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Association Phenomena. 5. Association of Cations with Nucleoside Di- and Triphosphates Studied by ^{31}P NMR[1]

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Changes in ^{31}P NMR chemical shifts have been evaluated for NAD and ADP as a function of concentration of Na^+ and Mg^{2+} at pH 4 and for ATP with Mg^{2+}, Ca^{2+}, Ba^{2+}, and Zn^{2+} at pH 4 and pH 8. Analysis of the data yields stability constants for the complexes to each phosphate moiety: e.g. for Mg^{2+}, K (M^{-1}) = 25 and 40 to NAD and 16 and 14 to ADP. Analysis of the chemical shifts yields structural information about coordination. There appears to be a decided difference in the mode of binding Mg^{2+} between NAD and ADP based upon the shifts in the ^{31}P NMR resonances: Mg^{2+} coordination to NAD causes upfield shifts, but coordination to ADP causes downfield shifts. The NMR studies of ATP indicate that the α-, β-, and γ-phosphate moieties are involved in 1:1 dication/ATP association.

We have been able to use ^{31}P chemical shifts to evaluate previously undetermined association constants between phosphate anions and cations—both metal cations and organic cations. By using a standard, trimethyl phosphate, which does not complex significantly with cations but does respond to media effects on the ^{31}P chemical shift, we have been able to gain sufficient accuracy to use this method for the determination of association constants. Comparison of the results with known association constants indicates that the method is valid.[1d] Because of the importance of metal complexing to nucleoside di- and triphosphates, we now report application of this method to NAD, ADP, and ATP.

The interaction of cations with nucleoside di- and triphosphates, with regard to the strength and structure of coordination and the effects on phosphate reactivity, is a subject of considerable interest and importance.[2] Phosphoryl transfer is one of the most common classes of biochemical reactions; these reactions are catalyzed by kinases that have a uniform requirement for ATP/ADP and metal cations, usually Mg^{2+}.

There is also intrinsic chemical interest in metal complexing with these phosphates because of the multiple coordination sites on the purine, ribose, and phosphate moieties of nucleotide phosphates. Szent-Gyorgyi first proposed that metal cations are chelated by the phosphate chain and the adenine ring of ATP.[3] Subsequently, it was concluded that transition-metal cations such as Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, and Mn^{2+} formed such "back-bound" structures while alkaline-earth cations did not.[4–6] Another structural form taken on by nucleoside triphosphates is the bis-cation complex. The first association constant of Mg^{2+} to ATP^{4-} has been reported to be 11 200 M^{-1}, and the second, 59 M^{-1}, and for Mg^{2+}/ADP^{3-} the first and second association constants have been reported as 1620 and 9 M^{-1}, respectively.[7] The bis-cation complex can therefore become a species of significant concentration if the ratio of Mg^{2+} to nucleotide is greater than 1; this was kept in mind in our experimental design. Another complexity in association involves nucleotide stacking;[6] the intermolecular forces between heterocycles can be augmented by cations that neutralize the negative charges on the phosphates, thereby reducing electrostatic repulsions.

Suitably referenced NMR chemical shifts of nuclei other than ^{31}P have been used to determine equilibrium constants,[8,9] but ^{31}P chemical shifts have not been used as the basis of a quantitative method despite the wide qualitative use of ^{31}P chemical shifts in studies of nucleoside phosphates.[10–13]

Experimental Section

Trimethyl phosphate was distilled through a 2-ft column at 197 °C. Tetramethylammonium chloride was recrystallized three times from ethanol/water and dried under vacuum. All other salts were analyzed reagent grade chemicals. Nucleotides were obtained from Aldrich or Sigma and stored desiccated and below 0 °C. The nucleotides were converted to the tetramethylammonium salts by ion-exchange chromatography.[10] Aldrich 98.8% D_2O was diluted to 30% D_2O, distilled, and made CO_2 free prior to use by degassing with ascarite-treated N_2 for 30 min.

Methods. For studies of nucleoside diphosphates, two stock solutions were made; each contained 0.01 M trimethyl phosphate, 0.01 M nucleoside diphosphate, 0.1 mM EGTA (ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid), and 30% D_2O. One stock solution contained the background electrolyte, tetramethylammonium chloride, and the other contained the salt of interest. The pHs of these solutions were adjusted with tetramethylammonium hydroxide.

