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ENTHALPIES OF LIGATION OF Co^{2+} AND Cu^{2+} TO EPIMERIC THREONINES AND ISOLEUCINES

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ABSTRACT

The enthalpies of protonation of epimeric threonines and isoleucines were determined calorimetrically at 298.15 K in 0.1 mol dm^{-3} $NaNO_3$. The enthalpies of ligation of the same ligands with Co^{2+} and Cu^{2+} were determined under the same conditions.

INTRODUCTION

The complexes of natural forms of threonine (Thr) and isoleucine (Ile) with cobalt(II) and copper(II) are appreciably more stable than those formed by respective *allo* epimers[1, 2]. This difference in the stability, which does not correlate with the ligand configuration, is not easy to understand on the ground of the existing stability[1, 2] and spectral[2] data. Therefore a calorimetric investigation of these ligational reactions was undertaken.

EXPERIMENTAL

Chemicals

All reagents were of the analytical reagent grade (or equivalent) and were used without further purification provided the assay was >99%. The ligand solutions were standardised by potentiometric titration and the metal ion stock solutions by visual complexometric titration. All solutions contained a $NaNO_3$ background (0.1 mol dm^{-3}).

Calorimeter design

An isoperibol calorimeter of Lund type was built for the purpose of the present investigation. The constructional details can be seen from Fig. 1. The temperature was sensed by means of two 100 kΩ thermistors (ITT, type F 15) connected as two opposite legs of a symmetrical d.c. Wheatstone bridge energised from a constant-voltage power supply (7.2 V). The remaining bridge components were two high quality helipots (100 kΩ and 1 kΩ) and a 92 kΩ manganin resistor. The bridge output

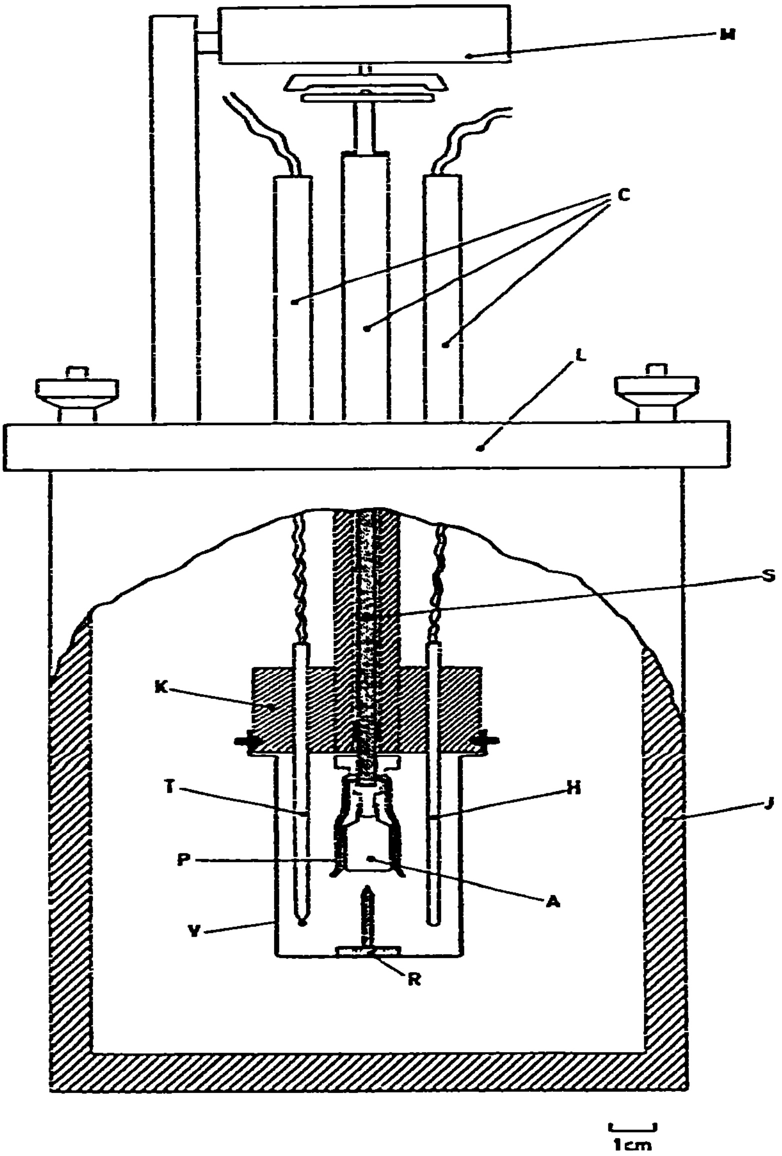

Fig. 1. Schematic drawing of the calorimeter used. A, moulded glass ampoule of ca. 1 cm^3 volume; C, stainless steel tubes accommodating stirrer, thermistors, heater, and nitrogen inlet; H, heater; J, outer can (cast aluminium); K, PTFE cover; L, outer lid (stainless steel); M, stirrer motor (synchronous, 250 min^{-1}); P, stirrer blades (stainless steel); R, ampoule breaking tip (stainless steel); S, stirrer shaft (fastened to the blades by means of two PTFE pieces); T, thermistor; V, calorimetric vessel (copper, chromium-plated from outside, epoxy-coated from inside; volume ca. 50 cm^3).

