Use of the Generalized Perturbation Theory to Predict the Interaction of Purine Nucleotides with Metal Ions†

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ABSTRACT: The generalized perturbation theory (Klopman, G. (1968), *J. Amer. Chem. Soc. 90*, 223) was used to predict the site(s) of protonation and divalent metal interaction on the bases of various purine nucleotides and to determine relative reactivity of different positions on the base. In general, the predictions agree well with available experimental data. It was found that, depending on the nature of the cation, the preferred position for coordination with the adenine ring is N-1 and N-7. Similar predictions were made for oxygen containing purines and in this case the possibility that metal ions may simultaneously interact with more than one site on the purine ring was evaluated.

In order to understand the role of divalent metal ions in enzymatic reactions involving nucleotides, attention has been focused on defining the structure of metal–nucleotide complexes in solution (Phillips, 1966; Izatt *et al.*, 1971) and in enzymatic systems (Mildvan and Cohn, 1970; Cohn and Reuben, 1971). The ultimate object of such studies is to understand the enzymatic mechanism involved in the utilization of the chemical energy of nucleotides. It is assumed that information obtained from studying metal–nucleotide complexes in solution will give some insight into why enzymes show specificity for a particular metal–nucleotide combination and the function(s) of the metal ion in the reaction.

There are two ways of bridging the gap between experimentally determined chemical and physical properties of the reaction components and interpretation and prediction of the mechanism of a reaction. The empirical method involves collecting a large body of data and fitting it into a pattern while the theoretical approach attempts to formulate a mathematical solution and uses selective experimental measurements to test its validity. Attempts have been made to fill this gap for simple organic systems with molecular orbital theory. The electronic structure of many compounds has been calculated, their reactions have been interpreted, and analogous reactions predicted. Molecular orbital calculations have been used to describe features of nucleotide structure such as the electron density, net charge of various atoms of the base, and the conformation about the glycosidic bond (Jordan and Pullman, 1968). Attempts have also been made to explain the relative ΔG values for the hydrolysis of various phosphate bonds including nucleoside polyphosphates (Boyd and Lipscomb, 1969; Alving and Laki, 1972).

The first molecular orbital calculation of the π-electron system of biologically important nucleotides was made in 1956 by Pullman *et al.* They used simple Hückel calculations to describe the energy levels and molecular orbital parameters for the purine (Pullman and Pullman, 1958) and pyrimidine (Pullman and Pullman, 1959) nucleosides and they correlated these calculations fairly well with experimental data on bond lengths and angles, basicity, and stability of the N-glycosidic bonds (Pullman and Pullman, 1963). These calculations, however, were unable to satisfactorily describe the reactivity of various nucleophiles or electrophiles with the base of the nucleotide, particularly the sites of protonation and interaction with various metal ions. More sophisticated programs and computers have been used more recently to incorporate the σ bonds into the calculations and to refine the molecular orbital parameters (Pullman and Pullman, 1968; Giessner-Prettre and Pullman, 1968; Pullman *et al.*, 1968; Boyd, 1969; Pullman, 1969). However, the latest treatments are still unable to satisfactorily predict why protonation occurs at N-1 of ATP while the metal ions Ni^{2+}, Mn^{2+}, and Co^{2+} interact at N-7 of the nucleotide base.

Molecular orbital parameters have been used to explain chemical reactivity by either static or dynamic methods. The former compares the initial nonreacting state with the earliest stage of molecular interaction while the latter compares the initial state to the transition state. The static methods have been most widely used because of difficulties in describing the transition state. Since the static method relates π-system reactivity to ground-state parameters, it is only valid with reactions having the same reaction curve energy profiles. If the transition state energy profile differences do not parallel the ground-state differences, the results will not be valid. Because of such limitations, the theory has been unable to adequately predict the site of electrophilic and nucleophilic reactions with various heterocyclic molecules.

Aside from molecular orbital approaches to chemical reactivity, there have been many theories based on empirical classifications. Pearson (1963) grouped acids and bases into soft and hard categories. Soft bases are nucleophiles with easily polarized valence electrons; hard bases have poorly polarized valence electrons. Hard acids are small in size, have a high positive charge, and are hard to polarize, while soft acids have the opposite properties. Pearson found that hard acids prefer to react with hard bases and soft acids with soft bases. Drago and Wayland (1965) extended these observations and correlated the preference for hard–hard or soft–soft interactions with the ability to form ionic or covalent bonds.

