Inorganica Chimica Acta, 55 (1980) 39–42

Stability Constants for some Transition Metal Complexes of Adenosine and 9-(β-D-Ribofuranosyl)purine in Aqueous Solution

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Received September 8, 1980

Stability constants for complexes of adenosine and 9-(β-D-ribofuranosyl)purine with cobalt(II), nickel(II), copper(II) and zinc(II) ions have been determined in aqueous solution at 298.2 K. Lower stabilities of the former complexes compared to the latter argue against formation of a chelate through coordination of a metal ion N7 and C6-NH_2 in adenosine. The structures of the complexes of 9-(β-D-ribofuranosyl)purine have been discussed on the basis of the downfield shifts that zinc(II) ion exert on the ^1H NMR signals of the purine protons.

Introduction

The role that C6-NH_2 plays in complexing of adenosine and its derivatives with metal ions has been one of the principal problems of studies dealing with interactions between cations and nucleic acid components in solution. Several lines of evidence suggest that silver(I) ion [1, 2], mercury(II) chloride [3, 4], and alkyl-mercurials [5–7] undergo, besides bonding to ring nitrogens, a monodentate coordination to the primary amino group of adenosine by proton displacement. In contrast, platinum(II) and palladium(II) ions bind exclusively to N1 and N7 of the base moiety [8–11]. The experimental results concerning the complexing of first-row transition metal ions seem to be open to various interpretations. Addition of diamagnetic metal ions in solutions of adenosine in dimethyl sulfoxide results in downfield shifts in the ^1H NMR signals of the amino protons, which has been interpreted as indicative of formation a chelate through metal bonding to N7 and C6-NH_2 of adenosine [12, 13]. Recent ^1H NMR relaxation studies on complexing of adenosine with copper(II) ion in D_2O have led to the same conclusion [14]. However, on the basis of earlier relaxation measurements [15, 16] and other spectroscopic observations [17] a monodentate coordination of copper(II) ion to N7 has been suggested, and this mode of interaction is widely approved [18–21]. In crystalline state the transition metal complexes of 9-substituted adenines show no indication of participation of C6-NH_2 in metal binding [22, 23]. The aim of the present study is to approach the problem by examining the effects that introduction of an amino group in position 6 of 9-(β-ribofuranosyl)purine exerts on stabilities of its transition metal complexes in aqueous solution.

Experimental

Materials

Of the nucleosides employed 9-(β-D-ribofuranosyl)purine was a product of Sigma Chemical Company, and it was used as received. Adenosine was purchased from Aldrich-Europe and crystallized from water before use. The metal perchlorates were products of G. Frederick Smith Chemical Company, and they were employed without further purification.

Titrimetric Measurements

A potentiostatic technique was applied to the determinations of the apparent protonation constants for adenoside and 9-(β-D-ribofuranosyl)purine. The measurements were performed at 298.2 K in aqueous solutions the ionic strength of which was adjusted to 1.0 mol dm^{-3} with sodium perchlorate. The appropriate salt solution (10 cm^3) of a known-concentration (0.02–0.1 mol dm^{-3}) was added to a thermostated vessel equipped with a Metrohm EA 121 combined electrode. The oxonium ion concentration was adjusted to the desired value by adding perchloric acid from an Agla micrometer syringe. The solution was agitated with a magnetic stirrer under nitrogen until the meter readings had settled, and a known amount of nucleoside (3–6 × 10^{-5} mol) was added. After complete dissolution perchloric acid was added to return the potential to its initial value. The volume of the reaction mixture increased less than 1% due to this addition. The apparent protonation constants, K (app.), were calculated *via* eqn. (1)

$$K(\text{app.}) = \frac{\Delta n(HClO_4)}{[H^+]\ \{n(L) - \Delta n(HClO_4)\}} \quad (1)$$

TABLE I. Apparent Equilibrium Constants, K(app.), for Protonation of 9-(β-D-ribofuranosyl)purine and Adenosine in Aqueous Solutions of Various Metal Perchlorates at 298.2 K.

M^{2+}	$[M^{2+}(tot.)]$[b] mol dm^{-3}	lg (K(app.)/dm^3 mol^{-1})[a]	
		9-(β-D-Ribofuranosyl)purine	Adenosine
–	–	2.46 ± 0.02	3.86 ± 0.01
Co^{2+}	0.10	2.18 ± 0.02	3.80 ± 0.02
Ni^{2+}	0.10	1.98 ± 0.02	3.76 ± 0.02
Cu^{2+}	0.02	2.27 ± 0.01	
	0.04	2.10 0.01	
	0.06	2.02 0.01	
	0.08	1.92 0.01	
	0.10	1.84 0.01	3.58 ± 0.01
Zn^{2+}	0.10	2.28 ± 0.02	3.79 ± 0.02