Association to nucleoside triphosphates is much stronger than to the diphosphates. For these studies a stock solution containing the background electrolyte, tetramethylammonium chloride, was prepared as for the nucleoside diphosphate studies except that the concentrations of trimethyl phosphate and nucleoside triphosphate were much higher, 0.025–0.1 M. A second stock solution, 1.0 M in the cation of interest and adjusted to the desired pH, was also prepared. Small volumes of the cation solution, less than 50 μL, were added to 3.0 mL of the nucleotide triphosphate stock solution to make the samples for the NMR study. The concentrations of all nucleotides were determined spectrophotometrically at pH 7.0:[14] (1) for ATP at 259 nm, ϵ = 15 400 M^{-1} cm^{-1}; (2) for ADP

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at 259 nm, $\epsilon = 15400$ M^{-1} cm^{-1}; (3) for β-NAD_{ox} at 260 nm, $\epsilon = 17800$ M^{-1} cm^{-1}.

Phosphorus-31 chemical shifts were determined relative to trimethyl phosphate at 23 ± 2 °C (sample) and 81.0 MHz on a Varian XL-200 spectrometer after 32 transients. The pulse width was 10 μs with a 2.0-s delay between pulses. Wide-band proton noise decoupling was employed, and 30% ^2H_2O allowed heteronuclear field frequency locking on deuterium. Resolution was 0.5 Hz or 0.006 ppm. Three spectra were recorded for each sample and averaged. Negative δ values indicate upfield shifts.

Calculation of Association Constants.[13] Since each phosphate moiety of each nucleotide rendered a unique ^{31}P NMR resonance, the apparent association constant to each phosphate moiety could be calculated. For nucleoside diphosphates, the association constant, K, is

$$K = C/M(P - C) \text{ or } 1/KM = (P/C) - 1 \tag{1}$$

where P, C, and M are the concentrations of total phosphate, metal-complexed phosphate, and metal ion, respectively. Because M is considerably greater than C, M approximates $M - C$. Since only a single sharp resonance was found for any of the phosphates, exchange of the cation was fast on the NMR time scale as expected for these cations. Therefore, the variable P/C can be related to observed chemical shift data, δ_{obsd}, from a time-averaged equation:

$$\delta_{obsd} = \delta_C(C/P) + \delta_P(P - C)/P \tag{2}$$

or, rearranging

$$P/C = (\delta_C - \delta_P)/(\delta_{obsd} - \delta_P) = \Delta\delta_{max}/\Delta\delta_{obsd} \tag{3}$$

which, with eq 1, gives

$$1/\Delta\delta_{obsd} = (1/M)(1/K\Delta\delta_{max}) + 1/\Delta\delta_{max} \tag{4}$$

This is a linear equation where $1/\Delta\delta_{obsd}$ is a function of $1/M$. The $\Delta\delta_{obsd}$ values at varying cation concentrations were analyzed graphically and by a weighted least-squares program where the weighting factor was $1/M^2$.[15] Standard deviations are $[\sum dev/(N - 2)]^{1/2}$. Standard deviations for slopes, σ_{DSL}, and for intercepts, σ_{DIN}, were determined by the usual methods.[15] Because K = intercept/slope, the standard deviation for the association constant, σ_K, is[15]

$$\sigma_K = [(\sigma_{DIN}/\text{slope})^2 + (\text{intercept} \times \sigma_{DSL}/\text{slope}^2)^2]^{1/2} \tag{5}$$

Because association of metal ions to ATP is much stronger than association to the nucleoside diphosphates and because two metal ions can associate to one ATP,[7] a different method is needed to determine and calculate the association constants. Therefore, the concentration of ATP was designed to be significantly greater than the concentration of cation, so that

$$K = C/P(M - C) \text{ or } M = C[(1/KP) + 1] \tag{6}$$

Substituting $P\Delta\delta_{obsd}/\Delta\delta_{max}$ for C and rearranging yield

$$\Delta\delta = M\Delta\delta_{max}/(P + K^{-1}) \tag{7}$$

As predicted by eq 7, chemical shifts were found to be linear in M at constant P, giving the two unknowns, $\Delta\delta_{max}$ and K for a given P value. Similar experiments were performed at various ATP concentrations. A reciprocal plot (eq 8) of the slopes, m, obtained from eq 7 vs. the total

$$M = \Delta\delta_{max}/(P + K^{-1}) \text{ or } 1/M = P/\Delta\delta_{max} + (1/K\Delta\delta_{max}) \tag{8}$$

ATP concentration yields a new slope, $\Delta\delta_{max}^{-1}$ for a line with an intercept of $K\Delta\delta_{max}^{-1}$. K was determined by measuring $\Delta\delta$ at $P = M$ and substituting $\Delta\delta/\Delta\delta_{max}$ for C/P (from eq 3) in eq 9. Standard deviations

$$K = C/(M - C)(P - C) = C/(P - C)^2 = (C/P)/P(1 - C/P)^2 \tag{9}$$

were calculated as described above (eq 5).[13] Although this method leads to small changes in chemical shifts, the accuracy is sufficient to determine the K's in most cases although with less accuracy than in the method used for nucleoside diphosphates.

