Thermodynamics of Formation of Complexes of Nickel(II) and Copper(II) with L- and DL-Serine and L- and DL-Threonine†

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Complexes formed between L- and DL-serine (Ser) and L- and DL-threonine (Thr) and H^+, Ni^{2+}, and Cu^{2+} at intermediate pH values have been studied potentiometrically at 25 °C and $I = 0.10$ mol dm^{-3} (K[NO$_3$]). The complexes of H^+ and Cu^{2+} have also been studied calorimetrically. Stereoselectivity in the Gibbs free-energy changes is insignificant. However, the standard enthalpy of formation of [Cu(L-SerO)$_2$] is more favourable than that of [Cu(L-SerO)(D-SerO)] (SerO = serininate) by a small but significant amount (1.7 kJ mol^{-1}). This stereoselectivity is explained by outer-sphere co-ordination between Cu^{2+} and un-ionized hydroxy-oxygen donor atoms. The presence of protonated complexes of both SerO and ThrO with Cu^{2+} at pH $\leqslant 4.8$ has been demonstrated.

POTENTIALLY tridentate amino-acid anions can be divided, arbitrarily, into two groups, those offering either a strong binding site in addition to the amino-acid carboxylate and amino-groups and those offering only a weak third site. Complexes of the first group have been relatively thoroughly investigated by both potentiometric and calorimetric techniques. In particular complexes of histidine (His) [1-3] and glutamic and aspartic acids [4,5] have been studied in order to gain information on the role of the third donor atom and the possibilities

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† No reprints available

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of stereoselectivity. It was found that, while all three donor atoms co-ordinate in all such cases, stereoselectivity was only significant when the third atom was typically ' soft ' as in His.

Serine (Ser) and threonine (Thr) are both biologically important amino-acids which interact strongly with Ni^{2+} and Cu^{2+}. For example, the ternary complex [Cu(L-HisO)(L-ThrO)] accounts for a significant proportion of the complexed labile Cu^{2+} in human serum and has therefore been studied extensively (HisO = histidinate, ThrO = threoninate). In spite of this the extent of co-ordination through the third donor atoms, the hydroxy-oxygens, is uncertain. In the solid state, the ThrO in [Cu(HisO)(ThrO)] does not co-ordinate through the hydroxy-group.[6] In solution, however, this ternary complex is significantly more stable than expected from the stabilities of the parent complexes.[7]

Stereoselectivity in the bis complexes of amino-acids may be expressed quantitatively by deviations from the statistically expected value of 4 for the equilibrium constant of reaction (1) (where + and − are used in

$$[M\{(+)\text{-}L\}_2] + [M\{(-)\text{-}L\}_2] \longrightarrow 2[M\{(+)\text{-}L\}\{(-)\text{-}L\}] \quad (1)$$

place of D and L to avoid confusion), *i.e.* stereoselectivity = (log β for $[M\{(-)\text{-}L\}_2]$) − (log β for $[M\{(+)\text{-}L\}\{(-)\text{-}L\}]$) +0.30. Stereoselectivity has not been found in complexes of simple bidentate amino-acids with transition-metal ions but is often present with tridentate amino-acids, particularly when the third donor atom is ' soft ' in character [8] as in His [3] or methionine.[9] We have therefore made a detailed study of the complexes of optically active and racemic Ser and Thr with Ni^{2+} and Cu^{2+} using both potentiometric and, with Cu^{2+}, calorimetric techniques in an attempt to find out whether the ionized forms of these ligands can be tridentate and whether they show any stereoselectivity.

EXPERIMENTAL

The amino-acids were obtained from the Sigma Chemical Co. (SIGMA grade) and were analyzed to confirm their purity.

Complex-formation constants were calculated from potentiometric titrations, changes in pH being followed with a glass electrode calibrated in terms of hydrogen-ion concentrations.[3] Values for the formation constants were calculated with the aid of the MINIQUAD computer program.[10] Enthalpy changes were calculated from calorimetric measurements carried out in the LKB 8700 reaction calorimeter, as described previously.[3] All measurements relate to 25 °C and an ionic strength of 0.02 mol dm^{-3} ($K[NO_3]$).

