Monatshefte für Chemie 125, 1083–1089 (1994)

Monatshefte für Chemie
Chemical Monthly

Transition Metal Complexes of N,N-Dimethylthreonine: Stability and UV/Vis Spectra*

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Summary. Acidity (dehydronation†) constants of N,N-dimethylthreonine (*DMT*) and stability constants of its complexes with Cu^{+2}, Ni^{+2}, and Co^{+2} were determined in aqueous solution by means of potentiometric titration. UV/Vis spectra were also taken during the titration. It is suggested that *DMT* acts as a bidentate ligand toward copper(II) by engaging either (a) amino and carboxyl groups (in [Cu(*DMT*)] and [Cu(*DMT*)$_2$]) or, (b) upon dehydronation, amino and hydroxyl groups (in [Cu(*DMT*)H$_{-1}$], [Cu(*DMT*)$_2$H$_{-1}$], and [Cu(*DMT*)$_2$H$_{-2}$]). It is suggested that the coordination in threoninato and *allo*-threoninato complexes is similar to that described under (a).

Keywords. Copper(II), aminoacidato complexes; N,N-dimethylthreonine, complexes with Cu(II); Speciation; Stability constants; UV/Vis spectra.

Übergangsmetallkomplexe von N,N-Dimethylthreonin: Stabilität und UV/Vis-Spektren

Zusammenfassung. Dehydronierungskonstanten des N,N-Dimethylthreonins (*DMT*) sowie die Stabilitätskonstanten seiner Komplexe mit Co^{+2}, Ni^{+2} und Cu^{+2} in wässriger Lösung wurden mittels potentiometrischer Titration bestimmt. Auch UV/Vis Spektren wurden während der Titration aufgenommen. Die Daten zeigen, daß in den [Cu(*DMT*)]- und [Cu(*DMT*)$_2$]-Komplexen die Amino- und Carboxylgruppen koordiniert sind, während in den dehydronierten Komplexen ([Cu(*DMT*)H$_{-1}$], [Cu(*DMT*)$_2$H$_{-1}$] und [Cu(*DMT*)$_2$H$_{-2}$]) die Donorgruppen $-NH_2$ und $-OH$ sind.

Introduction

Chelates of threonine with transition metals, especially with cobalt(II), nickel(II), and copper(II), have been extensively studied. Particular attention has been paid to the possible coordination of the threonine hydroxyl group. Early investigators

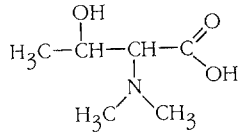
* Based upon Master of Science thesis submitted to the University of Zagreb, Croatia by *B. Blagović*
† In compliance with IUPAC 1990 Rules, the terms 'hydron' and 'hydronation' are used throughout, instead of the more usual 'proton(ation)' which has been given a more specific meaning (^{1}H)

[1, 2] suggested that there is no coordinative link between the hydroxyl group of threonine (or other β-hydroxy-α-amino acids) and the central atom. Instead, they postulated the formation of an intramolecular hydrogen bond between hydroxyl and amino groups.

Contrary to these views, *Gergely* and coworkers [3] inferred from their thermochemical data (variation of ligational enthalpy with temperature) that these amino acids were potential tridentates, capable of binding copper(II) by using the oxygen atom of the β-hydroxyl group as the third ligating atom. Summarizing a larger data body, *Martin* [4] suggested that serine and threonine were weak tridentates toward nickel(II) and cobalt(II), being even weaker toward copper(II). However, ^{1}H NMR spectral data [5] did not support this assumption. *Simeon et al.* [6, 7, 8] proposed the existence of hydrogen bridge(s) between threonine hydroxyl groups and apical water molecule(s) but were not able to offer a conclusive proof of their hypothesis.

Even X-ray diffractometric data for $[Cu(Thr)_2]$ did not help much to resolve this controversy. The coordination around copper in $[Cu(Thr)_2]$ was found to be distorted square-pyramidal [9], with ligands in a *trans* orientation (average Cu–O 196 ± 1 pm, average Cu–N 194 ± 2 pm), the fifth ligating atom being the carboxyl oxygen of another threonine molecule (Cu–O 248 pm); Cu–O contact (294 pm), with the carboxylate oxygen of still another threonine molecule on the opposite apex should also be mentioned. So far, no such polymeric species was detected in solution.

