Nucleoside Sites for Transition Metal Ion Binding

R. BRUCE MARTIN

Chemistry Department, University of Virginia, Charlottesville, Virginia 22901

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To the everlasting embarrassment of organic chemistry, by early 1953 it had not yet described definitively the dominant tautomeric structures that occur in the four nucleic bases of DNA. This deficiency caused base-pairing problems in formulation of the DNA double-helical structure.[1] However, since 1953 the predominant tautomeric forms and proton binding sites of the common nucleic bases have been confirmed by a variety of methods.[2,3] Figure 1 shows the predominant tautomeric forms of the nucleic bases. Though the predominant tautomeric forms and proton binding sites in nucleosides have been described, the problem of metal ion binding sites remains. This Account aims to describe the results of recent solution experiments that identify the favored nucleoside and nucleotide binding sites for several metal ions. The conclusions have wide application; for example, many enzymes acting on DNA and RNA contain Zn^{2+}.

In their nucleoside derivatives the pyrimidine bases are substituted at N1 and the purine bases at N9. Table I lists both the nucleic base proton binding sites and the corresponding pK_a values for the common nucleic bases, nucleosides, and their 5'-monophosphates.[4] The pyrimidine bases and their nucleosides both undergo protonation and deprotonation at N3. For the purine bases, however, N9 is a potential proton binding site, and hence there are differences in the basic properties of purine bases and purine nucleosides. For this reason this Account is limited to 9-substituted purines such as occur in nucleosides. When the N9 substituent is a D-ribofuranose the purine nucleosides are adenosine, inosine, and guanosine. The $pK_a = -1.6$ value listed in Table I for the very weakly basic N7 site of adenosine results from recent research.[5]

In pyrimidine bases metal ion binding occurs predominantly at N3.[4] For uracil and thymine metal ion binding is inhibited by the proton at N3 and the binding is often pH dependent in neutral solutions. Unless deprotonated in quite basic solutions exocyclic amino groups are not metal ion binding sites.[4]

Purine nucleosides display a dichotomy between binding at N1 or N7. The proton greatly favors N1. The presence of the 6-amino group in adenosine results in $pK_a \simeq 4$ at N1, while in the two 6-oxopurines, inosine, and guanosine, $pK_a \simeq 9$ at N1 (Table I). Thus in slightly acidic and neutral solutions both N1 and N7 remain unprotonated in adenosine while in the 6-oxopurines only N7 is unprotonated and metal ions must compete with the proton for binding at N1. As a result, on these relative basicity grounds, in acidic solutions a metal ion N1/N7 binding ratio will be greater in adenosine than in the 6-oxopurines, where N7 metal ion binding is favored. In neutral and basic solutions when the pH becomes high enough, depending upon the metal ion, the metal ion displaces the proton at N1. Under these conditions the 10^3-fold greater ratio of intrinsic N1 to N7 basicities in the 6-oxopurine nucleosides than in adenosine suggests a greater likelihood of metal ion coordination at N1 in the former than in adenosine.

Also significant are the relative metal ion N1 to N7 binding strengths for each nucleoside which are different from the proton and for each metal ion. The remainder of this Account elaborates how these problems have been solved for several metal ions in solution.

Crystal structure determinations[6] have given an exaggerated impression of the extent of metal ion binding to N7 in purine nucleosides and nucleotides. For two reasons, one advertent and one inadvertent, crystals have been collected from acid solutions which protonate N1 and favor metal ion binding at N7. To avoid precipitation of metal ion hydroxides, solutions are often acidic when made up. In concentrated unbuffered solutions, protons displaced from N1 on only a fraction of the ligands will render the solution more acidic, forcing the majority of remaining metal ions to coordinate at N7.

Palladium(II) Complexes

Resolution of the N7 vs. N1 dichotomy in purine nucleosides and their phosphates begins in studies of the complex (diethylenetriamine)palladium(II) ($dienPd^{2+}$). For our purpose this complex exhibits three important features. First, because the complex is diamagnetic, pronounced broadening and shifting of NMR peaks do not occur. Second, since the complex exchanges slowly between the N7 and N1 sites, the relative populations of all species in solution may be deduced from ^1H NMR spectra. Finally, as the tridentate ligand diethylenetriamine occupies three sites about the strongly square-planar Pd(II), only the fourth site remains free to undergo substitution at N1 or N7. Whether the fourth site in the free complex is occupied by H_2O, Cl^-, OH^-, or hydroxo bridge depends upon the Cl^- concentration, pH, and $dienPd^{2+}$ concentration in the solution.[7-9] With a pair of cis sites that may un-

Bruce Martin received his B.S. degree at Northwestern University and his Ph.D. in physical chemistry at the University of Rochester. Following postdoctoral appointments at Caltech and Harvard, in 1959 he joined the faculty at the University of Virginia where he is now Professor of Chemistry. His research has spanned wide areas in biophysical chemistry. His work with metal ions has included their interactions with amino acids, peptides, proteins, lipids, nucleosides, and nucleotides by a variety of physical techniques including multinuclear NMR.