Another approach for predicting chemical reactivity has been developed (Klopman and Hudson, 1967; Klopman, 1968). The generalized perturbation theory assumes that the

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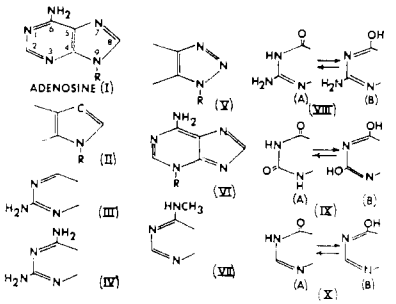

FIGURE 1: Compounds studied: (II) 7-deazaadenosine (tubercidin); (III) 2-aminopurine ribonucleoside; (IV) 2,6-diaminopurine ribonucleoside; (V) 8-azaadenosine; (VI) 3-isoadenosine; (VII) N^6-methyladenosine; (VIII-A) guanosine, lactam form; (VIII-B) guanosine, lactim form; (IX-A) xanthosine, lactam form; (IX-B) xanthosine, lactim form; (X-A) inosine, lactam form; (X-B) inosine, lactim form.

transition state of a reaction is formed by the mutual perturbation of the molecular orbitals of the reactants. The energy difference between the highest occupied orbital of the nucleophile or donor atom and the lowest empty orbital of the electrophile or acceptor atom characterizes the interaction. A large difference implies little charge transfer and is typical of a charge controlled or ionic interaction. This corresponds to the hard–hard interaction. When the two energy levels are approximately equal, there is strong electron transfer and overlapping of the interacting orbitals. This is a covalent or soft–soft interaction. Each type of reaction has its own perturbation equations and the energy of the interaction is calculated from the perturbation of each orbital of one species by each orbital of the other species. The effects of solvation are incorporated into this treatment by considering the change in the free energy ($\Delta H - T\Delta S$) of the solvation that occurs during a charge transfer reaction. The desolvation during the partial charge transfer that accompanies the coordination results from a steric inhibition of solvation as well as a modified distribution of charge among the reactants (Klopman, 1967). The general equation for the polyelectronic perturbation treatment of the free-energy change for the interaction between an electron donor (nucleophile) with an acceptor (electrophile) in a given solvent can be represented as follows

$$\Delta G_{total} = \text{(charge–charge interaction term)} + \text{(covalent interaction term)} = -q_r q_s \frac{\Gamma}{\epsilon} + \sum_{m}^{occ} \sum_{n}^{unocc} \left[\frac{2(C_r^m)^2 (C_s^n)^2 \beta^2}{E_m^* - E_n^*} \right]$$

where q_r is the charge on atom r in the nucleophile, q_s is the charge on atom s in the electrophile, Γ is the coulomb repulsion term, ϵ is the microscopic dielectric constant of the solvent, C_r^m and C_s^r are the molecular orbital coefficients of the respective occupied or unoccupied orbitals, E_m^* and E_n^* are the energy levels of the two perturbed orbitals in the solvent being used, and β is the resonance integral of the bond forming between the nucleophile and the electrophile. Parts of the free energy of desolvation appear in both terms of this equation *via* ϵ and E_m^* and E_n^*. For details see Appendix II in the paper by Klopman (1968). This theory has been successfully applied to the prediction of a number of nucleophilic additions and electrophilic substitutions on heterocyclic molecules (Klopman, 1968).

TABLE I: Parameters Used for Coulomb and Bond Integrals.

Group	Coulomb Integral h_x	Group	Bond Integral $K_{c_{sp^2}x}$
=Ċ=	0	C=C	1.0
=N=	0.5	C=N	1.0
—N̈R$_2$	1.5	C—N̈R$_2$ (or N̈HR)	0.8
=Ö	1.0	C—N̈H$_2$	1.0
—ÖH	2.0	C=Ö	1.0
		C—ÖH	0.8

In the work described below we have used the generalized perturbation theory to predict the most probable site of interaction of various metal ions and protons with the bases of different purine nucleotides and to compare the reactivity of metal ions with adenine nucleotides to their reactivity with other purine nucleotides.

