Stabilities of Mixed Metal Complexes with Biologically Active Colchiceine in Dioxan–Water and their Relation to Quantitative Softness Values: A New Approach

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Summary. Equilibria between colchiceine and protons or bivalent metal ions and mixed metal ions have been investigated potentiometrically. The method of Bjerrum and Calvin, as modified by Irving and Rossotti [1] has been used to find the values of n and pL. Stability constants have been calculated using NBAR and weighted least-squares programmes. The values of $S_{min} = \chi^2$ have also been calculated. The order of stability constants for bivalent metal complexes was found to be: Zn < Cu > Ni > Co > Cd > Mn > Mg and for mixed metal complexes: Cu–Ni > Cu–Zn > Cu–Cd. A linear relationship was observed between $\log K_1$ and the softness values of the complexes.

Keywords. Stability; Mixed metal complexes; Colchiceine; quantitative softness values.

Stabilitäten gemischter Metallkomplexe mit biologisch aktivem Kolchicein in Dioxan–Wasser-Mischungen und ihre Relation zu quantitativen Weichheitswerten: ein neues Konzept

Zusammenfassung. Es wurden Gleichgewichte zwischen Kolchicein und Protonen oder bivalenten Metallionen potentiometrisch untersucht. Zur Berechnung der Werte für n und pL wurde die Methode von Bjerrum und Calvin in der Modifikation von Irving und Rossotti genutzt, die Stabilitätskonstanten wurden mit NBAR und kleinsten-Fehlerquadrat-Methoden ermittelt. Die Werte für $S_{min} = \chi^2$ wurden ebenfalls berechnet. Die Reihung nach Stabilitätskonstanten ergab sich als: Zn < Cu > Ni > Co > Cd > Mn > Mg und für die gemischten Metallkomplexe: Cu–Ni > Cu–Zn > Cu–Cd. Es wurde eine lineare Beziehung zwischen $\log K_1$ und den Weichheitswerten der Komplexe beobachtet.

Introduction

Tublin is the main cellular target of antitumor and antimitotic drugs which perturb microtubule assembly. Among these tubulin ligands the three best known are colchicine, vinblastine and taxol, which bind to different sites on the protein. Colchicine binds to a single high affinity site of tubulin. Under nonassembly
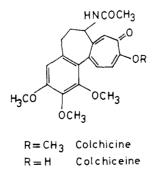
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conditions the binding of this ligand is not linked to protein self-association [2, 3], and the conformational changes induced in the system [4, 5] involve both colchicine [6] and tubulin [2]. Under assembly conditions, a morphologically abnormal polymerization of the tubulin-colchicine complex takes place [7, 8] with qualitative and apparent thermodynamic characteristics very similar to those of microtubule assembly [3]. It has been proposed that the abnormal polymerization of the tubulin-colchicine complex and the substoichiometric inhibition of the microtubule growth by this ligand [9, 10] are caused by a distortion of the normal interaction geometry between the tubulin protomers [3]. The primary cause of these effects is the binding of the drug to tubulin, which consists of processes not fully understood. The binding of colchicine to soluble tubulin is slow and the unoccupied binding site denatures rapidly, hampering equilibrium studies [11]. The colchicine-tubulin interaction is known to be strongly temperature-dependent. In fact, Van't Hoff enthalpy changes of $50-70\,\mathrm{kJ\,mol^{-1}}$ have been obtained [12–14]. The kinetics of binding of colchicine [Col] to tubulin (TB) consists of two parallel phases corresponding to two types of binding sites of which only the fast phase has been characterized [4, 5]. This phase conforms to a two-step mechanism: a fast reversible binding followed by a slow conformational change.

$$\mathrm{TB} + \mathrm{Col} \underset{}{\overset{K_1}{\rightleftharpoons}} \mathrm{TB \cdot Col} \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} (\mathrm{TB \cdot Col}). \tag{1}$$

The first step is weak binding and the backward rate constant of the second step is very small, which results in the overall high affinity formation of an essentially stable, fluorescent end product [4, 5].