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Table I
Base Acidity Constants for Nucleic Bases, Nucleosides, and 5′-Nucleoside Monophosphates

			pK_a near 25 °C and 0.1 M ionic strength		
base	nucleoside	site	base	nucleoside	monophosphate[a]
adenine	adenosine	N7		−1.6	
		N1	4.1	3.6	3.8
		N9	9.7	...	...
cytosine	cytidine	N3	4.5	4.3	4.4
hypoxanthine	inosine	N7	(1.9)	1.2	(1.3)
		N1	8.8	8.7	8.9
guanine	guanosine	N7	(2.9)	2.2	2.4
		N1	9.4	9.2	9.5
uracil	uridine	N3	9.5	9.3	9.6
thymine	thymidine	N3	9.8	9.6	9.9

[a] In addition the 5′-phosphate residue exhibits $pK_a \simeq 6.3$.

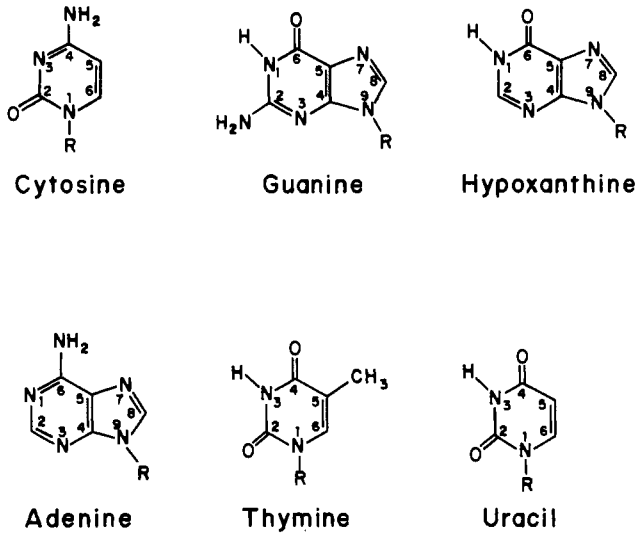

Figure 1. Structures of common nucleic bases. For the base the R group substituent is a hydrogen while in the nucleosides it is a ribose or deoxyribose sugar.

dergo substitution, $enPd^{2+}$ chelation reactions become more complex than $dienPd^{2+}$ coordination and are not discussed further here.[10-12] Dipeptides also form tridentate complexes leaving one available coordinating site in square-planar Pd(II).[13] Complexes of tridentate (dipeptide)palladium(II) with nucleotides[14,15] and other ligands[16,17] have given interesting results, but they are unrelated to the main subject of this Account.

Figure 2 shows a plot of the species distribution among the N1 and N7 sites of 5′-adenosine monophosphate. From pH 7 to 9, where both sites are deprotonated, the populations of complexes with $dienPd^{2+}$ coordinated to N1 and to N7 are nearly equal despite the 300 times greater basicity of the former site. The curves in Figure 2 are derived from pK_a values determined from ^1H NMR chemical shifts and stability constants estimated from peak areas. Similar analyses were also performed on $dienPd^{2+}$ complexes of adenosine, inosine, IMP, and GMP.[8]

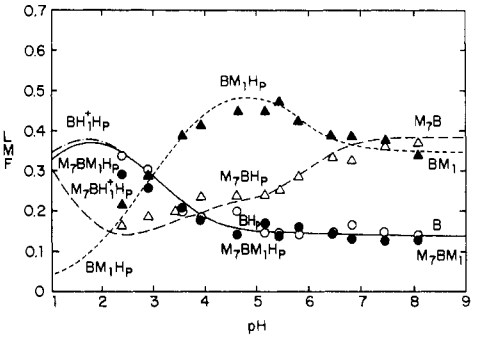

Figure 2. Species distribution of 5′-AMP binding of $dienPd^{2+}$ presented as ligand mole fraction (LMF) vs. pH with both total $dienPd^{2+}$ and total AMP at 0.10 M. From ref 8.