was led to a Burr–Brown Instrumentation Amplifier 3625C whose output was attenuated (if desired) and then either read on a 4.5 digit digital voltmeter (Systron Donner 7100 A) or recorded by means of a 10 mV strip-chart recorder (Unicam SP 22).

For the electrical calibration a ca. 20 Ω heater was spun from manganin wire and put into a thin-walled glass tube filled with paraffin oil. The heater was equipped with four leads, those serving to measure the potential drop across the heater being attached approximately half-way between the calorimeter and the cover of the outer can. The potential drop was measured by means of the DVM used for temperature monitoring. The heater was fed from a constant-voltage supply and the current through the heater was selectable in the limits of 50–150 mA. The time of heating was measured by a stop-watch (Gebrüder Haake), readable to 0.01 sec, which was coupled mechanically to the switch actuating the heater. The calorimeter was immersed in a well-stirred water bath whose temperature was controlled to ± 1 mK by means of a Tronac PTC-1000 A temperature controller. The temperature was 298.15 ± 0.1 K. Either solid or liquid reagent could be added by breaking the glass ampoule which was molded in order to ensure an easy breaking accompanied with a negligible heat effect.

Calorimeter check

The linearity of calorimeter response was checked by determining the effective heat capacity of the calorimeter ($1 < Q_{el}/J < 11$) filled with 35 cm^3 of water for varying Joule heats evolved by the heater. The corrected temperature rise, θ, was estimated by the Dickinson's method (in the electrical calibrations a factor of 0.5 was applied instead of 0.63 which was used in the chemical experiments). In a series of calibration runs a very satisfactory linear response was observed, the slope — which is equal to the effective heat capacity, $\varepsilon = Q_{el}/\theta$ — being (3.219 ± 0.008) J mV^{-1}. This corresponds to a sensitivity of 0.31 J mV^{-1}. The resolution of the calorimeter can then be estimated to be near 15 mJ (with a 10 mV recorder). It is possible to improve this figure to some extent by increasing the amplifier gain provided the temperature readings are made with the DVM and then processed numerically[3].

In order to check the accuracy of the calorimeter two reactions with very well known heat effects were performed, i.e. neutralisation of HNO_3 and NaOH and the dissolution of tris (hydroxymethyl) aminomethane (THAM) in 0.1 mol dm^{-3} HCl. For the first reaction (0.4 cm^3 of 0.2818 mol dm^{-3} HNO_3 was added to 25 cm^3 of 0.088 mol dm^{-3} NaOH) an enthalpy change of -57.36 ± 0.23 kJ mol^{-1} was obtained which, recalculated to the infinite dilution, gives $(\Delta H)^{\infty} = -55.80 \pm 0.23$ kJ mol^{-1} (the figures under ± sign are double standard errors of the mean), in excellent agreement with the generally accepted value of -55.805 ± 0.062 kJ mol^{-1} [4]. The molar enthalpy change for the dissolution of THAM (40 to 60 mg in 35 cm^3 0.1 mol dm^{-3} HCl) was found to be -29.63 ± 0.16 kJ mol^{-1}, which agrees well with Gunn's[5] value of -29.73 kJ mol^{-1}, considering the lower grade of THAM used in the present work (it is interesting to note that it is quite difficult to remove the moisture from THAM by drying at 110°C).

Experiment design and calculation

Two kinds of experiments were performed: (i) the measurements of the protonation enthalpies of the ligands, and (ii) the measurements of the ligational enthalpies. In the first group of experiments a precisely known amount of a neutral amino acid solution was put into the ampoule which was broken at a suitable instant and its contents mixed with an excess of NaOH. The initial and final pH values were measured (to 0.01) in order to enable the calculation of the concentration of HL and L^- species. In the other group of experiments a rather concentrated metal ion solution was put into the ampoule while the calorimetric vessel contained an alkaline ligand solution. The ligand concentration was varied in various runs so that at least two metal/ligand ratios were used for every system studied. The pH value of the calorimeter contents was measured before and after every run. Needless to say, every experiment was accompanied with an appropriate blank run. The concentration of any species in the final solution did not exceed 0.02 mol dm^{-3}.

