states are responsible for rapidly relaxing the [$^4T_1 \rightleftharpoons {}^2T_1$] equilibrium of states and thus make the decay of Ag(II) porphyrins radiationless. Analogously in Mo(V) porphyrins, $^2(\pi,d)$ excited states are likely to lie at lower energy than the [$^4T_1 \rightleftharpoons {}^2T_1$] manifold of states and thus provide pathways for relaxing them in 4 ns. In both Ag(II) and Mo(V) porphyrins, the spin-allowed relaxation of the low-energy ^{2}CT states to $^2S_0$ is likely to be much faster than their rate of population. Therefore the ^{2}CT states are not likely to be seen.

The possibility that the 4-ns lived transient seen in Figures 1 and 2 is due to recombination of photohomolysis products, $OMo^{IV}(TPP)$ and $\cdot OCH_3$, seems unlikely since this photoreaction has only been found[24] after irradiation into the Soret band at 455 nm, and we are exciting below this band at 527 nm. Also, in THF 75 min of excitation of an aerated solution at 455 nm failed to show any photoreaction. Photohomolysis should have produced a measurable loss of $OMo(TPP)OCH_3$ under these conditions.

Comparison of d^1 and d^9 Porphyrins. Picosecond kinetic and spectroscopic studies of Cu^{II}(OEP), Cu^{II}(TPP), and Cu^{II}(PP-IX-DME) as well as of Ag^{II}(PP-IX-DME) and Ag^{II}(MS-IX-DME) [MS-IX-DME = mesoporphyrin IX dimethyl ester] have been reported previously.[14–16,44] Many of these results are summarized in Table I, which also shows an apparent discrepancy concerning observation of the nanosecond-lived [$^4T_1 \rightleftharpoons {}^2T_1$] equilibrium of states in Ag(II) porphyrins. Specifically, it has been observed[15] in Ag^{II}(PP-IX-DME) but not in Ag^{II}(MS-IX-DME).[16] Also noted is the fact that the room temperature lifetimes of the [$^4T_1 \rightleftharpoons {}^2T_1$] manifold have not been well determined for Cu(II) and Ag(II) porphyrins. Other points of comparison among the $S = {}^1/_2$ metalloporphyrins in Table I concern the lack of observation of the initially populated $^2S_1(\pi,\pi^*)$ level for any of them and the large difference in tripquartet–tripdoublet equilibration rates (reaction 3) between the first-row Cu(II) porphyrin and the second-row Ag(II) and Mo(V) porphyrins. This increased equilibration rate for porphyrins with second-row transition metals could be due to increased spin–orbit coupling effects for these porphyrins relative to ones with first-row transition metals or it could be a consequence of ^{2}CT states mixing with the [$^4T_1 \rightleftharpoons {}^2T_1$] manifold of states.

The above results on open-shell metalloporphyrins contrast with those of closed-shell systems. In particular the initially formed $S_1(\pi,\pi^*)$ states in Zn(TPP) [with a first-row transition metal like copper] and $Sn(OEP)Cl_2$ [with a second-row transition metal like silver and molybdenum] have lifetimes of 2.7 ns[45] and ~500 ps,[14] respectively. The d^1 and d^9 open-shell porphyrins have quite different excited-state manifolds and as a consequence show much more rapid relaxation of their initially formed excited states ($\tau < 2$ ps) as well as more complicated decay routes for subsequently formed states.

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Registry No. $OMo(TPP)OCH_3$, 74751-79-4.

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Method for Determination of Phosphate Anion–Cation Association Constants from ^{31}P Chemical Shifts

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We report an experimental method that utilizes careful measurements of the changes in phosphorus-31 chemical shifts to determine association constants. Chemical shifts of phosphate monoanions and dianions, measured in the presence of cations and referenced to an internal standard, reflect chemical shift changes due to association and can be related quantitatively to the association constants. By this method we have obtained reliable values for dication–phosphate monoanion association constants; even the weak monocation–phosphate monoanion association constants have been measured. For example, the constants for association to dimethyl phosphate monoanion are 3.6 M^{-1} for Mg^{2+} and 0.29 M^{-1} for Na^+. Constants have been measured for the association of the dimethyl phosphate anions, the cAMP anion, the ethyl phosphate dianion, and the AMP dianion to Mg^{2+}, Ca^{2+}, Li^+, Na^+, K^+, and guanidinium ions.

