Nucleoside Complexing. A Charge Reversed Chelate Compound between Guanosine and Chloride Ion

Sir:

Metal ions mediate essentially every aspect of nucleic acid structure, function, and synthesis in living systems.[1] The difficulty inherent in specifically identifying binding sites of the metal to the nucleic acids has led to extensive studies of metal ion interactions with the constituent nucleosides and nucleotides.[1] Studies of metal interactions with the constituent heterocyclic bases are often carried out with nucleosides since nucleotides bond strongly through phosphate groups. Nonaqueous solvents are employed in such studies because the nucleosides are more soluble and the N–H pmr signals can be observed.

Numerous studies by at least three different groups[2-4] have been reported in this journal on the subject of the interaction of divalent diamagnetic metallic chloride salts (Zn, Cd, Hg, Mg, Ca, Sr, Ba) with guanosine in dimethyl sulfoxide (DMSO). As expected, Zn, Hg, and, perhaps, Cd form complexes as evidenced by changes in chemical shifts of all the guanosine base protons. However, it was totally unexpected that Ca^{2+} should complex.[2] This result was apparently substantiated by a careful, complete study of the interactions of four alkaline earth chlorides (Mg, Ca, Sr, and Ba).[3] One study on these alkaline earth metals concluded that guanosine or its derivatives formed stable complexes but that other nucleosides tested (adenosine) formed no complex.[2] Such a specific interaction, particularly of Ba, would be of great value in the heavy metal labeling of nucleic acids.[5-8] Additionally, if correct, these findings would mean that the concepts developed[1] for metal ion–nucleic acid bonding must be partly revised. We have, therefore, investigated these phenomena and find that an altogether different, and perhaps more intriguing, explanation is required.

We believe the correct interpretation is that *chloride* ion binds to guanosine. This novel finding requires that previous studies of binding of metallic chlorides in DMSO be reevaluated. Further, we find that interactions occur even in 26% water–74% DMSO (v/v) for 0.75 M [NEt₄]Cl and 0.15 M guanosine. Thus, interactions of this type may be of transient importance even in aqueous solution. The relative shifts from the free guanosine of the N_1H and C_2NH_2 protons are not affected by water, but in greater than 10% water the N_1H resonance is no longer observable.

Prior to describing our experiments in more detail, we will briefly summarize previous data. The most noteworthy aspect of the behavior of the N_1H and C_2NH_2 proton signals is that the resonances shift downfield smoothly upon addition of alkaline earth salt, with only

one resonance for each group being observed and with no decrease in intensity. These shifts are characterized by being equal in sign and magnitude for both resonances, reaching upper limits of 40–50 Hz (60 MHz instrument) dependent to some extent on the cation.[3] The guanosine derivative *O*-2′,3′,5′-triacetylguanosine exhibits the same behavior.[2] Stability constants determined from the chemical shifts are larger for the alkaline earth–guanosine complexes (*ca.* 10–50 M^{-1}) than for the zinc triad guanosine complexes (*ca.* 0.2–3 M^{-1}).[3] For the alkaline earth salts, no shift of the C_8H resonance was found whereas substantial shifts of this resonance were found with Zn, Cd, and Hg.[3,4] The major discrepancy between published articles[2,3] is that when care was taken to exclude moisture, $MgCl_2$ was found to produce shifts,[3] whereas in the original study[2] it was reported to have no effect.[9] The similar shifts of the N_1H and C_2NH_2 pmr signals were explained by a physically unrealistic model supported by an elaborate calculation.[3]

We have been able to produce this shift behavior with $CaCl_2$ but also with [NEt₄]Cl and LiCl, which are quite soluble, as well as with NaCl and KCl, which are poorly soluble in DMSO. Again, characteristically, the C_8H resonance does not shift appreciably. In addition, the salt K_2PtCl_4 reacts with guanosine to give an additional set of pmr signals for N_1H, C_8H, and C_2NH_2 at τ −1.38, 1.35, and 3.07, respectively (*vs.* TMS), which we attribute to a Pt(II)–guanosine complex bonded through N_7. Addition of $CaCl_2$ to this solution (to saturation) also causes shifts of the NH_2 and N_1H proton resonances with a relative ratio of $\sim$1.03 (20.5/20 Hz).

