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Complexing of Inosine and Guanosine with Divalent Metal Ions in Aqueous Solution

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Stability constants for the complexes of inosine and its monoanion with some alkaline earth metal and 3d transition metal ions have been determined in aqueous solution at 298.2 K. Comparison of the results with the corresponding data obtained with 1- and 7-methylinosines and guanosine indicates that N7 constitutes the preferential binding site in inosine and N1 in its monoanion. The relative complexing-abilities of different purine nucleosides is briefly discussed.

Introduction

The complexing with metal ions of nucleic acids, and their monomeric constituents nucleosides and nucleotides, has been the object of numerous investigations during the past decade [1–6]. Application of various spectroscopic techniques, including NMR, UV, ORD, and Raman measurements, has given valuable information about the structures of the complexes that the monomeric units form in aqueous solution [3, 4]. With purine nucleosides for example, coordination takes place at N1 and N7, the preferential binding site being determined not only by the substituents in the purine ring but also by the acidity of the medium and the nature of the metal ion [4]. Furthermore, direct chelation between N7 and the oxo or amino substituent at C6 has been suggested by some authors [7–12], and disputed by others [3, 4].

The studies concerning the stabilities of the metal complexes of purine nucleosides in aqueous solution are much more limited [1, 13]. In addition, the comparisons between the reported formation constants are difficult, because the determinations have usually been carried out under very different conditions. As stated by Marzilli in his review [3]: "there exists presently a great need for relatively extensive investigations of formation constants performed under a given set of conditions". We have previously reported on the complexing of 9-(β-D-ribofuranosyl)-purine and its 6-amino derivative, adenosine with some 3d transition metal ions at the ionic strength of 1 mol dm^{-3} at 298.2 K [14]. Introduction of an amino group in position 6 of the purine ring destabilized the complexes formed in acidic solutions, though the basicity of the ligand is at the same time increased. Accordingly, formation of a chelate through coordination at N7 and C6-NH$_2$ of adenosine was considered improbable. The investigations are now extended to inosine and guanosine, bearing an oxo group at C6, and the N-methylated derivatives of the former (for the structures see Schemes 1 and 2).

Experimental

Materials

Of the nucleosides employed inosine and guanosine were purchased from Aldrich-Europe, and 1- and 7-methylinosines from Sigma Chemical Company. The metal perchlorates were the products of G. Frederick Smith Chemical Company. All the reagents were used as received.

Titrimetric measurements

The protonation constants, K_2 in Schemes 1 and 2, for inosine, 1-methylinosine, and guanosine were determined by the modified potentiostatic technique described earlier [14]. The same method was applied to obtain the apparent equilibrium constants, defined by eqn. (1), for the protonation of these compounds in the presence of divalent metal ions.

$$K_2(\text{app.}) = \frac{[LH_2^+]}{[H^+]([LH] + [LHM^{2+}])} \qquad (1)$$

The apparent equilibrium constants, defined by eqn. (2), for the displacement of a proton by a metal ion in inosine were measured in various salt solutions

$$K_3(\text{app.}) = \frac{[LM^+][H^+]}{[M^{2+}]([LH] + [LHM^{2+}])} \qquad (2)$$

by the following procedure. The appropriate salt solution (20 cm^3) having a known concentration

TABLE I. Apparent Equilibrium Constants, K_2(app.), for Protonation of Inosine and 1-Methylinosine in Aqueous Solutions of Various Metal Perchlorates at 298.2 K.[a]

M^{2+}	$[M^{2+}]$ /mol dm^{-3}	lg $\{K_2(app.)/dm^3\ mol^{-1}\}$[b]	
		Inosine	1-Methylinosine
–	–	1.39 ± 0.02[c]	1.39 ± 0.01[d]
Mg^{2+}	0.20	1.26 ± 0.02	1.32 ± 0.04
Ca^{2+}	0.20	1.28 ± 0.01	1.40 ± 0.02
Ba^{2+}	0.20	1.31 ± 0.03	1.40 ± 0.05
Mn^{2+}	0.10	1.36 ± 0.04	
Co^{2+}	0.10	1.17 ± 0.06	1.30 ± 0.03
Ni^{2+}	0.10	1.06 ± 0.05	1.11 ± 0.02
Cu^{2+}	0.025	1.24 ± 0.04	
	0.050	1.09 ± 0.08	
	0.10	0.90 ± 0.04	0.85 ± 0.04
Zn^{2+}	0.10	1.20 ± 0.02	1.31 ± 0.03

[a] The ionic strength was adjusted to 1.0 mol dm^{-3} with sodium perchlorate. [b] Refers to eqn. (1). [c] In lit. 1.5 [15] and 1.2 [16]; the ionic strength not indicated. [d] In lit. [17] 1.2 at 85 °C; the ionic strength not indicated.