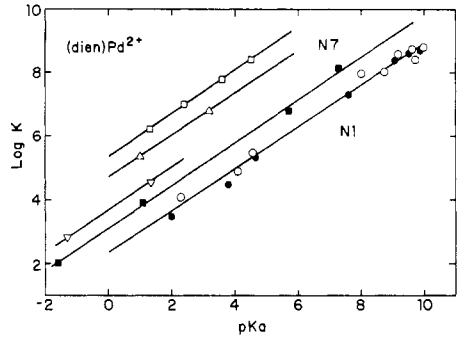

Figure 3. Log stability constant for $dienPd^{2+}$ binding vs. pK_a for binding site in a variety of ligands. Circles represent binding to pyridine-like nitrogens: closed circles for nucleosides and open circles for 5′-mononucleotides. Solid squares represent base line for purine N7 binding. The pair of inverted triangles refer to 5′-AMP, the pair of normal triangles to inosine, and the open squares of the topmost line to 5′-IMP and 5′-GMP. (Reprinted with permission from: Kim, S. H.; Martin R. B. *Inorg. Chim. Acta* **1984**, *91*, 11. Copyright 1984, Elsevier.)

Figure 3 summarizes a large number of $dienPd^{2+}$ stability constant determinations with a variety of ligands.[7] Five straight lines appear in the log stability constant vs. pK_a plot. All five straight lines display virtually identical slopes of 0.67 ± 0.02. The lowest straight line passes through points for 6 pyrimidine N3 nitrogens cytidine, uridine, thymidine, and their 5′-monophosphates and for 10 purine N1 nitrogens adenosine, inosine, AMP, IMP, GMP, and the corresponding species of each of the 5 ligands with a $dienPd^{2+}$ bound at N7. The 16 points spanning 8 pK_a units fit well on a single line. Thus points for N3 of pyrimidine nucleosides and their monophosphates fall on the same straight line as points for N1 of purine

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nucleosides and their monophosphates. Figure 3 shows that the same straight line accommodates the closed circles for nucleosides and the open circles for their 5′-monophosphates. It also does not matter whether a dienPd^{2+} is already coordinated at N7. Thus for dienPd^{2+} binding at the pyridine-like nitrogens, N3 of pyrimidine nucleosides and nucleotides and N1 of purine nucleosides and nucleotides, the stability constants correlate with the basicity of the binding site.

In contrast to the simple dependence on basicity of dienPd^{2+} binding to pyridine-like N1-type nitrogens, its binding to the imidazole-like N7 nitrogens of purines indicates that additional factors may augment the stability. The second lowest line through the four filled squares of Figure 3 passes through points for adenosine with and without a proton at N1, benzimidazole, and 1-methylimidazole. This line representing the base line for N7 binding includes two points for adenosine. The two inverted triangles in Figures 3 represent points for 5′-AMP. Their straight line lies 0.5–0.6 log unit above the N7 base line. The two normal triangles in Figure 3 correspond to points for inosine, which rises 1.6 log units above the N7 base line. The topmost line through the open squares in Figures 3 passes through points for 5′-IMP and 5′-GMP. This line lies 0.7 log unit above the inosine line; about the same difference is observed between 5′-AMP and adenosine. Thus presence of a 6-oxo group on a purine base enhances dienPd^{2+} binding at N7 (but not N1) by 1.6 log units. Independently, presence of a 5′-monophosphate group augments binding at N7 (but not N1) by 0.5–0.7 log units. What structural features are responsible for the dienPd^{2+}–N7 binding enhancements of 1.6 log units due to a 6-oxo group and 0.5–0.7 log unit due to a 5′-monophosphate?

The origins of the two enhancements are clarified by investigations of nucleoside and 5′-mononucleotide binding to (1,1,4,7,7-pentamethyldiethylenetriamine)-palladium(II) (pmdienPd(II)) where five methyl groups substitute for all nitrogen-bound hydrogens in coordinated dien.[7] Presumably due to steric hindrance, stability constants for pmdienPd^{II} are 0.7–1.9 log units weaker than those for dienPd^{II}. Binding of pmdienPd^{2+} to pyrimidine N3 and purine N1 sites yields a straight line of slope 0.80, slightly steeper than the 0.67 slope with dienPd^{2+}. Binding of pmdienPd^{2+} to adenosine, benzimidazole, and 1-methylimidazole, representative of N7 binding to purines, yields a slope of 0.79 displaced 1.2 log unit to stronger binding than the pmdienPd^{2+}–purine N1 line.

For the pmdienPd^{2+} complexes of inosine, IMP, and GMP, the stability constants fall 2.3, 2.0, and 1.9 log units, respectively, above pmdienPd^{2+}–N7 base line. Thus in constrast to the dienPd^{2+} results in Figure 3, there is no additional enhancement between a N7-bound purine nucleoside and its 5′-mononucleotide with pmdienPd^{2+}. Additionally the point for AMP–pmdienPd^{2+} N7 binding falls on the N7 base line, which contains the point for adenosine. These results are consistent with a 0.4–0.5 log unit promotion of the phosphate deprotonation upon dienPd^{2+} binding to N7 of AMP, IMP, and GMP[8] and the lack of promotion upon binding of pmdienPd^{2+}.