Experimental Section

The derivation and use of the generalized perturbation theory to predict chemical reactivity have been presented in detail (Klopman and Hudson, 1967; Klopman, 1968). It is used in this paper to evaluate the interaction of cations with the purine nucleosides shown in Figure 1. The perturbation energies were obtained by first determining the molecular orbital coefficients for these compounds from simple Hückel molecular orbital calculations and then applying these coefficients to the perturbation equations. For the Hückel molecular orbital calculations, the parameters used to modify the coulomb integral α_0 and bond integral β_0 for the heteroatom x, where $\alpha_x = \alpha_0 + h_x\beta_0$ and $\beta_{cx} = K_{cx}\beta_0$, are given in Table I (Streitwieser, 1961). The perturbation energy for each site on the molecule is calculated as a function of the metal or electrophile orbital energy. For a particular metal ion characterized by a specific orbital energy, the site of the molecule showing the greatest perturbation energy by the metal ion will be the most favorable site for interaction with it.

For metal ion complexes with purine nucleotides that have an oxygen atom available at C-6, there is evidence that a metal ion may simultaneously bind to two sites on the base (Tu and Friederich, 1968). To evaluate this type of binding, we determined the difference in perturbation energy between the independent and synchronous interaction of the metal ion at two sites. For sites a and b on the molecules, this difference would be

$$\Delta E = E_{ab} - (E_a + E_b) = \frac{2\Sigma(C_a^i + C_b^i)^2}{-E_n^* + E_i^*} - \frac{2\Sigma(C_a^i + (C_b^i)^2}{-E_n^* + E_i^*} = \frac{4\Sigma C_a^i C_b^i}{-E_n^* + E_i^*}$$

where E_a and E_b are the independent perturbation energies at sites a and b, E_{ab} is the synchronous perturbation at sites a and b, C_a and C_b are the Hückel coefficients for orbital i, E_n^* is the energy level of the metal ion and E_i^* is the energy level of the ith orbital.

The pK_a values were determined spectrophotometrically as described by Shugar and Fox (1952).

TABLE II: Orbital Energy Values for Some Metal Ions.

Metal Ion	Metal Orbital Energy (eV)	Pearson's Classification[a]
Mg^{2+}	2.42	H
Ca^{2+}	2.33	H
Sr^{2+}	2.21	H
Ba^{2+}	1.89	
Cr^{2+}	0.91	
Fe^{2+}	0.69	B
Mn^{2+}	0.59	
Co^{2+}	0.56	B
H^{+}	0.42	H
Ni^{2+}	0.29	B
Cu^{2+}	−0.55	B
Zn^{2+}	−0.97	B
Cd^{2+}	−2.04	S
Cu^{+}	−2.30	S
Ag^{+}	−2.28	S
Hg^{2+}	−4.64	S

[a] H, hard; B, borderline; S, soft.

Results and Discussion

The perturbation energies from the interaction of metal ions at different sites on the purine nucleotides are dependent on the orbital energy of the metal ion or its hardness–softness properties. Klopman (1968) was able to establish a theoretical energy scale for the hardness and softness of various metal ions. The orbital energy of a particular metal ion is a function of the ionization potential, charge, hydrated ionic radius, and dielectric constant. Table II lists his calculated values for many metal ions of interest in aqueous solution along with values we have calculated for Mn^{2+}, Co^{2+}, and Zn^{2+}. With the exception of H^{+}, the calculated values agree very well with Pearson's empirical classification and provide a method for quantifying the hardness or softness of a metal ion. The soft metal ions have the greatest negative orbital energy values while hard metal ions have the greatest positive ones. The energy orbital values given in Table II fall into three groups. Metal ions with energies greater than 2 eV are hard acids while those with energies less than −2 eV are soft acids. The divalent metal ions Mg^{2+}, Ca^{2+}, and Ba^{2+} are hard acids; Fe^{2+}, Mn^{2+}, Cl^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} are borderline acids; and Cd^{2+}, Ag^{2+}, and Hg^{2+} are soft acids. The energy values for the metal ions are dependent on the dielectric constant. In order to determine whether the *relative* hardness or softness of Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, or Mg^{2+} would be different in nonaqueous solvents, we have plotted the energy values of these metal ions against the reciprocal macroscopic dielectric constant (Figure 2). It is clear that the orbital energy values for the range of dielectric constants from 2 to 80 still fall into the hard and borderline classifications for these metal ions.

