II. One would expect excited species II to have more "nonbonding" electron density on the nitrogen hydrogen bonded to water than on the nitrogen bonded to ethanol. By acquiring such a distribution upon going to the excited state, the molecule maintains as much as possible the stability due to the strong hydrogen bond with water while only sacrificing stability due to a weak hydrogen bond with ethanol. The result is a large excited-state stabilization energy on going from species I to species II and therefore a small blue shift in the spectrum of species II relative to species I. Although these arguments are not based on any theoretical evidence we feel that they are qualitatively sound. It is possible that the small shift upon going from species I to species II is in some way associated with the dipole moment of species II.[11] If this were so we would expect the peak of species II to be shifted as the dielectric constant of the solvent changes. Since we observe no shift in the species II peak as the water concentration changes or upon addition of up to 10^{-1} *M* LiCl to the solvent, we can rule out dipole moment contributions to the shift.

Low Phosphorescence Yield of Species II. The small phosphorescence quantum yield of species II relative to species I indicates either a smaller quantum yield of intersystem crossing from the excited singlet state or a more rapid rate of intersystem crossing from the triplet for species II. The latter can be ruled out since the lifetime of species II is similar to that of species I and because the protonated and deuterated species II have the similar lifetimes even though they have different phosphorescence yields. It is known that a $^3\pi\pi^*$ state lies between the lowest $^1n\pi^*$ and $^3n\pi^*$ states in pyrazine.[12] Upon going from species I to species II the energy gap between $^1n\pi^*$ and $^3\pi\pi^*$ should get larger and this may well result in a smaller intersystem crossing rate from the $^1n\pi^*$ state. The increased phosphorescence yield of species II in deuterated solvents is interesting and is probably associated with a reduction in the rate of internal conversion from the $^1n\pi^*$ state to the ground state. Internal conversion from $^1n\pi^*$ is known to be important in the azines.[13]

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Proton Magnetic Resonance Studies of Metal Complexation of Nucleosides in Dimethyl Sulfoxide

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Abstract: For the purpose of deciding whether the group IIa metal ions (Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}) and the group IIb ones (Zn^{2+}, Cd^{2+}, and Hg^{2+}) have different biochemical characters with respect to formation of the complexes with nucleosides, we have carried out proton resonance studies. The nucleosides used were adenosine (Ad) and guanosine (Gu), in dimethyl sulfoxide medium. The dependences of the proton shieldings on the metal concentrations have been analyzed to yield limiting shifts and formation constants of the 1:1 metal–nucleoside complexes in equilibrium, and different effects were detected between the group IIa and IIb ions. The changes of the proton nuclear shielding by formations of the metal complexes are ascribed to the electrostatic field effect originating from the charges of the metal ions. For the group IIa–Gu complexes, the distances of the ion from the binding sites were estimated as 3.7 Å, using the Buckingham–Musher equation.

The nucleosides are of prime biological importance, and proton magnetic resonance (pmr) studies on the binding of metal ions to these have been reported by a number of investigators.[2-6] Studies of metal complexes of nucleosides may serve to some degree as models for the nucleic acid and metal ion interactions. Wang and Li[4a] measured the pmr shifts of the systems in which the metal salt is $ZnCl_2$ and the nucleosides are adenosine (Ad), guanosine (Gu), cytidine (Cy), and uridine (Ur) in dimethyl sulfoxide (DMSO) medium, and estimated the formation constants of the 1:1 Zn–nucleoside complexes. Kan and Li[5] found the formation constants of the 1:1 mercury(II) complexes with Ad, Gu, and Cy in DMSO by the same technique. Jordan and McFarquhar[6] reported evidence concerning the existence of a 1:1 Ca^{2+}–Gu complex.

Wacker, *et al.*,[7] found that the nucleic acids extracted from natural products, especially RNA, contain a considerable amount of metals Mg, Ca, Sr, Ba,

