Binding Sites and Stabilities of Transition Metal Ions with Nucleosides and Related Ligands

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Stability constant logarithms for Ni^{2+}, Cu^{2+}, and Zn^{2+} binding at pyridine or purine N1 type nitrogens and imidazole or purine N7 type nitrogens display a linear relationship with pK_a for each metal ion and nitrogen type. The slopes of all lines vary only from 0.3 to 0.5. For all three aqueous metal ions and dienPd^{2+}, at the same pK_a, the stability constant for N7 binding is 0.8 to 1.2 log units stronger than for N1 binding. For neutral adenosine the N1 site is intrinsically 320 times more basic than the N7 site. However, for the above three aqueous metal ions the ratio of N1 to N7 bound adenosine complexes is projected to be 3, 2.5, and 1, respectively. Thus solutions of neutral adenosine and these aqueous metal ions contain comparable amounts of N1 metalated and N7 metalated complexes. Intrinsic protonation and metal ion stability constants are compared for adenosine, guanosine and inosine. N7 coordination in purine bases predominates at low pH and gives way to favored N1 coordination for dienPd^{2+}, Cu^{2+}, and Zn^{2+} between pH 1.5 to 2.7 for adenosine, pH 6.1 to 6.7 for inosine, and pH 6.9 to 7.5 for guanosine. Absolute chemical shifts for both tetramethylammonium ion and acetonitrile are found to be dependent on ionic strength in opposite senses. There seems to be no reliable internal chemical shift reference when ionic strength is varied.

Introduction

In our previous paper we have established that for dienPd^{2+}, plots of log K, where K is a stability constant, *versus* pK_a for the binding site yield good parallel, straight lines for the two types of nitrogens occurring in purine nucleosides [1]. Points for pyridine like pyrimidine N3 and purine N1 nitrogens fall on one straight line, while points for imidazole like purine N7 nitrogens fall on a parallel base line elevated 0.8 log units on the log K axis. The N7 but not N1 points for inosine are exceptional for dienPd^{2+} and fall 1.6 log units above the N7 base line.

Insights obtained in the log K *versus* pK_a analysis for dienPd^{2+} encourages us to attempt a similar treatment for the aqueous metal ions (Co^{2+}), Ni^{2+}, Cu^{2+}, and Zn^{2+}. There are no reliable estimates of the relative binding strengths of these metal ions at the N1 and N7 sites of adenosine. Pitfalls in the previously popular selective broadening and 'selective T_1' experiments with paramagnetic transition metal ions have been described [2–5]. By comparing the stability constants of a variety of ligands with pK_a of the binding site, we are able to estimate stability constants for aqueous metal ions at the N1 and N7 sites of adenosine.

Experimental

Stability constants of ligands with replaceable protons were determined potentiometrically in solutions containing excess ligand. Since stabilities of only 1:1 complexes were desired, only the early section of a titration curve, where no metal ion hydrolysis occurs in the absence of ligand, was analyzed. Both acidity constants and stability constants were evaluated by the SCOGS program [6].

For determination of stability constants for metal ion binding to N7 of purine nucleosides and for proton binding in H_2SO_4 to N7 of adenosine four cell ultraviolet difference spectra methods were used [7]. The N7 adenosine–metal ion binding experiments were performed at pH 1.5. Spectra were measured on a Cary 11 or a Cary 14R spectrophotometer. NMR chemical shift measurements were not used due to the following circumstance.

During the course of this study it was found that the chemical shift difference between internal references tetramethylammonium nitrate (TMA) and acetonitrile (CH_3CN) is a linear function of the ionic strength of added nitrate salts. Solutions were 14 m*M* in TMA and 100 m*M* in CH_3CN in D_2O. For no added salt and 0.5 to 2.5 *M* $NaNO_3$, 0.2 to 2.1 *M* KNO_3, 0.41 to 2.07 *M* $Zn(NO_3)_2$, and mixtures of these salts to give up to 7 *M* ionic strength, a plot of the chemical shift difference in ppm between internal TMA and CH_3CN in the same solution obtained on a Varian EM390 spectrometer yields for 13 points a

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straight line, $\Delta\delta = 1.108(1) - 0.0099(4)\,\mu$, where the numbers in parentheses represent one standard deviation, and the correlation coefficient $r = -0.989$. To bring the diverse salts into a single correlation it is necessary to use ionic strength as the independent variable; molarity is unsatisfactory. Thus at least up to 7 *M* ionic strength, the chemical shift difference between internal TMA and CH_3CN decreases with increasing ionic strength by 0.010 ppm per mole ionic strength of added nitrate salts.