Due to the terminal methyl groups, rotation of pyrimidines, benzimidazole, and purines is restricted in pmdienPd^{2+} complexes, and two rotamers are evident in proton magnetic resonance spectra. With benzimidazole and purine nucleosides and 5′-nucleotides there is an approximately 2:1 mole ratio of the two rotamers. Nuclear Overhauser effect experiments and chemical shift analysis permit identification of all peaks for pmdien methyl groups and aromatic ring protons.[7]

In summary, plots of stability constant logarithms vs. pK_a for dienPd^{2+} binding to a variety of nitrogen heterocycles yield straight lines, all of 0.67 slope. Points for binding at pyridine like purine N1 and pyrimidine N3 nitrogens in nucleosides and 5′-mononucleotides fall on a single straight line. The base line for binding at imidazole like purine N7 nitrogens is 0.8 log unit stronger than for N1 binding. N7 binding to purine bases with a 6-oxo grup is enhanced by 1.6 log units above the N7 base line. The presence of a 5′-phosphate group enhances N7 binding (but not N1 binding) by 0.5–0.7 log unit. Weaker binding occurs with pmdienPd^{2+} and the straight line slopes are 0.79. The N7 base line rises 1.2 log units above the N1 line. Presence of the 6-oxo group enhances pmdien binding by 2.3 units. There is no enhancement of pmdienPd^{2+} binding to N7 due to the 5′-phosphate of nucleotides.

The lack of enhancement due to a 5′-phosphate for pmdienPd^{2+} in contrast to a consistent 0.5–0.7 log unit enhancement for AMP, IMP, and GMP complexes of dienPd^{2+} strongly suggests intramolecular hydrogen bonding from a coordinated dien nitrogen to the phosphate group in a macrochelate. This conclusion is supported by the 0.4–0.5 log unit acidification of the phosphate deprotonation with dienPd^{2+} but not with pmdienPd^{2+}.

Perhaps surprisingly the high enhancement of inosine N7 binding is actually greater for pmdienPd^{2+} at 2.3 log units than for dienPd^{2+} at 1.6 log units. This comparison rules out hydrogen bonding from nitrogen-bound hydrogens on chelated dien to the 6-oxo group as the cause of enhancement with dienPd^{2+}. Consistent with this conclusion a crystal structure of the dienPt^{2+} complex of guanosine shows N7 coordination and no or a very weak dien to 6-oxo hydrogen bond.[18] A crystal structure of dichlorobis(1-methylcytosine)palladium(II) does not support stabilizing interactions between Pd and either exocyclic oxo or amino groups.[19] To be reviewed below, the aqueous metal ions Ni^{2+}, Cu^{2+}, and Zn^{2+} exhibit no binding enhancement at N7 of 6-oxopurines.

Investigations of Pd(II) complexes have proven extremely useful for establishing a thermodynamic base line for the much more slowly reacting Pt(II) complexes, including antitumor Pt(II) complexes.[5,9,20–22] A similar enhanced binding for antitumor *cis*-(amine)PtCl$_2$ complexes to N7 of guanosine, to that found for dienPd^{2+} to 6-oxopurines, accounts for the observed favored Pt binding at guanosine N7 from among the naturally occurring nucleoside sites. The order of bindings sites shows preference of guanosine N7 over other sites.[4,8,9,22] It is not necessary to postulate N7–O6 chelation by the *cis*-(amine)platinum(II) complexes, nor is it likely to occur.[4,10,12,23] Kinetic factors also favor binding of Pt(II)

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Table II
Downfield ^{1}H NMR Chemical Shifts in 5′-Mononucleotides upon Phosphate Deprotonation[a]

species	AMP	GMP	IMP
ligand	0.138	0.106	0.123
dienPd^{2+} at			
N1	0.139		
N7	0.267	0.256	0.249
both	0.262	0.270	0.273
pmdienPd^{2+} at			
N1	0.153		0.140
N7	0.197	0.140	0.140
	0.149[b]	0.062[b]	
both		0.146	0.178
		0.056[b]	0.065[b]

[a] In ppm from ref 7 and 8. [b] Two isomers. High-field isomer listed first.

at N7 in 6-oxopurines. Once bound at N7 the relatively inert Pt sticks and migration to protonated N1 is difficult.[9]

Wrongway Chemical Shifts

Usually deprotonation of a compound leads to upfield shifts in ^{1}H NMR spectra. (In contrast, there is much less regularity in ^{13}C NMR.[23]) A curious result of the studies on 5′-mononucleotides is the significant "Wrongway" downfield shift of H8 upon phosphate deprotonation in the pH 6 region.[8,24] Results for several systems are collected in Table II. (The downfield shifts are usually greater than the H8 upfield shifts caused by N1 deprotonation.) The extent of the downfield shift is comparable for AMP, GMP, and IMP, with the first tending to the largest shifts. Metalation at N1 induces little change in the shift compared to free ligand. Metalation at N7 by dienPd^{2+} at least doubles the wrongway shift, while pmdienPd^{2+} causes a smaller increase. In ATP the wrongway shift is reduced to 0.035 ppm.[25] A small 0.025 ppm wrongway shift also occurs at H2 in AMP.[8] In all other cases the H2 shift is upfield.