Kaitner et al. [10] determined the crystal structure of a copper complex of *L*-N,N-dimethylthreonine (*DMT*, see Formula) and found that the coordination around copper in the $[Cu(DMT)_2]$ complex was also distorted square pyramidal, with *trans* ligation (average Cu–O 192.8 ± 0.2 pm, average Cu–N 204.9 ± 0.4 pm), the fifth (apical) ligand being a water molecule (Cu–O 220.6 pm). Having in view the well-known 'plasticity' of the copper(II) coordination polyhedron which can easily be distorted it seems unsafe to postulate the existence of such a low-symmetry structure in solution.



Therefore, it seemed useful to study the stability of *DMT* complexes with three transitional metal ions (Co^{+2}, Ni^{+2}, Cu^{+2}), as well as their electronic spectra, expecting that the additional steric hindrance, introduced near the coordination site, would affect the complex stability and acidity constants in a decipherable way.

Results and Discussion

Equilibrium constants determined in the present work are collected in Table 1. Each of these values was obtained by averaging the results from several titrations, according to the criteria stated above.

Species distributions, *i.e.* relative abundances ($\alpha_{qnp} = [M_qL_nH_p]/c_M$) of Co^{+2}, Ni^{+2}, and Cu^{+2} complexes, plotted *vs.* p[H] are shown in Figs. 1, 2, and 3, respectively. It is seen therefrom that, at the physiological *pH* of *ca.* 7.4, uncomplexed

Table 1. Cumulative stability constants ($\lg\beta^0_{qnp}$) of N,N-dimethylthreonine (HL) complexes ($M_qL_nH_p$) with Co^{+2}, Ni^{+2}, and Cu^{+2} at $T = 298.2$ K in aq. KCl (0.1 mol/l); standard errors, in units of the last decimal place, are given in parentheses

Species	H^+	Co^{+2}	Ni^{+2}	Cu^{+2}
$[HL]$	8.849(7)	–	–	–
$[H_2L]^+$	10.31(21)	–	–	–
$[ML]^+$	–	2.65(6)	3.49(2)	5.50(4)
$[MLH_{-1}]$	–	n.d.	−5.64(4)	−2.10(2)
$[MLH_{-2}]^-$	–	−14.73(?)	n.d.	n.d.
$[ML_2]$	–	n.d.	n.d.	9.80(5)
$[ML_2H_{-1}]^{-1}$	–	n.d.	n.d.	1.6(2)
$[ML_2H_{-2}]^{-2}$	–	n.d.	n.d.	−8.2(3)

n.d.: species was not detected; (?): uncertain estimate

Co^{+2}(aq) and Ni^{+2}(aq) are predominant species, while in the case of Cu^{+2} the major species are $[Cu(DMT)]$ and $Cu(DMT)_2]$.

It can be seen that the precision of some of the constants is markedly lower than would be expected. For instance, the value of $\lg\beta_{012}$ has an unusually high standard error of ± 0.21. However, the carboxyl group in DMT molecule is so acidic ($pK_a = 1.46$ – see below) that it is largely ($\geqslant 80\%$) dehydronated, even at the very beginning of the titration ($p[H] \geqslant 2$). Fortunately, the imprecision in this constant does not

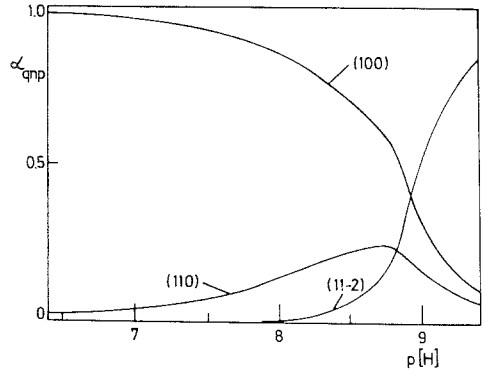


Fig. 1. Species distribution in the system Co^{+2} + N,N-dimethylthreonine; $\alpha_{qnp} = [M_qL_nH_p]/c_M$