Introduction

Due to the important biochemical roles of phosphates and polyphosphates and their bound metal ions, the interactions of phosphate anions and cations encompass a set of phenomena that are relevant to many biological processes.[1,2] The association of cations to phosphates in aqueous solution alters phosphate chemical properties, including their reactivity[2] and acidity.[3] It is becoming clear that metal and organic cations affect nucleic acid structures and, therefore, such nucleic acid functions as transcription and translation.[1] The salient features of the phosphate–cation complex structure with regard to all of these effects are the sites of coordination and the strengths of association of the cations. Cation–phosphate stability constants are therefore essential to a valid interpretation of biological and model studies.[3,4] In this paper, we report a method for the determination of previously unmeasured weak association constants. The method has even given approximate quantitative results for the weak association between mono-

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Table I. Variations in the ^{31}P NMR Chemical Shifts of AMP^{2-} with Increasing Concentrations of Mg^{2+} [a]

$[MgCl_2]$	$[(CH_3)_4NCl]$	$\delta_{AMP^{2-}}$	δ_{TMP}	δ_{obsd}	$\Delta\delta_{obsd}$	$\Delta\delta_{obsd}^{-1}$	$[Mg^{2+}]^{-1}$
0.000	0.200	5.713	5.051	0.662			
0.0200	0.160	5.518	5.047	0.471	0.191	5.24	50.0
0.0400	0.120	5.417	5.038	0.379	0.283	3.53	25.0
0.0500	0.100	5.385	5.038	0.347	0.315	3.17	20.0
0.0667	0.0667	5.342	5.032	0.310	0.352	2.84	15.0
0.100	0.0000	5.271	5.016	0.255	0.407	2.46	10.0

[a] $[Cl^-] = 0.200$ M, pH 8.5, [EGTA] = 0.10 mM, $T = 21 \pm 1$ °C, CO_2 free, 30% D_2O, and $[AMP^{2-}] = [TMP] = 10$ mM. Concentrations are given in M and chemical shifts in ppm. TMP is trimethyl phosphate.

cations and phosphate monoanions.

Although ^{31}P NMR was used early in the development of NMR spectroscopy to study the effects of ionization and salts on the ^{31}P chemical shifts of ATP,[5] other workers have apparently failed to use ^{31}P chemical shifts to measure stability constants. ^{31}P NMR studies have been conducted to determine the precise site of binding of cations to the polyphosphate chain of ATP, but these studies have only produced qualitative results.[6-8] The ^{31}P NMR chemical shifts of the α- and β-phosphates of ATP have also been used as a probe for measuring the intracellular concentrations of free Mg^{2+} in a variety of tissues utilizing known association constants.[9]

Van Wazer et al. observed the effect of monocations on the ^{31}P NMR chemical shift of various ethyl phosphates.[10] They observed that tetramethylammonium ions induced an upfield shift while sodium ions induced a downfield shift. These results were explained in an empirical fashion using eq 1, where ΔX°,

$$\Delta\delta = 180\Delta X_0 - 147\Delta n_\pi - A\Delta\theta \qquad (1)$$

Δn_π, and $\Delta\theta$ represent change engendered by cation coordination in the electronegativity of the phosphoryl oxygens, the degree to which phosphorus electrons occupy d_π orbitals, and the O–P–O bond angles, respectively. The effect of $(CH_3)_4N^+$ was thought to be the result of perturbations of solvation around the phosphate ions; in contrast, sodium ions complex directly to the phosphate anions.

Despite these and other studies of phosphates using ^{31}P NMR and the use of NMR with other nuclei to measure stability constants, the size and complexity of ^{31}P chemical shifts have apparently discouraged workers from using such measurements to determine association constants. Yet, the biochemical importance of association constants of phosphate anions and cations makes reliable values for weak association constants, which are difficult to determine by other methods, important. After many early difficulties in this research, we were able to reliably determine even the weak association constants for phosphate monoanions and monocations.

Experimental Section

Materials. Trimethyl phosphate was distilled through a 2-ft column at 197 °C. The barium salt of dimethyl phosphate was prepared according to the method of Haake and Westheimer.[12] Barium ethyl phosphate was prepared from commercial ethyl phosphoric acid and barium hydroxide. The barium salts were converted to the tetramethylammonium salts by ion-exchange chromatography.[5] Guanidinium chloride and tetramethylammonium chloride were purchased from Aldrich (95% and 97%, respectively) and recrystallized three times from an ethanol–water solution and dried under vacuum. All other materials were analyzed reagent grade.