[a]Refers to eqn. (1). The ionic strength was adjusted to 1.0 mol dm^{-3} with sodium perchlorate. [b]Stands for the sum concentration of complexed and free metal ions.

where $[H^+]$ stands for the equilibrium concentration of oxonium ion, n(L) is the total amount of nucleoside added, and $\Delta n(HClO_4)$ is the amount of perchloric acid needed to return the potential to its initial value. At the oxonium ion concentrations employed, *viz.* 2–6 × 10^{-4} mol dm^{-3} with adenosine and 1–7 × 10^{-3} mol dm^{-3} with 9-(β-D-ribofuranosyl)purine, the values obtained for K (app.) were independent of $[H^+]$.

^{1}H NMR Measurements

The ^{1}H NMR spectra were recorded in D_2O (>99.7%) on a Jeol JNM-PMX60 spectrometer at the normal probe temperature. The concentration of nucleoside was 0.2 mol dm^{-3}. Methanol was employed as an internal standard.

Results and Discussion

Table I records the apparent equilibrium constants, defined by eqn. (1), for protonation of adenosine and 9-(β-D-ribofuranosyl)purine in various salt solutions at 298.2 K. The values obtained for lg(K(app.)/dm^3 mol^{-1}) in the absence of divalent metal ions, *viz.* 2.46 ± 0.02 for 9-(β-D-ribofuranosyl)purine and 3.86 ± 0.01 for adenosine, are in satisfactory agreement with those reported in the literature [18, 24], taking the differences in the experimental conditions into account. With both nucleosides the values of K(app.) most probably refer to protonation of N1 of the purine moiety [18, 25]. Inspection of Table I reveals that addition of cobalt(II), nickel(II), copper(II), or zinc(II) perchlorates in the reaction mixture decrease the values of K(app.). This is expected considering the complexing of nucleosides with metal ions. Attachment of a positively charged metal ion to one of the purine nitrogens lowers the electron density at other potential binding sites. Accordingly, binding of a proton to the same molecule becomes difficult, even though different atoms were preferred in proton and metal ion binding. In other words, it seems reasonable to assume that equilibria (2) and (3) prevail at pH values near the pK_a values of the protonated nucleosides,

$$L + H^+ \rightleftharpoons LH^+ \quad (2)$$

$$L + M^{2+} \rightleftharpoons LM^{2+} \quad (3)$$

unless the metal ion concentration is extremely high. In the latter case formation of species LM_2^{4+} can become quantitatively significant. Formation of complexes containing more than one ligand molecule can also be neglected, since the measurements have been carried out in solutions where the total concentration of ligand is small compared to that of M^{2+}. Consequently, the apparent protonation constant, K(app.), can be expressed by eqn. (4) where

$$K(\text{app.}) = \frac{[LH^+]}{[H^+]([L] + [LM^{2+}])} = \frac{K(LH^+)}{1 + K(LM^{2+})[M^{2+}]} \quad (4)$$

$K(LH^+)$ and $K(LM^{2+})$ denote the equilibrium constants for eqns. (2) and (3), respectively, and $[M^{2+}]$ is the equilibrium concentration of the metal ion. Equation (4) can be easily transformed to eqn. (5), which

$$\frac{K(LH^+)}{K(\text{app.})} = K(LM^{2+})[M^{2+}] + 1 \quad (5)$$

TABLE II. Formation Constants, $K(LM^{2+})$, for the Complexes of 9-(β-D-ribofuranosyl)purine and Adenosine with Some Transition Metal Ions in Aqueous Solution at 298.2 K.

M^{2+}	lg ($K(LM^{2+})$/dm^3 mol^{-1})[a]	
	9-(β-D-Ribofuranosyl)purine	Adenosine
Co^{2+}	1.00 ± 0.08 (0.57)[b]	0.2 ± 0.2 (−0.30)[c]
Ni^{2+}	1.31 ± 0.06 (0.93)	0.4 ± 0.2 (−0.17)
Cu^{2+}	1.50 ± 0.04 (0.98)	0.96 ± 0.05 (0.84)
Zn^{2+}	0.7 ± 0.1 (0.27)	0.2 ± 0.2 (−0.28)

[a]The equilibrium constant for eqn. (3). The ionic strength was adjusted to 1.0 mol dm^{-3} with sodium perchlorate. [b]The values in parentheses refer to complexes of 9-(1-ethoxyethyl)purine at the ionic strength of 0.6 mol dm^{-3} at 313.2 K [26]. [c]The values in parentheses refer to complexes of adenosine in concentrated salt solutions (up to 3 mol dm^{-3}) at 298 K [27].