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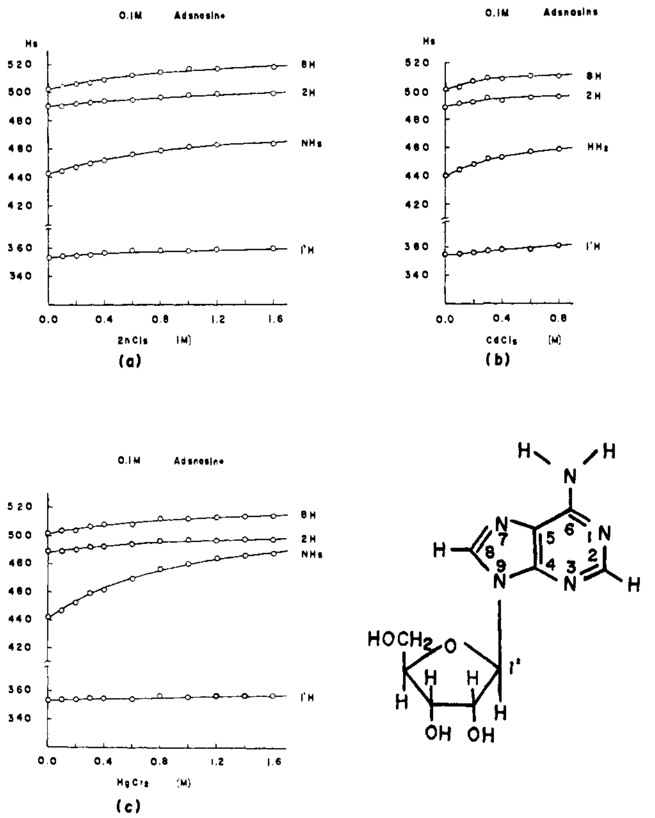

(a)

(b)

(c)

Figure 1. Pmr frequencies of adenosine protons in DMSO solutions containing 0.1 *M* adenosine and varying concentrations of the IIb metal ions: (a) $ZnCl_2$, (b) $CdCl_2$, (c) $HgCl_2$.

Zn, etc. For the purpose of deciding whether the group IIa elements (alkali earth metals) and the group IIb ones have different effects on the interactions with nucleosides, we have carried out extensive pmr studies on these systems using $MgCl_2$, $CaCl_2$, $SrCl_2$, and $BaCl_2$ as group IIa metal salts, $ZnCl_2$, $CdCl_2$, and $HgCl_2$ as group IIb ones, and Ad and Gu as nucleosides. DMSO was also used as the solvent because it provides adequate solubility to both nucleosides and metal salts without proton exchange.

Calculation of Formation Constants of Metal Complexes

The downfield shifts of proton resonances in the heterocyclic aromatic ring on metal complexation have been observed by several researchers.[8,9] The amount of the shifts may serve as a measure of the strength of the interaction between a metal ion and a nucleoside. The cause of the shifts will be examined later in our discussion.

If one may assume 1:1 metal–nucleoside complexation, as has been assumed in other literatures,[4a,5,6] the observed frequency of a ring proton of a nucleoside in the presence of a metal is a weighted average of the characteristic frequencies of the free and complexed molecules, which are designated as ν_f and ν_c, respectively, and the observed frequency is given by the equation[4a,9]

$$\nu = \frac{B_0 - (\mathrm{MB})}{B_0}\nu_f + \frac{(\mathrm{MB})}{B_0}\nu_c = \nu_f + \frac{(\mathrm{MB})}{B_0}(\nu_c - \nu_f) \qquad (1)$$

where B_0 is the initial concentration of base, (MB) is the equilibrium concentration of the metal complex, and ν_f is taken to be the frequency of 0.1 *M* nucleoside in the absence of metal. The equilibrium constant of the reaction

$$\mathrm{M} + \mathrm{B} \rightleftharpoons \mathrm{MB} \qquad (2)$$

is given by the equation

$$K = \frac{(\mathrm{MB})}{(M_0 - (\mathrm{MB}))(B_0 - (\mathrm{MB}))} \qquad (3)$$

where M_0 is the initial concentration of a metal ion. The observed frequencies of a ring proton of the nucleoside on adding the metal salt enable one to obtain the values of *K* and ν_c of the metal complex. We could determine the best fitted values of *K* and ν_c by computer simulation technique using the least-squares method. This method is described in the Appendix.

Experimental Section

Materials. Adenosine and guanosine were obtained from Sigma Chemical Co. and dried by pumping in a vacuum desiccator over silica gel for several days. DMSO was purified twice by vacuum distillations after drying over calcium hydride. Commercial grade $CaCl_2$, $SrCl_2$, and $BaCl_2$ were dried in an oven before using. Anhydrous $ZnCl_2$ and $CdCl_2$ were obtained by heating in the quartz vessel filled with dry hydrochloric gas. Anhydrous $MgCl_2$ was synthesized by heat decomposing magnesium–ammonium double salt, using $MgCl_2 \cdot 6H_2O$ as the starting material.[10] Anhydrous $HgCl_2$ was reagent grade and used without further purification.