Methods. Two stock solutions were made. Each contained 30% D_2O (made CO_2 free by bubbling Ascarite-treated N_2 through the solution for 30 min), 0.01 M trimethyl phosphate, 0.01 M phosphate diester or monoester, and 0.1 mM EGTA (ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid). The latter was added to avoid paramagnetic broadening of NMR signals. EGTA functions well in this role due to its greater affinity for paramagnetic cations than for Ca^{2+} or Mg^{2+}.[13] One stock solution contained the background electrolyte, tetramethylammonium chloride, and the other contained the chloride salt of the cation of interest. The pH of the stock solutions was adjusted with tetramethylammonium hydroxide.

Phosphorus-31 chemical shifts were recorded on a Varian XL-200 spectrometer after 32 transients. Wide-band proton noise decoupling was employed, and 30% ^2H_2O allowed heteronuclear field-frequency locking on deuterium. The resolution was 0.5 Hz or 0.006 ppm. Three spectra were recorded for each sample, and the chemical shifts were averaged. The temperature of the samples was measured to be 21 ± 1 °C after removal from the NMR probe.

If the concentration of cation–phosphate anion complex is represented by *C*, the total phosphate anion concentration is represented by *P*, and the total cation concentration, *M*, equals the free-cation concentration (because it is much larger than the total phosphate concentration), the association constant, *K*, is

$$K = \frac{C}{(P - C)M} = \frac{1}{(P/C - 1)M} \qquad (2)$$

Since only a single sharp resonance was found for any of the phosphates, exchange of the cation was fast on the NMR time scale. Therefore, the variable *P*/*C* can be related to chemical shift data:

$$\delta_{obsd} = \frac{C}{P}\delta_C + \frac{P - C}{P}\delta_P \qquad (3)$$

$$\frac{P}{C} = \frac{\delta_C - \delta_P}{\delta_{obsd} - \delta_P} = \frac{\Delta\delta_{max}}{\Delta\delta_{obsd}} \qquad (4)$$

where δ_C is the chemical shift of the complex, δ_P is the chemical shift of the uncomplexed anion, and δ_{obsd} is the observed chemical shift of the phosphate anion in reference to the standard. Therefore, substituting into the expression for *K* and rearranging give

$$\frac{1}{\Delta\delta_{obsd}} = \frac{1}{K\Delta\delta_{max}}\frac{1}{M} + \frac{1}{\Delta\delta_{max}} \qquad (5)$$

This is an equation of the form $y = mx + b$, where $1/\Delta\delta_{obsd}$ is a function of $1/M$. A set of data, $\Delta\delta_{obsd}$ at varying cation concentrations, was solved by a weighted least-squares program, where the weighting factor was M^{-2}. The calculated intercept divided by the calculated slope yielded *K*. Standard deviations are $(\sum(\text{deviations})^2/(N - 2))^{1/2}$.[16]

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Table II. Variations in the ^{31}P NMR Chemical Shifts of the Dimethyl Phosphate Monoanion, DMP$^-$, with Increasing Concentrations of Na$^+$ [a]

[NaCl]	[(CH$_3$)$_4$NCl]	δ_{DMP^-}	δ_{TMP}	δ_{obsd}	$\Delta\delta_{obsd}$	$\Delta\delta_{obsd}^{-1}$	[Na$^+$]$^{-1}$
0.000	2.00	4.804	5.094	0.290			
0.200	1.80	4.850	5.101	0.251	0.039	26	5.00
0.333	1.67	4.879	5.107	0.228	0.062	16	3.00
0.500	1.50	4.921	5.117	0.196	0.094	11	2.00
1.00	1.00	5.028	5.143	0.115	0.175	5.7	1.00
2.00	0.00	5.194	5.226	0.082	0.258	3.9	0.500

[a] [Cl$^-$] = 2.00 M, pH 7.0, [EGTA] = 0.10 mM, T = 21 ± 1 °C, CO$_2$ free 30% D$_2$O and [DMP$^-$] = [TMP] = 10 mM. Concentrations are given in M and chemical shifts in ppm. TMP is trimethyl phosphate.

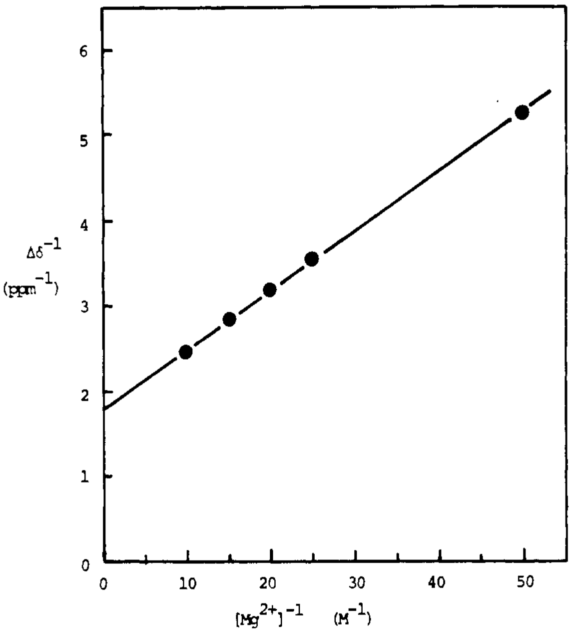

Figure 1. Plot of the data in Table I.