The relative shift data for chloride and bromide salts are given in Table I. The salt LiCl gave maximum

Table I. Ratio[a] of Shifts of the NH_2 to N_1H Proton Resonances of Guanosine (0.2 M) in DMSO

| Cation	Chloride	Bromide
Li	1.05	3.1[c]
Na	1.05	$\sim$3[d]
K	$\sim$1	$\sim$3[d]
Mg	1.08, 0.93[b]	
Ca	1.06, 1.02[b]	
Sr	0.99[b]	
Ba	1.09, 1.09[b]	$\sim$3[d]
Et₄N	1.09	
Bu₄N		2.9[c]

[a] Actual values calculated from our data, although error will vary from $\sim$5 to perhaps 20% for some of the bromide salts. $T = 29.5°$. [b] From ref 3, maximum shifts. [c] Maximum shift 20 Hz. [d] Relatively small shifts of $\sim$9 Hz for NH_2 and 3 Hz for NH resonances.

shifts of 50 and 48 Hz for the NH_2 and N_1H proton resonances, respectively. Perchlorate salts (Na, Li) and nitrate salts (Ca, Mg, Sr, Ba, Na) gave no appreciable shifts. Taken together, these results imply involvement of the anion and rule out significant cation interactions with guanosine as well as nonspecific ionic strength effects. For the alkaline earth metal ions previously studied,[2,3] the interaction of the anionic chloride with guanosine is the only interaction which is causing chemical shifts. For the zinc triad,[3] the observed shifts can be attributed to a combination of (1)

(1) G. L. Eichhorn, "Inorganic Biochemistry," G. L. Eichhorn, Ed., Elsevier, Amsterdam, 1973, Chapters 33 and 34.

(2) F. Jordan and B. Y. McFarquhar, *J. Amer. Chem. Soc.*, **94**, 6557 (1972).

(3) S. Shimokawa, H. Fukui, J. Sohma, and K. Hotta, *J. Amer. Chem. Soc.*, **95**, 1777 (1973).

(4) S. M. Wang and N. C. Li, *J. Amer. Chem. Soc.*, **90**, 5069 (1968); L. S. Kan and N. C. Li, *ibid.*, **92**, 281, 4823 (1970).

(5) J. W. Wiggins and M. Beer, *Anal. Chem.*, **44**, 77A (1972).

(6) F. P. Ottensmeyer, E. E. Schmidt, and A. J. Olbrecht, *Science*, **179**, 175 (1973).

(7) T. J. Kistenmacher, L. G. Marzilli, and C. H. Chang, *J. Amer. Chem. Soc.*, **95**, 5817 (1973).

(8) L. G. Marzilli, T. J. Kistenmacher, and C. H. Chang, *J. Amer. Chem. Soc.*, **95**, 7507 (1973).

(9) We had no difficulty observing shifts with $MgCl_2$, without taking the stringent precautions employed in ref 3.

Cl⁻ interacting with uncoordinated guanosine, (2) Cl⁻ interacting with coordinated guanosine, and (3) the co-ordination of guanosine to the metal. This complexity was obviously not recognized when the stability constants were calculated,[2,3] and, thus, such constants are inaccurate.

We believe that the charge reversed chelate complex (I), in which two positive centers chelate a negative ion,
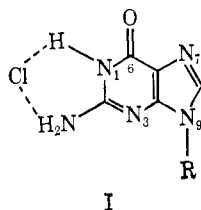
I

is formed. Our reasons for suggesting this particular model are as follows. (1) Downfield shifts are expected from hydrogen bonding.[10] (2) Only when a chelate is possible are large shifts seen. (No large shifts were observed for adenosine.)[2,3] (3) Almost identical interactions have been observed in the solid.[11] (4) Both N₁H and C₂NH₂ pmr signals shift substantially and simultaneously. The shifts level off simultaneously,[3] and this is inconsistent with one-point attachment. (5) The interaction is observed in solvents (DMSO, M-pyrol) which do not solvate anions, whereas water decreases the interaction. (6) Anions which do not accept hydrogen bonds readily do not show this interaction. (7) Modified guanosine derivatives which leave the six-membered ring unchanged (the triacetyl[2] and platinum derivatives) exhibit the same effect. (8) The Cl⁻ ion is remote from the C₈H group, and the pmr signal of this group is not expected to shift.

If the effect is due entirely to chloride ion, then we must explain the different stability constants found for the four alkaline earth salts.[3] We attribute these differences to (1) failure to maintain constant ionic strength, (2) variation in moisture content of the solution,[12] (3) data analysis based on an incorrect model, and (4) ion pairing. If we assume a one-to-one chloride ion–guanosine complex, we calculate[13] $K = 2.8$

(10) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, p 400.