Whether it is TMA or CH_3CN that is mainly responsible for the decreasing chemical shift difference with increasing ionic strength was resolved by measuring chemical shifts on the same solution in a permanent magnet (Varian EM390 at 90 MHz) and a superconducting solenoid (Nicolet 360 at 360 MHz) NMR spectrometers. The different geometric requirements for cylindrical tubes in the two kinds of spectrometers give rise to a different dependence of the observed chemical shift on bulk magnetic susceptibility, X [8]. For iron core magnets where the magnetic field is applied perpendicular to the sample tube axis the observed chemical shift is given by

$$\delta_{ic} = \delta_{int} + 2\pi(X_{ref} - X_{sample})/3$$

where δ_{int} is the intrinsic chemical shift after allowance for bulk magnetic susceptibility corrections. For superconducting solenoids which apply a magnetic field parallel to the sample tube axis the observed chemical shift is given by

$$\delta_{sc} = \delta_{int} - 4\pi(X_{ref} - X_{sample})/3$$

Elimination of the bulk magnetic susceptibility term between the two equations yields for the intrinsic chemical shift

$$\delta_{int} = (\delta_{sc} + 2\delta_{ic})/3$$

Table I tabulates the observed chemical shifts for TMA and CH_3CN against a tetramethylsilane external reference for a solution containing no salt and two salt solutions. The intrinsic chemical shifts of both TMA and CH_3CN in each solution are calculated from the last equation. The calculated difference in intrinsic chemical shifts between TMA and CH_3CN appears in the third to last row of Table I. These calculated differences are in close agreement with those in the last row that are predicted from the least squares analysis of the directly observed differences. The agreement supports the consistency of the analysis.

Least squares analysis of the 3 points for the intrinsic chemical shifts yields $\delta_{int}(TMA) = 3.236(1) - 0.0058(4)\,\mu$ with a correlation coefficient, $r = -0.998$ and $\delta_{int}(CH_3CN) = 2.128(1) + 0.0046(4)\,\mu$ with a correlation coefficient $r = 0.996$. The combined results yield for the TMA–CH_3CN chemical shift difference, $\Delta\delta_{int} = 1.108 - 0.010\,\mu$, in agreement with the directly observed difference for 13 points discussed above.

TABLE I. TMA and CH_3CN Chemical Shifts (ppm) on Superconducting and Iron Core NMR Spectrometers[a].

	No salt	2.0 *M* KNO_3	1.5 *M* $Zn(NO_3)_2$
TMA			
δ_{sc}	2.466	2.420	2.313
δ_{ic}	3.622	3.626	3.660
δ_{int}	3.237	3.224	3.211
CH_3CN			
δ_{sc}	1.357	1.337	1.252
δ_{ic}	2.512	2.538	2.596
δ_{int}	2.127	2.138	2.148
$\Delta\delta_{int}$[b]	1.110	1.086	1.063
Ionic strength	0	2.0	4.5
$\Delta\delta_{int}$[c]	1.108	1.089	1.064

[a]Tetramethylsilane used as an external reference. [b]Shift difference between TMA (3rd row) and CH_3CN (6th row). [c]From least squares fit of directly measured chemical shift difference (see text).

The conclusion of the internal reference analysis is that the intrinsic chemical shifts of both TMA and CH_3CN are dependent upon the ionic strength of the medium. Per mole ionic strength of added salt, the chemical shift of TMA moves upfield 0.0058 ppm, while that of CH_3CN moves downfield 0.0046 ppm. Thus neither the positively charged ion nor the neutral molecule provide a good internal reference in the presence of varying amounts of added salt. No organic molecules were added in these experiments.

Results and Discussion

Stability Constants

Stability constants for aqueous Ni^{2+}, Cu^{2+}, and Zn^{2+} binding to ligands with pyridine and imidazole type nitrogens appear in Table II. Most values agree well with other values in the literature [9, 10]. The first 4 ligands in Table II contain a pyridine like nitrogen atom similar to N1 in purines. The log K_1 *versus* pK_a plot in Fig. 1 shows that for Cu^{2+} and Zn^{2+} (open points) the points fall on good straight lines. As indicated after the first 4 ligands in Table II, the slope of the lines for both metal ions is 0.42–0.45 ±0.01.