RESULTS

Formation constants for the complexes of L- and DL-Ser and L- and DL-Thr with H^+, Ni^{2+}, and Cu^{2+} are given in Table 1. Under the conditions used (pH <10) there was no detectable ionization of the hydroxy-group of the amino-acids and the experimental data were satisfied to a very high precision by assuming formation of the normal stepwise complexes only, *i.e.* $[ML]^+$, $[ML_2]$, and $[ML_3]^-$, with the probability of a monoprotonated complex with Cu^{2+}.

It can be assumed that complexes containing only the D hand of the ligand will be identical in thermodynamic properties to those with the L hand. If this is so, thermodynamic constants for *meso* complexes can be readily calculated from the ' overall ' constants calculated by using the racemic mixtures of the ligands.[11] Since values calculated for the optically active and racemic ligands are effectively identical, stereoselectivity is not significant and the formation constants for the *meso* complexes will differ from those for the optically active complexes by statistical factors only.[11]

TABLE 1

Complex-formation constants at 25 °C and $I = 0.10$ mol dm^{-3} ($K[NO_3]$). Standard deviations, σ values, are in parentheses

	L-Ser	DL-Ser	L-Thr	DL-Thr
log β_{HL}	9.074(3)		8.974(3)	
log β_{H_2L}	11.02(1)		10.95(1)	
log β_{CuL}	7.858(3)	7.862(5)	7.947(3)	7.940(5)
log β_{CuL_2}	14.428(3)	14.437(6)	14.613(4)	14.601(7)
log β_{NiL}	5.137(4)	5.320(5)	5.467(9)	5.45(1)
log β_{NiL_2}	9.743(4)	9.755(5)	10.040(9)	10.02(1)
log β_{NiL_3}	12.73(1)	12.68(2)	13.18(1)	13.13(2)
Literature values [15]				
log β_{HL}	9.10		8.98	
log β_{H_2L}	11.25		11.22	
log β_{CuL}	7.93		8.02	
log β_{CuL_2}	14.48		14.72	
log β_{NiL}	5.43		5.42	
log β_{NiL_2}	9.96		9.95	

Since stereoselectivity in the formation constants (Δ*G*) of the complexes with Ni^{2+} was insignificant, it was also assumed to be insignificant in the enthalpy (Δ*H*) changes.[3] However, this assumption does not necessarily hold with Cu^{2+} since, with the HisO ion, stereoselectivity in both Δ*H* and Δ*S* act in opposite directions with the result that there is a coincidental cancelling of stereoselective effects producing no significant stereoselectivity in Δ*G*.[3] Complex formation with Cu^{2+} was therefore studied calorimetrically and the results found, together with those for the heats of formation of the monoprotonated amino-acids, are given in Table 2. Copper(II), unlike Ni^{2+}, does not form a tris complex when the ligand : metal ratio is $\leqslant 2.1 : 1$. It was therefore possible to select conditions for the calorimetric reactions to ensure effectively complete formation of the bis complexes, $[CuL_2]$, allowing accurate calculation of values for ΔH_{1-2}, the overall heat of formation of the bis complex.

DISCUSSION

The results found confirm that, in the pH region studied (<pH 8.5), hydroxy- and polynuclear complexes are not important. This is in agreement with the calorimetric

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[10] A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.

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work of Thornton and Skinner,[12] and recent potentiometric studies.[2,13] From published constants the maximum percentage of the species $[CuL_2H_{-1}]$ at the end of the calorimetric experiments would be 1.5. Hence the hydroxy-groups can be considered as un-ionized throughout the study. Ionization of the hydroxy-protons at pH >8.5 may be the source of differences in previously published enthalpy changes.

Table 2

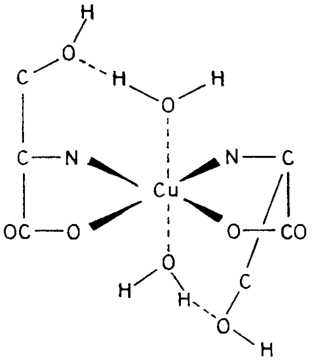
Thermodynamic data (kJ mol^{-1}) for the complexes of serine and threonine with H^+ and Cu^{2+} at 25 °C and $I = 0.10$ mol dm^{-3} ($K[NO_3]$). Values for ΔH in parentheses are standard deviations of the mean calculated from seven replicate calorimetric determinations