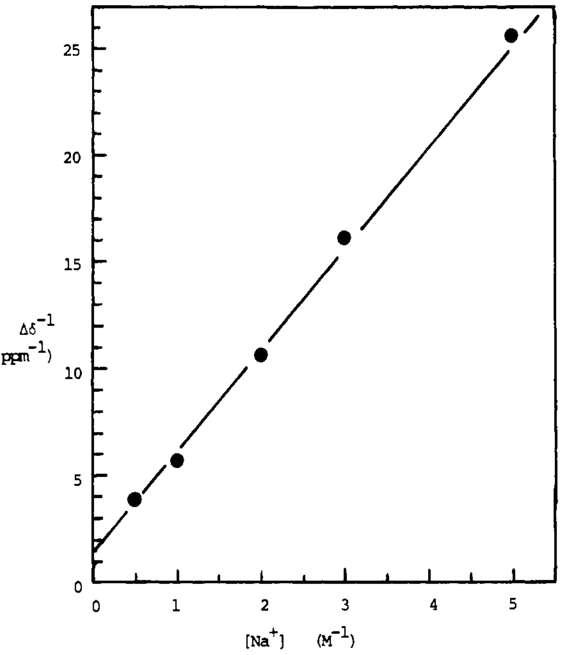

Figure 2. Plot of the data in Table II.

Table III. Data for Determination of the Association Constant for Mg^{2+} and AMP^{2-} according to Eq 6[a]

$M/\Delta\delta_{obsd} - P/\Delta\delta_{max}$, M ppm^{-1}	$(\Delta\delta_{max} - \Delta\delta_{obsd})^{-1}$, ppm^{-1}	$M/\Delta\delta_{obsd} - P/\Delta\delta_{max}$, M ppm^{-1}	$(\Delta\delta_{max} - \Delta\delta_{obsd})^{-1}$, ppm^{-1}
0.088	2.70	0.172	4.76
0.124	3.58	0.228	6.45
0.141	4.05		

[a] $\Delta\delta_{max}$ is taken from Figure 1, and other parameters are taken from Table I.

However, for the association of dications with phosphate dianions, experimental conditions mandated that the total cation concentration could not approximate the free-cation concentration. That is, association was strong enough that a significant proportion of the total cation concentration was the complex concentration. The limiting change in chemical shift ($\Delta\delta_{max}$) could still be determined as described above. The association constant is $K = C/(M - C)(P - C)$. Substituting and rearranging give an equation from which K may be obtained by least squares:[16]

$$\frac{1}{\Delta\delta_{max} - \Delta\delta_{obsd}} = K\left(\frac{M}{\Delta\delta_{obsd}} - \frac{P}{\Delta\delta_{max}}\right) \qquad (6)$$

Results

Tables I and II present typical data as obtained from the NMR experiments. The examples shown are data for association of Mg^{2+} to AMP^{2-} (Table I), for which a stability constant has been measured previously by another method,[18] and data for a previously unknown stability constant, that of the association of Na$^+$ to the dimethyl phosphate monoanion, DMP$^-$ (Table II). The analyses of the data in Tables I and II, as described in the Experimental Section, are given in Figures 1 and 2, respectively. Because association between Mg^{2+} and AMP^{2-} is relatively strong, an appreciable portion of the Mg^{2+} can be complexed at low Mg^{2+} concentration. For example (Table I), at 0.0200 M Mg^{2+} and with an assumed association constant of 25 M^{-1}, approximately 15% of the Mg^{2+} is complexed. Thus, the total [Mg^{2+}] is not the free [Mg^{2+}] and the slope of the double-reciprocal plot in Figure 1 is greater than it should be. This leads to an underestimation of the stability constant. However, the intercept is an extrapolation to infinite [Mg^{2+}] and is therefore free of this limitation. The true association constant is determined by eq 6. The data and corresponding graphical analysis are presented in Table III and Figure 3, respectively.