It was pointed out above that the behavior of H^{+} appears to be anomalous. Pearson (1963) considered it to be a hard acid because it is small, highly charged, poorly polarized, and binds bases primarily through ionic forces. The calculations shown in Table II on the other hand predict it to be a borderline acid. To provide an explanation for this apparent discrepancy, we determined the relationship between the hardness–softness, *i.e.*, orbital electronegativity, of a variety of cations and the amount of charge transfer that may occur

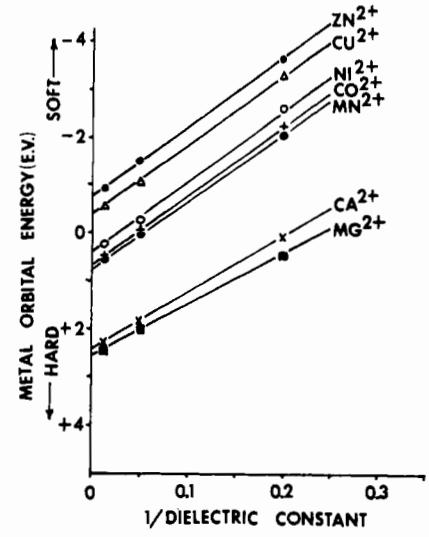

FIGURE 2: Variation of metal ion orbital energy with dielectric constant.

during the formation of the transition state of a reaction. This can be done by varying the function describing the amount of charge transfer. If the reaction is almost completely charge controlled as in an ionic type of interaction there will be little charge transfer whereas in the formation of a covalent bond there will be a maximum charge transfer between the two reacting species. The resulting relationship is shown in

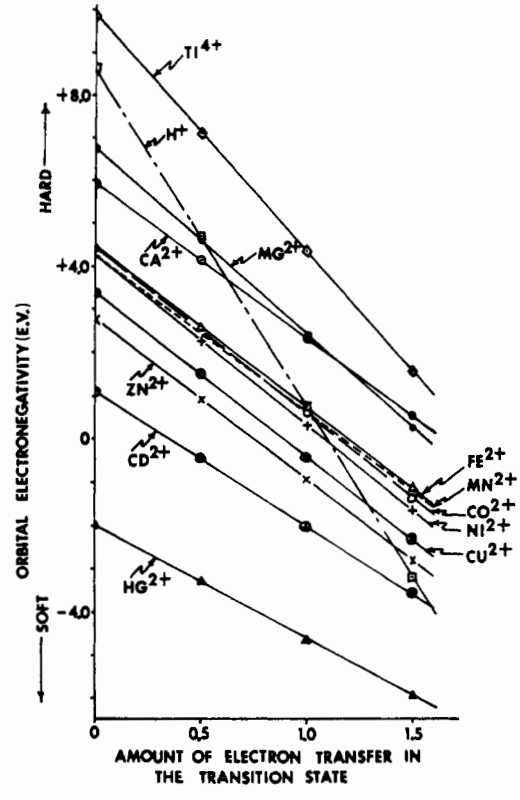

FIGURE 3: Variation of orbital electronegativity with the amount of electron transfer occurring in the formation of the transition state. The relationship is derived from the following equation (Klopman, 1968): orbital energy $= -IP + b^2(IP - EA) + 14{:}338(X/R)\cdot(1 - 1/\epsilon)(q - 2b^2x)$, where IP = ionization potential in electron volts; EA = electronic affinity in electron volts; $\epsilon = 80$ = dielectric constant of water; R = ionic radius + 0.82; $x = q - (q - 1)(0.75)^{1/2}$; q = charge of the cation; b^2 = one-fourth of the total charge transferred to the cation in the transition state.