Pmr Measurements. The pmr spectra were obtained by the field sweep with a JEOL 3H-60 spectrometer in DMSO at 21 ± 0.2°. All samples were prepared inside the sealed drybox which was kept under a slight positive pressure of dry nitrogen. The frequencies were measured with respect to tetramethylsilane (TMS) as the internal standard to within ±0.2 Hz. The concentration of nucleosides was 0.1 *M* in all samples.

Results

The pmr spectra of adenosine and guanosine in DMSO and their peak assignments have been reported by several investigators.[11,12] The effects of the metal salts of group IIa and IIb elements on frequencies of these nucleosides were observed.

(A) Adenosine–Metal Complexes. The effects of the group IIb metal salts, namely, $ZnCl_2$, $CdCl_2$, and $HgCl_2$, on frequencies of 0.1 *M* adenosine in DMSO are shown as (a), (b), and (c) of Figure 1, respectively. The formation constant (*K*) and the limiting frequency (ν_c) of the adenosine–metal complexes were evaluated by the least-squares method using a FACOM 270-20/30 computer. All lines describing the frequency changes of the protons were drawn by the X–Y plotter of the computer. The agreement of the simulated lines with the observed frequencies is quite satisfactory, and this fact verifies the assumption of the 1:1 complexation. We defined a limiting shift of a proton as ($\nu_c - \nu_f$) and denoted it as $\Delta\nu$. The values of *K* and $\Delta\nu$ of the adenosine complexes are shown in Table I. The *K* values listed in this paper are for the protons which yield the largest limiting shifts in each complex.

The large changes in proton shieldings caused by addition of the metals indicate the binding sites of the metals in the complexes. The largest change of the chemical shift of the NH_2 protons in Ad by adding the

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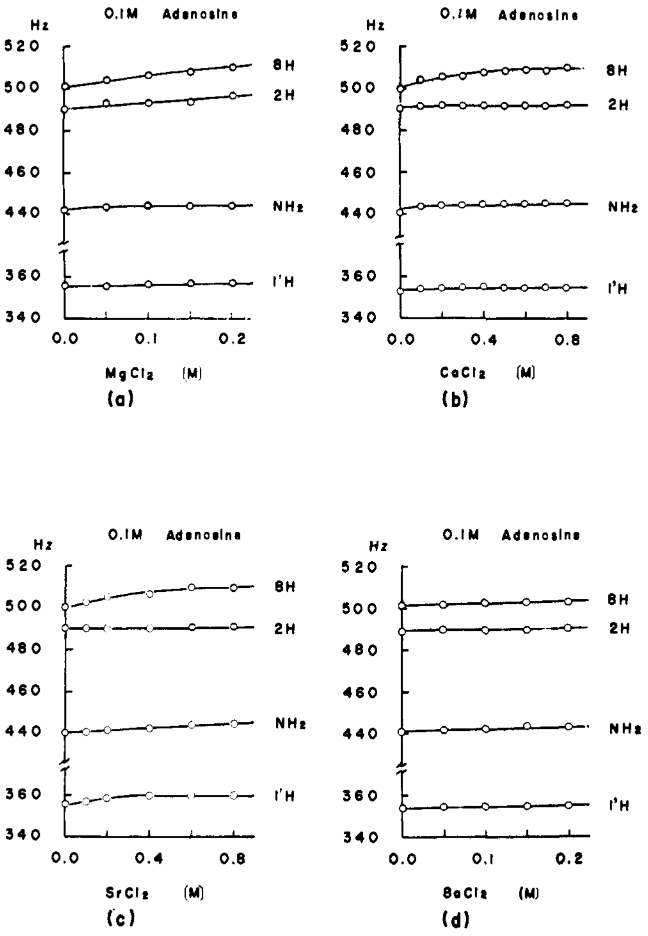

Figure 2. Pmr frequencies of adenosine protons in DMSO solutions containing 0.1 *M* adenosine and varying concentrations of the IIa metal ions: (a) $MgCl_2$, (b) $CaCl_2$, (c) $SrCl_2$, (d) $BaCl_2$.