Table IV gives a compilation of the results of this NMR study. The calculated maximal changes in ^{31}P chemical shifts ($\Delta\delta_{max}$) and the association constants (K) are presented. Negative $\Delta\delta_{max}$ values indicate an upfield perturbation of chemical shift with binding, while positive $\Delta\delta_{max}$ values indicate a corresponding downfield change in chemical shift. The standard deviations for these determinations are the values within parentheses. The average of the standard deviations is about 13%. In addition, the $\Delta\delta_{max}$ and K values are often within experimental error of values for similar complexes. That is, for AMP^{2-}, for example, the $\Delta\delta_{max}$ and K values for association to metal monocations are all within experimental error of each other. Therefore, these values may only be accurate to one significant figure.

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Table IV. Results of the ^{31}P NMR Study[d]

		Mg^{2+}	Ca^{2+}	$C(NH_2)_3^+$	Li^+	Na^+	K^+
dimethyl phosphate anion (pH 7; [Cl^-] = 2.00 M)	$\Delta\delta_{max}$	−0.225 (0.002)	−0.14 (0.04)[a]	0.85 (0.07)	0.94 (0.08)	0.74 (0.09)	1.3 (0.3)
	K (M^{-1})	3.6 (0.1)	4 (1)	0.19 (0.02)	0.22 (0.02)	0.29 (0.02)	0.15 (0.03)
$cAMP^-$ (pH 7; [Cl^-] = 2.00 M)	$\Delta\delta_{max}$	−0.775 (0.006)	−0.162 (0.001)	1.05 (0.02)	2.7 (0.2)	3.1 (0.2)	1.8 (0.3)
	K (M^{-1})	0.82 (0.09)	3.66 (0.09)	0.221 (0.005)	0.081 (0.004)	0.076 (0.005)	0.11 (0.02)
ethyl phosphate dianion (pH 8.5; [Cl^-] = 0.200 M)	$\Delta\delta_{max}$	−0.518 (0.006)	−0.30 (0.01)[a]	0.20 (0.01)[b]	0.16 (0.01)[b]	0.20 (0.05)[b]	0.20 (0.03)[b]
	K (M^{-1})	39 (5)	32 (5)	10 (1.4)	3.5 (0.3)	2.2 (0.6)	2.0 (0.3)
AMP^{2-} (pH 8.5; [Cl^-] = 0.200 M)	$\Delta\delta_{max}$	−0.562 (0.002)	−0.3030 (0.0002)	0.20 (0.01)[b]	0.13 (0.01)[b]	0.15 (0.01)[b]	0.11 (0.05)[b]
	K (M^{-1})	27 (2)	25 (1)	12 (1)	4.0 (0.4)	3.5 (0.3)	5 (0.3)

[a] Some precipitate present in samples. [b] [Cl^-] = 1.00 M. [c] Guanidinium ion. [d] The concentration of the phosphate anion equals the concentration of trimethyl phosphate, which is 0.01 M, [EGTA] = 100 μM, [Cl^-] is kept constant with tetramethylammonium chloride, T = 21 ± 1 °C, 30% D_2O, CO_2 free. Values in parentheses are standard deviations. Negative values of $\Delta\delta$ indicate association engenders upfield perturbation of chemical shift. Positive $\Delta\delta_{max}$ values indicate a downfield change in chemical shift.

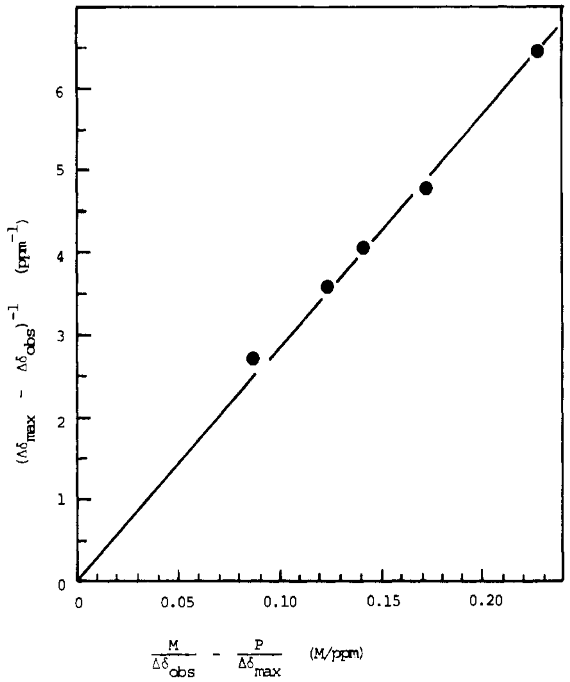

Figure 3. Plot of the data in Table III.