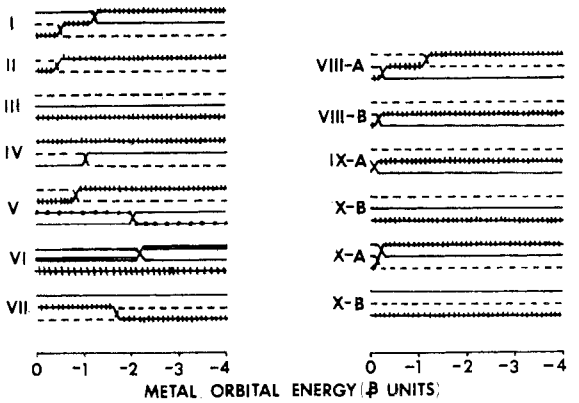

FIGURE 4: Relative perturbation energies of ring nitrogens as a function of metal ion orbital energy. The roman numeral with each set of lines refers to the compounds shown in Figure 1. The figure was constructed by calculating the perturbation energies for the indicated positions in each of the compounds at a number of different metal orbital energy values. The relative order of reactivity decreases from top to bottom for each set of lines. The hardness of the metal ion increases as the metal orbital energy expressed in β units becomes more negative: (-|-|-|-) N-1; (- - -) N-3; (—) N-7; (●-●-●) N-8; (=) N-9.

Figure 3. It should be emphasized that this figure shows the relative hardness–softness scale for a variety of metal ions for the entire possible range of reaction conditions. It is interesting that with a single exception the same categories are maintained. In an ionic interaction, where the charge transfer is zero, Mg^{2+} is hard, the transition metals are borderline, and Cd^{2+} and Hg^{2+} are soft. When the reaction becomes charge transfer controlled all metal ions become softer but the same categories are maintained. The one exception is H^+ which goes from a very hard acid in an ionic interaction to a very soft acid in a covalent interaction. The relative position of H^+ in the hardness–softness scale will therefore greatly depend on the nature of the reaction. The apparently anomalous position of H^+ in Table II therefore stems from the fact that these values were calculated for the condition in which one electron is transferred in the transition state, whereas Pearson's empirical classification is based on experimental circumstances in which H^+ is taking part in ionic interactions.

Since the greatest reactivity occurs between hard acids and hard bases or between soft acids and soft bases, it is possible that the relationship between the amount of electron transfer in the transition state and the orbital electronegativity shown in Figure 3 may provide some insight into the interaction of metal ions with the base of nucleosides and nucleotides. The interaction of most metal ions and the bases of *nucleosides* is weak if it occurs at all (Phillips, 1966; Izatt *et al.*, 1971; Glassman *et al.*, 1973). However, when inorganic triphosphate is included in the metal ion–*nucleoside* solution there is a significant interaction of the metal ion with the base (Glassman *et al.*, 1973). It is currently unknown how phosphates (and perhaps other ligands as well) affect the properties of the metal ion to allow it to interact with the base. One possibility is that the phosphate ligand alters the hardness–softness properties of the metal ion so that the orbital electronegativity of the metal ion is more compatible with the orbital electronegativity of a site on the nucleotide base and thereby facilitates the reaction. Another possibility is that interaction of the phosphate bound metal with a specific position of the adenine ring results from a preferred conformation about the glycosidic bond rather than from differences in orbital electronegativity of available sites in the adenine ring. This is very unlikely for the following reason. Both Ni^{2+} and Mn^{2+} form backbound 1:1 complexes with ATP in which the metal is bound to N-7 through a bridging water molecule. When the nitrogen at position 7 is replaced with a carbon atom there appears to be little interaction with the purine ring in the case of Mn^{2+}, whereas Ni^{2+} now binds at N-1 and/or N-3 (Glassman *et al.*, 1971). The most obvious explanation is that Ni^{2+} is capable of interacting at more than one site whereas Mn^{2+} may not be. Since there is no reason to assume the binding of the metal ion to the phosphate residues will influence the conformation about the glycosidic bond the most obvious interpretation is that the site of interaction with the purine part of the molecule stems from the properties of the available sites *per se*.

The possible sites of protonation and interaction with metal ions for the nucleotide bases shown in Figure 1 are at the nitrogen and oxygen atoms. Figure 4 shows the predicted sites of interaction on the various nucleotide bases as a function of metal ion energy values. The most probable site of interaction, the site with the highest perturbation energy, is at the top of the set. The metal orbital energies are expressed in β units; hard metal ions have the most positive metal orbital energy (most negative coefficients of β). It is not possible to correlate the metal energies given in β units in Figure 4 directly with the calculated metal energy values in electron volts shown in Table II. As was stated above, the metal orbital negativity is dependent on the nature of the reaction. It is influenced by the amount of charge transfer in the transition state, by the properties of the other reactant, by the solvent, and by other conditions which are contained in the term β units. At this point, the theory is not advanced enough to account for these conditions. The metal orbital energy scale given in Figure 4 can be used to separate the reactivity of hard metal ions from soft ones but cannot be used to give the reactivity of specific metal ions.