TABLE II. Formation Constants, K_4, for the Complexes of Inosine and 1-Methylinosine with Some 3d Transition Metal Ions in Aqueous Solution at 298.2 K.[a]

M^{2+}	$[M^{2+}]$ mol dm^{-3}	lg $(K_4/dm^3\ mol^{-1})$[b]		
		Inosine	1-Methylinosine	[c]
Co^{2+}	0.10	0.8 ± 0.2	0.4 ± 0.3	1.0
Ni^{2+}	0.10	1.1 ± 0.2	1.0 ± 0.1	1.3
Cu^{2+}	0.025	1.2 ± 0.2		
	0.050	1.3 ± 0.2		
	0.10	1.3 ± 0.1	1.4 ± 0.1[d]	1.5
Zn^{2+}	0.10	0.7 ± 0.1	0.3 ± 0.3[e]	0.7

[a] The ionic strength was adjusted to 1.0 mol dm^{-3} with sodium perchlorate. [b] See Schemes 1 and 2. [c] The corresponding values for 9-(β-D-ribofuranosyl)purine from Ref. 14. [d] In lit. [18] 1.36 at 358 K; the ionic strength adjusted to 0.4 mol dm^{-3} with sodium perchlorate. [e] Complexing not detected [18].

(0.1–0.2 mol dm^{-3}) was added in a constant-temperature vessel (298.2 K) equipped with a Metrohm EA 121 combined electrode. The solution was agitated under nitrogen, and the oxonium ion concentration was adjusted to the desired initial value (pH 4–5) with perchloric acid. A known amount of inosine (0.05–0.3 mmol) was introduced in the solution. After complete dissolution sufficient sodium hydroxide (0.01–0.05 mol dm^{-3}) was added from an Agla micrometer syringe to maintain the initial meter reading. The volume of the reaction mixture increased by less than 1 per cent during the titration. The addition of inosine did not affect the meter reading in the absence of divalent metal ions. The equilibrium constants, K_3(app.), were calculated by eqn. (3). Here n(NaOH) denotes the amount of sodium hydroxide needed to maintain the initial pH,

$$K_3(\text{app.}) = \frac{n(\text{NaOH})[H^+]}{\{n(\text{LH, tot.}) - n(\text{NaOH})\}[M^{2+}]} \qquad (3)$$

and hence the amount of the complex LM^+ formed, and n(LH, tot.) the total amount of inosine added. The remainder of these two quantities, indicated in the denominator of eqn. (3), is equal to the sum of n(LH) and n(LHM^{2+}), since the amount of the diprotonated ligand LH_2^+ is negligible under the experimental conditions. The equilibrium concentration $[M^{2+}]$ of the metal ion is essentially the same as its total concentration.

The equilibrium constants, K_3 in Scheme 2, for the corresponding reactions of 7-methylinosine were determined analogously, but the ligand was added as an 0.15 mol dm^{-3} aqueous solution having the same oxonium ion concentration and ionic strength as the salt solution. Under these conditions 7-methylinosine is almost completely converted to its monocation.

Results and Discussion

Table I summarizes the apparent equilibrium constants, K_2(app.) defined by eqn. (1), for protonation of inosine and 1-methylinosine in aqueous solutions of various metal perchlorates at 298.2 K. The protonation constants, K_2 in Schemes 1 and 2, obtained in the absence of divalent metal ions agree satisfactorily with the values reported in the literature, taking into account the differences in the experimental conditions [15–17]. Addition of alkaline earth metal cations to the reaction mixture has little effect on K_2(app.). The situation is the same with manganese(II) ion, but the other 3d transition

