

## THERMODYNAMICS OF ION ASSOCIATION: TRANSITION METAL COMPLEXES OF *N*-ISOBUTYROYL-L-LYSINE

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### ABSTRACT

Calorimetric measurements have been made at 25°C in aqueous 0.1 M NaClO<sub>4</sub> for the formation of the mono- and bis-*N*-isobutyroyl-L-lysine complexes with copper(II), nickel(II), cobalt(II) and zinc(II). These data have been used in conjunction with previously determined stability constants, to calculate  $\Delta G$ ,  $\Delta H$  and  $\Delta S$  values for both protonation and metal complexation. These are discussed in terms of the nature of the metal ligand binding. All thermodynamic data are in agreement with the formation of glycine-like mono- and bis-chelates involving the  $\alpha$ -amino and carboxyl groups.

### INTRODUCTION

The transition metal amino acid and peptide complexes are simple models for the mode of binding of metal ions in proteins and enzymes and have thus received much recent attention. Numerous papers have been devoted to the determination of the stability constants of these complexes by potentiometric titrations. Comparatively, few precise values of the enthalpy changes accompanying these reactions have been obtained.

However, such values are of great importance, as they give a direct insight into the mode of bonding between the metal ions and the ligand and allows the correlation between the strength of the bonding and the nature of the donor atoms.

Moreover, the combination of the enthalpy change of the reaction with the free enthalpy change derived from stability constants, allows the calculation of the entropy changes associated with the reaction. This provides additional information about the structure of the complexes and the role played by the solvent.

For a long time,  $\Delta H$  values were derived from the temperature dependence of the stability constants. Unfortunately, these values are submitted to

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unacceptable uncertainties and direct calorimetric measurements have become the most reliable method.

This paper reports a microcalorimetric investigation of the enthalpy change associated with the complex formation between some divalent metal ions (Cu(II), Ni(II), Co(II) and Zn(II)) and *N*- $\epsilon$ -isobutyroyl-L-lysine  $(\text{CH}_3)_2\text{CHCONH}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{COOH}$ , an amino acid derived from L-lysine. Previously reported values of the formation constants are used to calculate all the thermodynamic parameters of the reactions which are compared with those of parent compounds.

## EXPERIMENTAL

### *Materials*

*N*- $\epsilon$ -Isobutyroyl-L-lysine (NIBL) was synthesized and purified as described elsewhere [1]. Copper(II), nickel(II), cobalt(II) perchlorates and zinc(II) nitrate (Merck or Fluka) were reagent grade chemicals. Metal solutions were analyzed by passing through an Amberlite IR 120 ion-exchange resin in the acid form and titrating the resulting acid solution with standard sodium hydroxide.

Sodium hydroxide solutions were standardized against oxalic acid and perchloric acid solutions against sodium carbonate.

All measurements were made at constant ionic strength of 0.1 M sodium perchlorate (Merck).

### *Methods*

All measurements were carried out at  $25 \pm 0.01^\circ\text{C}$ . Calorimetric measurements were made with a Calvet type batch microcalorimeter (Setaram, France). Inox cells (100 ml) containing a glass or inox inner tube of varying volume were used. This allows the mixing of solutions with variable volume ratios. Before being introduced into the apparatus, cells were preheated at a temperature as close as possible to the inner temperature of the calorimeter. Then, when thermal equilibrium was attained, the calorimeter was rocked two or three times to ensure complete mixing of the solutions. The heat signal was amplified and recorded as a function of time. The heat effect (in J) was obtained after integration of the experimental curve and calibration.