Table I. Association Constants for Metal Cations and Nucleoside Diphosphate Dianions Determined by ^{31}P NMR[a]

		β-NAD(M^{n+})				β-NAD(M^{n+})	
		$\Delta\delta_{max}$	K, M^{-1}			$\Delta\delta_{max}$	K, M^{-1}
P_1	Na^+	1.1 (0.1)	0.54 (0.06)	P_1	Mg^{2+}	−0.12 (0.03)	25 (8)
P_2	Na^+	1.7 (0.6)	0.3 (0.1)	P_2	Mg^{2+}	−0.06 (0.02)	40 (14)

		ADP(M^{n+})		ADP(M^{n+})$_2$	
		$\Delta\delta_{max}$	K, M^{-1}	$\Delta\delta_{max}$	K, M^{-1}
P_α	Na^+	1.8 (0.2)	0.52 (0.09)	not obsd	
P_β	Na^+	3.2 (0.6)	0.31 (0.07)	not obsd	
P_α	Mg^{2+}	0.137 (0.004)	16.2 (0.6)	2.8 (0.2)	0.69 (0.08)
P_β	Mg^{2+}	0.98 (0.05)	13.6 (0.9)	7.6 (0.1)	0.76 (0.02)

[a] The concentration of nucleoside diphosphates and trimethyl phosphate was 0.0111 M with [EGTA] = 0.1 mM, [Cl^-] = 0.500 M (held constant with $(CH_3)_4NCl$), pH 7, and T = 21 °C. See Experimental Section for method and other conditions.

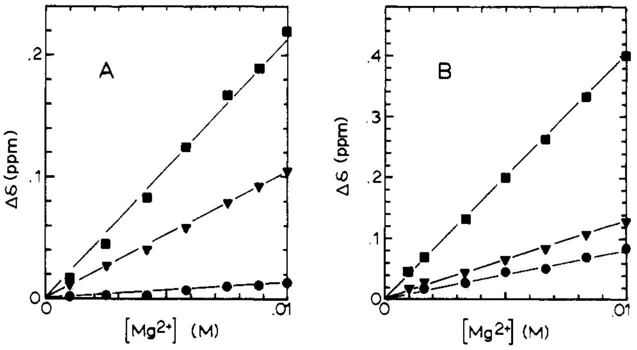

Figure 1. Plots of the change in ^{31}P NMR chemical shifts (ppm) of ATP at pH 4.0 (A) and ATP at pH 8.2 (B) with increasing concentration of Mg^{2+} (M). The concentrations of ATP and trimethyl phosphate were 0.09 M in A and 0.10 M in B. [Cl^-] was held to 0.100 M with $(CH_3)_4NCl$ and [EGTA] = 0.1 mM. T = 22 and 21 °C for A and B, respectively. The circles, squares, and triangles are the α-, β-, and γ-phosphates of ATP, respectively. Similar data were obtained at the other ATP concentrations shown in Figure 2.

Results

Diphosphates. The ^{31}P NMR resonances of each phosphate moiety of NAD and ADP were observed; chemical shifts, referenced to trimethyl phosphate, were used to determine the association constants according to eq 4. Table I presents the results of the nucleoside diphosphate dianion associations to Na^+ and Mg^{2+}. The association of Na^+ to both the NAD and ADP dianions yields stability constants that are surprisingly similar to those for the association of Na^+ to monophosphate monoanions: 0.3 and 0.5 vs. 0.29 M^{-1}.[1d]

The Mg^{2+} data show that association to the diphosphate dianion of NAD is very similar to Mg^{2+} association to the ethyl phosphate dianion of AMP^{2-} (the monophosphate association constants are 39 and 27 M^{-1}, respectively[1d]). Furthermore, the directions of the changes in the ^{31}P chemical shifts of NAD and the monophosphates are upfield, as are the chemical shift changes for Mg^{2+} complexes of monophosphates. In contrast, Mg^{2+} association to ADP yields lower stability constants and shows a downfield ^{31}P chemical shift.