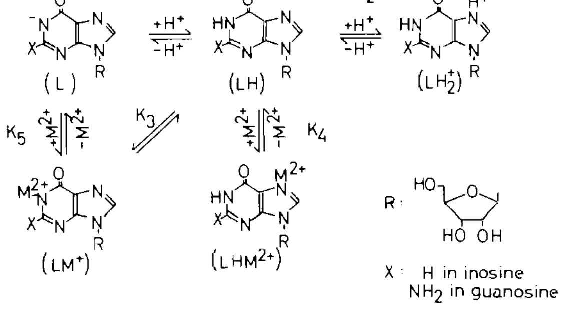
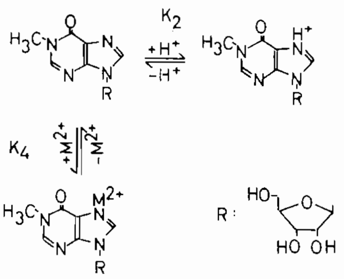
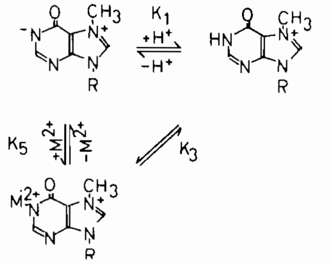


TABLE III. Apparent and Real Equilibrium Constants, K_3(app.) and K_3, for Displacement of a Proton by a Metal Ion from Inosine and the Monocation of 7-Methylinosine in Aqueous Solution at 298.2 K[a], and the Formation Constants, K_5, for the Complexes of the Anionic Inosine.

M^{2+}	$[M^{2+}]$ mol dm^{-3}	lg K_3 (app.)[b]	lg K_3[c]	lg (K_5/dm^3 mol^{-1})[d]	lg K_3[e]
		Inosine			7-Me-Ino
Co^{2+}	0.10	−6.8 ± 0.1	−6.6 ± 0.1	2.1	−5.7 ± 0.1
Ni^{2+}	0.10	−6.3 ± 0.1	−5.9 ± 0.2	2.8	−5.6 ± 0.1
Cu^{2+}	0.10	−4.6 ± 0.1	−4.2 ± 0.1	4.5	−3.8 ± 0.1
Zn^{2+}	0.10	−6.5 ± 0.1	−6.3 ± 0.2	2.4	−5.7 ± 0.1

[a] The ionic strength was adjusted to 1.0 mol dm^{-3} with sodium perchlorate. [b] Refers to eqn. (2). [c] See Scheme 1. [d] See Scheme 1. The value of lg(K_1/dm^3 mol^{-1}) = −8.7 has been used in the calculations. [e] See Scheme 2.





metal ions investigated retard considerably the protonation of both inosine and its 1-methyl derivative, most probably due to the competitive complexing of the compounds with metal ions. As described earlier [14] the formation constants K_4 in Schemes 1 and 2 for the 1:1 complexes of the neutral ligands with the metal ions can be calculated from the apparent protonation constants by eqn. (4).

$$K_4 = \frac{[LHM^{2+}]}{[LH][M^{2+}]} = \frac{1}{[M^{2+}]}\left\{\frac{K_2}{K_2(\text{app.})} - 1\right\} \tag{4}$$

The results obtained are given in Table II. The formation constants observed for inosine are of the same magnitude as those for 1-methylinosine, in agreement with the widely approved suggestion that under acidic conditions N7 constitutes the preferential binding site in inosine [1–4]. With both compounds the stabilities of the 3d transition metal complexes obey the Irving-Williams order, which has also been observed to be the case with 9-(β-D-ribofuranosyl)-purine and adenosine [14]. As seen from Table II, the complexing-ability of inosine is comparable to that of 9-(β-D-ribofuranosyl)purine, and hence slightly greater than that of adenosine [14].

Table III records the apparent equilibrium constants K_3(app.), defined by eqn. (2), for the displacement of a proton from inosine by a metal ion in aqueous solution at 298.2 K. Substitution of $[LHM^{2+}]$ from eqn. (4) into eqn. (2) yields eqn. (5). Accordingly, the real equilibrium constants

$$K_3(\text{app.}) = \frac{[LM^+][H^+]}{[M^{2+}][LH](1 + K_4[M^{2+}])} \tag{5}$$

for the displacement reaction, K_3 in Scheme 1, can be calculated by eqn. (6). These constants are also listed in Table III, as are the corresponding constants for the monocation of 7-methylinosine. The

$$K_3 = K_3(\text{app.})(1 + K_4[M^{2+}]) \tag{6}$$

equilibrium constants K_3 obtained with inosine are of the same order of magnitude as those observed for the corresponding 7-methyl derivative, as would be expected if the coordination occurs in both ligands with the displacement of the proton at N1. The complexing-efficiencies of different metal ions obey the Irving-Williams order in both series. For inosine the pK_a value of 8.7 has been reported at 298.2 K [1]. Consequently, the logarithmic formation constants K_5 listed in Table III are obtained for the 1:1 complexes of the monoanion of inosine with various metal ions. They agree satisfactorily with the values reported in the literature [15], *viz.* 2.6, 3.3 and 5.0 for the cobalt(II), nickel(II) and copper(II) complexes at 293.2 K, taking into account the differences in the temperature and the ionic strength. The 3d transition metal complexes of the inosine anion are thus more stable (by a factor of 10^2) than those of the neutral ligand.