In a typical experiment, a known volume of a NIBL and metal ion solution at a given initial pH ( $\text{pH}_i$ ) was placed in one compartment of the cell. A known volume of  $\text{HClO}_4$  or  $\text{NaOH}$  solution was introduced into the second compartment. After the experiment, the final pH of the mixture was carefully measured ( $\text{pH}_f$ ). From the stability constants of each complex, previously determined [2] and using the COMSOL computer program [2],

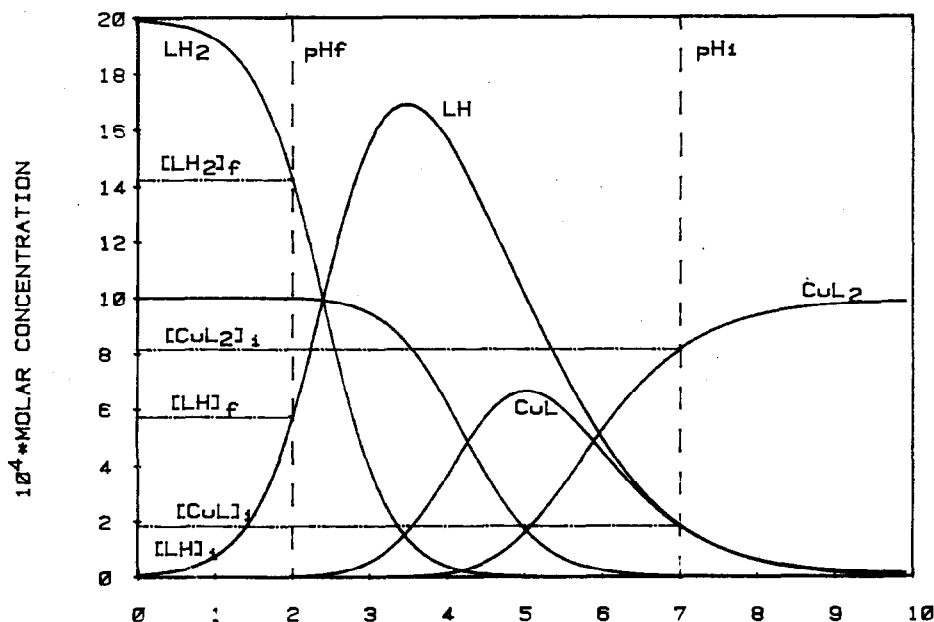


Fig. 1. Distribution curve for the NIBL-Cu(II) system ( $[NIBL] = 1.10^{-3}$  M;  $[Cu] = 1.10^{-3}$  M).

the distribution curves of the different species as a function of pH were calculated. From these curves the concentration change of each species between  $pH_i$  and  $pH_f$  could be calculated (see Fig. 1 for example).

The pH measurements were made with a Tacussel Minisis 5000 precision pH-meter ( $\pm 0.01$  pH unit) with a TBHS glass electrode and a C8 calomel reference electrode. The pH-meter was carefully standardized before and after each experiment because most of the precision on the heat effects depends on the precision of the pH change. Potassium hydrogenphthalate and borax buffers were used for the standardization.

The microcalorimeter was calibrated both by Joule effect using special calibration cells and by measuring the heat of dilution of sodium chloride solution [3].

## Results

The recorded heat effect during an experiment includes a variety of terms: the heat of dilution of the two solutions ( $q_d^L$  for the dilution of the ligand/metal solution;  $q_d^R$  for the dilution of the reagent,  $HClO_4$  or  $NaOH$ ), the heat of neutralization of  $H^+$  or  $OH^-$  (formation of water molecules)  $q_{OH}$ , the heats of neutralization (or protonation) of the ligand,  $q_{LH}$  and  $q_{LH_2}$ ,

and the heats of formation (or destruction) of the complexes (two complexes ML and  $ML_2$  for NIBL [2])  $q_{ML}$  and  $q_{ML_2}$ .

$$q = q_d^L + q_d^R + q_{OH} + q_{LH_2} + q_{ML} + q_{ML_2} \quad (1)$$

The heats of dilution are dependent both on the initial concentrations of the reagents and on the dilution ratio (final volume/initial volume). The heat of dilution of NIBL/Cu mixtures in 0.1 M  $NaClO_4$  and of  $HClO_4$  in 0.1 M  $NaClO_4$  were determined in separate experiments. These heat effects are very weak for the dilute solutions used in our study.