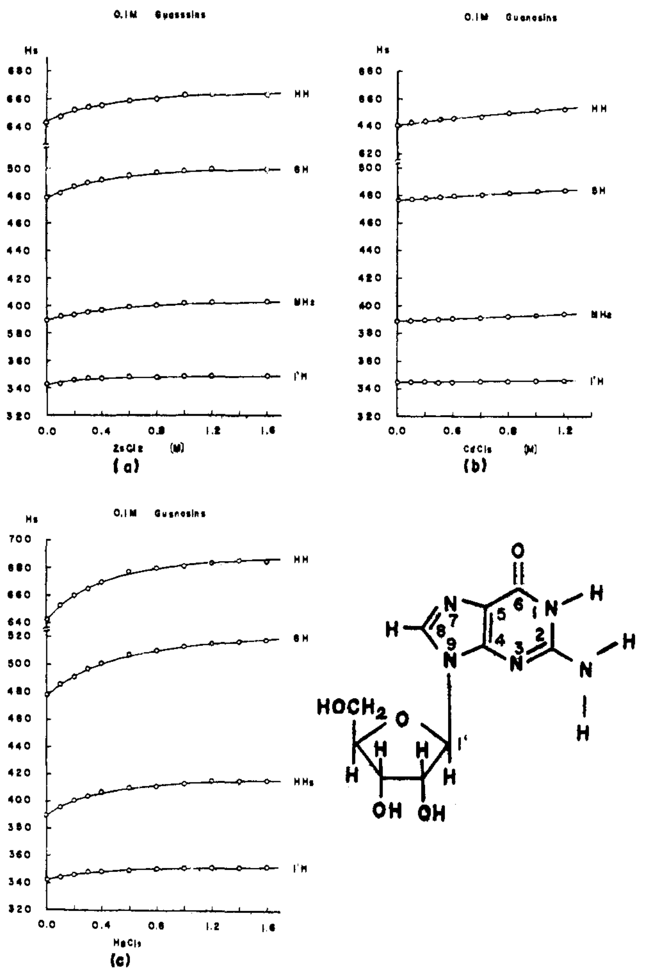

Figure 3. Pmr frequencies of guanosine protons in DMSO solutions containing 0.1 *M* guanosine and varying concentrations of the IIb metal ions: (a) $ZnCl_2$, (b) $CdCl_2$, (c) $HgCl_2$.

Table I. Experimentally Determined Parameters for Adenosine-Metal Complexes[a]

		IIa				IIb		
		Mg	Ca	Sr	Ba	Zn	Cd	Hg
$\Delta\nu$, Hz	8H	28.7	10.5	15.3	0	28.7	12.1	22.3
	2H		0	0	0	20.9	9.1	18.5
	NH_2				0	36.6	26.0	79.5
	1'H	0	0	7.7	0	10.0		0
K, M^{-1}		3.14	6.42	2.75	0	1.03	3.42	1.04

[a] Equilibrium constants are given only for the protons that have the largest limiting shifts in each adenosine-metal complex. The positive sign of the $\Delta\nu$ indicates the lower field shift. The uncertainty of the K and $\Delta\nu$ values seems to be about 10%.

salts of any metals in the IIb group, as shown in Figure 1, means that NH_2 is the preferred binding site in the IIb-Ad complexes.

The pmr frequencies of the protons of 0.1 *M* adenosine in DMSO as a function of varying concentration of the IIa metal salts, $MgCl_2$, $CaCl_2$, $SrCl_2$, and $BaCl_2$, are shown as (a), (b), (c), and (d) of Figure 2, respectively. The IIa metal salts except $BaCl_2$ influence the chemical shifts of the adenosine protons. The largest effects on the shift of the Ad 8H, as shown in (a), (b), and (c) of Figure 2, indicate that the binding site in the IIa-Ad complexes is 7N, but the strength of the interaction is rather weak. Figure 2d shows that $BaCl_2$ does not form a complex with adenosine. The values of K and $\Delta\nu$ of the adenosine complexes are listed in Table I.

(B) Guanosine-Metal Complexes. The spectra of (a), (b), and (c) of Figure 3 show changes of the chemical shifts of the guanosine protons in the 0.1 *M* DMSO solution on adding IIb metal salts. As shown in (a), (b), and (c) of Figure 3, it is found that all of the IIb metal ions have nearly the same effects on the NH, 8H, and NH_2 protons in guanosine. The same effects on all of the base protons seem to indicate that a IIb metal ion may exist above center of the base plane of guanosine, and the ion may be at the same distances from the NH, 8H, and NH_2 protons. The values of K and $\Delta\nu$ are listed in Table II.

