

# Chelate Formation of Riboflavine with Yttrium (III), Lanthanum (III), and Cerium (III)

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## Riboflavine Chelates, Metal Chelates, Stability Constants

Stepwise stability constants of the interactions of 7,8-dimethyl (ribo-2,3,4,5-tetrahydroxypentyl) isoalloxazine (riboflavine) with Y(III), La(III) and Ce(III) have been determined at 35 and 45 °C and ionic strength of 0.05 M using Bjerrum-Calvin pH titration technique. The values of free energy ( $\Delta G$ ), enthalpy ( $\Delta H$ ) and entropy changes ( $\Delta S$ ) have been reported.

Trace elements appears to play an important part in cancer and trace element requirement is a consequence of this disease. Rechenberger<sup>1</sup> has demonstrated experimentally that the iron and copper content of the blood serum are always disturbed and he showed that cancer elevates the copper level of the serum in all cases, and depresses the iron content to a level well below average, except in acute leukaemia. Voisin<sup>2</sup> in his studies on cancer and soil, lays great stress on the important role that copper is supposed to play in cancer.

Halme<sup>3</sup> suggested that zinc accumulation may also be an important factor in man, and he pointed out that in his native Finland 35 per cent of the drinking waters contain high levels of zinc. Certainly, upsets in the zinc metabolism have been observed in other forms of cancers. On the other hand, some trace elements have, on occasions, been shown to exert an inhibitory influence on the cancer development. Thus, manganese malate inhibits new growths of tumours in rats and mice. Apparently it alters the metabolism of tumour and tends to promote the normal aerobic respiration of cells, in place of the pathological anaerobic respiration of cancer cells<sup>4</sup>. Other workers<sup>5,6</sup> have shown that copper compounds can have inhibiting effect on tumour formation. Metal salts and metal chelates including zinc complex of riboflavine-like compound<sup>7,8</sup> also inhibit the tumour growth. At present there is little doubt that trace elements do play a part in the development of, as well as in the resistance, to this lethal disease.

The transition metal ions having a pronounced tendency for chelate formation are associated with

vitamins, protein, enzymes, and nucleic acids and have been found to alter the composition in cancer cell. Also these carcinogenic (abnormal) metal ions may replace essential metals from the normal specific enzyme with similar electronic configuration. The application of metal for metal replacement (*i. e.* metal ions as probes) technique to the problems of elucidating metal-binding sites depends upon the fact that the electronic properties of the metal ion may be made available to enzymes having metal ions without these electronic properties by metal for metal replacement. Thus a main group metal may be replaced by a transition metal ion. There is a tremendous interest at present in the probes of this type. For successful use of a probe metal ion it must fill the same site as the original metal ion. A useful guide here is whether or not biological activity of the molecule is maintained. If this is so, then the new metal ion is assumed to be bound at the same site. The ionic radius of the metal ion is an important consideration. The probe metal must also have similar requirement in terms of preferred stereochemistry of the metal binding site and the nature of the binding ligands. In this connection, the metal for metal replacement of  $\text{Ca}^{2+}$  by lanthanide<sup>3+</sup> ions and that of  $\text{Fe}^{3+}$  by  $\text{Gd}^{3+}$ ;  $\text{Tb}^{3+}$ ,  $\text{Eu}^{3+}$ ,  $\text{Er}^{3+}$ ,  $\text{Ho}^{3+}$  have been used in the study of biological systems<sup>9</sup>. The lanthanide elements are particularly useful as there is the possibility of having a series of probes differing only slightly in ionic size.

Since riboflavine 7,8 dimethyl (ribo 2,3,4,5-tetrahydroxypentyl) isoalloxazine has a chelating tendency, and more information is needed about the chelates formed with carcinogenic metal ions under physiological conditions, for an understanding of the chelate hypothesis in cancer problem. A comparison of  $K_n$ ,  $\Delta G$ ,  $\Delta H$  and  $\Delta S$  and thereby the free

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metals in equilibrium with the chelate may give good information. Also since some metal salts and metal chelates are used as anticancer compounds, the metal chelates formed under different physiological pH conditions may be used for the inhibition of tumour.

No information is, however, available on the chelation of rare earth metal ions with riboflavine. Though the chelates of this compound with Cu(II), Ni(II), Zn(II), Co(II), Cd(II), Fe(II), Mn(II) and Mg(II) have already been studied by Albert<sup>10</sup> and those of Al(III), Cr(III), Be(II), Pb(II) and U(VI) by Nayan and Dey<sup>11</sup>. It was, therefore, thought desirable to study the chelating behaviour of some of the rare earth elements with riboflavine.

The present work describes the determination of stepwise proton-ligand stability constants and metal-ligand stability constants using Bjerrum-Calvin technique<sup>12, 13</sup> as used by Irving and Rossotti<sup>14</sup>. The overall changes in free energy of formation ( $\Delta G$ ), enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) have been evaluated at 35, 45 °C and in ionic strength of 0.05 M.