The  $q_{OH}$  contribution was calculated from the pH variation (giving the concentration change of hydroxyl ions  $\Delta[OH]$ ) and the heat of formation of water  $\Delta H_{H_2O}$ .

$$q_{OH} = \Delta H_{H_2O} (-\Delta[OH]) \quad (2)$$

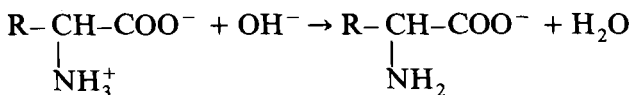
We used the value  $\Delta H_{H_2O} = -56.39 \text{ kJ mol}^{-1}$  valid for 0.1 M  $NaClO_4$  medium [4] ( $\Delta H_{H_2O} = -56.48 \text{ kJ mol}^{-1}$  in 1 M  $NaClO_4$  [5]).

The corrected heat effect  $q_c$  was then calculated by:

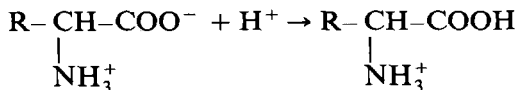
$$q_c = q - q_d^L - q_d^R - q_{OH} \quad (3)$$

### Heats of protonation

The heat of protonation of the  $NH_2$  group of NIBL was first measured by adding sodium hydroxide to a solution of the amino acid in the zwitterionic form.



The heat of protonation of the carboxyl group of NIBL was determined in the same way using perchloric acid as the reagent:



Experimental data are given in Table 1.

For reaction (4) the mean value is  $-11.56 \pm 0.09 \text{ kJ mol}^{-1}$ . Taking into account the heat of formation of water, the value  $\Delta H_{LH} = -44.83 \pm 0.09 \text{ kJ mol}^{-1}$  is obtained for the protonation of the amino group:



TABLE 1

Heats of neutralization of NIBL with sodium hydroxide or perchloric acid (in aqueous 0.1 M NaClO<sub>4</sub>; 298 K)<sup>a</sup>

$C_{LH} \times 10^3$ (mol dm <sup>-3</sup> )	$C_{NaOH} \times 10^2$ (mol dm <sup>-3</sup> )	$C_{HClO_4} \times 10^2$ (mol dm <sup>-3</sup> )	pH <sub>i</sub>	pH <sub>f</sub>	$\Delta n_{LH} \times 10^5$ (mol)	$-q_c$ (J)	$-\Delta H$ (J mol <sup>-1</sup> )
1.003	1.515	—	6.36	11.24	1.940	0.224	11 537
1.003	1.515	—	6.36	11.25	1.941	0.225	11 615
2.345	1.515	—	6.41	10.11	3.886	0.444	11 422
2.345	1.515	—	6.43	9.82	3.320	0.388	11 683
10.03	—	2.012	6.36	1.88	16.060	0.034	209
10.03	—	2.012	6.36	1.24	18.512	0.035	192
23.45	—	2.012	6.36	1.61	41.178	0.091	221
23.45	—	2.012	6.36	1.37	43.382	0.092	213

<sup>a</sup>  $C_{LH}$ , Total concentration of ligand;  $C_{NaOH}$ , molar concentration of sodium hydroxide;  $C_{HClO_4}$ , molar concentration of perchloric acid; pH<sub>i</sub>, initial pH; pH<sub>f</sub>, final pH;  $\Delta n_{LH}$ , variation of the number of moles of ligand in the LH form;  $q_c$ , corrected heat effect;  $\Delta H$ , molar enthalpy.

The mean value for the protonation of the carboxyl group is  $\Delta H_{LH_2}^H = -209 \pm 8 \text{ J mol}^{-1}$ .



and for overall reaction:



$$\Delta H_{LH_2} = -45.04 \text{ kJ mol}^{-1}.$$

### Heats of complexation

Starting from a NIBL–metal ion mixture, a sodium hydroxide solution was added to achieve varying metal–ligand complexations (ligand to metal ratios ranging from 1 to 5 were used in these experiments). Then this solution was mixed with excess perchloric acid (final pH around 1) and the heat effect was measured. In a series of experiments, the initial pH was varied to have various amounts of the ML and ML<sub>2</sub> complexes. In the case of copper, for example, Fig. 1 shows that pH<sub>i</sub> should be adjusted between 4 and 8. In each experiment the concentrations of the L, LH and LH<sub>2</sub> species also changed. Then, the corrected heat effect  $q_c$  is given by:

$$q_c = q_{LH_2} + q_{LH} + q_{ML} + q_{ML_2} \quad (9)$$

As  $\Delta H_{LH}$  and  $\Delta H_{LH_2}$  are known and the variations in the number of moles of LH and LH<sub>2</sub> are obtained from the distribution curve (Fig. 1), it results that:

$$q_{LH_2} = \Delta H_{LH_2} \Delta n(LH_2)$$

$$q_{LH} = \Delta H_{LH} \Delta n(LH) \quad (10)$$

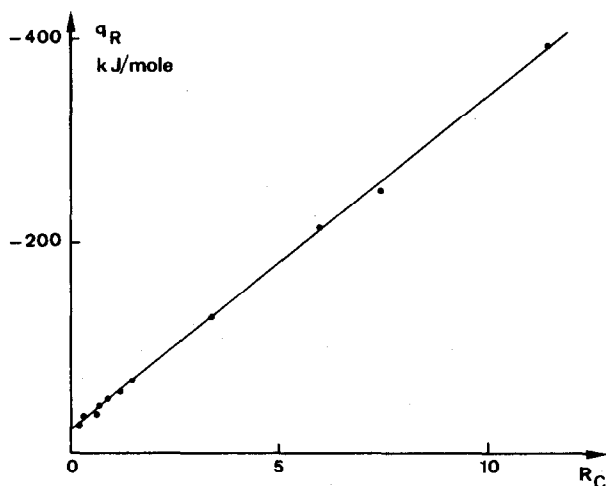


Fig. 2. Plot of  $q_R = f(R_C)$  (see text) for the NIBL-Cu(II) system.

and

$$q'_c = q_c - q_{LH_2} - q_{LH} = \Delta n(\text{ML}_2) \Delta H_{\text{ML}_2} + \Delta n(\text{ML}) \Delta H_{\text{ML}}$$

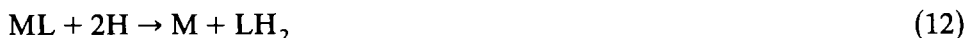
where  $\Delta n(\text{ML}_2)$  and  $\Delta n(\text{ML})$  are the variations of the number of moles of each complex and  $\Delta H_{\text{ML}_2}$  and  $\Delta H_{\text{ML}}$  are the corresponding enthalpy changes. Thus:

$$q_R = q'_c / \Delta n(\text{ML}) = \Delta H_{\text{ML}} + \Delta H_{\text{ML}_2} \Delta n(\text{ML}_2) / \Delta n(\text{ML}) \quad (11)$$

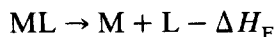
Values of  $\Delta H_{\text{ML}}$  and  $\Delta H_{\text{ML}_2}$  are then obtained as the intercept and slope of the curve  $q_R = f(R_C)$  with  $R_C = \Delta n(\text{ML}_2) / \Delta n(\text{ML})$ .

Figure 2 shows a typical curve obtained for the NIBL-copper system.

$\Delta H_{\text{ML}}$  corresponds to the reaction:



which is the sum of:



and



The heat of formation  $\Delta H_{\text{F}}$  of the ML complex is then obtained from:

$$\Delta H_{\text{F}} = \Delta H_{\text{LH}_2} - \Delta H_{\text{ML}}$$

$\Delta H_{\text{ML}_2}$  is for reaction:



which is the sum of:

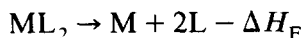


TABLE 2

Thermodynamic values for the complexes of NIBL with  $H^+$ ,  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Co^{2+}$  and  $Zn^{2+}$  ( $t = 298\text{ K}$ ;  $0.1\text{ M NaClO}_4$ )