The effects of the IIa metal salts on the chemical shifts of 0.1 *M* guanosine in DMSO are shown in (a)-(d) of Figure 4. One may find that all the metals of the IIa show the same effects to the Gu protons in which the Gu-NH and Gu-NH_2 signals are shifted downfield to an equal extent but Gu-8H and Gu-1'H signals do not move. These results indicate that the binding sites of the IIa metals to guanosine are 1N and NH_2. Since the 8H and 1'H are further away from the assumed binding site in the IIa-Gu complexes, one would expect that the frequencies of these protons would remain unchanged. The values of K and $\Delta\nu$ are listed in Table II. The equilibrium constants of the IIa-Gu

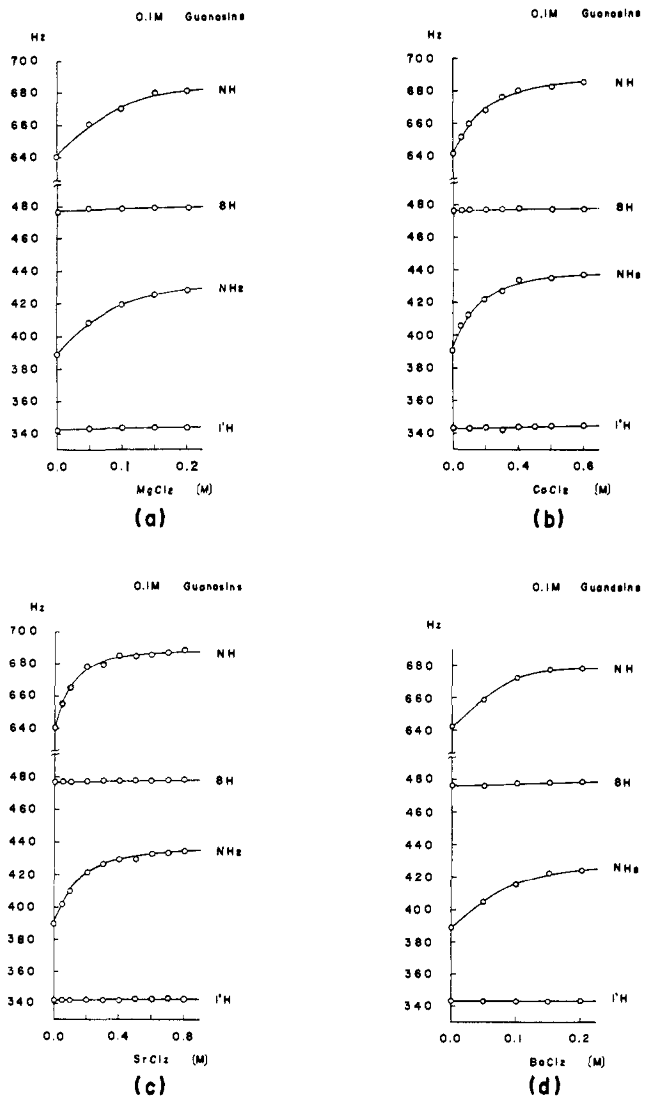

Figure 4. Pmr frequencies of guanosine protons in DMSO solutions containing 0.1 M guanosine and varying concentrations of the IIa metal ions: (a) $MgCl_2$, (b) $CaCl_2$, (c) $SrCl_2$, (d) $BaCl_2$.

Table II. Experimentally Determined Parameters for Guanosine–Metal Complexes[a]

		IIa				IIb		
		Mg	Ca	Sr	Ba	Zn	Cd	Hg
$\Delta\nu$, Hz	NH	46.8	50.6	50.1	39.0	27.3	67.0	55.9
	8H	0	0	0	0	25.7		53.1
	NH_2	43.3	51.5	49.4	42.7	17.7		34.2
	1'H	0	0	0	0	8.1	0	12.5
K, M^{-1}		42.2	11.4	20.9	50.5	2.25	0.206	2.76

[a] Equilibrium constants are given only for the protons that have the largest limiting shifts in each guanosine-metal complex. The positive sign of the $\Delta\nu$ indicates the lower field shift. The uncertainty of the K and $\Delta\nu$ values seems to be about 10%.

complexes are much larger than those of the IIb–Gu ones.