Wrongway downfield shifts also occur at H6 upon phosphate deprotonation in the pyrimidine 5′-mononucleotides CMP and UMP. The downfield shifts are 0.125 ppm in CMP and 0.146 ppm in UMP.[11] These values are comparable to the H8 shifts for the purine nucleotides listed atop Table II. In the enPd^{2+} complexes with two ligands bound to the metal ion the H6 shifts increase to 0.138 and 0.172 ppm for the two isomers with CMP and decrease to 0.089 ppm for UMP.

In considering the source of the H8 wrongway downfield phosphate deprotonation shifts listed in Table II, it is possible to rule out several explanations. Occurrence and even magnification of the downfield shift upon metalation at N7 eliminates a hydrogen bond between the protonated phosphate (assumed as shielding) and N7 as the cause. Comparable magnitudes of the shifts in AMP on one hand and GMP and IMP on the other rule out a phosphate–C6 substituent interaction, since 06 is weakly basic in the 6-oxopurines and the 6-amino group is weakly acidic in adenosine.[4] It is as if there is a weak H8 to deprotonated phosphate interaction. One does not like to invoke a hydrogen bond from a carbon-bound hydrogen. However, H8 is weakly acidic: it undergoes exchange with solvent water which is accelerated by metalation at N7.[26]

None of the previous explanations are applicable to CMP and UMP which also exhibit wrongway downfield shifts of comparable magnitude to the purine nucleotides. Preferred is a single explanation common to both pyrimidine and purine nucleotides. Addition of a phosphate to a nucleoside to give a 5′-nucleotide results in *deshielding* of H8 in purines and H6 in pyrimidines.[8,11,24] There is a still greater deshielding of these hydrogens upon phosphate deprotonation. Nucleotides exist in two conformations about the glycosyl bond with the anti conformation favored.[4,24] In this conformation the N1–C6 bond of pyrimidines and the N9–C8 bond of purines projects onto or near the sugar ring. An explanation of the wrongway downfield shifts common to both pyrimidine and purine nucleotides is that the phosphate deprotonation increases the preference for the anti conformation about the glycosyl bond.

First-Transition-Row Metal Ions

Unlike Pd(II), alkali- and alkaline-earth-metal ions and metal ions of the first-transition row favor interaction with the phosphate rather than the nucleic base of nucleotides.[4] Therefore, to favor binding to the nucleic base, we now limit our discussion to nucleosides. Under some conditions, such as high pH, chelation of a metal ion may occur to the *cis*-2′,3′-dihydroxy groups of the ribofuranose sugar. In uridine there is no deprotonated nitrogen and in dimethyl sulfoxide solutions ionized *cis*-2′,3′-dihydroxy groups interact with the copper acetate dimer.[27] In most aqueous solutions first-transition-row metal ions interact predominantly with the nucleic base portion of nucleosides.

With its diamagnetic and slow-exchange properties, Pd(II) allows direct determination of its distribution among ligand sites from peak intensities in ^{1}H NMR spectra. These advantages are lost when investigating the first-transition-row metal ions because they exchange rapidly among ligand sites and in most cases are paramagnetic as well. Selective broadening and changes in other relaxation parameters are not reliable indicators of paramagnetic metal ion binding sites in the nucleic bases.[4,28–30] However, what we have learned from Pd(II) about the excellent correlations between stability constants and basicity may be applied effectively to first-transition-row metal ions.

Figure 4 shows a plot of log K_1 vs. pK_a for Cu^{2+} binding to 15 3- and 4-substituted pyridines and for Ni^{2+} to 8 of the same ligands. The filled circles refer to seven pyridines with aliphatic substituents and the open circles to six pyridines with aromatic or heterocyclic substituents. Points for the two kinds of substituents fall on a single straight line for each metal ion. The least-squares straight line slope is 0.46 ± 0.02 for 13 Cu^{2+} points and 0.27 ± 0.02 for 8 Ni^{2+} points. The

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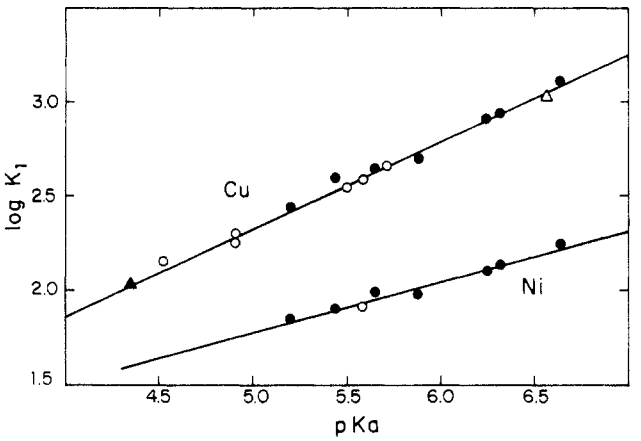