Figure 4 predicts that soft metal ions react with N-7 of adenosine (I) while harder metal ions react with N-1. Both hard and soft metal ions would complex preferentially to N-3 of 2-aminopurine ribonucleoside (III), to N-1 of 2,6-diaminopurine (IV) ribonucleoside, and to N-7 of N^6-methyladenosine (VII). Soft metals will interact with N-7 of isoadenosine (VI) and with N-3 of 8-azaadenosine (V) and tubercidin (II). Hard metals will interact with N-1 of tubercidin (II) and 8-azaadenosine (V) and with N-9 of isoadenosine (VI).

In general, the predictions derived from Figure 4 agree well with experimental observations except for the oxygen containing nucleosides. Figure 4 implies that soft metal ions interact with N-3 of guanosine (VIII) and xanthosine (IX) and N-7 of inosine (X) and that hard metal ions interact with N-1 of the lactam form of guanosine (VIII-A) and inosine (X-A), with N-3 of xanthosine (IX) and the lactim form of guanosine (VIII-B), and with N-7 of the lactim form of inosine (X-B). The results in Figure 4 are derived from perturbation calculations that assumed the metal ion interacts at only one site on the ring and predict that the oxygen containing purine nucleotides will interact with metal ions at N-1 or N-3 of the base. Possibly the molecular orbital parameters used for inosine (X), guanosine (VIII), and xanthosine (IX) are incorrect or the presence of an enolic oxygen at C-6 allows for a synchronous perturbation and the formation of a five-membered chelate structure involving both the enolic oxygen and N-7.

Tu and Freiderich (1968) concluded from conductometric,

TABLE III: Differences in Energy between the Synchronous Perturbation of Two Sites and the Summation of Independent Perturbations of the Same Two Sites on the Nucleotide Base.

Compd	Metal Orbital Energy (β Units)	Group at C-2	Group at C-6	ΔE^a (β Units)		
I	−0.2		NH_2	−1.21[b]		0.12[f]
	+1			−0.36[c]		0.04[g]
II	−0.2		NH_2	−1.26[b]		
	+1			−0.36[c]		
III	−0.2	NH_2		−1.40[b]		
	+1			−0.32[c]		
IV	−0.2	NH_2	NH_2	−1.08[b]	−1.60[d]	0.58[f]
	+1			−0.54[e]	−0.36[e]	−0.06[g]
V	−0.2		NH_2	−1.20[b]		0.24[f]
	+1			−0.36[c]		−0.02[g]
VIII-A	−0.2	NH_2	=O	−0.96[b]	−1.96[d]	0.96[f]
	+1			−0.32[c]	−0.40[e]	−0.02[g]
VIII-B	−0.2	NH_2	OH			0.50[f]
	+1					0.02[g]
IX-A	−0.2	=O	=O			0.35[f]
	+1					−0.04[g]
IX-B	−0.2	OH	OH			0.42[f]
	+1					0.02[g]
X-A	−0.2		=O			0.64[f]
	+1					−0.02[g]
X-B	−0.2		OH			0.40[b]
	+1					0.04[g]

[a] The more positive the value of ΔE the more favored the synchronous perturbation. [b] N-1. [c] C-6. [d] N-3. [e] C-2. [f] N-7. [g] C-6.

potentiometric, and spectrophotometric titrations and from infrared spectroscopy that Cu^{2+} complexation with guanosine and inosine involves both N-7 and the enolic oxygen at C-6. In order to evaluate synchronous binding at two sites we have also calculated the energy difference between independent and synchronous perturbations at sites N-1 plus the C-6 substituent, N-3 plus the C-2 substituent, and N-7 plus the C-6 substituent of these compounds. The sum of the energies from the independent perturbations at both sites was compared to the energy from the synchronous perturbation at both sites. The calculations were made for metal orbital energy values of +0.2 and −1.0 β unit corresponding to soft and hard metal ions and the results are shown in Table III. The more positive the value of ΔE the more likely it is that synchronous interaction is favored. Synchronous binding at N-1 plus the C-6 substituent and at N-3 plus the C-2 substituent to form four-membered rings is energetically much less favorable than independent binding at the two sites for all cases shown and is probably an electronically forbidden process. However, calculations based on synchronous binding at N-7 plus the C-6 substituent of guanosine, inosine, xanthosine, and 2,6-diaminopurine ribonucleoside with soft metals (β = +0.2) to form five-membered rings are more favorable and this probably indicates binding could occur at both sites.