Table V. Some Literature Data on Cation-Phosphate Association Constants (M^{-1}) for Comparison with Table II (Standard Deviations in Parentheses)

	Mg^{2+}	Ca^{2+}	Li^+	Na^+	K^+
phosphate monoanion	6[a]	11.9 (0.6)[b]			
AMP^{2-}	34 (3)[c]	25 (2)[c]	4.1 (0.4)[d]	2.2 (0.2)[d]	1.8 (0.3)[d]

[a] Reference 5 ($(CH_3O)_2PO_2^-$). [b] Reference 6 ($H_2PO_4^-$). [c] Reference 19a. [d] Reference 19b.

The comparison of our association constants in Table IV and the results of other workers in Table V is quite good, especially for the AMP^{2-} data, where, except for the case of Mg^{2+}, the association constants are within experimental error of each other. It must also be noted that for their monocation–AMP^{2-} study, Smith and Alberty used tetraethylammonium chloride as the background electrolyte.[18] Furthermore, all the values of Smith and Alberty for AMP^{2-} are consistent with their extensive study of mono- and divalent cations and their associations to phosphate dianions and polyphosphates.

The phosphate monoanion values in Table V are representative of the sparse data for these complexes. The Mg^{2+} value, 6 M^{-1}, is for association to the dimethyl phosphate anion. It was measured by a competitive spectrophotometric technique based on the strong affinity of the dye 8-hydroxyquinolone for Mg^{2+}. No indication of experimental error was given by Kluger et al.[20] The Ca^{2+} value is for association to HPO_4^-.[21] In this experiment, the ionic strength varied and a very small range of concentrations was examined.

An important aspect of the experimental procedure that contributed to the accuracy of our NMR method was the use of an internal reference, trimethyl phosphate, a neutral water-soluble phosphate, possessing a ^{31}P chemical shift ~5 ppm downfield with respect to the deuterium-locked NMR

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signal. While the dipole moment of trimethyl phosphate may be strong enough to allow for some interaction with cations, the amount of association should be negligible in comparison to the anion–cation associations of interest. However, such an internal standard is subject to solvation effects. Therefore, chemical shift variations of the phosphate anions referenced to trimethyl phosphate depended solely upon the degree of association. In addition, the use of an internal standard compensated for small variations in the data due to artifacts introduced by the NMR spectrometer. NMR data for identical, but individually prepared, solutions obtained on different days show some variation. However, these differences are reduced to the level of spectrometer resolution when the chemical shifts are referenced to the trimethyl phosphate chemical shift.

Another aspect of the experimental procedure that increased accuracy was the use of EGTA (ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid) to reduce line broadening due to trace paramagnetic impurities. The EGTA stability constants are orders of magnitude higher for trivalent or divalent paramagnetic cations than for the diamagnetic cations studied.[13] An EGTA concentration of 0.1 mM was insufficient to significantly alter the effective diamagnetic cation concentrations but was found to be an effective concentration for elimination of paramagnetic line broadening.

Following the protocol of Sillēn,[17] we chose a constant chloride ion concentration maintained by the appropriate addition of tetramethylammonium chloride as the constant ionic medium in order to avoid drastic changes in activity coefficients. Neither the tetramethylammonium ion[18] nor the chloride ion[19] is considered to complex to phosphate or metal ions in aqueous solutions. The constant chloride ion concentration controlled cation activity. Although we did observe that high concentrations (1–4 M) of tetramethylammonium ions do engender upfield changes in phosphate anion chemical shifts, the magnitude of these changes are negligible when compared to the effects of the other cations studied under comparable conditions.

The change in the $\Delta\delta_{\text{obsd}}$ for association of Mg^{2+} with the dimethyl phosphate monoanion was small over a wide range of $(CH_3)_4N^+$ concentrations. For 1 M Mg^{2+} solutions in which Cl^- and $(CH_3)_4N^+$ varied 2–4 M and 0–2 M, respectively, $\Delta\delta_{\text{obsd}}$ only varied from 0.37 to 0.38 ppm. In contrast, for 1 M $(CH_3)_4N^+$ solutions in which Cl^- and Mg^{2+} varied 2–4 M and 0.5–2 M, respectively, $\Delta\delta_{\text{obsd}}$ varied from 0.29 to 0.43 ppm. Similarly, for a 1 M Na^+ solution in which both Cl^- and $(CH_3)_4N^+$ varied from 2 to 4 M, $\Delta\delta_{\text{obsd}}$ changed from 0.17 to 0.20 ppm. For 1 M $(CH_3)_4N^+$ in which both Cl^- and Na^+ varied from 2 to 4 M, $\Delta\delta_{\text{obsd}}$ varied from 0.17 to 0.40 ppm.