Results for the last 6 ligands in Table II refer to binding at an imidazole type nitrogen. Slopes of good straight lines for filled points in Fig. 1 appear at the bottom of Table II as well as the slope for Ni^{2+} points which are not plotted in Fig. 1.

In contrast to the results for dienPd^{2+} in ref. 1, where points for inosine fall 1.6 log units above the N7 base line, all points for the last 6 ligands in Table

TABLE II. Equilibrium Constant Logs for Binding of Aqueous Ions[a].

	pK_a	Ni^{2+}	Cu^{2+}	Zn^{2+}
Cytidine	4.34	0.95	2.04	0.56
Pyridine[b]	5.47	1.88	2.56	0.99
7-CH_3-inosine	6.57		3.04	1.43
Inosine (N1)[c]	8.7	2.8		2.4
Slope (N1 type)			0.45	0.42
Adenosine (N7)	−1.56		0.16	−0.89
1-CH_3-inosine[c]	1.4	1.0	1.4	0.3
Inosine (N7)[c]	1.4	1.1	1.3	0.34[a]
Guanosine (N7)[c]	2.33	1.4	1.9	0.87[a]
Benzimidazole[d]	5.63		3.50	
1-CH_3-imidazole	7.39	3.44	4.61	2.98
Slope (N7 type)		0.40	0.50	0.44

[a]Unless otherwise stated in 1.0 *M* $NaClO_4$ at 21 °C, this study. [b]In 0.5 *M* $LiClO_4$ at 25 °C. From G. Faraglia, F. J. C. Rossotti and H. S. Rossotti, *Inorg. Chim. Acta, 4*, 488 (1970). [c]In 1.0 *M* $NaClO_4$ at 25 °C. From H. Lonnberg and P. Vihanto, *Inorg. Chim. Acta, 56*, 157 (1981). [d]Average of values at 0.16 ionic strength and 20 and 25 °C. From C. J. Hawkins and D. D. Perrin, *J. Chem. Soc.*, 1351 (1962) and T. J. Lane and K. P. Quinlan, *J. Am. Chem. Soc., 82*, 2994 (1960).

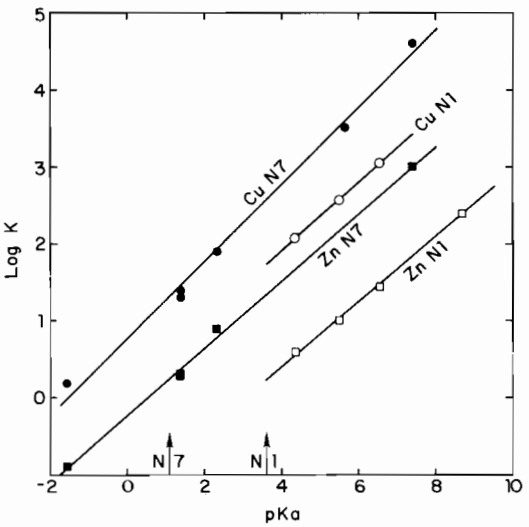


Fig. 1. Stability constant logarithms *versus* pK_a values for N1 type and N7 type sites tabulated in Table II. Circles refer to Cu^{2+} and squares to Zn^{2+}. Open symbols represent N1 type sites and closed symbols N7 type sites. Arrows at the bottom of the figure are placed at intrinsic pK_a values for neutral adenosine, 1.1 for N7 and 3.6 for N1. The two N1 lines terminate at $pK_a = 3.6$.

II fall on a single line for each aqueous metal ion. Evidently there is little if any increase in stability due to hydrogen bond formation from coordinated water to O6 of inosine and guanosine.

For all straight lines in Fig. 1 and slopes in Table II the straight line correlations with a standard deviation of only 1–4% of the slope value are remarkable considering that a variety of sources are used for the constants and which span nearly 9 pK_a units in the cases Cu^{2+} and Zn^{2+} binding at the N7 imidazole type nitrogen.