Figure 4. Log stability constant vs. pK_a for Cu^{2+} and Ni^{2+} binding to ligands containing pyridine-type nitrogens. For both metal ions the seven filled circles from low to high pK_a refer to the following aliphatic substituted pyridines: 3-CH_2OH, none, 4-CH_2OH, 3-CH_3, 4-CH_3, 3,5-dimethyl, and 3,4-dimethyl.[31] For Cu^{2+} the six open circles refer to the following aromatic or heterocyclic substituted pyridines: 3-(2-thienyl), 3-(3-thienyl), 3-phenyl, 4-phenyl, 4-(2-thienyl), and 4-(3-thienyl).[32] For Ni^{2+} only a point for 4-(2-thienyl) has been determined.[33] On the Cu^{2+} line the filled triangle at pK_a = 4.34 refers to cytidine and the open triangle at pK_a = 6.57 to 7-methylinosine.[5]

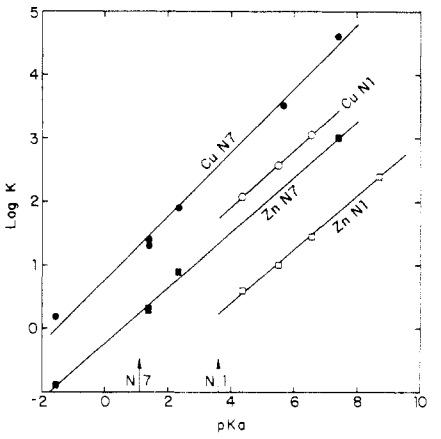

Figure 5. Stability constant logarithms vs. pK_a values for N1-type and N7-type nitrogens. Circles refer to Cu^{2+} and squares to Zn^{2+}. Open symbols represent N1-type sites and closed symbols N7-type sites. From low to high pK_a, the sequence of N7-type points is adenosine, 1-methylinosine and inosine (both pK_a = 1.4), guanosine, benzimidazole, and 1-methylimidazole. The sequence of N1-type points is cytidine, pyridine, 7-methylinosine, and inosine. 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. (Reprinted with permission from: Kim, S. H.; Martin, R. B. *Inorg. Chim. Acta* **1984**, *91*, 19. Copyright 1984, Elsevier.)

respective correlation coefficients, r, are 0.994 and 0.98. The triangular points for Cu^{2+} binding to cytidine and 7-methylinosine are within 0.02 log unit of the 13-point least-squares line. This result further supports the view that the purine N1 nitrogen acts as 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.[31,32] Even with two ortho substituents the point for cytidine fits the Cu^{2+} correlation to within 0.01 log unit. 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 to the line, the ortho oxo group in cytidine does not appear to strengthen Cu^{2+} (or Zn^{2+}) binding by chelation. These results appear to stand in contrast to several crystal structure determinations of Cu(II) complexes of cytosine and cytidine that show a normal Cu-N3 bond length and a much weaker, longer apical chelation to O2.[4,6,34] The resolution is that if the Cu to O2 interaction occurs in solution it does not contribute measurably to stability.

The above results are extended in Figure 5, which shows plots of log K vs. pK_a for binding to ligands with N7-type or N1-type nitrogens for Cu^{2+} and Zn^{2+}. The Cu^{2+}–N1 line in Figure 5 lies in the same position as the Cu^{2+} line in Figure 4. In Figure 5 the three lowest lines exhibit slopes from 0.42 to 0.46 while the Cu^{2+}–N7 line is somewhat steeper with a slope of 0.50. For all straight lines in Figure 5 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, which span nearly nine pK_a units in the cases Cu^{2+} and Zn^{2+} binding at the N7 imidazole type nitrogen.

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 0.8–1.2 log units stronger than for N1 binding.

In contrast to the results for dienPd^{2+} in Figure 3 where points for inosine fall 1.6 log units above the N7 base line, all points in Figure 5 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 06 of inosine and guanosine.