The difficulties encountered for a rigorous equilibrium characterization of the tubulin-colchicine interaction led to the study of the similar ligand colchiceine. Colchicine on acid hydrolysis under very mild conditions gives colchiceine and methanol. Colchiceine contains hydroxyl and carbonyl groups in favourable positions which makes it an excellent chelating agent. The binding capability of the ligand has been determined potentiometrically. Although some heteronuclear mixed complexes of common biofunctional ligands have been synthesized [15, 16] and the analytical importance of this kind of ternary compounds has been shown [17–19], the driving forces for mixed-metal complex formation remain obscure. Inspite of the contribution of the thermodynamic approach to elucidate the characteristics of coordination of two different ligands around one metal ion, only recently the formation constants of ternary complexes of δ-hydroxylysine containing two dissimilar metal ions have been determined [20].

R = CH$_3$ Colchicine
R = H   Colchiceine

A literature survey reveals that there in no work reported on the binding capability of colchiceine with bivalent metal ions, and the stabilities of mixed-metal complexes with common biofunctional ligands have been determined only in aqueous solutions. As we are already working on biologically active ligands [21], in this work we report the stability constants of bivalent metals and mixed-metal complexes with colchiceine in dioxan-water mixture.

Experimental Part

Colchiceine was prepared by the method of Leete and Nemeth [22]. A colchiceine solution was prepared in freshly distilled dioxan. The solutions of bivalent metal ions were standardised by conventional methods. Me_4NOH $(TMAH)$ (E. Merck, A. G., Darmstadt) in 75% dioxan (aqueous) was used as titrant. Its solution was standardised with oxalic acid. $HClO_4$ was standardised with standard Na_2CO_3 and diluted to the required molarity with double distilled water. $NaClO_4$ (Riedel) was used to maintain the ionic strength. Dioxan A.R. (B.D.H) was purified by the method given by Vogel [23].

A digitial pH-meter (ECIL, model pH 5652) in conjunction with a glass electrode was used for pH measurements. The pH-meter was standardised before performing the titration. All measurements were made at a definite temperature maintained constant by using an MLW (F.R.G.) (NBE type) thermostat. A PC/XT computer was used for most of the calculations. pH and volume corrections were applied for getting the values of stability constants.

Titration

Investigations were carried out at different ionic strengths (0.1, 0.05, 0.025 and 0.01M) keeping the temperature constant at 20 °C. For each set of experiments, the final volume was made up to 20 cm^3. Each set was titrated against 0.05M $TMAH$. The titrations were carried out in covered double-walled glass cells in an atmosphere of N_2 which was presaturated with the solvent (dioxan in H_2O) before being passed into the reaction solution.

Calculations

From the titration curves the values of $\bar{n}$ and pL have been calculated using the NBAR programme (developed for calculating the $\bar{n}$ and pL values using the Irving and Rossotti method). The corresponding values of stability constants have been calculated using a weighted least squares programme. The weighted least squares treatment determines that the set of β_n values which makes the function

$$U \left\{ U = \sum_{n=0}^{N} (y - x - nz)\beta_n x^n \right\}$$

nearest to zero by minimizing

$$S \left\{ S = \sum_{i=1}^{I} U^2 (x_i y_i z_i) \right\}$$

with respect to the variation in β_n. We report the S_{min} values for the different metal complexes. S_{min} has the same statistical distribution as χ^2 with K degrees of freedom and S_{min} can be equated to χ^2. Klopman [24] has given the following equation for deriving the softness values (En^{2+}) of metal:

$$En^{2+} = IPn - b^2(IPn - EAn) - \frac{X_s(C_s)^2}{R_s}\left(1 - \frac{1}{E}\right)[q_s - 2b^2 X_s(C_s)^2]$$

where, En^{2+} is the softness of metal, IP the ionization potential of the atom, EA the electron affinity, $a^2 = 3/4, b^2 = 1/4, C = 1, E$ is the dielectric constant, R_s the radius of the atom whose softness is to be determined, q is the charge on the atom, $x = q - (q - 1)k$, and $k = 0.75$. The softness values so derived have been termed as "effective softness" and are designated by symbols En^{2+}(eff) for a metal. These values are approximate because the quantities used for deriving them are themselves based on approximations.