The straight line for Cu^{2+} binding at N1 in Fig. 1 is confirmed by literature results for binding at 13 3- and 4-substituted pyridines at 25 °C. For each of the following 7 pyridines the first value is the pK_a and the second value the stability constant logarithm for Cu^{2+} binding [11]: pyridine, 5.44, 2.60; 3-methyl, 5.88, 2.70; 4-methyl, 6.24, 2.93; 3,4-dimethyl, 6.64, 3.11; 3,5-dimethyl, 6.31, 2.94; 3-CH_2OH, 5.19, 2.43; and 4-CH_2OH, 5.66, 2.65. A plot of log K_1 *vs.* pK_a for these 7 pyridines yields a least squares slope of 0.45 ± 0.03, an ordinate value of 1.7 at $pK_a = 3.6$, and a correlation coefficient r = 0.992. The excellent correlation is strengthened by inclusion of 6 pyridines with an aromatic or heterocyclic substituent. Again the reported respective pK_a and stability constant logarithm for Cu^{2+} binding are as follows [12]: 3-phenyl, 4.92, 2.25; 4-phenyl, 5.49, 2.54; 3-(2-thienyl), 4.52, 2.15; 4-(2-thienyl), 5.59, 2.57; 3-(3-thienyl), 4.92, 2.30; and 4-(3-thienyl), 5.71, 2.67. The log K_1 *versus* pK_a plot for the 13 meta and para substituted pyridines yields an excellent straight line with least squares slope 0.46 ± 0.02, an ordinate value of 1.7 at $pK_a = 3.60$, and a correlation coefficient r = 0.994. All these results conform closely to the straight line for Cu^{2+} binding at N1 in Fig. 1. The points for cytidine and 7-methylinosine in Table II and Fig. 1 are within 0.02 log units of the least squares line through the 13 substituted pyridines. Pyridines with substituents that are aliphatic and aromatic (3- and 4-phenyl) yield values that fit the same straight line as does 7-methylinosine with two fused rings. These correlations confirm that the N1 nitrogen in purines is a 'pyridine' type nitrogen.

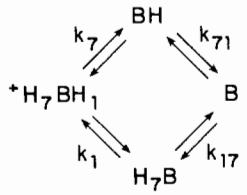
Ortho substituted pyridines do not fit the above correlation but give values 1.2 to 1.5 log units to weaker Cu^{2+} binding [11, 12]. Even with two ortho substituents the point for cytidine fits the Cu^{2+} correlation to within 0.01 log units. Evidently the ortho oxo and amino groups in cytidine are not as hindering for Cu^{2+} binding as methyl and larger groups in other ortho substituted pyridines. On the other hand, due to the close fit, the ortho oxo group in cytidine does not appear to strengthen Cu^{2+} (or Zn^{2+}) binding by chelation.

For Ni^{2+} binding to the 7 meta and para substituted pyridines given above the respective stability constant logarithms are as follows [11]: 1.91, 1.97, 2.09, 2.26, 2.13, 1.85, and 1.97. For 4-(2-thienyl)-pyridine the value is 1.91 [13]. For these 8 ligands a plot of log K_1 *versus* pK_a yields a least squares straight line of slope 0.27 ± 0.02, a correlation coefficient r = 0.98, and ordinate values of 1.4 and 1.6

at $pK_a = 3.6$ and 4.34, respectively. The latter pair of values, meant to apply to cytidine, projects a stability constant logarithm 0.6 log units greater than that of 0.95 in Table II. Possibly an ortho effect is operating with cytidine in the complex with Ni^{2+}, which is more strictly hexacoordinate than Cu^{2+} or Zn^{2+}.

Microconstants

Purine bases substituted at N9 such as the nucleosides adenosine, guanosine, and inosine exhibit an ambivalency for binding of protons and metal ions at the nitrogens N1 and N7. The intrinsic tendency of N1 and N7 as binding sites may be formulated by the microconstant, Scheme I.



Scheme I.

For all three nucleosides the main monoprotonated species is BH_1, and the upper pathway to the unprotonated base, B, predominates. The intrinsic tendency of the base to add a proton at N1 is given by k_{71} and at N7 by k_{17}.