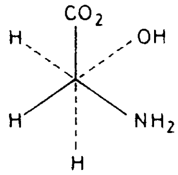
	L-Ser	DL-Ser	L-Thr	DL-Thr
$-\Delta H_{HL}$	43.28(7)		42.24(5)	
$-\Delta G_{HL}$	51.77(2)		51.20(2)	
$T\Delta S_{HL}$	8.5		9.0	
$-\Delta H_{CuL_2}$	53.59(4)	52.76(3) *	53.13(11)	52.91(9)
$-\Delta G_{CuL_2}$	82.32(2)	82.32(2)	83.37(2)	83.37(4)
$T\Delta S_{CuL_2}$	28.7	29.5	30.2	30.4
Literative values [15]				
$-\Delta H_{HL}$	42.68		40.92	
$-\Delta G_{HL}$	51.92		51.75	
$T\Delta S_{HL}$	9.2		10.8	
$-\Delta H_{CuL_2}$	51.46		48.53	
$-\Delta G_{CuL_2}$	83.70		82.73	
$T\Delta S_{CuL_2}$	32.2		34.2	

* The calculated value for formation of the *meso* complex [Cu(L-SerO)(D-SerO)] is $-\Delta H_{1-2} = 51.92$ kJ mol^{-1}; hence $\Delta(\Delta H_{1-2}) = 1.7$ kJ mol^{-1}.

The question of participation of the hydroxy-groups of Ser and Thr in their co-ordination to transition-metal ions has received much attention. Such co-ordination is not supported by crystal structures,[14] but several thermodynamic studies, including the results reported here, show a marked relative increase in the stability of the hydroxyamino-acid complexes compared to alkyl-substituted glycines.[15,16] This has been interpreted as suggesting a weak metal–un-ionized hydroxy bond,[15] a finding supported by e.s.r. parameters for the 1 : 1 copper complexes [17] and the circular dichroism (c.d.) spectra of nickel complexes.[18] However, some more recent spectroscopic studies suggest that, in neutral solution, the ligands co-ordinate in a glycine-like manner, the hydroxy-group only co-ordinating after ionization.[13,19]

In alkaline solution (pH >10) after ionization of the hydroxy-proton, both Ser and Thr would be expected to act as tridentate ligands. Since stereoselectivity in bis amino-acid complexes appears to be restricted to tridentate co-ordination, and to be absent from complexes with glycine-like co-ordination,[9] significant stereoselectivity may be found at high pH when the hydroxy-proton is ionized. However, the additional co-ordination site is ' hard ' rather than ' soft ' in character. At near-neutral pH values (the biologically significant region), however, stereoselectivity would be expected only if tridentate behaviour were significant and if the hydroxy-oxygen donor were rather ' softer ' than an O^- donor atom. Results reported in Table 1 give no indication of stereoselectivity in ΔG but values for ΔH_{1-2} in Table 2 show a small, but significant, preferential enthalpy change for optically active $[Cu(L\text{-}SerO)_2]$ with a compensating difference in the entropy changes. It is interesting to note that the preferential formation of the optically active bis serinate complex is comparable to that with histidinate.[3] This observation may be taken as evidence for tridentate co-ordination by the SerO, the ligand molecules bonding glycine-like in the equatorial plane of the copper, with *cis* co-ordination, and the hydroxy-groups interacting weakly with the apical co-ordination sites. However, the geometry required for such a genuinely tridentate ligand would impose considerable steric strain. An alternative arrangement could involve co-ordination of the hydroxy-groups in the secondary co-ordination sphere of the metal ion [21] (see diagram).





The protons of co-ordinated water molecules are more acidic than in free water molecules and the hydroxy-group in SerO is electron rich and less acidic than in water. These conditions are favourable to the formation of a strong hydrogen bond. The strength of such a hydrogen bond would be *ca.* 15—20 kJ mol^{-1} and so could account readily for the stereoselectivity found. The serinate ligand can also be regarded as effectively tridentate. This type of outer-sphere co-ordination also explains spectroscopic results incompatible with inner-sphere co-ordination of the hydroxy-group.