Table 1. Stability constants of bivalent metal complexes of colchiceine at different ionic strengths

Metal ion	Weighted least squares method			S_{min}
	$\log K_1$	$\log K_2$	$\log \beta_2$	
$\mu = 0.1M$ $NaClO_4$, Temp. $= 20 \pm 0.5\,°C$				
H^+	8.41	–	–	–
Cu(II)	7.73	7.67	15.40	0.00963
Zn(II)	7.60	7.00	14.60	0.00080
Ni(II)	7.46	6.61	14.07	0.02653
Co(II)	7.28	6.30	13.58	0.01983
Cd(II)	6.62	6.38	13.00	0.01588
Mn(II)	5.89	5.71	11.60	0.31414
Mg(II)	5.22	4.15	9.37	0.00239
$\mu = 0.05M$ $NaClO_4$, Temp. $= 20 \pm 0.5\,°C$				
H^+	8.77	–	–	–
Cu(II)	8.05	6.85	14.90	0.01485
Zn(II)	7.90	6.90	14.80	0.02060
Ni(II)	7.61	7.25	14.86	0.03030
Co(II)	7.44	6.49	13.93	0.00269
Cd(II)	6.91	6.16	13.07	0.26143
Mn(II)	6.22	4.83	11.05	0.02260
Mg(II)	5.64	4.02	9.66	0.02335
$\mu = 0.025M$ $NaClO_4$, Temp. $= 20 \pm 0.5\,°C$				
H^+	8.98	–	–	–
Cu(II)	8.13	7.33	15.46	0.00590
Zn(II)	8.05	7.20	15.25	0.00164
Ni(II)	8.00	7.34	15.34	0.05434
Co(II)	7.59	6.06	13.65	0.00321
Cd(II)	7.25	6.03	13.28	0.05751
Mn(II)	6.59	5.03	11.62	0.01011
Mg(II)	5.72	4.76	10.48	0.07034
$\mu = 0.01M$ $NaClO_4$, Temp. $= 20 \pm 0.5\,°C$				
H^+	9.22	–	–	–
Cu(II)	9.03	8.00	17.03	0.01091
Zn(II)	8.50	7.98	16.48	0.10724
Ni(II)	8.79	8.20	16.99	0.03585
Co(II)	8.39	7.76	16.15	0.00132
Cd(II)	7.44	6.30	13.74	0.05515
Mn(II)	6.88	6.04	12.92	0.13798
Mg(II)	5.93	4.72	10.65	0.12516

Results and Discussion

Order of Stability Constants

The order of the stability constants of the metal complexes with colchiceine on the basis of quantitative values of softness is as follows: Zn < Cu > Ni > Co > Cd > Mn > Mg.

The order is in good agreement with that found by Mellor and Maley [25] and Irving and Williams [26, 27]. In all the systems, the values of $\log K_1 > \log K_2$. The $\log K_1, \log K_2, \log \beta_2$ and S_{min} values at different ionic strengths are given in Table 1. The results show that the stability of the metal chelates increases regularly from Mn to Cu and falls again at Zn. Furthermore, the stability order is independent of the ligand. Minor deviations can be predicted on theoretical grounds. The regularity of

Table 2A. $\bar{n}$ and pL values of mixed metal complexes of colchiceine in 75% dioxan-water medium at $\mu = 0.1 M$ NaClO$_4$

$\bar{n}$	pL values		
	Cu–Ni	Cu–Zn	Cu–Cd
0.4	9.26	8.90	–
0.5	9.04	8.70	7.64
0.6	8.84	8.48	7.43
0.7	8.62	8.28	7.18
0.8	8.42	8.10	6.98
0.9	8.30	7.94	6.83
1.0	8.05	7.76	6.73
1.1	7.80	7.58	6.63
1.2	7.53	7.38	6.48
1.3	7.25	7.14	6.32
1.4	7.00	6.88	6.22
1.5	6.74	6.67	6.12
1.6	6.50	6.43	6.04
1.7	6.22	6.14	5.94
1.8	5.96	5.92	5.84
1.9	–	–	5.73
2.0	–	–	5.59

Table 2B. Stability constants of mixed metal complexes of colchiceine at 20 °C and ionic stength of 0.1M NaClO$_4$

Metal ion	Weighted least squares method			S_{min}
	$\log K_1$	$\log K_2$	$\log \beta_2$	
Cu–Ni	8.29	6.94	15.23	0.35667
Cu–Zn	8.09	6.47	14.56	0.56317
Cu–Cd	7.07	6.20	13.27	0.05694

this stability sequences can be correlated with a monotonic decrease in the ionic radii, and a monotonic increase in the second ionization potential which, in passing from Mn to Cu, may be taken to indicate that either coordination has not altered the electronic ground state of the metal ions or that any modifications are of secondary importance. $\bar{n}$ values greater than 2.0 have not been obtained for these chelates. We therefore conclude that not more than two chelates, i.e. 1:1 and 1:2 ($M:L$), are formed in each system.