### Materials and Methods

Solutions of riboflavine (E. Merck) was prepared in aqueous potassium hydroxide. The solutions of La(NO<sub>3</sub>)<sub>3</sub> · 6 H<sub>2</sub>O, Y(NO<sub>3</sub>)<sub>3</sub> · 4 H<sub>2</sub>O and Ce<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> ·

8 H<sub>2</sub>O were prepared in glass distilled water from Analar grade.

'Systonix' pH meter with a glass and calomel electrode assembly was used to record the changes in pH and measurements were checked before and after the titrations with standard buffers. The titrations were carried out at 35 ± 0.1 °C and 45 ± 0.1 °C in a constant temperature water-bath.

### Procedure

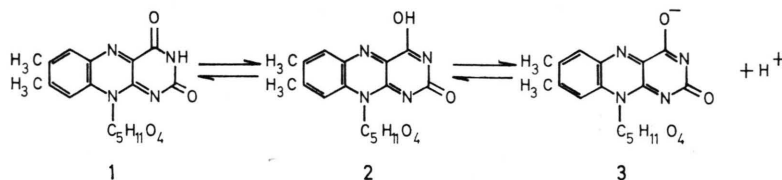
The mole ratio of metal to ligand was kept 1:5 in order to fulfill the maximum co-ordination number of metal ions. The following mixtures were prepared: Mixture A: 5 ml of 0.01 M HNO<sub>3</sub>; mixture B: A + 20 ml of 0.0025 M riboflavine; mixture C: B + 5 ml of 0.002 M metal ion.

The ionic strength was adjusted by adding KNO<sub>3</sub> solution, keeping the total volume 50 ml in each case. The mixture A, B and C were titrated against standard alkali potentiometrically. The plots of the volume of alkali used versus pH of solutions were of usual shape.

## Results and Discussion

### Proton-ligand stability constant

Though riboflavine (**1**) does not have a phenolic group, the tautomeric form (**2**) contains phenolic hydrogen<sup>10</sup> which dissociates as shown in (**3**).



To calculate the proton ligand formation constant, the average number of protons associated with the ligand ( $\bar{n}_A$ ) at different pH values were calculated using the acid and ligand titration curves. According to Irving and Rossotti<sup>14</sup>

$$\bar{n}_A = y T C_{L_0} - \frac{(v'' - v')(N^0 + E^0)}{(V^0 + v')} \bigg/ T C_{L_0}, \quad (1)$$

where  $v'$ ,  $v''$  are the volume of alkali in acid and ligand titration to reach the same pH value.  $V^0$ ,  $E^0$ ,  $N^0$  are the total volume of the titrating mixture, total concentration of acid, total concentration of alkali respectively,  $y$  is the number of dissociable protons. The values of proton-ligand stability constants, which were determined by a. interpolation

and half  $\bar{n}$  values and b. average value method, are in agreement (Table I).

Table I. Stability constants of riboflavine chelates ( $\mu = 0.05$  M).

Cation	T [°C]	log $K_1$	log $K_2$	log $K_3$	log $\beta_3$
H <sup>+</sup>	35	9.95			
	45	9.60			
La(III)	35	6.28	5.25	4.30	15.83
	45	6.29	5.85	5.50	17.64
Ce(III)	35	7.00	6.45	6.09	19.52
	45	6.86	6.09	5.83	18.78
Y(III)	35	6.44	6.06	5.45	17.95
	45	7.00	6.33	6.20	19.53

### Metal-ligand stability constant

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

$$K_n = \frac{C_{ML_n}}{C_{ML_{n-1}} C_L} \dots (n=1, 2, \dots n).$$

The stability constants were obtained by plotting average number of ligands attached per metal ion ( $\bar{n}$ ) versus free ligand exponent ( $pL$ ). The  $\bar{n}$  and  $pL$  values were calculated using the equations

$$\bar{n} = \frac{(v''' - v'') (N^0 + E^0)}{(V^0 + v') \bar{n}_A T C_{M_0}}$$

$$pL = \log_{10} \left[ \frac{\sum_{n=0}^j \beta_n^H \left( \frac{1}{\text{antilog } B} \right)^n}{T C_{L_0} - \bar{n} T C_{M_0}} \frac{V_0 + v''}{V^0} \right]$$

where  $T C_{M_0}$  is total concentration of metals  $\beta_n^H$  overall proton-ligand stability constant,  $B$  is the pH, and other terms have usual meanings.

The formation curves (Figs 1–3) were obtained by plotting  $\bar{n}$  against  $pL$  of metal chelates with riboflavine. The values of stability constants were obtained by various computational methods<sup>15</sup> a. at half  $\bar{n}$  values, b. interpolation at various  $\bar{n}$  values which are in close agreement. The average values of the metal ligand stability data at ionic strength of 0.05 M are reported in Table I. The error limits are  $\pm 0.05$  for  $\log K_n$  values.