There is excellent X-ray crystallographic evidence obtained with adenine hydrochloride (Cochran, 1951), AMP (Kraut and Jensen, 1963), and ATP (Kennard *et al.*, 1970) that protonation occurs at N-1. Bullock and Jardetsky (1965) examined the protonation of various purines and purine nucleosides by nuclear magnetic resonance (nmr). They found that protonation of adenosine leads to a greater chemical shift of the H-2 proton while with inosine there is a greater shift of the H-8 proton leading them to conclude that protonation of adenosine is at N-1 while with inosine it is at N-7. Crystallographic studies have also shown that guanosine (Broomhead, 1951) and xanthosine (Staab and Mannschreck, 1962) are protonated at N-7. There is evidence from several laboratories showing that Zn^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, and Cu^{2+} interact at N-7 with adenosine nucleotides (Cohn and Hughes, 1962; Sternlicht *et al.*, 1965; Glassman *et al.*, 1971; Berger and Eichhorn, 1971) but proton and ^{15}N magnetic resonance studies failed to show any interaction with Mg^{2+} and Ca^{2+} (Happe and Morales, 1966). Kuntz and Swift (1973) have examined the effect of Mg^{2+} on the longitudinal relaxation spectrum of ATP in aqueous solution. They concluded that Mg^{2+} has a significant effect on the relaxation time of H-2 but not on H-8. This implies that Mg^{2+}, a hard metal ion, interacts with N-1 and/or N-3 of ATP, a conclusion predicted by our calculations. Kuntz (1973) also concluded that Zn^{2+} interacts at N-7 and

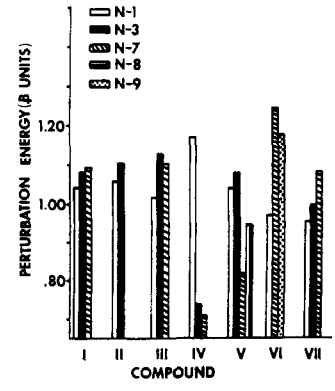

FIGURE 5: Perturbation energy of ring nitrogens for a soft metal ion with an orbital energy of 0 β unit.

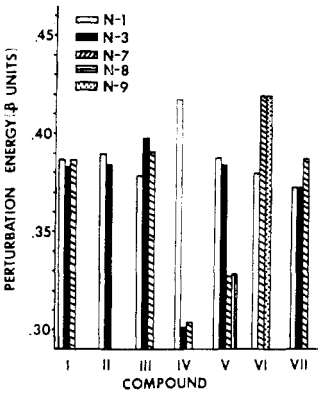

FIGURE 6: Perturbation energy of ring nitrogens for a hard metal ion with an orbital energy of $-2.0\,\beta$ units.

possibly N-1 and/or N-3 of ATP and at N-7 of ITP. He found no interaction of Ca^{2+} with ATP but described one with N-7 and C-6 of ITP. Jordan and McFarquhar (1972) have demonstrated the formation in dimethyl sulfoxide of a 1:1 Ca^{2+}–guanosine complex in which the metal ion appears to be bound to N-3 and/or N-1. This result is also consistent with our predictions for guanosine. However, our calculations are still unable to account for the protonation of guanosine at N-7.

Nuclear magnetic resonance studies have shown that Ni^{2+} (Glassman *et al.*, 1971) and Cu^{2+} (Berger and Eichhorn, 1971) broaden the H-2 resonance peak more than the H-8 peak of tubercidin 5′-triphosphate indicating that these ions are bound to N-1 or N-3.