TABLE IV. Apparent Equilibrium Constants, K_2(app.), for Protonation of Guanosine in Aqueous Solutions of Various Metal Perchlorates, and Formation Constants, K_4, for its Complexes with Some 3d Transition Metal Ions at 298.2 K.[a]

M^{2+}	$[M^{2+}]$ /mol dm^{-3}	lg{K_2(app.)/dm^3 mol^{-1}}[b]	lg{K_4/dm^3 mol^{-1}}[c]
–	–	2.33 ± 0.02[d]	
Mg^{2+}	0.20	2.31 ± 0.05	
Ca^{2+}	0.20	2.36 ± 0.01	
Ba^{2+}	0.20	2.30 ± 0.01	
Co^{2+}	0.10	2.01 ± 0.05	1.0 ± 0.1
Ni^{2+}	0.10	1.77 ± 0.02	1.4 ± 0.1
Cu^{2+}	0.10	1.36 ± 0.06	1.9 ± 0.1[e]
Zn^{2+}	0.10	2.12 ± 0.02	0.8 ± 0.1

[a] The ionic strength was adjusted to 1.0 mol dm^{-3} with sodium perchlorate. [b] Refers to eqn. (1). [c] See Scheme 1. [d] In lit. 1.9 (T = 298.2 K, I = 0) [19], 2.17 (T = 298.2 K, I = 0.1 mol dm^{-3}) [20] and 2.20 (T = 293.2 K, I = 1.0 mol dm^{-3}) [21]. [e] In lit. [21] 2.15 (T = 293.2 K, I = 1.0 mol dm^{-3}).

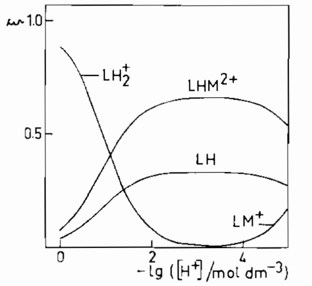


Fig. 1. Extent of protonation and complexation of inosine at different oxonium ion concentrations in 0.10 mol dm^{-3} aqueous solution of copper(II) perchlorate at 298.2 K. The ionic strength was adjusted to 1.0 mol dm^{-3} with sodium perchlorate. For the structures of LH, LH_2^+, LHM^{2+} and LM^+ see Scheme 1. The total ligand concentration is less than 0.01 mol dm^{-3}.

The expressions for the equilibrium constants K_2, K_3 and K_4, together with the conservation eqn. (7), enable the construction of the distribution

$$[LH(tot.)] = [LH] + [LH_2^+] + [LHM^{2+}] + [LM^+] \quad (7)$$

diagram for various species of inosine as a function of oxonium ion concentration in the acidity region where the concentration of the deprotonated inosine L^- can be neglected. In Fig. 1 such a diagram has been presented for the copper(II) complexes of inosine. Complexing of the anionic ligand becomes quantitatively significant only above pH 4, *i.e.* under conditions where the metal ion begins to form basic salts and precipitate as hydroxide.

The apparent protonation constants K_2(app.) for guanosine in the presence of divalent metal ions and the formation constants K_4 for its 1:1 metal ion complexes in aqueous solution at 298.2 K are listed in Table IV. The limited solubility of guanosine in neutral aqueous solutions prevented the measurements of the equilibrium constants for the displacement of the N1 proton by a metal ion. Comparison of the data in Tables II and IV indicates that the 3d transition metal complexes of neutral guanosine are slightly more stable than those of inosine. This finding is compatible with the proposal that with both ligands N7 serves as the coordination site, the small stability difference reflecting the greater basicity of this site in guanosine. If coordination could take place at N1 or N3, introduction of an amino group at the adjacent carbon on going from inosine to guanosine would be expected to profoundly affect the stabilities of the metal complexes.

In summary the results of the present paper, together with those reported earlier [14], indicate that the stabilities of the 3d transition metal complexes of purine nucleosides decrease in the order: the monoanion of inosine, guanosine, 9-(β-D-ribofuranosyl)purine, inosine, adenosine. With each nucleoside the relative complexing-efficiencies of different metal ions are rather similar, the only exception being the large stability difference between the copper(II) and the other transition metal complexes of adenosine.

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