Formation of such a hydrogen bond would mean the hydroxy-group of co-ordinated SerO adopting the less favourable co-ordination *gauche* to both the CO_2^- and

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NH_2 groups (see diagram). Such a conformation is found[14] in crystals of $[Cu(SerO)_2]$ but is absent in crystals of $[Ni(SerO)_2]$.[20] If a tridentate interaction of the hydroxy-group *via* a hydrogen bond is responsible for stereoselectivity in $[Cu(SerO)_2]$, then the disruption of such a bond by the steric or inductive effects of the β-methyl substituent of Thr could result in the lack of observable stereoselectivity in $[Cu(ThrO)_2]$.

Complex Formation by the Zwitterion.—For a typical bidentate amino-acid the zwitterion, HL, has a wide pH range of existence. Since the amino-group is protonated, the zwitterion has only limited donor ability, co-ordinating in an acetate-like manner to form a monoprotonated complex of formula $[M(HL)]^{2+}$ (*i.e.* a 111 species). Such complexes are to be expected only at low pH. If they are included in the calculation of formation curves ($\bar{n}$ against pL) the species are only significant when the value of $[H^+]K_{M(HL)}{}^{ML}$ approaches unity, *i.e.* when log $K_{M(HL)}{}^{ML} \approx$ pH. In general the inclusion of protonated complexes of amino-acids in the formation curves will only cause deviation below $\bar{n} \approx 0.5$.

Formation constants of protonated copper–amino-acid complexes have been calculated by a number of workers. The complexes were found to be weak (comparable to acetate complexes) and unimportant above pH 4. Protonated complexes have, however, always been disregarded with SerO and ThrO. We therefore carried out careful titrations at low pH and found that protonated complexes are significant between Cu^{2+} and both ligands. Inclusion of the $[Cu(HL)]^{2+}$ species produced a clear improvement in the statistics of the fit between experimental and calculated quantities and gave consistent values for $\beta_{Cu(HL)}$ over a number of titrations. Calculated constants are given in Table 3. At pH 3.5 the $[Cu(HL)]^{2+}$ species of both Ser and Thr bound *ca.* 20% of the Cu^{2+} ions in a 2 : 1 ligand : Cu^{2+} mixture, and are far more important species than the analogous glycine complex.

The relative affinity of the amino-acid carboxylate groups for protons and metal ions is examined by comparison of the values for log K_{H_2L} − log $K_{Cu(HL)}$ given in Table 3. For Ser and Thr these values are negative indicating a greater affinity for Cu^{2+} than H^+. These anomalously large values for log $K_{Cu(HL)}$ for the hydroxy-amino-acid complexes cannot be explained by the (−*I*) inductive effect of the α-OH groups since this should weaken the Cu–O bond. The explanation is, therefore, likely to be participation of the hydroxy-groups in chelate formation. Support for such bidentate co-ordination is found in a comparison of the log $K_{M(HL)}{}^{HL}$ values with the formation constants of the complex of Cu^{2+} with 2-hydroxypropionic acid, when very similar results are found.

TABLE 3

Formation of protonated complexes of Cu^{2+} with glycine, L-serine, and L-threonine at 25 °C and $I = 0.10$ mol dm^{-3} ($K[NO_3]$)

	log-$\beta_{Cu(HL)}$	log-$K_{CuL(HL)}{}^{HL}$	log K_{H_2L} − log $K_{Cu(HL)}$
L-Ser	11.41(6)	2.40(6)	−0.46
L-Thr	11.54(6)	2.56(6)	−0.58
Gly	11.05(3)	1.45(3)	0.85
2,2′-Dimethylglycine	11.5 [a]	1.2	1.1
	log β_{CuL}		
Acetic acid	1.89 [b]		
2-Hydroxypropionic acid	2.49 [c]		

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[b] R. S. Kolat and J. E. Powell, *Inorg. Chem.*, 1962, **1**, 293.
[c] H. Thun, W. Guns, and F. Verbeck, *Analyt. Chim. Acta*, 1967, **37**, 332.

Neglect of the $[Cu(HL)]^{2+}$ complex with Ser and Thr may be partly responsible for the discrepancy between values for enthalpy changes obtained by enthalpy titration[15] and by calorimetric methods independent of stepwise formation constants[11] as used here. It would, of course, lead to great inaccuracy in the enthalpy changes determined from the temperature variation of the stability constants.[21] The presence of copper–hydroxy-oxygen bonding in the monoprotonated complex suggests that it is also likely to be present in the simple complexes $[ML]^+$ and $[ML_2]$.

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