Discussion

Buckingham, Schaefer, and Schneider[13] have divided the extramolecular effects on the shielding of a proton in a solution into four terms: a bulk susceptibility, a solvent anisotropy, a van der Waals, and an electrostatic field term. The first two terms, the bulk susceptibility and the solvent anisotropy, are constant in this case since all the metal ions are diamagnetic and spherical. Yonemoto[14] predicted that a van der Waals deshielding of a proton at distance ρ from an ion is proportional to $[\langle\Sigma_i x_i^2\rangle/a_0^2]\rho^{-6}$. The van der Waals shifts will be small for this system because all the metal ions are not so large and this term falls away rapidly with the distance ρ from the ion. Therefore, the electrostatic field term is considered to predominate, and only this effect will be discussed in greater detail in this paper.

Estimation of the Distance between the Ion and the Binding Sites. The Buckingham–Musher equation[15,16] of the X–H proton shifts by the electrostatic fields predicts field dependence as

$$\delta\sigma_E = -a \times 10^{-12}E_z - b \times 10^{-18}E^2 \qquad (4)$$

where the positive z direction is taken from X to H in the X–H bond, and $\delta\sigma_E$ is the proton shielding change caused by the electric field E. The parameter a, which may be related to the bond polarizability, has been evaluated theoretically[14-16] and experimentally[15,17-19] for the C–H bonds as about 3 esu^{-1}. The coefficient of E^2, b, is usually assumed to have the hydrogen-atom value,[20] 0.738 esu^{-2}.

A metal ion binding to a nucleoside gives rise to a spherical symmetric electrostatic field. If the distance from the ion to the proton is ρ Å, and the angle between the C–H and H–ion vectors is θ, the change of the proton shielding on the formation of the complex is predicted to be

$$\delta\sigma_E\,(\text{ppm}) = 28.8\rho^{-2}\cos\theta - 68.0\rho^{-4} \qquad (5)$$

since in this case all the metal ions are divalent. We will use this effect for the quantitative estimation of the distances between the ion and the binding sites from the limiting shifts observed for the IIa–Gu complexes.

According to our result the binding sites in the IIa–Gu complexes are 1N and NH_2. The position of a group IIa metal ion of the guanosine complex cannot be determined uniquely from the shifts of NH and NH_2 protons. The two amino protons are equivalent because of sufficiently fast rotation about the C–N bond in guanosine base. Therefore, only two independent equations are derived from eq 5, and so the position of the ion referring to three-dimensional coordinates cannot be fixed from the two equations. We may now assume that the ion is perpendicular to the base plane at the middle point between the two nitrogen atoms, 1N and NH_2, because the NH and NH_2 protons are shifted downfield to an equal extent. Besides, we shall assume that the electrostatic effect on the N–H proton shift is the same as that for the C–H proton shift.[21] Thus, using eq 5 and the X-ray crystallographic data of guanosine,[22] for $\Delta\nu$ = 50 Hz in the N–H proton of the

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IIa–Gu complexes, one finds the distance ρ between the ion and the N–H proton of 3.8 Å.[23] The perpendicular distance from the IIa metal ion to the guanosine base plane will be estimated as 3.5 Å. The distances from the ion to the two nitrogen atoms, 1N and NH_2, will both be calculated as 3.7 Å. The estimated distance between the ion and the binding sites is 3.7 Å.

Schulman, *et al.*,[24] carried out the relaxation studies of pmr of Ni^{2+}, Co^{2+}, and Mn^{2+} complexes with ATP and from the results they estimated the distance from the ion to the 8H proton, that is, the nearest proton from the ion, as 3.5 Å. The agreement between the values estimated from the two independent ways is very good. However, the following points are noteworthy with regard to the validity of our estimation. First, the effect of the counterion, which was neglected in our estimation, will reduce the field at the hydrogen to some value smaller than our assumed field, namely $2e/\rho^2$, and the actual distance may be smaller than the estimated value. It seems unfeasible to calculate the counterion effect exactly in the concentrated ionic solution, but Green and Martin[19] have reported that the counterion effect was no greater than 50% of the primary ion effect in their experiment. Second, we have assumed the dielectric constant of vacuum ($\epsilon = 1$) for the space intervening between the ion and the proton. This is not necessarily so, but the $\epsilon = 1$ is reasonably considered to be much better than the bulk value of the DMSO, $\epsilon = 45$, in this case by the reason of the dielectric saturation effect.[25–28] The actual dielectric constant may be something between 1 and 45, and a greater value of ϵ would decrease the field at the proton and give the shorter value for the distance. But the following consideration convinces us that the reduction of the distance due to the dielectric constant is rather small. The dielectric saturation theory predicts that the dielectric constant in infinite strength of the electric field will decrease to n^2 asymptotically,[25,27] in which n is the optical refractive index. Using the refractive index of DMSO of 1.48, we compute an effective dielectric constant of $\epsilon = 2.2$ in the neighborhood of the ion in DMSO medium. The local field strength, E, acting on the molecule at the short distance ρ from the ion will be expressed as