The two relevant observations to be made with respect to the data discussed above are the relative magnitudes and directions of the changes in chemical shifts in the various media. First, it is obvious that $(CH_3)_4N^+$ has a far smaller effect than even Na^+. Second, at higher $[(CH_3)_4N^+]$ there are greater upfield and downfield perturbations of chemical shifts in the presence of Mg^{2+} and Na^+, respectively. As already mentioned, $(CH_3)_4N^+$ alone engenders upfield changes in phosphate chemical shifts.[10] Therefore, one would expect increasing $(CH_3)_4N^+$ concentration to at least diminish the magnitude of the downfield change in chemical shift induced by the weakly associating sodium ion if $(CH_3)_4N^+$ does indeed specifically interact with phosphate anions. Since the chemical shift perturbations of both Mg^{2+} and Na^+ are enhanced by increasing $(CH_3)_4N^+$ concentrations, it appears that $(CH_3)_4N^+$ is relatively inert to phosphate anion complexation, as previously observed.[18]

These results indicate to us that as one drastically alters the ionic character of the NMR solutions at these high ionic strengths, the cause for the observed differences in chemical shifts in differing ionic media can be traced to changes in the fundamental properties of the solvent (these include ionic strength, dielectric constant, and the availability of waters of solvation, among others) rather than any specific interaction of Cl^- or $(CH_3)_4N^+$. These solvent effects are compensated for by holding the $[Cl^-]$ constant to keep cation activity coefficients relatively constant and referencing chemical shifts to an internal standard, trimethyl phosphate (see above).

Discussion

The factors that affect ^{31}P NMR chemical shifts include ionization,[11] cation complexation,[10] O–P–O bond angles,[23] torsional angles,[24] temperature,[25] and solvent.[26] Each of these effects can induce changes in chemical shifts on the order of parts per million. Van Wazer and Letcher concluded that the predominant electronic effects came from the phosphorus p-orbital occupation of the σ-bonds and from the phosphorus d-orbital occupation of the π-bonds. These factors were interrelated with the electronegativity of the ligands and the bond angles at phosphorus.[27] Empirically, however, ^{31}P chemical shifts appear to be anomalous; the well-documented effect of phosphate ionization causes a downfield shift of the ^{31}P resonance despite the fact that ionization must increase the electron density on the oxygens and phosphorus.[5]

Although all the monocations in Table II, as might be expected, cause a downfield shift in the ^{31}P chemical shifts of both mono- and dianionic phosphates, the tetramethylammonium ion, Mg^{2+}, and Ca^{2+} cause upfield shifts.

Empirically, it appears to us that the relation of chemical shifts to bond angles, as proposed by Gorenstein,[23,24] is most useful in understanding the chemical shifts of the phosphates that we have studied. Ionization of phosphates increases the electrostatic repulsion between oxygens and increases the bond angles. The same effect should be expected when a monocation forms an inner-sphere complex with a phosphate anion and disrupts the solvation to water molecules including the strong hydrogen bonds between the phosphorus oxygens and water molecules. Both of these phenomena cause downfield chemical shifts. In contrast, the hydrophobic effect of a tetramethylammonium ion should cause increased strength of hydrogen bonding in the solvation shell due to the iceberg effect,[28] a decrease in the O–P–O bond angles, and an upfield chemical shift, as observed. Dications have strong electrostatic interactions with two oxygens in a phosphate monoanion or two to three oxygens in a phosphate dianion. The electrostatic force can be expected to cause a compression of bond angles between the phosphate oxygens in agreement with the observed upfield shift. Evidence for this argument is found in the literature: crystallographic data show that for dication–monophosphate association bond angles decrease[29] and for monocation–monophosphate association and monophosphate ionization bond angles increase.[30,31] Finally, it should be noted that we had attempted to measure the association constant for Ba^{2+}–monoester and –diester complexes. Although solubility problems prevented the collection of enough data to make a

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reliable assessment of this association constant, we observed a downfield shift in contrast to Mg^{2+} and Ca^{2+}. The crystallographic literature predicts this also. Bond angles increase in Ba^{2+} salts.[32] Apparently, the greater cationic radius of Ba^{2+} (1.35 Å) as compared to the radii of Ca^{2+} and Mg^{2+} (0.99 and 0.65 Å) expands the O–P–O bond angle.