The excellent log K vs. pK_a correlations provide part of the basis for estimation of N1/N7 metal ion binding ratios. All that remains is to estimate the intrinsic pk_a for proton binding at the nucleic base nitrogens. All the common nucleic bases protonate first predominantly at N7 then at N1 with representative constants presented in Table I. We also need to know the pk_{17} value for deprotonation from the N7 site when the N1 site is not protonated. We set up the standard microconstant scheme for two acidic groups with four microconstants.[5] For the decisive additional item of required information, we use 7-methylinosine with pk_1 = 6.57 and 7-methylguanosine with pk_1 = 7.2 as models for deprotonation from N1 in an N7 protonated species. From the properties of a cyclic system we obtain pk_{17} as 3.2 and 4.1 for deprotonation at N7 in inosine and guanosine, respectively, when N1 is not protonated.[5]

From these results we find the intrinsic tendency to bind a proton at N1 over N7 as $10^{5.6}$ and $10^{5.1}$ 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 mono-

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Scheme I

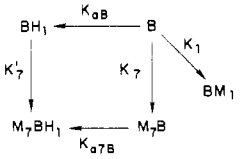

Table III
Stability Constant logs for Scheme I[a]

	K_{aB}	K_1	K_7'	K_7	K_{a7B}
		Cu^{2+}			
adenosine	3.6	1.7	0.2	1.3	2.5
inosine	8.8	4.0	1.3	2.4	7.7
guanosine	9.2	4.2	1.9	2.8	8.3
		Zn^{2+}			
adenosine	3.6	0.2	−0.9	0.2	2.5
inosine	8.8	2.4	0.3	1.2	7.9
guanosine	9.2	2.6	0.9	1.6	8.5
		$dienPd^{2+}$			
adenosine	3.89	4.5	2.0	3.9	2.0
inosine	9.06	8.33	5.34	6.80	7.60

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protonated 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.

A similar but slightly more involved argument leads to $pk_{17} = 1.1$ for adenosine.[5] The intrinsic tendency for adenosine to bind a proton at N1 over N7 becomes $10^{2.5}$. Thus the intrinsic basicity ratio of N1 over N7 is about 10^3 times greater in the 6-oxopurines than in adenosine.

It is now possible to estimate all the equilibrium constants shown in Scheme I. K_{aB} and K_7' are determined directly. Metal ion binding constant K_1 is determined either directly or from the interaction of pK_{aB} with the appropriate straight line in Figure 5. For adenosine the $pK_{aB} = 3.6$ value is designated by the arrow labeled N1 on the abscissa in Figure 5. Extension of the arrow to the Zn–N1 line gives $\log K_1 = 0.2$. Metal ion binding constant K_7 for binding at N7 with N1 neither protonated nor metalated is estimated from Figure 5 and the pk_{17} values discussed above. For adenosine, the $pk_{17} = 1.1$ is indicated by the arrow labeled N7 in Figure 5. Extension to the Zn–N7 line yields $\log K_7 = 0.2$. This value is identical to $\log K_1$ and thus Zn^{2+} distributes evenly between the N1 and N7 sites in neutral adenosine. The last constant in Scheme I, pK_{a7B} is evaluated from knowledge of the other three constants in the cycle. The constants of Scheme I for several systems are collected in Table III. For $dienPd^{2+}$ the values follow from direct observation of peak intensities in ^1H NMR spectra as indicated above. Limited results for Co^{2+} suggest that it behaves similarly to Zn^{2+}.[5]

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 neutral adenosine bind metal ions with comparable strength. Solutions composed of neutral adenosine and metal ions contain

Table IV
Neutral Adenosine Stability Constants[a]

	$\log k_1$	$\log K_7$	$[BM_1]/[M_7B]$
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
$dienPd^{II}$	4.5	3.9	4
$dienPd^{II}$ [b]	4.87	4.51	2.3
$dienPt^{II}$ [b]			0.5

[a] Reprinted with permission from: Kim, S. H.; Martin, R. B. *Inorg. Chim. Acta* **1984**, *91*, 19. Copyright 1984, Elsevier. [b] Observed values for 5′-AMP at pH 5.

Table V
Intrinsic log ([N1]/[N7]) Binding Ratios[a]

	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

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both N1-metalated and N7-metalated complexes. The 3:1 N1/N7 ratio for adenosine and aqueous Ni^{2+} is consistent with a switch to greater N7 coordination to help account for the 11 times greater stability of $HOP_3O_9Ni^{2-}$.[35] The last two entries in Table IV for 5′-AMP show that $dienPt^{2+}$ favors N7 relative to N1 4.5 times more than $dienPd^{2+}$.[5]

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.[5] 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.[4] The ratio, R, of N7 to N1 bound metal ion is given by

$$R = \frac{[N7]}{[N1]} = \frac{[M_7BH_1] + [M_7B]}{[BM_1]} = \frac{1}{K_1}\left[\frac{(H)K_7'}{K_{aB}} + K_7\right]$$

where the last equality is derived from the definitions of the equilibrium constants in Scheme I. Binuclear complexes with metal ions at both N1 and N7 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 most metal ions N1 coordination dominates at pH

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Table VI
Crossover pH Values from N7 to N1 Coordination[a]

	adenosine	inosine	guanosine
CH_3Hg^+ [b]		4.3	5.6
$dienPd^{2+}$	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] Reprinted with permission from: Kim, S. H.; Martin, R. B. *Inorg. Chim. Acta* **1984**, *91*, 19. Copyright 1984, Elsevier. [b] Calculated in ref 4 from results of: Simpson, R. B. *J. Am. Chem. Soc.* **1964**, *86*, 2059.