$$E = \frac{\epsilon + 2}{3} \frac{2e}{\epsilon \rho^2} \qquad (6)$$

From this equation the dielectric effect in DMSO is not greater than 50% in the vicinity of the ion. Third, we have neglected the electron-transfer mechanism from the nucleoside base to the ion. Li, *et al.*,[4a,8,9] have reported that the downfield shifts of the resonances of the heterocyclic aromatic ring protons upon protonation or metal complexation were ascribed to extensive π-electron redistribution.[29,30] The extensive π-electron redistribution needs a large mixing with the unoccupied p orbital of the metal ion. The magnitude of mixing of the orbitals is expected to be proportional to the resonance integral between the orbitals of the metal ion and the base. The resonance integral decreases abruptly as the distance increases and so the electron-transfer effect, namely the extensive π-electron redistribution, will be small if the distance between the ion and the binding site is larger than the diameter of the ion. Besides, in our experiment the shifts at ring protons decrease as the protons become separated from the binding sites by a greater number of bonds. This fact would be in contrast to the well-known π-orbital delocalization, for the ion would affect all of the base protons if the extensive π-electron redistribution was a mechanism of the lower field shifts.

Conclusions

The interaction of a IIa or a IIb metal ion with a nucleoside molecule, either an adenosine or a guanosine molecule, in DMSO solvent appears to be interpreted in terms of the 1:1 complex equilibrium, in which the nucleoside molecule displaces presumably one solvent molecule from the primary solvation shell of the ion.

The IIa metal ions and the IIb ones show quite different effects on the nucleoside regarding binding sites, limiting shifts, and equilibrium constants, but the ions of the same group are very similar in the effects. These facts permit us to suppose a correlation between the differences in the binding sites of the metal ions of the IIa and IIb group and the fact that the IIa and IIb ions have entirely different biochemical characteristics. It is concluded that the binding sites of the adenosine complexes are NH_2 for the IIb metal ions and 7N for the IIa ones. But Ba^{2+} does not bind merely to adenosine. The strength of the interaction in the IIa–Ad complexes is rather weak. As for the guanosine complexes, the IIb metal ions do not bind to the particular site but above the center of the base plane, and the IIa ones bind to both 1N and NH_2. The equilibrium constants of the IIa–Gu complexes are much larger than those of the IIb–Gu ones.

The simple electrostatic field model, using the field of $2e/\rho^2$, accounts adequately for the proton shielding change and enables one to calculate successfully the position of the ion relative to the nucleoside molecule. Our estimation of the distance from the ion to the binding sites in the IIa–Gu complexes is consistent with a larger bound or limit of 3.7 Å, which is quantitatively in accordance with the previous estimates for the ATP complexes.[24]

Since our experiment reveals the position of an ion relative to a molecule in the primary solvation shell of the ion, it appears feasible to use it to study the structure of solvation complexes.

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Appendix

The values of the equilibrium constant (K) and the limiting frequency (ν_c) were evaluated by the least-squares method. The least-squares method used in our experiment is described below.

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[23] The distance ρ calculated from the Laszlo–Musher equation of ref 21 was found to be 3.9 Å. This value agrees sufficiently close to the value of 3.8 Å in the text.

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[30] T. Schaefer and W. G. Schneider, *Can. J. Chem.*, **41**, 966 (1963).

The proton frequency of the complex is represented as a function of the two unknown parameters, K and ν_c. The values of the parameters are determined from the observed proton frequencies at the various concentrations of a metal ion. Let the most reliable values of the K and ν_c be denoted as K^0 and $\nu_c{}^0$, respectively, and the approximate values to these as K' and $\nu_c{}'$. The corrections for the parameters to be determined are given as

$$\begin{aligned} \delta K &= K^0 - k' \\ \delta\nu_c &= \nu_c{}^0 - \nu_c{}' \end{aligned} \qquad \text{(A1)}$$

where the corrections are assumed to be sufficiently smaller than the parameters themselves.