The stability order of heterobinuclear copper(II)-colchiceine complexes with nickel(II), zinc(II), and cadmium(II) in dioxan-water mixture at 20 °C and ionic strength 0.1 M is also according to the softness values i.e.: Cu–Ni > Cu–Zn > Cu–Cd. $\bar{n}$, pL and the values of stability constants are given in Tables 2A and 2B.

Table 3. Er(Mn–Zn) and δH values for complexes of colchiceine

Parameters[a]	Metal ions				
	Mn^{2+}	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
$Log K_1$	7.25	7.90	8.40	8.60	8.50
ΔF	9.78	10.66	11.33	11.60	11.47
ΔF_r	–	0.88	1.55	1.82	1.69
ΔH_H	–	43.00	62.00	63.00	47.00
ΔH_L	–	43.88	63.55	64.82	48.69
$[(n-5)/5]\,Er$	–	19.47	29.21	38.95	–
δH	–	24.41	34.34	25.87	–

[a] ΔF = free energy change = $2.303\,RT\,\log K_1$ (where R, T and K_1 have the usual significance) at $T = 293$ K; ΔF_r = change in heat content formation of the complex in solution; ΔH_H = heat of hydration of the metal ion; ΔH_L = heat of complexation referring to the metal ion in the gaseous state and the ligand in solution; n = number of electron in the 3d orbital; $[(n-5)/5]\,Er$ = lattice energy difference for Zn^{2+} and Mn^{2+} complexes; δH = thermodynamic stabilization energy; $\log K_1$ = values used in the above calculations have been obtained by extrapolating the $\log K_1$ vs. $\sqrt{\mu}$ plot to zero ionic strength

Table 4. Calculated softness values of cations

M^{2+}	IP_n	EAn	Orbital energy (eV)	Rs ($rb + 0.82$)	Desolvation in 75% dioxan-water mixture	En^{2+}(eff) (eV)
Zn	17.96	9.39	15.82	1.57	14.60	−1.22
Cu	20.29	7.72	17.15	1.54	14.89	−2.26
Ni	18.15	7.63	15.51	1.51	15.18	−0.33
Co	17.05	7.86	14.76	1.56	14.70	−0.06
Cd	16.90	8.99	14.93	1.79	12.81	−2.11
Mn	15.64	7.43	13.60	1.63	14.07	0.47
Mg	15.03	7.64	13.18	1.48	15.49	2.31

Table 5. Softness values for mixed metal complexes

Complex	$En^{2+}(\text{eff})(\text{Cu}-M)$
Cu–Ni–Col	1.93
Cu–Zn–Col	1.04
Cu–Cd–Col	0.15

Evidence for mixed metal complex formation was obtained by comparing the experimental titration curves obtained with solutions containing Cu^{2+}, Ni^{2+}, Zn^{2+} or Cd^{2+} and the ligand with those calculated on the assumption that no heterobinuclear species are present in solution. It was found that the trend of experimental curves is not explained by assuming mixed metal complex formation.

The different 'soft' and 'hard' character of metal ions may explain the trend of stability constants, since nickel(II), zinc(II), and cadmium(II) respond in a different way to the double σ and π bonding character of ligand.

On the basis of formation of heterobinuclear species it can be asserted that the existence of this type of complexes does not need the presence of two complexing sites of different donor properties, as previously suggested [28], and that probably the species distribution relative to complexes of essential metal ions with ligands in biofluids [29] may be affected by the presence of heteronuclear ternary species.

Effect of Ionic Strength

In the present studies it has been observed that the value of the dissociation constant of the ligand (pK_a) and the stability constants decrease with increasing ionic strength of the medium (μ), which is in agreement with the Debye–Hückel equation. Thermodynamic stability constants were obtained by extrapolating the plot of $\log K_1$ vs. $\sqrt{\mu}$ to zero ionic strength.

Thermodynamic Parameters

Orders of enthalpies, free energies and ligand field stabilization energies (Table 3) have been found to be: Mn < Co < Ni < Cu > Zn; Mn < Co < Ni < Cu > Zn and Co < Ni > Cu, respectively.

Acknowledgement

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