

In order to confidence in completeness of complex formation reaction between molybdenum (VI) and amino acid, the complex solutions initially were placed in water bath (25±0.1°C) for one night. Because of high speed of reaction, all complex solutions were investigated after 15 min. Ionic strength of all solutions were kept constant at I=0.15 using sodium perchlorate. The acidic constants of amino acid were determined at ionic strength of 0.15 M of sodium perchlorate and at 25°C by potentiometric method using standard solution of NaoH. Formation equilibrium of complex of molybdenum (VI) with l-histidine was investigated using polarimetry determinations by Job method.

2.4. Determination of acidic constants of l-histidine

L-histidine is an amino acid containing the polar group of R with positive charge that contains an extra amine group (imidazole ring) in addition of α-carboxyl groups. The equilibrium reaction of dissociation of this amino acid can be shown by relations stated as below:

$$H_3L^{2+} \Rightarrow H_2L^{+} + H^{+} \quad K_1 = \frac{[H_2L^{+}][H^{+}]}{[H_3L^{2+}]} \quad (1)$$

$$H_2L^{+} \Rightarrow HL + H^{+} \quad K_2 = \frac{[HL][H^{+}]}{[H_2L^{+}]} \quad (2)$$

$$HL \Rightarrow L^{-} + H^{+} \quad K_3 = [L^-][H^+]/[HL] \quad (3)$$

where K_1 and K_2 are ionization constants of amino acid. The acidic dissociation constants of l-histidine were investigated by pH potentiometric technique. The reactions of complex formation can be investigated by polarimetry method in which at least one of the reactants or complex has optical activity. In this regard at first the optical activity of ligand at various amounts of pH was investigated. The results are listed in table 1.

For determining a pH range at which the maximum amount of complex is formed, a solution consist of metal and ligand with equal concentrations was prepared and variation of optical rotation of solution versus variation of pH showed that the maximum amount of complex is formed at pH=5.8. The results are summarized in table 2.

Table 1. Obtained data from determination of optical rotation of l-histidine solution (0.05M) at various pH and 25°C, I=0.15M, Naclo$_4$

pH	αL	**pH**	αL
10.70	0.487	6.50	0.925
10.50	0.501	6.25	0.701
9.75	0.524	6.00	0.460
9.50	0.604	5.80	0.305
9.25	0.779	5.50	0.142
9.00	1.000	5.25	0.053
8.89	1.103	5.00	0.032
8.79	1.150	4.75	0.080
8.36	1.252	4.50	0.131
7.50	1.251	4.25	0.164
7.25	1.250	4.00	0.180
7.00	1.149	3.86	0.187
6.75	1.072	3.75	0.202

Table 2. optical rotation variation of solution containing Mo ion (0.05M) and *l*-histidine (0.05M) with l-histidine solution (0.05 M) versus pH, T=25°C, I=0.15M,Naclo$_4$

pH	$\Delta\alpha$	**pH**	$\Delta\alpha$
8.00	0.089	6.00	0.971
7.75	0.110	5.75	0.954
7.50	0.146	5.50	0.820
7.25	0.380	5.25	0.582
7.00	0.613	5.00	0.361
6.75	03717	4.75	0.180
6.50	0.793	4.50	0.041

2.5. Determination of the stability constant of complex using Job method

In order to determine the component ratios in complex and calculate it's stability constant by Job method and using polarimetry, a series of solutions with equal total concentration of ligand and metal with molar fraction of metal between 0-1 were prepared and the optical rotation of these solutions were determined at pH=5.8. The results show that there is a maximum at molar fraction of 0.5 for molybdenum (VI) indicates that formation of complex between molybdenum (VI) and l-histidine is of 1:1 kind. So we can indicate the reaction of complex formation between molybdenum (VI) and l-histidine by relation 4:

$$M_0O_4^{2-} + L^- + 2H^+ \Rightarrow M_0O_3L^- + H_2O$$

$$K = {M_0O_3L^-}\Big/{[M_0O_4^2][L^-][H^+]^2} \qquad (4)$$

The molar rotation coefficient of complex (k$_c$) was obtained from determining the optical rotation degree (α) at pH=5.8 for molybdenum (VI) solution with concentration of 0.131 M and l-histidine with concentration of 0.0056 M and ionic strength of 0.15 M of sodium perchlorate. The determined rotation amount is α =0.102. Amounts of coefficients of $K_{H_2L^+}$ and K_{HL} can be calculated using the optical ratio ion curve of ligand versus pH in a pH region at which concentration of these species are maximum and we can ignore other species. At pH values of 3.9 and 7.6, amounts of $K_{H_2L^+}$ and K_{HL} can be calculated using the relations 5:

$$K_{H_2H^+=\frac{\alpha H_Z L^+}{[H_2L^+]}} \qquad K_{HL} = \frac{\alpha HL}{[HL]} \qquad (5)$$

Amounts of $[L^-]$ and $[M_0O_4^{2-}]$ can be calculated using relations 6 and 7:

$$[L^-] = \frac{k_3[HL]}{[H^+]} \qquad (6)$$

$$[M_0O_4^{2-}] = T_{M_0O_4^{2-}} - [M_0O_3L^-] \qquad (7)$$

Amount of stability constant at two concentrations of $(T_M + T_L) = 0.03$ and $(T_M + T_L) = 0.05$ was calculated using relation 4 (T_L is the total concentration of amino acid of l-histidine).