The macroconstants determined by titration are related to the microconstants in the above scheme by

$$K_{a1} = k_7 + k_1 \quad \text{and} \quad K_{a2}^{-1} = k_{71}^{-1} + k_{17}^{-1}$$

For adenosine, inosine, and guanosine in H_2O [5], $pK_{a2} = 3.6$, 8.8, and 9.2, respectively. For the same three nucleosides $pK_{a1} = -1.56$, 1.0, and 2.1. Since the upper pathway predominates for the three nucleosides, $K_{a1} = k_7$ and $K_{a2} = k_{71}$.

From the properties of a cyclic system we have

$$K_{a1}K_{a2} = k_7k_{71} = k_1k_{17}$$

There are 4 microconstants and only 3 independent equations. An additional item of information is required to evaluate k_{17} in order to asses the intrinsic tendency for proton binding at N7.

As a model for the k_1 deprotonation we use the acidity constants for 7-methylinosine, $pk_1 = 6.57$ (this research) and 7-methylguanosine, $pk_1 = 7.2$ [14]. The interaction difference $pk_{71}-pk_1$ becomes 2.2 for inosine and 2.0 for guanosine. The interaction difference measures the extent of acidification of N7 due to presence of a proton at N1 and *vice versa*. Only pk_{17} remains to be evaluated from the above equations to obtain $pk_{17} = 3.2$ and 4.1 for inosine and guanosine, respectively.

The intrinsic tendency to bind a proton at N1 over N7 may be expressed as the difference $pk_{71} - pk_{17} =$ 5.6 and 5.1 for inosine and guanosine, respectively. Thus for the two 6-oxopurines, the N1 site possesses intrinsically more than 10^5 times greater basicity than the N7 site. At any pH, the ratio of molar concentrations of N1 to N7 monoprotonated species is about 10^5. Note that the above difference is not the same as the difference between the two successive acidity macroconstant logs, $pK_{a2} - pK_{a1}$, because pK_{a1} refers to further protonation of a species already bearing a proton at N1.

Since 7-methyladenosine is unstable its acidity constant has not been determined. Coordination of dienPd at N7 acidifies the N1 proton by 1.89 log units in adenosine and by 1.46 log units in inosine [15]. The purine ring in adenosine appears to possess a more sensitive interaction than the one in inosine, so that we add the above difference of 0.43 log units to the inosine proton interaction difference of 2.2 log units to obtain 2.63 log units for the proton interaction difference in adenosine. On this basis for adenosine we estimate $pk_{17} = -1.56 + 2.63 = 1.1$, for the intrinsic acidity of the adenosine N7 site. The difference $pk_{71} - pk_{17} = 3.6 - 1.1 = 2.5$, so that the intrinsic tendency for adenosine to bind a proton at N1 over N7 is $10^{2.5}$, which is the ratio of N1 to N7 monoprotonated species at all pH values. Thus the intrinsic basicity ratio of N1 to N7 sites is about 10^3 times greater in the 6-oxopurines than in adenosine.

Adenosine

The good straight line slopes in Fig. 1 and Table II may be used to predict stability constants for binding of the aqueous metal ions to N1 and N7 of free base adenosine. The intrinsic acidity constants for binding at the free base adenosine are measured as $pK_{aB} = 3.6$ at N1 and estimated, as described above, as $pk_{17} = 1.1$ at N7. These values are represented by the small vertical arrows labeled N7 and N1 near the abscissa in Fig. 1. Table III shows the log K_1 and log K_7 stability constants estimated from the intersection of the extended N7 arrow with the N7 slopes and the extended N1 arrow with the N1 slopes.

The projected log K_1 and log K_7 for Cu^{2+} and Zn^{2+} with neutral adenosine in Table III tend to be greater than values reported in the literature [10, 16, 17]. All the literature analyses invoked only a single complexed species. The comparable values of K_1 and K_7 for adenosine indicate that a multiplicity of complexes occur: M_7BH_1, M_7B, BM_1, and M_7BM_1. The presence of complexes in addition to the one being considered serves to withdraw ligand and metal ion from solution resulting in lower apparent stability constants than the real constants for binding at a single site. A detailed analysis suggests that simultaneous allowance of all of these complexes (except M_7BM_1) with B and BH_1 free ligand species yields expressions for the observed apparent constants that are not constant over all conditions employed. Since

TABLE III. Stability Constant Logs for Scheme II.