>3. At pH >4.5 the [N1]/[N7] molar ratio for adenosine is given by the antilog of the values in Table V.

Conclusions

The strong log stability constant vs. pK_a correlations provide the vehicle for estimating metal ion stability constants at nucleic base sites of established pK_a. The results for the N1 to N7 binding ratio in purine nucleosides are summarized in Tables V and VI. For adenosine Table V shows that the N1 to N7 binding ratio is 320 for the proton, 3 for Ni^{2+}, 2.5 for Cu^{2+}, and 1.0 for Zn^{2+}. Thus Zn^{2+} is distributed equally between the adenosine N1 and N_7 sites in neutral solutions.

N7 coordination in purine nucleosides predominates at low pH and gives way to favored N1 coordination at higher pH. Table VI shows for the three aqueous metal ions that the crossover pH for N7 to N1 coordination occurs from pH 2.1 to 2.7 for adenosine, pH 6.1 to 7.1 for inosine, and pH 6.9 to 7.8 for guanosine. Thus for all three purine nucleosides aqueous metal ion binding at both the N7 and N1 sites is important in neutral solutions.

The conclusions should be applicable for metal ion interactions to nucleic bases in single-stranded polynucleotides. For double-stranded helices, N3 of pyrimidines and N1 of purines are blocked by specific base pairing, paving the way for relatively greater N7 interactions with metal ions.

Polymer Excluded Volume and the Renormalization Group

KARL F. FREED

The James Franck Institute and the Department of Chemistry, The University of Chicago, Chicago, Illinois 60637

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Roughly half of the American chemical industry is involved with polymers, and the study of polymers is obviously relevant to the understanding of a variety of biological systems. Theories of polymer properties in solution and the melt pose complicated mathematical problems because polymers have long-range cooperative interactions of both intramolecular and intermolecular character. Whereas these problems were untractable for many years, new theoretical developments now enable the approximate description of wide variety of large-scale polymer properties in a manner which can be tested by experiment.

An important aspect of the practical utilization of polymers and the understanding of their properties centers upon the determination of their physical properties in the limit of infinite dilution. No complete description of more concentrated solutions is possible until this foundation is laid. Experimental methods of polymer characterization include light scattering, osmometry, sedimentation, viscometry, etc. When the polymer concentration c approaches zero, osmometry provides a determination[1] of the molecular weight M of the polymer, while small-angle light scattering yields the polymers' radius of gyration R_G. The limiting slope of light-scattering intensity vs. c in the zero-angle limit gives the polymer second virial coefficient[1] A_2 as a measure of the effective volume that a polymer excludes to others. The translational diffusion coefficient D is written for $c \rightarrow 0$ using Stokes' and Einstein's laws[2] in terms of a hydrodynamic radius[1] R_H as $D = kT/6\pi\eta_0 R_H$, where T is the absolute temperature, k is Boltzmann's constant, and η_0 is the solvent viscosity. If η is the viscosity of the polymer solution, the intrinsic viscosity $[\eta] = \lim_{c\rightarrow 0} (\eta - \eta_0)/c\eta_0$ gives another estimate of the volume occupied by a single polymer chain.[1]

All of these large-scale measurable properties of polymers provide different measures of the overall size and shape of the polymer. They are frequently observed to vary with polymer molecular weight with a power law form KM^b, where the proportionality factors K and the exponents b are dependent on polymer, solvent, and temperature. It is the goal of a comprehensive theory to explain this variation of K and b with system and temperature over the full experimentally accessible range.

Theoretical Models

For the description of large-scale polymer properties like R_G, A_2, D, and $[\eta]$, it suffices to employ apparently simple models,[1,3] which capture the essential large

Karl F. Freed was born in Brooklyn and received his B.S. in Chemical Engineering from Columbia University and his Ph.D. from Harvard University as a student of Bill Klemperer. After a year as a NATO postdoctoral fellow with Sam Edwards at the University of Manchester (England), he joined the faculty at the University of Chicago, where he is now Professor of Chemistry and Director of the James Franck Institute. He is the holder of Sloan, Guggenheim, and Dreyfus Fellowships, as well as the recipient of the Marlow Medal of the Faraday Division of the Chemical Society and the ACS Pure Chemistry award. Freed's current research, in addition to the statistical mechanics of polymers, involves theories of photodissociation, molecular relaxation and collision dynamics, and molecular electronic structure.

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