Stabilities of Vanadyl Complexes with Methionine, Phenylalanine, and Threonine

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Stepwise formation constants corresponding to 1:1 vanadyl complexes with methionine, phenylalanine and threonine have been determined at 25 °C, and at various ionic concentrations, *viz.* 0.01, 0.1 and 0.3 M, maintained by the addition of potassium chloride solution. Thermodynamic stability constants have been obtained by extrapolation of $\log K$ values to zero ionic strength. Logarithms of the overall stability constants ($\log K^{(u=0)}$) are 7.72 for methionine, 7.70 for phenylalanine and 7.44 for threonine complexes. The corresponding free energy changes (ΔG^0) are −10.53, −10.51, −10.15 kcal·mol^{-1} respectively.

Threonine, methionine and phenylalanine are biologically important substances as these are essential for body growth.

A better insight into metal-protein interaction can be obtained by studying the equilibria between metal ions and amino acids. The stability constants of vanadyl ion (VO^{2+}) with some amino acids have been reported in literature[1–3]. Since vanadyl ion has been found to form stable complexes with amino acids, it can, therefore, affect the environment. No information about the interaction of this ion with threonine, methionine and phenylalanine is available. It was, therefore, desirable to carry out investigation on the stability of vanadyl ion complexes with these amino acids. The present paper deals with the determination of thermodynamic metal-ligand stability constants by the method of Bjerrum-Calvin[4, 5] as adopted by Irving and Rossotti[6].

Materials and Methods

Apparatus

'Systronix' pH meter was used with a glass and calomel electrode assembly. The solutions were maintained at 25 ± 0.1 °C in an electronically controlled water thermostat.

Materials

The amino acids, L-phenylalanine, L-threonine and DL-methionine were of E. Merck pure grade and were dried at 100 °C for 1 h before use. One molar standard solution of KCl (Analar, B.D.H.) prepared by direct weighing and was employed to maintain the ionic strength constant. A solution of potassium hydroxide (Analar, B.D.H.) was prepared and standardized against oxalic acid. The hydrochloric acid (Analar, B.D.H.) solution was standardized against standard alkali. Twice distilled water free from carbon dioxide was used for preparing all the solutions.

Standard V(IV) solution

Solution of vanadyl sulphate (0.05 M) was prepared by dissolving appropriate amount of vanadyl sulphate (B.D.H.) in double distilled water. It was standardized by titration with 0.05 M $KMnO_4$ in hot solutions until a permanent pink colour was obtained. The reaction proceeded according to the following equation:

$$5\,VO^{2+} + MnO_4^- + 6\,H_2O \rightarrow 5\,HNO_3 + Mn^{2+} + 7\,H^+.$$

pH-titration method

The ratio of metal to ligand was kept 1:5 in order to fulfil the maximum co-ordination number of metal. The mixtures containing (A): 2 ml of 0.005 M HCl, (B): A + 2 ml of 0.05 M ligand, (C): B + 2 ml of 0.01 M metal ion were prepared. The ionic strength was maintained at 0.01, 0.1 and 0.3 M by KCl solution keeping total volume 20 ml. These mixtures were titrated individually against the standard alkali. A curve between pH and volume of the alkali required to reach the corresponding pH change was plotted. The shapes of titration curves were as usual.

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Results and Discussion

Proton-ligand stability constants

The average number of protons associated with the ligand ($\bar{n}_A$) at different pH were calculated, utilizing acid and ligand titration curves, by the formula

$$\bar{n}_A = y\,T\,C\,L_0 - \frac{(v'' - v')\,(N^0 + E^0)}{(V^0 + v')} \Big/ T\,C\,L_0\,,$$

where v', v'' are the volumes of alkali required to reach the same pH in acid and ligand titration curves respectively; y is the number of dissociable protons attached to the ligand; and N^0, E^0, $T\,C\,L_0$ and V^0 are the normality of alkali, strength of acid (in a total volume of 20 ml), total concentration of ligand and total volume of titrating solution, respectively. The values of proton-ligand stability constants, calculated from the formation curves according to Irving and Rossotti [6], are $\log K_1^{\mathrm{H}}$ 9.65, $\log K_2^{\mathrm{H}}$ 2.30 for methionine, $\log K_1^{\mathrm{H}}$ 9.41, $\log K_2^{\mathrm{H}}$ 2.18 for threonine and $\log K_1^{\mathrm{H}}$ 9.52, $\log K_2^{\mathrm{H}}$ 2.22 for phenylalanine. These values were obtained by extrapolation of the curves of $\log K^{\mathrm{H}}$ *versus* $\mu^{\frac{1}{2}}$ to zero ionic strength and the results agree with the literature values [7].

Metal-ligand stability constants

The nth metal-ligand stability constant (K_n) in the stepwise complex formation is given by

$$K_n = \frac{C_{\mathrm{ML}_n}}{C_{\mathrm{ML}_{n-1}}}\,\frac{1}{C_\mathrm{L}} \quad (n = 1, 2, \ldots n)\,.$$

The formation constants were obtained by plotting a graph between average number of ligands attached per metal ion ($\bar{n}$) and free ligand exponent ($p\,L$). The values of $\bar{n}$ and $p\,L$ were calculated using the equations

$$\bar{n} = \frac{(v''' - v'')\,(N^0 + E^0)}{(V^0 + v')\,\bar{n}_A \cdot T\,C_{\mathrm{M}_0}}$$

$$p\,L = \log_{10}\left[\frac{\sum\limits_{n=0}^{n=j} \beta_n^{\mathrm{H}} \left(\dfrac{1}{\text{antilog B}}\right)^n}{T\,C_{\mathrm{L}_0} - \bar{n}\,T\,C_{\mathrm{M}_0}}\;\frac{V_0 + v'''}{V^0}\right]$$

where $T\,C_{\mathrm{M}_0}$ is total concentration of metal, β_n^{H} overall proton-ligand stability constant, B is the pH, and other terms have usual meanings.