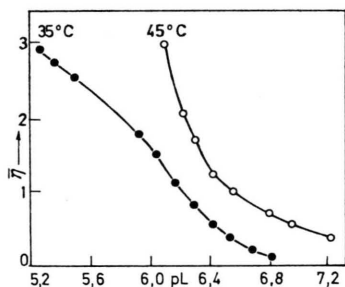


Fig. 1. Formation curves for Y(III) chelates.

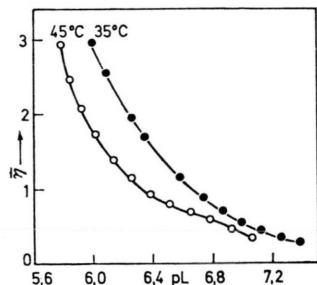


Fig. 2. Formation curves for Ce(III) chelates.

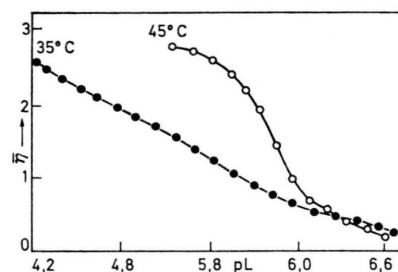


Fig. 3. Formation curves for La(III) chelates.

An examination of the titration curves indicated that the metal titration curves are well separated from ligand titration curves showing that liberation of protons is due to complexation. The chelate formation begins from pH 5.80, 5.25, 5.50 in the case of La(III), Ce(III) and Y(III) respectively. The formation curves (Figs 1–3) indicate that in all cases, the values of  $\bar{n}$  approach 3. It is thus clear that for all these systems, three chelates  $ML$ ,  $ML_2$ , and  $ML_3$  are formed ( $L \equiv$  riboflavine).

From the data (Table I) it is observed that the formation of chelates is more favourable at higher temperature for La(III) and Y(III) ions, while the stability is decreased at higher temperature for cerium (III) chelates. It is also observed that values of  $\log K_1$  for La(III) chelates are equal at 35 and 45 °C viz. 6.28 and 6.29 respectively, thus indicating that stabilities are enhanced only during the second and third steps of complexation as the values  $\log K_2$  are 5.25 and 5.85 at 35 and 45 °C. The values of  $\log K_3$  are 4.30 and 5.50 at 35 and 45 °C respectively. This is because the formation of more rings results in an increase in the stabilities. The observed stability order for  $\log \beta_n$  values is given by Y(III) > Ce(III) > La(III) at 35 and 45 °C with exception of higher stability for Ce(III) than Y(III) at 35 °C. The interpretation of the trends in stability constant data cannot be accounted for by any single factor. The stability order Y > Ce > La is the same as increasing radii of these ions and is also justified by considering  $Z^2/r$  values for these metal ions. The greater stability of cerium chelates as compared with yttrium at 35 °C is justified on the basis of their second ionization potential.

### Thermodynamic parameters

The following relationships were used to derive the thermodynamic parameters:

$$\Delta G = -RT \ln \beta$$

$$\frac{d(\ln \beta)}{dT} = \frac{\Delta H}{RT^2}$$

and

$$\Delta G = \Delta H - T \Delta S.$$

The values of overall changes in free energy of formation ( $\Delta G$ ), enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) are summarized in Table II. The accuracy of  $\Delta H$

Table II. Thermodynamic parameters of riboflavine chelates ( $\mu=0.05$  M).

Metal ion	T [°C]	$\Delta G$ [Kcal/mol]	$\Delta H$ [Kcal/mol]	$\Delta S$ [cal/deg/mol]
La(III)	35	-22.32	81.0	335.6
	45	-25.67		335.6
Ce(III)	35	-27.52	-33.1	-18.2
	45	-27.33		-18.2
Y(III)	35	-25.31	70.1	311.8
	45	-28.42		311.8

values is  $\pm 2.0$  Kcal/mol and that of  $\Delta S$  values is  $\pm 7$  cal/deg/mol.

The data reported in Table II show negative values of  $\Delta H$  and  $\Delta S$  for Ce(III) chelates. It is

only the  $\Delta H$  which is favourable for chelation. It is also evident that the values of  $\Delta H$  are positive for riboflavine chelates of lanthanum and yttrium which means that the formation of such chelates take place with absorption of energy. In spite of these unfavourable enthalpy changes, the chelates are stabilized by very large entropy increase. The values of  $\Delta S$  (335.6) for La(III) and (311.8) for Y(III) chelates have resulted not only from charge neutralization during chelation but also from disruption of primary hydration sphere and freeing of water molecules surrounding the metal ions. This is supported by the fact that chelation is favoured by entropy change in terms of originally bound water molecules<sup>16</sup> and further that majority of rare earth metal ion chelates are entropy stabilized.

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