The calculation of the enthalpy changes for the two consecutive ligation reactions

$$M^{2+} + L^- = ML^+ \qquad \Delta_{01}H \qquad (1)$$

$$ML^+ + L^- = ML_2 \qquad \Delta_{12}H \qquad (2)$$

were calculated from the experimental data (analytical concentrations, overall and dilution heats, pH) and the previously determined stability constants[2] as described by Barnes and Pettit[6].

The calculation of ΔG and ΔS from the stoichiometric stability constants, K_s^c, and ligational enthalpies deserves a short comment on the solute standard states. Conventionally, the solute standard state is defined as that of a hypothetical solution of unit concentration (c° = mol dm^{-3}) possessing the properties of an ideal solution which are believed to be approached in the limit of infinite dilution. Besides this, the standard pressure (most commonly P° = 101.325 kPa) and the solvent have to be specified. Such a choice does not seem to be very practical in the frequently encountered case when the thermodynamic measurements are made with solutions which contain an excess of an "inert" electrolyte in order to keep the conventional activity coefficients approximately constant. If, instead of water, a 0.1 mol dm^{-3} $NaNO_3$ solution is regarded as the solvent the definition of the standard state has to be modified: this will be the state of an ideal solution of unit concentration (c°), at standard pressure, in 0.1 mol dm^{-3} $NaNO_3$ (in our case). This standard state will be denoted by superscript † in order to distinguish it from the conventional one (denoted by $^\circ$). If the conventional activity coefficients are virtually kept constant by means of the $NaNO_3$ excess the relative activities of any reacting species B (defined in terms of the new standard state) should be nearly equal to c_B/c° if $c_B \ll [NaNO_3]$. It was, in fact, observed that both K_s^c and ΔH values do not depend, within experimental error, on the concentration of any reacting species. Therefore it was considered legitimate to identify the observed values of $\Delta_s H$ and $c^\circ K_s^c$ with $\Delta_s H^\dagger$ and $K_s^\dagger$ thus enabling the calculation of $\Delta_s G^\dagger$ and $\Delta_s S^\dagger$.

RESULTS

Besides the enthalpy of water formation from H^+ and OH^- it is necessary to determine the enthalpy of ligand protonation. As the experiments were conducted

TABLE 1

PROTONATION ENTHALPIES OF EPIMERIC THREONINATES AND ISOLEUCINATES AT 298.15 K IN 0.1 MOL DM^{-3} NaNO$_3$

L$^-$	$-\Delta_1 H^a$ (*kJ mol*$^{-1}$)
(L-Thr)$^-$	41.6 (4)
(L-*allo*-Thr)$^-$	42.06 (8)
(L-Ile)$^-$	43.2 (5)
(L-*allo*-Ile)$^-$	43.5 (6)

[a] The figures in parentheses are the double standard errors of the mean (in the units of the last decimal place).

TABLE 2

STEPWISE LIGATIONAL GIBBS FUNCTIONS, ENTHALPIES AND ENTROPIES OF Co^{2+} AND Cu^{2+} COMPLEXES WITH EPIMERIC THREONINES AND ISOLEUCINES AT 298.15 K IN 0.1 MOL DM^{-3} NaNO$_3$

Complex	$-\Delta G^\dagger$ (*kJ mol*$^{-1}$)	$-\Delta H^\dagger$ (*kJ mol*$^{-1}$)	$\Delta S^\dagger$ (*J K*$^{-1}$ *mol*$^{-1}$)
Co (L-Thr)$^+$ Co (DL-Thr)$^+$	24.3	10.8	45
Co (L-Thr)$_2$ Co (DL-Thr)$_2$	22.4	8.1	48
Co (L-*allo*-Thr)$^+$	22.8	8.2	49
Co(L-*allo*-Thr)$_2$	18.3	4.8	45
Co (L-Ile)$^+$	26.2	18.5	26
Co (L-Ile)$_2$	24.8	3.5	71
Co (D-*allo*-Ile)$^+$	23.4	12.6	36
Co(D-*allo*-Ile)$_2$	19.2	4.2	50
Cu (L-Thr)$^+$ Cu (DL-Thr)$^+$	45.9	25.6	68
Cu (L-Thr)$_2$ Cu (DL-Thr)$_2$	39.3	20.7	62
Cu (L-*allo*-Thr)$^+$	42.6	23.1	65
Cu (L-*allo*-Thr)$_2$	37.0	18.8	61
Cu (L-Ile)$^+$	48.5	26.2	75
Cu (L-Ile)$_2$	41.6	18.3	78
Cu (D-*allo*-Ile)$^+$	46.2	24.0	74
Cu (D-*allo*-Ile)$_2$	39.7	18.0	73

at high enough pH values only the first step of ligand protonation had to be taken into account ($L^- + H^+ = HL$) and therefore the enthalpies of binding the second proton were not determined. The first protonation enthalpies are collected in Table 1, together with respective double standard errors of the mean. The value for L-Thr (-41.6 kJ mol^{-1}) agrees well with the existing literature data[7-9] which range from -40.9 to -41.8 kJ mol^{-1}.