	K_{aB}	K_1[a]	K_7'	k_{17}	K_7[b]	K_{a7B}[c]
Cu^{2+}						
Adenosine	3.6	1.7	0.2	1.1	1.3	2.5
Inosine	8.8	4.0	1.3	3.2	2.4	7.7
Guanosine	9.2	4.2	1.9	4.1	2.8	8.3
Zn^{2+}						
Adenosine	3.6	0.2	−0.9	1.1	0.2	2.5
Inosine	8.8	2.4	0.3	3.2	1.2	7.9
Guanosine	9.2	2.6	0.9	4.1	1.6	8.5
dienPd^{2+} [d]						
Adenosine	3.89	4.5	2.0		3.9	2.0
Inosine	9.06	8.33	5.34		6.80	7.60

[a]From pK_{aB} and Fig. 1. [b]From pk_{17} and Fig. 1. [c]Calculated from cyclic system in scheme II. [d]All observed values in D_2O from ref. 15 except log K_7' for adenosine which is corrected.

interpretation of observed apparent equilibrium constants of Cu^{2+} and Zn^{2+} with neutral adenosine is hazardous, the most reliable stability constants for N1 and N7 binding in adenosine appear to be the projected values in Table III.

Limited results for Co^{2+} allow only tentative conclusions. Under the same conditions as those of Table II we obtain log K = 2.77 for 1-methylimidazole. Combination with the stability constants for nucleosides [9] suggests stability constants similar to Zn^{2+} at both N1 and N7 sites. Also as for Zn^{2+}, the projected binding at the N1 and N7 sites of neutral adenosine is of nearly equal strength.

The reactions of Pt(II) are about 10^5 times slower than those of Pd(II) [18]. For dienPd^{2+} binding to AMP at pH 5 the N1 to N7 mole ratio is about 2.3 [15]. We attempted to find the equilibrium [N1]/[N7] binding ratio of dienPt^{2+} to 5'-AMP at pH 5.1. The solution, initially with mainly M_7BH_p complex, sat for two months during which time it was heated at 50° for 13 days. Leveling off in the slow growth of the proton NMR peaks due to the BM_1 complex suggests that equilibrium may have been obtained. The last ratio of BM_1H_p to M_7BH_p complexes is 0.5. Thus Pt(II) favors N7 relative to N1 about 4.5 times more than Pd(II).

A summary of stability constants for binding of several metal ions to the N1 and N7 sites of adenosine appears in Table IV. The last column of the table gives the molar ratio of N1 to N7 protonated and metalated complexes. Except for the proton the ratio ranges from 0.5 to 4 with values centering near unity. Thus we conclude that the N1 and N7 sites of netural adenosine bind metal ions with comparable strength. Solutions composed of neutral adenosine and metal ions contain both N1 metalated and N7 metalated complexes. The 3 to 1 N1 to N7 ratio for

TABLE IV. Neutral Adenosine Stability Constants.

	Log K_1	Log K_7	$[BM_1]/[M_7B]$
H^+	3.6	1.1	320
Ni^{2+}	1.4	0.9	3
Cu^{2+}	1.7	1.3	2.5
Zn^{2+}	0.2	0.2	1
dienPd(II)[a]	4.5	3.9	4
dienPd(II)[a,b]	4.87	4.51	2.3
dienPt(II)[b]			0.5

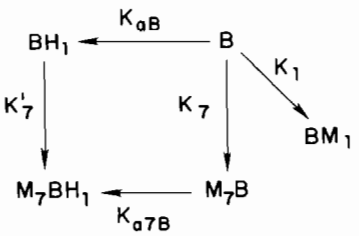
[a]Observed values from ref. 15. [b]Observed values for AMP at pH 5.

adenosine and aqueous Ni^{2+} is consistent with a switch to more N7 coordination to help account for the 11 times greater stability of $HOP_3O_9Ni^{2-}$ [19].