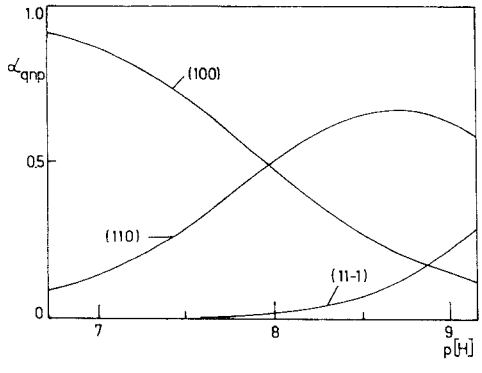


Fig. 2. Species distribution in the system Ni^{+2} + N,N-dimethylthreonine; $\alpha_{qnp} = [M_qL_nH_p]/c_M$

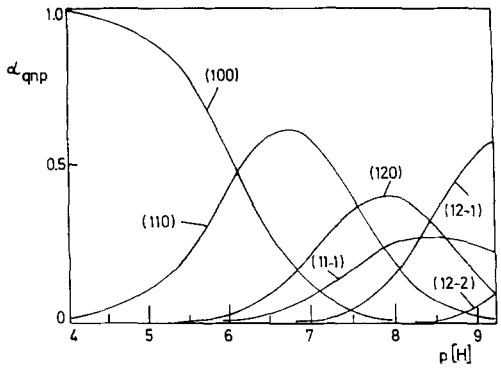


Fig. 3. Species distribution in the system Cu^{+2} + N,N-dimethylthreonine; $\alpha_{qnp} = [M_qL_nH_p]/c_M$

affect the values of other equilibrium constants. Stability constants of two dehydronated copper complexes, $[CuL_2H_{-1}]^-$ and $[CuL_2H_{-2}]^{-2}$, are also less precise than the rest (*cf.* Fig. 3). This is easy to explain in the case of $[CuL_2H_{-2}]^{-2}$ since it is a minor species. However, in the case of $[CuL_2H_{-1}]^-$, which is a major species, the problem is of kinetic nature. At higher p[H] values the system's response to the titrant addition was rather sluggish, making it difficult to see whether the equilibrium state had been achieved. This difficulty was still more pronounced in the systems with Co^{+2} and Ni^{+2}.

As can be seen from Table 2, the peaks in the UV/Vis spectra can be classified into four groups (A, B, C, D). The peaks belonging to the first group (A) (appearing at $\approx$210 nm) can be assigned to the ligand carboxyl n-π^* transition. They were only very slightly bathochromically shifted with increasing p[H]. Peaks classified as B (230...250 nm) and C (286...292 nm) appeared only in the presence of metal ions, provided p[H] was sufficiently high, showing either a slight bathochromic shift with increasing p[H] (B) or almost no shift at all (C). These two peaks are, in all probability, of charge-transfer (*CT*) origin. Peak D, seen only in copper solutions,

Table 2. Positions of peaks ($\lambda_{max.}$/nm) in UV/Vis absorption spectra during titration

System	p[H]	A	B	C	D
DMT	2...12	204	–	–	–
Co^{+2} + *DMT*	7.7	212	230	290	–
	8.6	212	230	290	–
Ni^{+2} + *DMT*	7.5	212	230	290	–
	8.5	212	230	290	–
Cu^{+2} + *DMT*	6.6	208	248	–	710
	7.5	210	244	284	620
	7.8	208	248	280	620
	8.0	210	246	286	620
	9.1	212	250	290	600
	9.9	212	252	292	600
	10.6	212	250	286	560

was strongly p[H] dependent, exhibiting a very intensive hypsochromic shift with increasing p[H] (710... 560 nm). This peak, obviously signalling a ligand-field (*LF*) d–d transition, had a very low intensity compared to the A, B and C peaks.

The absence of *LF* peaks in cobalt and nickel solutions is easy to explain: these peaks are very low and the concentrations of complexes in these systems are lower than in the systems containing copper. Since the spectra were taken during the titrations, the experimental conditions (optimized for potentiometry) were not favourable for accurate absorbance measurements; so it was not possible to resolve the complex spectral curves into the spectra of individual species as was done with CD spectra of threoninates [8]).