The generalized perturbation theory can also be used to compare the relative reactivity of metal ions with adenine nucleotides *vs.* nucleotides containing purine analogs of adenine. In Figures 5 and 6, the calculated perturbation energy values for the possible binding sites on the base are given for metal energy values of 0 and $-2.0\,\beta$ units. These energy levels correspond to soft and hard metal ions, respectively. The values shown in these figures are the calculated perturbation energies for interaction at the available sites in each of these compounds. The larger the perturbation energy the greater probability that an interaction will occur at a given site in the molecule. It is also possible to compare whether the interaction of the metal ion with adenosine is stronger or weaker than with any of the indicated analogs of adenosine.

Figure 5 predicts that soft metal ions complex more strongly to N-3 of 2-aminopurine ribonucleoside (II), N-1 of 2,6-diaminopurine ribonucleoside (IV), and N-7 of isoadenosine (VI) than to N-7 of adenosine (I). It would be interesting to correlate the ability to bind a soft metal at N-7 with enzymatic reactivity in systems requiring the metal bound at N-7 of the metal–nucleotide substrate. For a hard metal ion (β less than -2), Figure 6 predicts that N-1 of 2,6-diaminopurine ribonucleoside (IV) and N-7 or N-9 of 3-isoadenosine (VI) would react more readily than either N-1 or N-7 of adenosine (I).

Insufficient information is presently available to permit evaluation of the relative reactivities of adenine nucleotide analogs with metal ions. Very few of the binding constants of these compounds with metal ions are known but it is possible to evaluate the relative reactivity regarding protonation by examining the available pK_a data. If a direct relationship is assumed between pK_a and the ability of a proton to interact with the base, it should be possible to predict relative pK_a values for the compounds shown in Figure 6. Such a prediction would depend on the assumed site of protonation and the hardness of the proton in each specific case (see Figure 3).

TABLE IV: pK_a Values of Purine Nucleosides.

Compound	pK_a	Reference
Isoadenosine	5.5	Leonard and Laursen (1965)
Tubercidin	5.2	Duvall (1963)
Tubercidin	5.0	This paper
N^6,N^6-Dimethyladenosine	4.5	Sober (1968)
2,6-Diaminopurine ribonucleoside	4.2	This paper
N^6-Methyladenosine	4.0	Sober (1968)
Adenosine	3.6	This paper
Adenosine	3.5	Sober (1968)
2-Aminopurine ribonucleoside	3.5	This paper
N^6-Methyladenosine	3.5	This paper
8-Azaadenosine	2.8	Lynch *et al.* (1969)

The pK_a values culled from the literature along with some of our own values are shown in Table IV. If it is assumed that isoadenosine (VI) is protonated at N-9, N^6-methyladenosine (VII) at N-7, 8-azaadenosine (V) at N-7 or N-8, and all the others at N-1, then there is very good agreement between the predicted and experimental values. The single exception is tubercidin (II) where the observed pK_a is greater than would be predicted and a more complicated explanation is required in this case. The prediction that protonation may occur elsewhere than at N-1 in purine derivatives is not unreasonable since guanosine is most probably protonated at N-7 (Shapiro, 1968).

Several lines of investigation are in process in our laboratory to determine the validity of the above predictions. The best way to evaluate the site of interaction of a metal ion with a nucleotide base is by nuclear magnetic resonance. This technique works best for paramagnetic metal ions but can be used with diamagnetic metals if the interaction is strong enough. It has been shown that the interaction of Ni^{2+} and adenosine is increased when triphosphate is added to the Ni^{2+}-adenosine solution (Glassman *et al.*, 1973). It is possible that the order of binding constants for metal ions and metal ions with triphosphate and adenine analogs is predicted by the above calculations. The cations Ca^{2+}, Mg^{2+}, and Ni^{2+} have been shown to form 2:1 metal–ATP complexes (Mohan and Rechnitz, 1972; Frey *et al.*, 1972; Glassman *et al.*, 1973). Several lines of evidence suggest that both metal ions in 2:1 metal–ATP complexes interact with the adenine ring. Glassman *et al.* (1973) presented spectrophotometric evidence for the formation of a Ni_2–ATP complex and provided reasons why both nickel atoms are probably interacting with the adenine. It is possible that the relative order of binding constants of these 2:1 metal–nucleotide complexes for a series of adenosine analogs could be predicted by the generalized perturbation theory.

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