Reaction	$\log \beta$ or $\log k$ (from ref. 2)	$-\Delta G$ ( $\text{kJ mol}^{-1}$ )	$-\Delta H_F$ ( $\text{kJ mol}^{-1}$ )	$\Delta S$ ( $\text{J mol}^{-1}$ )
$L + H \rightarrow LH$	9.42	$54.05 \pm 0.06$	$44.83 \pm 0.09$	$30.9 \pm 1.5$
$LH + H \rightarrow LH_2$	2.40	$13.77 \pm 0.06$	$0.21 \pm 0.06$	$45.5 \pm 1.5$
$L + 2H \rightarrow LH_2$	11.82	$67.82 \pm 0.12$	$45.04 \pm 0.10$	$76.4 \pm 3.0$
$L + Cu \rightarrow CuL$	8.00	$45.90 \pm 0.06$	$26.84 \pm 0.20$	$64.0 \pm 3.0$
$CuL + L \rightarrow CuL_2$	6.80	$39.01 \pm 0.06$	$29.43 \pm 0.20$	$32.1 \pm 1.6$
$2L + Cu \rightarrow CuL_2$	14.80	$84.91 \pm 0.12$	$56.27 \pm 0.40$	$96.1 \pm 4.6$
$L + Ni \rightarrow NiL$	5.21	$29.89 \pm 0.18$	$20.90 \pm 0.20$	$30.2 \pm 1.8$
$NiL + L \rightarrow NiL_2$	4.29	$24.61 \pm 0.12$	$18.81 \pm 0.20$	$19.5 \pm 1.0$
$2L + Ni \rightarrow NiL_2$	9.50	$54.50 \pm 0.30$	$39.71 \pm 0.40$	$49.7 \pm 2.8$
$L + Co \rightarrow CoL$	4.26	$24.44 \pm 0.18$	$13.54 \pm 0.15$	$36.6 \pm 2.3$
$CoL + L \rightarrow CoL_2$	3.12	$17.90 \pm 0.12$	$11.70 \pm 0.15$	$20.8 \pm 1.1$
$2L + Co \rightarrow CoL_2$	7.38	$42.34 \pm 0.30$	$25.24 \pm 0.30$	$57.4 \pm 3.4$
$L + Zn \rightarrow ZnL$	4.89	$28.06 \pm 0.30$	$11.29 \pm 0.25$	$56.3 \pm 2.8$
$ZnL + L \rightarrow ZnL_2$	3.40	$19.51 \pm 0.30$	$9.61 \pm 0.25$	$33.2 \pm 1.7$
$2L + Zn \rightarrow ZnL_2$	8.29	$47.57 \pm 0.60$	$20.90 \pm 0.50$	$89.5 \pm 4.5$

and



In the same way  $\Delta H_F$  is given by:  $\Delta H_F = 2\Delta H_{LH_2} - \Delta H_{ML_2}$ .

All the thermodynamic values obtained for the complexes of NIBL with  $H^+$ ,  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Co^{2+}$  and  $Zn^{2+}$  are given in Table 2. The formation constants  $\log \beta$  or equilibrium constants  $\log K$  were determined previously [2].

## DISCUSSION

### Heats of protonation

The heat of protonation of the amino group of NIBL is high and reflects the formation of a highly covalent bond between nitrogen and proton. It is close to values reported for other amino acids such as glycine and alanine ( $-45.94$  and  $-49.20$  [6]), proline and hydroxyproline ( $-47.44$  and  $-43.22$  [7]), valine ( $-47.46$  [8]) ornithine and lysine from which NIBL is derived ( $-47.08$  and  $-47.25$  for the  $\alpha\text{-NH}_2$  [9]).

Enthalpies of protonation of  $\alpha\text{-NH}_2$  in dipeptides have similar values [10].

The corresponding entropy change  $\Delta S$  is similar to those observed for other amino acids [11]. It results from the formation of a zwitterionic species with a partial charge neutralization which leads to the release of water molecules.

The enthalpy term contributes for the most part to the free enthalpy of this protonation.

The enthalpy of protonation of the carboxylate group is very low as is usual for this type of reaction [4,12]. On the contrary, the entropy change is high and results from complete neutralization of two charged species with the liberation of a high number of electrostricted water molecules. In this case, the entropy term is the major contributor to the free enthalpy change.