Stability constants reported for CH_3Hg^+ binding to N1 type nitrogens in the nucleosides cytidine, inosine, guanosine, uridine [20], and thymidine [21], and pyridine [22], when plotted as in Fig. 1 yield a straight line of slope 0.87 ± 0.08 and an intercept at $pK_a = 3.6$ of log $K_1 = 3.7$. For N7 type nitrogens in inosine and guanosine [20], 1-methylimidazole, imidazole, and methylmercuryimidazole [23], the plot yields an excellent straight line of slope 0.52 ± 0.01 and an intercept at $pK_a = 1.1$ of log $K_7 = 3.8$. If binding of CH_3Hg^+ to N7 of inosine and guanosine is not enhanced the slopes for N1 and N7 binding of CH_3Hg^+ show the greatest difference in a log K vs. pK_a plot. The values of log $K_7 = 3.8$ and log $K_1 = 3.7$ at the intrinsic pK_a values of neutral adenosine suggests that there is significant CH_3Hg^+ binding at both N1 and N7 sites. This postulated N7 mercurated adenosine complex possesses the appropriate characteristics and provides a more reasonable alternative than the one offered for an additional mercurated complex needed to fit the results in a proton exchange study [24].

Intrinsic Stability Constants

It is practical to use the information furnished in Figure 1 and Table III to evaluate all the equilibrium constants in Scheme II.



Scheme II.

Equilibrium constant logarithms appear in Table II for several metal ions and nucleosides. Log K_1 and log K_7 values are derived from intersection of pK_{aB} and pK_{17}, respectively, with the appropriate line in

Fig. 1. From the properties of a cyclic system $pK_{a7B} = pK_{aB} + \log K_7' - \log K_7$, completing Table III. The difference $pK_{aB} - pK_{a7B} = 0.7$ to 1.1 for the aqueous ions with all 3 nucleosides corresponds to the acidification of the N1 proton by a metal coordinated ion at N7. This range is reasonable and as anticipated is less than the 2.0–2.6 log unit interaction produced by the much more polarizing proton.

Sufficient information is available for $dienPd^{2+}$ so that the results at the bottom of Table III are all derived from direct observations [15].

Intrinsic [N1]/[N7] binding ratios may be derived from the $\log K_1 - \log K_7$ difference in Table III. The results reported as the log of the binding ratio are tabulated in Table V for several metal ions and nucleosides. The values for CH_3Hg^+ are only estimated from the straight line plots and the appropriate acidity constants described in the last section. The proton and CH_3Hg^+ favor N1 over N7 bonding about 10^3 times more in the 6-oxopurines than in adenosine. $DienPd^{2+}$, Cu^{2+}, and Zn^{2+} favor N1 over N7 about 10 times more in the 6-oxopurines than in adenosine.

Due to the greater basicity of N1 over N7 in all three purine nucleosides and to the relative metal ion binding strengths at each site, a change in dominant binding site from N7 to N1 occurs as the pH increases. The pH at which the changeover occurs is termed the crossover pH [5]. The ratio, R, of N7 to N1 bound metal ion is given by

$$R = \frac{N(7)}{N(1)} = \frac{[M_7BH_1] + [M_7B]}{[BM_1]} = \frac{1}{K_1}\left[\frac{(H)K_7'}{K_{aB}} + K_7\right]$$

where the last equality is derived from the definitions of the equilibrium constants in Scheme II. Binuclear complexes with metal ions at both N(1) and N(7) are not considered in this treatment. At the crossover pH_c, R = 1 and we have

$$pH_c = pK_{aB} + \log K_7' - \log(K_1 - K_7).$$

Calculated values for the crossover pH_c appear in Table VI. The results in Table VI indicate that for adenosine with all metal ions N1 coordination dominates at pH > 3. At pH > 4.5 the [N1]/[N7] molar ratio for adenosine is given by the antilog of the values in Table V.

TABLE V. Intrinsic Log ([N1]/[N7]) Binding Ratios.

	Adenosine	Guanosine	Inosine
H^+	2.5	5.1	5.6
CH_3Hg^+	−0.1	2.7	3.3
$dienPd^{2+}$	0.6		1.5
Ni^{2+}	0.5	0.8	1.0
Cu^{2+}	0.4	1.4	1.6
Zn^{2+}	0.0	1.0	1.2

TABLE VI. Crossover pH Values from N7 to N1 Coordination.

	Adenosine	Inosine	Guanosine
CH_3Hg^+ [a]		4.3	5.6
$dienPd^{2+}$ [b]	1.5	6.1	
Ni^{2+}	~2.1	7.1	7.8
Cu^{2+}	2.3	6.1	6.9
Zn^{2+}	2.7	6.7	7.5

[a]Calculated in ref. 5 from results of R. B. Simpson, *J. Am. Chem. Soc., 86*, 2059 (1964). [b]From ref. 15.

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