The association constants presented in Table IV are relatively small. They represent a difference of several kilocalories per mole between the unassociated and associated species. This results from association, not between naked ions but between strongly solvated species.[33,34] Therefore, association involves the replacement of several water molecules by one ion. The difference in the free energy changes between equilibria with equilibrium constants of 0.1 and 30, for example, is less than 3.5 kcal/mol. This indicates that the total solvation of the divalent ions is comparable in energy to the Coulombic attraction of divalent counterions. In this regard, it is interesting that our values in Table IV for dication–phosphate monoanion association are very similar to the values in Tables II and III for monocation–phosphate dianion association.

The previously unmeasured stability constants for association of monocations with the dimethyl phosphate monoanion and the cAMP monoanion appear to be within experimental error of 0.2 and 0.1 M^{-1}, respectively. These values are about an order of magnitude less than both monocation–dianion and dication–monoanion association constants which, in turn, are about an order of magnitude less than the dication–dianion association constants. The nucleoside phosphate, cAMP monoanion, has consistently lower metal cation association constants than the dimethyl phosphate monoanion and different $\Delta\delta_{\max}$ values. In contrast, the stability constants and $\Delta\delta_{\max}$ values of ethyl phosphate and AMP are nearly all within experimental error of each other. This is most likely due to some effect of the six-membered phosphate-containing ring. Possible causes include an abnormal O–P–O angle or the water molecule that is known to be bound to the 2′-hydroxyl and phosphoryl oxygens.[4]

The results of guanidinium ion association demonstrate little difference between guanidinium ion and metal cations. There is consistency of the $\Delta\delta_{\max}$ and K values for dimethyl phosphate and cAMP association. We previously measured the constant for association of the guanidinium ion to bis(nitrophenyl) phosphate and determined K to be 0.18 M^{-1} by a kinetic method.[35] This is in excellent agreement with our NMR results in Table II. Dianion association to the guanidinium ion shows an appreciable increase in affinity over that to the metal monocations. This is presumably due to the ability of guanidinium ions to hydrogen bond and thereby enhance the electrostatic interaction.

The results in Table IV indicate our NMR method is revealing inner-sphere complexation. This conclusion is drawn from the specific effects individual cations bring about. The most notable of these effects is the change in direction of the chemical shift incurred upon binding monocations vs. dications. The $\Delta\delta_{\max}$ values for dications are similar but very different from those for monocations. The relative K values also fit for changes in charges of the complexing ions as mentioned above. In fact, it seems likely that a major advantage of this method is the much larger effects of inner-sphere complexation over outer-sphere effects that may interfere in other methods for the determination of association constants.

Future applications of this method will include an examination of the sites of coordination of cations to nucleoside polyphosphates and a measurement of constants for association to polynucleotides.

Registry No. DMP$^-$, 7351-83-9; cAMP$^-$, 62906-31-4; AMP^{2-}, 6042-43-9; Mg, 7439-95-4; Ca, 7440-70-2; Li, 7439-93-2; Na, 7440-23-5; K, 7440-09-7; C(NH$_2$)$_3$$^+$, 25215-10-5; ethyl phosphate, 57919-10-5.

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Low-Frequency IR and Raman Spectra of Mn(CO)$_3$(η^5-C$_5$H$_5$) and Re(CO)$_3$(η^5-C$_5$H$_5$). Study of Dynamic Disorder

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Low-frequency Raman (5–200 cm^{-1}) and infrared spectra (20–200 cm^{-1}) of Mn(CO)$_3$(η^5-C$_5$H$_5$), Re(CO)$_3$(η^5-C$_5$H$_5$), and the deuterated (d_5) derivative of the Mn complex have been reported in the 5–300 K temperature range. A complete assignment for the low-frequency modes is proposed; their temperature dependence is discussed in terms of anharmonicity and vibrational dephasing associated with large amplitude motions. A potential barrier of 10 kJ mol^{-1} at 300 K is deduced from the torsional frequency; it is compared with the activation energy found by quasi-elastic neutron scattering and by NMR.

I. Introduction

This work has been undertaken as a part of a general study of the mechanism involved in the order–disorder phase transitions occurring in metallocenes and arene metal carbonyls.[1-8] From Raman and neutron scattering we have shown that aromatic rings are able to undergo jumps in the solid state and that the associated potential barrier is between 4 and 16 kJ mol^{-1} for most of these compounds; these values agree generally well with NMR results. We have recently used incoherent quasi-elastic neutron scattering (IQNS) to study the

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