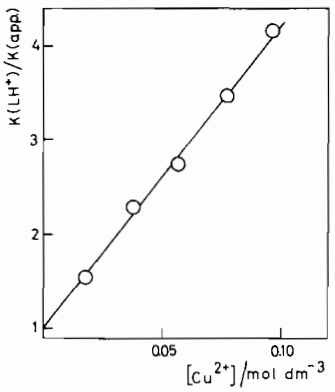

Fig. 1. The effect of copper(II) ion on the apparent protonation constant for 9-(β-D-ribofuranosyl)purine at 298.2 K. The concentration of the uncomplexed copper(II) ion has been calculated as $[M^{2+}] = [M^{2+}\text{(tot.)}] - \{[L\text{(tot.)}] - [L] - [LH^+]\}$.

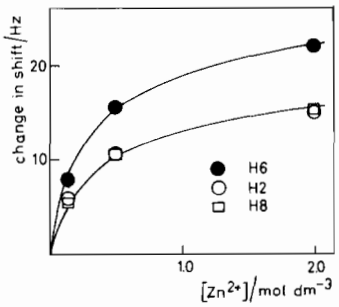

Fig. 2. The effect of zinc(II) ion on the ^{1}H NMR shifts of the purine protons of 9-(β-D-ribofuranosyl)purine in D_2O.

indicates that plotting the ratio of $K(LH^+)/K$(app.) against $[M^{2+}]$ should yield a straight line with the slope equal to $K(LM^{2+})$ and the intercept equal to unity. Figure 1 clearly shows that this is the case as far as complexing of 9-(β-D-ribofuranosyl)purine with copper(II) ion is concerned. Accordingly, the approximations made in the context of the derivation of eqn. (4) seem to be justified. Table II summarizes the formation constants, $K(LM^{2+})$, calculated *via* eqn. (4) for the transition metal complexes of adenosine and 9-(β-D-ribofuranosyl)purine. The values reported in the literature for the corresponding complexes of 9-(1-ethoxyethyl)purine [26] and adenosine [27] are included in the table for reference. The values obtained in the present work for the complexes of adenosine are somewhat larger than those determined earlier [27], but at least part of this difference may be a consequence of the completely different ionic composition of the solutions employed in the determinations. It is, however, reassuring that the relative complexing abilities of different metal ion roughly correlate. The agreement between the relative stabilities of the complexes of purine derivatives is even better, although the values refer to different temperatures and ionic strengths.

The order of the complexing efficiencies of various metal ions is expected both with adenosine and 9-(β-D-ribofuranosyl)purine. The stabilities of the complexes increase from Co^{2+} to Ni^{2+} and Cu^{2+}, and decrease thereafter on going to Zn^{2+}. Accordingly, the Irving–Williams order seems to be obeyed. With each metal ion the complex of 9-(β-D-ribofuranosyl)purine is considerably more stable than that of adenosine. In other words, introduction of an amino group in position 6 of the purine ring makes the attachment of metal ions less favorable. This is just the opposite of what is expected on the basis of polar effects. As an electropositive substituent, a primary amino group increases the electron density at the potential binding sites of purine ring, facilitating the binding of positively charged particles. For example, the basicity of adenosine is far greater than that of 9-(β-D-ribofuranosyl)purine (Table I). Most probably the observed destabilization of the metal complexes is a consequence of the C6-NH_2 forming a steric obstacle to coordination of a metal ion. It is not quite clear which is the preferential

binding site in 9-(β-D-ribofuranosyl)purine. Addition of zinc(II) perchlorate in the solution of this compound in D_2O results in a downfield shift in the ^1H NMR signals of all the purine protons, the signal for H6 being shifted more than those for H2 and H8 (Fig. 2). A possible explanation for this observation is that N1 and N7 are approximately equally favorable as binding sites. Thus two different types of 1:1 complexes would be formed. Accordingly, the signals for H2 and H8 would be affected only by the zinc(II) ions coordinated to the adjacent nitrogens, N1 and N7, respectively, whereas the signal for H6 would be shifted by both N1 and N7 bound metal ion. The lower stabilities of the adenosine complexes compared to complexes of 9-(β-D-ribofuranosyl)purine could hence be accounted for by the steric hindrance of the C6-NH_2 to binding of metal ions to N1.

On the basis of the preceding discussion, it seems highly improbable that complexing of adenosine with transition metal ions could occur with formation of a chelate through coordination to N7 and C6-NH_2. Formation of such a chelate structure would be expected to make the complexes of adenosine more stable than those of 9-(β-D-ribofuranosyl)purine. As indicated above, this is, however, not the case.

Acknowledgements

The authors wish to thank Dr. Antti Vesala for many helpful discussions. The financial aid from the Finnish Academy, Division of Sciences, is gratefully acknowledged.

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