Ligand Acidity Constants. By inspecting dehydronation (acidity) constants of two diastereomeric threonines (Thr, *L-allo*-Thr) and *DMT* (see Table 3), it can be readily seen that all three threonines are less basic than their parent amino acid [12], α-aminobutyric acid (*AABA*), because of negative inductive effect exerted by the OH group. On the other hand, owing to the positive inductive effect of the methyl group, methylated amino acids would be expected to be more basic than their unsubstituted counterparts [13]. It is surprising to see that N,N-dimethylation did not affect pK_{az} of the threonine amino group, but increased the acidity of the rather distant carboxylic group by a factor of approx. 30. This finding can hardly be explained without additional data.

Complex Stability and Spectra. As seen from Table 1, the chelates formed by N,N-dimethylthreonine are less stable (by a factor of > 100) than the corresponding threoninates [8]. Ligand basicities being approximately equal, this stability difference can safely be attributed to the steric hindrance exerted by two N-methyl substituents.

Since no reliable data on pK_a values for N,N-dimethylthreoninato complexes of cobalt(II) and nickel(II) could be obtained (except for the dehydronation of $[Ni(DMT)]^+$), further discussion will be limited to copper(II) complexes.

Dehydronation constants of copper *bis*-(N,N-dimethylthreoninato) complexes (see Table 4) are generally lower than those of its threonine analogues [8]: the acidity of $[Cu(DMT)_2]$ ($pK_a = 8.2$) is higher than that of either $[Cu(Thr)_2]$ ($pK_a = 9.75$) or $[Cu(allo\text{-}Thr)_2]$ ($pK_a = 9.68$). Likewise, $[Cu(DMT)_2H_{-1}]$ is a stronger acid ($pK_a = 9.8$) than either $[Cu(Thr)_2H_{-1}]$ ($pK_a = 10.57$) or $[Cu(allo\text{-}Thr)_2H_{-1}]$ ($pK_a = 10.55$) whose dehydronation happens in a high p[H] range where usually the dehydronation of coordinated water takes place.

Table 3. Comparison of ligands' dehydronation constants

	pK_{a1}	pK_{a2}	$pK_{a1} + pK_{a2}$	Ref.
L-N,N-Me_2Thr	1.46	8.85	10.31	[a]
L-Thr	2.20	8.89	11.09	[8]
L-allo-Thr	2.11	8.83	10.94	[8]
AABA[b]	2.27	9.97	12.24	[12]

[a] Present work; [b] α-Aminobutyric acid

Table 4. Dehydronation constants pK_{qnp} of copper(II) *L*-N,N-dimethylthreoninates $[Cu_qL_nH_p]$, compared to threoninate complexes

Species	*DMT*	*L*-Thr	*L-allo*-Thr
$[CuL]^+$	7.60	–	–
$[CuL_2]$	8.2	9.75	9.68
$[CuL_2H_{-1}]^-$	9.8	10.57	10.55
Ref.	[a]	[8]	[8]

[a] This work

Similarly, $[Cu(DMT)]^+$ is a weak acid ($pK_a = 7.6$), stronger than $[Ni(DMT)]^+$ ($pK_a = 9.13$), while no acid properties of their threoninato analogues, $[M(Thr)]^+$ and $[M(allo\text{-}Thr)]^+$ ($M = Co^{+2}$, Cu^{+2}) could be detected [5]. The stronger acidity of N,N-dimethylthreoninato complexes compared to the threoninates may be taken as an indication of alcoholic –OH coordination (with simultaneous hydron liberation), in contrast to the threoninates Such bidentate species seem to be stereochemically possible.

The $p[H]$ dependence of the *LF* band position (*cf.* Table 2) in the visible spectrum of copper-containing *DMT* solutions follows a pattern (hypsochromic shift with increasing alkalinity) similar to that of the analogous peaks in CD spectra of threoninates [8]. If the conclusion on the OH participation in the bonding deduced in the preceding paragraph is correct (that is if the OH group is coordinated in *DMT* complexes and the opposite is true for threoninates), the observed spectral pattern should be interpreted in a more cautious way than is frequently done (possibly by analogy to peptide complexes): it seems that the hypsochromic shift of the copper(II) *LF* band reflects any change in the complex geometry or bonding, not only the change in ligating group(s).