The ligational heats are shown in Table 2. The values for copper threoninates can again be compared to those reported by others[7-10]: $-\Delta_{01}H$ values range between 22 and 23 kJ mol^{-1}, slightly lower than the value determined in the present work (24.3 kJ mol^{-1}). On the contrary, the literature data for $-\Delta_{12}H$ are almost invariably higher than our value except the value determined by Sharma ($\Delta_{01}H + \Delta_{12}H = -41.4$ kJ mol^{-1})[10]. However, regarding the uncertainties introduced by not only calorimetric and analytical errors but also by those in the pH measurement and in the stability constants, the agreement may be taken as reasonable. It is rather difficult to assess the accuracy of our (as well as others') data. Even the precision is not easy to estimate because only a limited number of runs (2–4) was made for any particular system. As all the ligational enthalpies are of a similar magnitude the precision may be estimated by considering the deviations of particular ΔH values from the respective means: the r.m.s. error based upon all the values from Table 2 amounts to ±0.13 kJ mol^{-1}, which is a very satisfactory figure probably even too good to be realistic.

The ligational entropies, also shown in Table 2, were calculated from the respective enthalpies and the stability constants obtained by potentiometric titration[2]. The latter are reliable within ca. ±0.05 log units (±0.29 kJ mol^{-1} in ΔG°) which, combined with a typical uncertainty of ±0.13 kJ mol^{-1} for calorimetric data, indicates that the uncertainty of ΔS values should be approximately ±2 J K^{-1} mol^{-1}.

DISCUSSION

The greater basicity of isoleucine with respect to threonine may clearly be seen by comparing not only the pK values[2] but also the protonation enthalpies: the protonation of either Ile or *allo*-Ile is by roughly 1.5 kJ mol^{-1} more exothermic than the protonation of Thr or *allo*-Thr. This is evidently due to the inductive effect of the OH group in the Thr molecule. The difference in the basicity is further reflected in the stability constants and the ligational enthalpies: the ligation of Ile to either Co^{2+} or Cu^{2+} to form an ML complex is considerably more exothermic than Thr ligation. For ML_2 complexes some exceptions from this regularity can be found in the case of Co^{2+}.

Furthermore, it can regularly be observed that the ligation of natural epimers is infallibly more exothermic than the analogous reactions of *allo*-forms which parallels the relationships in the stability constants[2]. On average, the ligation of natural forms of Thr and Ile is more exergonic by 3 (±0.5) kJ mol^{-1} and more exothermic by 2.3 (±0.7) kJ mol^{-1} than is the case with the *allo*-forms. By inspecting the stepwise ligational entropies no regularity can be observed, possibly owing to the accumulation of experimental errors in this quantity. The cumulative ligational entropies of ML_2 complexes are, however, almost equal without respect to the ligand configuration. The stability difference is hence caused by the enthalpy term. In order to explain the greater stability of complexes with natural epimers Krause et al.[1] have tentatively assumed the existence of a hydrogen bridge in the Thr molecule connecting OH and

NH_2 groups. The observed differences in $\Delta G^{\dagger}$ and $\Delta H^{\ddagger}$ values are probably too small to support such an assumption. Of course, the behaviour of Ile complexes cannot be explained in this way. A plausible explanation is still harder to find if one considers that the absolute configuration of the natural form of threonine (2R, 3S) is different from that of natural isoleucine (2R,3R). Therefore it seems that there can hardly be a unique explanation for the greater stability of complexes with natural epimers. By considering the crystal structures of Thr and Ile complexes, however, some tentative suggestions can yet be given. Freeman et al.[11] have determined the crystal structure of CuHisThr complex (the structures of CuThr or $CuThr_2$ do not seem to have been published so far). On the grounds of their data one might conclude that, in the solution, a Cu–OH (or Co–OH) interaction could take place, though a direct contact is not established but a H_2O molecule is rather interposed. Such kind of bonding was detected by Larsson in his IR studies of glycolate complexes with heavy metals[12]. If present, such bonding should be sensitive to the configuration of the 3-C atom.

On the other hand, by considering the crystal structure of $Cu(Ile)_2$ complex[13] one can imagine that the lower stability of *allo*-Ile complexes with respect to those formed by natural isoleucine could be caused by a hindered rotation around the C_2–C_3 axis.

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