For the ligand-metal complexes with Cu(II), Ni(II), Co(II) and Zn(II), all the  $\Delta H$  values are highly negative and the  $\Delta S$  values highly positive, which both contribute to a high negative free enthalpy change. In all cases, the enthalpies of complexation are similar to those of amino acids which form glycine-like complexes [4,6-10,12-14]. Thus, the formation of a five-membered chelate ring between the  $\alpha$ -amino and the carboxyl group of NIBL is very likely [1,2].

For the NIBL-copper, values are very close to those reported for glycine-copper [4]. The enthalpy terms for the first and second step of complexation are nearly equal and the entropy term of the second step is half the value for the first one. This is due to a loss of configurational entropy. In this case the variation in the free enthalpy change between the two steps is entirely caused by the entropy change.

For the complexes with nickel, cobalt and zinc, the addition of the second ligand ( $ML + L \rightarrow ML_2$ ) is less exothermic. This is probably the result of increased steric hindrance caused by the bulky chain of NIBL;  $\Delta S$  also decreases from the first to the second step as for copper. Both the enthalpy and entropy terms contribute to the decrease of the free enthalpy change [16].

In agreement with the Irving-Williams series ( $Co < Ni < Cu > Zn$ ),  $\Delta H$  decreases from copper to nickel, zinc and cobalt for the two steps, whereas  $\Delta S$  decreases from copper to zinc, cobalt and nickel. The higher  $\Delta H$  and  $\Delta S$  values obtained for the copper complexes are related to its  $d_9$  configuration and the Jahn-Teller effect. The tetragonal distortion of the octahedral symmetry causes a shortening of the  $xy$  bonds and a stretching of the  $z$  bonds. Thus the Cu-N bonding is more covalent and more exothermic. In addition, the net charges of metal and ligand are better balanced, lowering the electrostriction between charges and the dipoles of water molecules, and increasing the solvent entropy [15].

All the experimental results of Table 2 support the formation of glycine-like chelates and rules out the participation of the amide bond in the formation of the complexes.

According to previous works [17-18], it is possible to split the enthalpy



TABLE 3  
Values of  $\Delta n$ ,  $\Delta H_e$ ,  $\Delta H_c$  and  $\Delta H_0$  for NIBL complexes

Complex	$\Delta n$	$\Delta H_e$ (kJ mol <sup>-1</sup> )	$-\Delta H_c$ (kJ mol <sup>-1</sup> )	$-\Delta H_0$ (kJ mol <sup>-1</sup> )	Mean
CuL	1	7.71	33.5	33.5	34.05
CuL <sub>2</sub>	2	12.90	69.2	34.6	
NiL	1	5.04	25.9	25.9	25.2
NiL <sub>2</sub>	2	9.23	48.9	24.5	
CoL	1	5.54	19.1	19.1	18.3
CoL <sub>2</sub>	2	9.84	35.1	17.5	
ZnL	1	7.10	18.4	18.4	17.5
ZnL <sub>2</sub>	2	12.37	33.3	16.6	

term  $\Delta H$  into two parts, dependent and independent of temperature:

$$\Delta H_c = \Delta H - \Delta H_e \quad (16)$$

According to ref. 17, the temperature independent contribution,  $\Delta H_c$  is almost entirely responsible for the observed heat changes. The temperature-dependent term  $\Delta H_e$ , sensitive to the environment, especially to the dielectric constant of the solvent is given by:

$$\Delta H_e = (T - a)(\Delta S + \Delta nR \ln 55.5) \quad (17)$$

where  $a$  is the temperature coefficient of the dielectric constant of the solvent (219 K for water) and  $\Delta n$  is the change in the number of particles during the reaction.

Table 3 gives the values of  $\Delta H_e$ ,  $\Delta H_c$  and  $\Delta H_0$  (per mol of ligand) for each complex.

The value  $\Delta H_0$  represents the heat for the formation of one metal-oxygen (carboxylate) and metal-nitrogen bond;  $\Delta H_0$  decreases from copper to nickel, cobalt and zinc. The value obtained for nickel is in reasonable agreement with those of previous works (23.68 kJ mol<sup>-1</sup> [8] and 24.6 kJ mol<sup>-1</sup> [17]). Our results also show that for the four metal ions,  $\Delta H_c$  is the main contribution to the experimental enthalpy change.

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