(11) T. J. Kistenmacher and T. Shigematsu, observed for adenine dihydrochloride, *Acta Crystallogr.*, in press. We note that interactions between halide ions and purine bases normally involve in plane hydrogen bonds. However, guanosine hydrobromide involves a charge-transfer type interaction (crystallographic evidence) between bromide and the six-membered ring: P. Tougard in "The Purines. Theory and Experiment," E. D. Bergmann and B. Pullman, Ed., Academic Press, New York, N. Y., 1972, p 217. We cannot exclude such an interaction in our studies, but we feel the hydrogen bond interaction is more consistent with our results.

(12) We find that the effect is not very sensitive to water in the concentration ranges 2–25% water but diminishes gradually; however, all the salts, guanosine, and the solvent are hygroscopic. It seems advisable that future studies involving metal ion interactions with nucleosides employ partially aqueous systems, rather than deal with the uncertainties of a small but unknown amount of water. Addition of water to a 0.2 M guanosine solution (DMSO) had the following effects: (a) the N₁H proton resonance shifted downfield (∼2–3 Hz) and broadened and became lost in the noise as the water content reached 10% by volume and (b) the NH₂ and C₈H proton resonances did not shift up to 40% by volume water. This last solution was supersaturated. Guanosine precipitated before more water was added but after the spectrum was recorded.

(13) The calculation of the equilibrium constants in ref 2 requires several assumptions. These are that the chemical shifts of both the free guanosine and the complex guanosine pmr signals are not dependent on (1) the concentration of these species and (2) the concentration of the CaCl₂ salt. Most of these assumptions cannot be checked. However,

M^{-1}. The observation[2] of increased acidity of guanosine induced by CaCl₂ merely requires that deprotonated guanosine form a calcium complex.

We believe that the amino group is rotating rapidly on the pmr time scale because its resonance remains relatively sharp. If dissociation of guanosine from I is rapid, the magnitude of the effect of Cl⁻ on the shift of the amino proton resonance represents an average value for the two nonequivalent positions. Thus, the equivalent shifts of both the N₁H and the NH₂ pmr resonances of guanosine upon formation of I are coincidental. This reasoning is supported by the three times greater shift of the pmr signal of the NH₂ group than of the N₁H group in guanosine upon formation of the bromide analog of I.

Salt effects on biopolymers were recently reviewed[14] and are not completely understood. Geiduschek[15] has found that the melting temperature of DNA's can vary as much as 60° by varying salt concentrations and the phenomenon is due mainly to the anions. The magnitude of the effect is dependent on the base composition of DNA. It is interesting to note that the influence of anions on stabilizing DNA follows the order Cl⁻, Br⁻ > I⁻ > ClO₄⁻ > SCN⁻, and we find that effectiveness of these anions in causing shifts follows this same order. Our results provide evidence that specific interactions between anions and bipolymers are possible.

in the absence of CaCl₂, the guanosine resonances *are* concentration dependent.[2,4] Strictly speaking, the calculations[2] have assumed Δ_t should be a constant (Δ_t = total shift difference between complexed and free guanosine). This may not be true unless the effect of CaCl₂ concentration exactly balances the change in chemical shifts attending the decrease in free guanosine concentration. Alternatively, the concentration dependence of the shift of the amino group of the complex must accomplish this result. Neither of these possibilities is likely. If we use the data in ref 2 and assume that Δ_t = 46 Hz and the [ClG⁻] = (Δ_0/46 Hz) × 0.282 M, the equilibrium constant for Cl⁻ + G ⇌ ClG⁻ can be calculated to be 3.9 ± 0.5 M^{-1}. If we calculate this equilibrium constant using the data in ref 2 and a method of varying Δ_{max} for NH₂ similar to that in ref 2, we calculate $K = 2.82 ± 0.09\ M^{-1}$ (Δ_{max} = 52.5 Hz). The calculation performed as in ref 3 gives $K = 2.83$, Δ_{max} = 52.4 Hz.

(14) P. H. von Hippel and T. Schleich, *Accounts Chem. Res.*, 2, 257 (1969).

(15) K. Homaguchi and E. P. Geiduschek, *J. Amer. Chem. Soc.*, 84, 1329 (1962).

Chien-Hsing Chang, Luigi G. Marzilli*
Department of Chemistry, The Johns Hopkins University
Baltimore, Maryland 21218
Received January 18, 1974

Long-Range ¹³C–¹H Coupling Constants. I. Cyanopyridines

Sir:

Until now, ¹³C–¹H coupling constants have been measured either from the satellite peaks of proton nmr spectra[1] or directly from ¹³C nmr spectra with the aid of computers of average transients.[2] Hence

(1) K. Tori and T. Nakagawa, *J. Phys. Chem.*, 68, 3163 (1964).
(2) K. Takahashi, T. Sone, and K. Fujieda, *J. Phys. Chem.*, 74, 2765 (1970).