The formation curves (Figs 1 – 3) for vanadyl-amino acid complexes show that $\bar{n}$ does not exceed unity indicating the formation of only 1:1 complexes in the pH range 3.50 – 4.75 under the present experimental conditions. It has also been observed that beyond pH 4.75, the complexes were found to be unstable in the sense that there is an abrupt fall in pH, possibly due to hydrolysis. It is thus indicated that the complex formation is facilitated in acidic medium. The formation of 1:1 complexes of vanadyl ion with L-asparagine, β-alanine, L-alanine, glycine, L-leucine, L-proline, DL-serine, and DL-valine had already been reported by Farooq *et al.* [2].

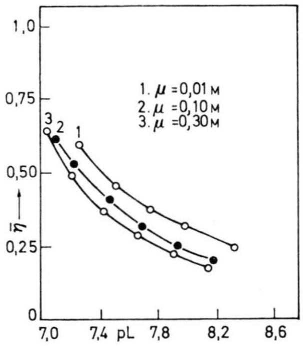

Fig. 1. Formation curves for threonine complexes.

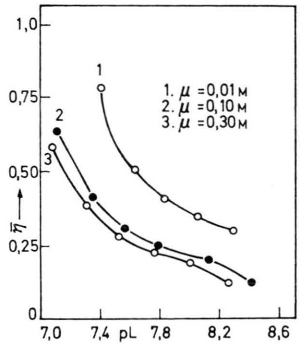

Fig. 2. Formation curves for phenylalanine complexes.

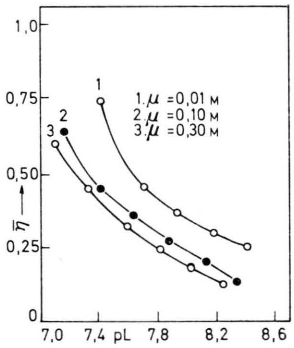

Fig. 3. Formation curves for methionine complexes.

The values of $\log K_1$ have been determined by a plot of $\log \frac{1-\bar{n}}{\bar{n}}$ *vs* pL for $\bar{n} < 1$. At the points falling on the straight line, the relationship[8]

$$\log K_1 = pL - \log \frac{1-\bar{n}}{\bar{n}} \text{ holds good.}$$

Average values of stability constants are reported in Table I which are in agreement with those obtained by Bjerrum half $\bar{n}$ method[4]. The values of the stability constants reported in third column of Table I are the concentration stability constants.

Table I. Stability constants and free energy of formation of vanadyl-amino acid complexes at 25 °C.

Amino acid	Ionic strength	$\log K_1$	$\log K_1^{\mu=0}$	$-\Delta G^0$ [kcal/mol]
DL-methionine	0.01	7.64		
	0.10	7.32	7.72	10.53 ± 0.2
	0.30	7.25		
L-phenylalanine	0.01	7.60		
	0.10	7.25	7.70	10.51 ± 0.2
	0.30	7.15		
L-threonine	0.01	7.40		
	0.10	7.25	7.44	10.15 ± 0.2
	0.30	7.19		

The thermodynamic stability constants have been obtained by extrapolation of plots of $\log K_1$ *versus* $\mu^{\frac{1}{2}}$ to zero ionic strength[9] and their values are given also in Table I.

The free energy changes (ΔG^0) were calculated by the formula:

$$\Delta G^0 = -2.303\,R\,T \log K^{\mu=0}$$

where $K^{\mu=0}$ is the formation constant at zero ionic strength.

The values are reported in Table I.

It is seen that the stability constants and the standard free energies of formation are not very different from each other and they are in the decreasing order, *i. e.* methionine > phenylalanine > threonine. The error limits are ±0.05 for $\log K_n$ values.

The effect of increasing ionic strength on the stability constants is also self evident because the ionic activity should decrease. Accordingly in the case of these amino acids, the stability constant decreases with the increase in the value of μ. The slopes of the curves obtained by plotting $\log K_1$ against $\mu^{\frac{1}{2}}$ for complexes of methionine and phenylalanine are -1 in each case, whereas the slope in the case of threonine system is -0.5. Thus it is seen that the decrease in stability is same with increasing ionic strength for methionine and phenylalanine systems. However, for threonine system, the corresponding decrease in stability is less.

It is known[10] that various amino acid anions which commonly occur in aqueous systems of plant and animals, as well as in soil, form stable, soluble chelates of essential metals. As vanadium(IV) is found to form 1:1 chelates with these amino acids and those already reported by Farooq *et al.*[2] and chelate formation is favourable in acidic medium. Therefore, it is suggested that amino acid anions and sufficient additional water of hydration satisfy the co-ordination requirement of the metal ion and are present under acidic soil condition. Thus it is possible that roots of plant secrete and release small amounts of amino acids which solubilize and transport trace metals. The exact nature of these materials is not yet known but amino acids most certainly are chelating agents and have sufficient affinity for essential metal ions to form stable, soluble complexes under soil conditions.

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