2.6. Determination of the stability constant of complex by spectrophotometry

The stability constant of complex of Mo (VI) and l-histidine has been investigated using continuous variations (Job) method and by spectrophotometry determinations at pH=5.8 and at wavelength range of 220-300nm. The plots are dispensable based on molar absorption of ligand and also the molar absorption coefficient of complex is much more than molar absorption coefficient of metals that effects on the calculation of stability constant.

Based on obtained data from determining the absorption of prepared solutions in Job method, the summit of plot appears at molar fraction of 0.5 related to metal, means that the ratio of components in complex of Mo (VI) with l-histidine is 1:1 that confirms the polarimetry data. The crystal of molybdate in solid state has a tetrahedral shape, but molybdate ion is polymerized in strong acidic solutions, but in weak acidic, neutral and basic solutions has octahedral shape and can be in forms of $H_2M_0O_4, HM_0O_4^-$ and $M_0O_4^{2-}$. These forms are in equilibrium with each other.

The reaction of complex formation between Mo (VI) and l-histidine can be shown by relation 8, because the spectrophotometry determinations were carried out in a pH region in which the molybdenum ion was the major species in the solution:

$$M_0O_4^{2-} + L^- \Leftrightarrow M_0O_3L^- + H_2O$$

$$K_{for} = \frac{[M_0O_3L^-]}{[M_0O_4^{2-}][L^-]} \qquad (8)$$

Using obtained results of Job curves, the molar absorption coefficient of complex can be calculated in metal molar fraction of 0.05 (assume that all metal exist in complex). And in various wavelengths we can calculate the absorption coefficient of complex using beer lambert relation:

$$A_{M_0O_3L^-} = \in_{M_0O_3L^-} [M_0O_3L^-] \qquad (9)$$

The molar absorption coefficients of metal and ligand are calculated at various wavelengths using this method at molar fraction of 1 and 0. Using relation 9, the concentration of complex at maximum point of Job curves, its molar absorption and its molar absorption coefficient and absorption at two maximum points are determined. The concentration of remainder metal and ligand can be calculated by relations 10 and 11:

$$[M_0O_4^{2-}] = T_{M_0O_4^{2-}} - [M_0O_3L^-] \qquad (10)$$

$$[L^-] = T_L - [M_0O_3L^-] \qquad (11)$$

where $[L^-]$ is concentration of remainder ligand? With replacing the related amounts in relation 8, the formation constant of complex of Mo (VI) with l-histidine is calculated. For determining the stability constant, we use the relation 12:

$$M_0O_4^{2-} + L^- + 2H^+ \Leftrightarrow M_0O_3L^- + H_2O$$

$$K_{st} = \frac{[M_0O_3L^-]}{[M_0O_4^{2-}][L^-][H^+]^2}, K_{st} = \frac{K_{for}}{[H^+]^2} \qquad (12)$$

Also the formation constant of complex has been calculated at pH values of 6.0, 6.3 and 6.8 for complex solution with total concentration of 0.012M of ligand and metal at various wavelengths. Results are summarized in table 3.

Table 3. The calculated formation constants using spectrophotometry data for solution of Mo (VI) ion with l-histidine at four pH (T_M+T_L) = 0.012M (Naclo$_4$)

Log K for pH	265	270	275	280nm
5.80	5.98	6.14	6.25	6.28
6.00	5.52	5.69	5.80	5.84
6.30	5.10	5.25	5.38	5.43
6.80	4.29	4.52	4.58	4.61

3. RESULTS AND DISCUSSION

pH of histidine solution is about 7.6 and considered pka is placed in two side of this region of pH, so the titration was carried out once with per chloric acid (0.1M) until pH=3 and once with NaoH (0.1M) at pH range of 3-11.8 to attain a complete plot for second and third pka of l-histidine. The first pka doesn't have a determinative role for calculating the stability constant. According to plots of there are two maximum in volumes of 0.53 and 4.35 ml of added NaoH(0.1M), which are related to the pH values of 4.49 and 7.62 respectively. The average of these two pH is 6.05 at which the concentration of two equilibrated species (H_2L^+, HL) are equal, so at this pH, pka is equal to pH of solution also for second pka we can calculate it's value.

$$H_3L^{2+} \xleftrightarrow[pk_1=1.82]{} H_2L^+ \xleftrightarrow[pk_2=6.05]{} HL \xleftrightarrow[pk_3=9.19]{} L^-$$