The available data apparently lead to the conclusion that *DMT* acts as a bidentate ligand toward copper(II) by engaging either amino and carboxyl groups in $[Cu(DMT)]$ and $[Cu(DMT)_2]$) or, upon dehydronation, amino and hydroxyl groups (in $[Cu(DMT)H_{-1}]$, $[Cu(DMT)_2H_{-1}]$, and $[Cu(DMT)_2H_{-2}]$). The latter way of ligation is not likely to be operative in threoninato and *allo*-threoninato complexes.

Experimental

Chemicals. *L*-N,N-dimethylthreonine was synthesized by reductive condensation of *L*-threonine with formaldehyde and purified by a two-step fractional crystallisation from ethanolic solution. Details of the procedure are given elsewhere [10]. Other chemicals were of analytical reagent grade (or equivalent) and were not further purified. Water was first deionized and then distilled twice in an all-glass still.

Potentiometric Titrations. The solutions to be titrated contained *DMT* ($c_L \approx 3$ mmol/l), HCl (*ca.* 6 mmol/l), KCl (0.1 mol/l) and (except in the titrations for pK_a determination) $M(NO_3)_2$ ($M = Co^{+2}$, Ni^{+2}, Cu^{+2}; $c_M = c_L/x$, $x \approx 1, 2, 3, 6$). Stock solutions of metal salts were standardized complexometrically. A carbonate-free NaOH solution (0.1 mol·l^{-1}, with the same KCl background), standardized against potassium hydrogen phtalate), served as the titrant.

The titrations were carried out at 25 ± 0.05 °C under nitrogen (pre-purified to remove O_2 and CO_2). The equilibrium e.m.f. of the cell, consisting of Radiometer glass (G 202B) and calomel (K 401) electrodes, was measured to the nearest 0.1 mV by means of a Radiometer pHM 64 digital voltmeter; this instrument was calibrated against a certified *Weston* cell. The cell response was calibrated in terms of p[H] (cologarithm of hydron concentration – see Ref. [8] for details).

Because of limited amount of the ligand, the spectra were taken at preselected p[H] values during the titration by means of a Hewlett-Packard HP 8452A diode array spectrophotometer, equipped with a flow-through cell and a peristaltic pump.

Computations. Stability constants (β_{qnp}) of generalized species $M_qL_nH_p$, are defined here as dimensionless quantities ($c = 1$ mol/l):

$$\beta_{qnp} = ([M_qL_nH_p]/[M]^q[L]^n[H]^p)\cdot(c^0)^{q+n+p-1}. \quad 1$$

For each model (*i.e.* for each set of assumed species), the initial estimates of β_{qnp} values were refined by means of the SUPERQUAD [11] program. Dehydronation (acidity) constants are generally defined as:

$$pK_a^{qnp} = -\lg\frac{\beta_{q,n,p}}{\beta_{q,n,p-1}}. \quad 2$$

The criteria for accepting a model were the following: (1) the values of error parameters (standard errors of the constants' estimates, variance about the final regression curve, σ^2, as well as χ^2) had to be low but, having in view imperfections in the algorithm, were not required to pass a formal statistical test; (2) an estimate of β differring by $\geqslant 2$ standard deviations from the common weighted mean was considered an irregular outlier and was removed (*Chauvenet*'s criterion); (3) complex species whose concentration in a titration never reached $0.1c_M$ were considered marginal and were omitted from the model; (4) the model had to be chemically sensible (for instance, if $\beta_{n+1}/\beta_n > \beta_n/\beta_{n-1}$, the existence of ML_{n+1} was considered questionable, *etc.*).

As a check of the apparatus and methods, pK_a values of glycine were redetermined, with very satisfactory results: $pK_{a1} = 2.37(8)$, $pK_{a2} = 9.56(7)$.

Acknowledgments

Financial support by the Ministry of Science of the Republic of Croatia and US DHHS is gratefully acknowledged.

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Received August 5, 1993. Accepted (revised) September 20, 1993