Let the frequencies measured in varying concentrations of an ion be

$$\nu_1,\ \nu_2,\ \ldots,\ \nu_n \qquad \text{(A2)}$$

The residuals, v, between the observed and calculated frequencies are expressed as

$$v_i = \nu_i - f_i(K^0, \nu_c{}^0) \qquad i = 1, 2, \ldots, n \qquad \text{(A3)}$$

where f_i is the function corresponding to the proton frequency at the initial concentration, M_{0i}, of the ion. Since the corrections are assumed to be small, $f_i(K^0, \nu_c{}^0)$ of eq A3 can be expanded in the power series of δK and $\delta\nu_c$ in the vicinity of K' and $\nu_c{}'$. If one neglects the higher order terms of more than one, eq A3 is written as

$$v_i = \Delta\nu_i - (\partial f_i/\partial\nu_c)_{\nu_c{}'K'}\delta\nu_c - (\partial f_i/\partial K)_{\nu_c{}'K'}\delta K \qquad \text{(A4)}$$

$$i = 1, 2, \ldots, n$$

where

$$\Delta\nu_i = \nu_i - f_i(K', \nu_c{}') \qquad \text{(A5)}$$

According to the theory of least squares, the corrections, δK and $\delta\nu_c$, must be determined to minimize the sum of squares of residuals. From this condition one can get the values of the corrections as the solution of the following normal equation

$$\begin{aligned} [\mathrm{AA}]\delta\nu_c + [\mathrm{AB}]\delta K &= [\mathrm{A}\Delta] \\ [\mathrm{AB}]\delta\nu_c + [\mathrm{BB}]\delta K &= [\mathrm{B}\Delta] \end{aligned} \qquad \text{(A6)}$$

where

$$\begin{aligned} [\mathrm{AA}] &= \textstyle\sum_i(\partial f_i/\partial\nu_c)^2 \\ [\mathrm{AB}] &= \textstyle\sum_i(\partial f_i/\partial\nu_c)(\partial f_i/\partial K) \\ [\mathrm{BB}] &= \textstyle\sum_i(\partial f_i/\partial K)^2 \\ [\mathrm{A}\Delta] &= \textstyle\sum_i(\partial f_i/\partial\nu_c)\Delta\nu_i \\ [\mathrm{B}\Delta] &= \textstyle\sum_i(\partial f_i/\partial K)\Delta\nu_i \end{aligned}$$

The corrections obtained from eq A6 are added to the parameters and the iteration proceeds to the convergency of the parameters. A Fortran IV computer program was written to do this.

Proton Nuclear Magnetic Resonance and Electron Spin Resonance Investigation of the Electronic Structure and Magnetic Properties of Synthetic Low-Spin Ferric Porphyrins

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Abstract: The proton nmr spectra of low-spin ferric bis(imidazole) complexes of a series of synthetic porphyrins have been recorded and analyzed. The satisfactory agreement between the observed relative shifts and the calculated relative geometric factors for the phenyl protons of $[\mathrm{TPPFeIm_2}^+]\mathrm{Cl}^-$ establishes that these shifts are wholly dipolar in origin and permits a quantitative separation of all other shifts into their contact and dipolar contributions. The flowing-helium esr spectra at X-band of dilute glasses of these complexes exhibit highly rhombic g tensors, with $g_1 \sim 2.9$, $g_2 \sim 2.3$, and $g_3 \sim 1.5$. The sizable in-plane magnetic anisotropy appears to be an intrinsic property of the porphine skeleton in low-spin ferric hemes and does not seem to depend on the symmetry of the peripheral substituent distribution. The averaging to zero of this in-plane anisotropy at ambient temperatures permits the determination of the axial anisotropy, which agrees within experimental error with the value derived from the experimental dipolar shifts of the meso phenyl protons. Deviations from Curie behavior are observed which appear to originate in the dipolar interaction. The resultant contact shifts are consistent with L → M π charge transfer from the highest filled π MO. Comparison of the present analysis with those of related complexes of natural heme suggests that the magnetic properties and bonding are very similar.

Since the initial observation[2a] of isotropically shifted proton resonances for the heme moiety in oxidized ferricytochrome *c*, nmr of paramagnetic macromolecules has developed[2,3] into a powerful new tool for investigating structure and structure–function relation-

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