AT pH=5.8, H_2L^+ and HL are two prevailing species and the concentration of other species are dispensable. Using obtained data from polarimetry determinations by Job method and existence relations, the stability constant of complex in two concentration of 0.05 and 0.03 M was calculated and amounts of log K_{st}=17.57 and 17.63 were obtained respectively. In spectrophotometry method using Job method and using obtained data and also existence relations, formation and stability constants of complex have been calculated. The formation constant of complex in concentration of 0.012M and at four pHs are listed in table 3. Results show that the formation constant of complex decreases upon increasing the pH and amount of complex decreases gradually and this issue confirms polarimetry data. The stability constants at four wavelengths have been calculated and results show that the stability constant decreases upon decreasing the wavelength. It seems that calculation uncertainty at higher wavelengths is less in comparison with lower wavelengths. So we have used a wavelength range from 265 to 285 in calculations. Calculations for two total concentrations of 0.01 and 0.006 M and at various wavelengths have been done. Results for $(T_L + T_M = 0.01)$ = 0.01 are presented in table 4.

Table 4. The calculated stability constants using spectrophotometry data for solution of Mo (VI) ion with l-histidine at PH=5.8, I=0.15M ($NaClo_4$), T_M=0.01M

	280	**275**	**270**	**265nm**
Log kst	17.86	17.81	17.73	17.59
4m	11.1	38.9	70.0	155.6
4c	282	358	450	582

In all Job plots we see a maximum at molar fraction of 0.5, indicates that ratio of 1:1 is the components ratio of complex and confirms the polarimetry data. Of course in more acidic solutions (4 < pH < 5), there is a maximum at molar fraction of 0.33 that is a reason for complex formation with ratio of 1:2 (ligand to metal). But at pH < 5, there are different species of Mo (VI) ion and we can't express our opinion about complex formation correctly.

According to the investigations, the core of complex of molybdenum (VI)-L-histidine has an octahedral structure of MoO_3 [2-9]. Also investigations show that at a pH range of 5-7, the Mo (VI) ion can't form complex with bidentate ligands [10], but about tridentate ligands there are investigations that confirm formation of complex between Mo (VI) and these ligands at mentioned range of pH. So we can conclude that L-histidine acts as a tridentate ligand. Of course formation of complex of Mo (VI) ion with bidentate ligands has been reported by Butcher but at more acidic region (pH < 5).

Investigations in this study showed that at 4 < ph < 5 using Job method, there is an absorption maximum at molar fraction of about 0.33; means that maximum absorption from molar fraction of 0.5 (related to metal) inclines towards less molar fractions upon decreasing the pH. This molar fraction indicates the ratio of 2:1 of ligand to metal. But because of limitations and also existence of various species of isopolymolybdates in this region of pH we can't attain a suitable conclusion. According to the previous investigations [11], we can conclude that the coordinable points of L-histidine in the investigated complex are oxygen of carboxyl group, nitrogen of α-amine and nitrogen (number 1) of imidazole ring; means that ligand has acted as a tridentate ligand.

According to the formation or stability constants of two complexes of L-histidine-molybdenum (VI) and methyl eternal-histidine-molybdenum (VI) we see that this amount for methyl external-histidine is less, that can be due to this fact that oxygen of ester group earns positive charge through binding to metal but about carboxyl group without L-histidine proton, oxygen discharged and definitely this compound is more stable than the first one in which oxygen has positive charge. So the complex acts tridentate and the binding points are the same as we mentioned previously.

Finally according to the all examples and calculations by polarimetry and spectrophotometry methods, the stability constant of this complex was calculated (17.6 ± 0.1) by polarimetry method and (17.8 ± 0.3) by spectrophotometry method. Amount of calculated stability constant based on spectrophotometry method confirms the same amount, obtained from polarimetry method.

4. REFERENCES

[1] Kielland, J, J. Am. chem. Soc., 59, 1675 (1937).

[2] Zare k., Lagrange P. and Lagrange J., J. chem. Soc. Dalton Trans., 1372(1979)

[3] Zare k., Lagrange P. and Lagrange J., Proceeding of the 568 (1979).

[4] Zare k., Lagrange P.and lagrange J., Proceeding of the 2nd International symposium on the mechanism of reactions in solution, 93 (1979).

[5] Zare k., lagrange P. and Lagrange J. 2nd International conference on the mechanisms of Reactions solution, Conterbury (England) July (1979).

[6] Gharib, F., zare, k., khorrami, S. A., J. chem. Eny. Data 40, 186-189 (1995).

[7] Gharib, F., zare, k., khorrami, S. A., J. chem. Eng. Data 38, 602-4 (1993).

[8] Gharib, F., zare, k., khorrami, S. A., J. Sci. Az-zahra Unive. In press (1995).

[9] Gharib, F., zare, k., Behju, A., J. chem. Eng Data 40, 1214-16 (1995)

[10]Spence, J., less, J., Inorg chem. 4, 3, 385 (1965).

[11]Mcdonald, c., Phillips, W., J. Am. chem. Soc., 85, 3736 (1963).