The data for association of a second equivalent of Mg^{2+} to ADP are characterized by lower association constants than those of monophosphate monoanion/dication complexes and downfield shifts (Table I).

ATP. The α, β, and γ resonances of ATP^{3-} and ATP^{4-} were all observed to shift downfield with increasing cation concentration. Figure 1 displays typical data for metal dication association to ATP trianion (pH 4, Figure 1A) and tetraanion (pH 8.2, Figure 1B). Similar data were obtained at the other ATP concentrations shown in Figure 2, which displays the data according to eq 8: $1/M$ plotted against the ATP concentration. The inverses of the slopes of these plots yield the $\Delta\delta_{max}$ values that allow calculation of the association constants from eq 9. Tables II and III give the $\Delta\delta_{max}$ and stability constant values for the association of Mg^{2+}, Ca^{2+},

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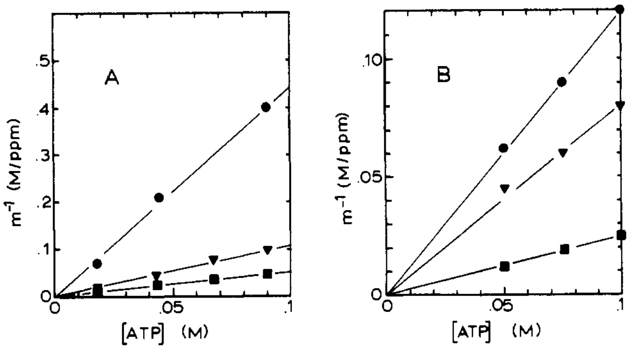

Figure 2. A plot of the inverse of slopes, m^{-1} (M/ppm), from plots of $\Delta\delta$ vs. [Mg^{2+}] (as in Figure 1) against varying [ATP] (M). A and B are the plots for ATP^{3-} and ATP^{4-}, respectively. The circles, squares, and triangles are the α-, β-, and γ-phosphates, respectively.

Table II. *K* and $\Delta\delta_{max}$ for Association of Metal Dications to ATP Trianion (See Figures 1 and 2 for Conditions)[a]

	Mg^{2+}	Ca^{2+}	Ba^{2+}	Zn^{2+}
		P_α		
$\Delta\delta_{max}$	0.22 (0.02)	0.29 (0.04)	0.48 (0.02)	0.53 (0.06)
K, M^{-1}	600 (200)	inadequate precision	280 (20)	600 (200)
		P_β		
$\Delta\delta_{max}$	1.92 (0.07)	2.0 (0.2)	1.9 (0.2)	2.9 (0.2)
K, M^{-1}	230 (30)	230 (80)	180 (30)	450 (30)
		P_γ		
$\Delta\delta_{max}$	0.92 (0.08)	0.89 (0.03)	1.0 (0.1)	2.2 (0.1)
K, M^{-1}	280 (50)	280 (20)	180 (30)	280 (40)
lit. *K*, M^{-1}	174 (4)[b] 31 (7)[c]	135 (3)[b] 41 (9)[c]	71 (2)[b]	468 (11)[b]

[a] Association constants determined from eq 9 and [ATP] = [metal dichloride] = 0.0110 M. [b] Taqui Khan, M. M.; Martell, A. E. *J. Phys. Chem.* **1962,** *66,* 10–15. [c] Smith, R. M.; Alberty, R. A. *J. Am. Chem. Soc.* **1956,** *78,* 2376–2380.

Table III. *K* and $\Delta\delta_{max}$ for Association of Metal Dications to the ATP Tetraanion (See Figures 1 and 2 for Conditions)

	Mg^{2+}	Ca^{2+}	Ba^{2+}	Zn^{2+}
		P_α		
$\Delta\delta_{max}$	0.83 (0.01)	0.7 (0.1)	0.71 (0.04)	0.71 (0.04)
K, M^{-1}	7000 (1000)			
		P_β		
$\Delta\delta_{max}$	4.0 (0.1)	3.3 (0.1)	3.0 (0.2)	3.8 (0.1)
K, M^{-1}	9000 (3000)			
		P_γ		
$\Delta\delta_{max}$	1.23 (0.08)	1.2 (0.2)	1.5 (0.2)	0.9 (0.2)
K, M^{-1}	4000 (1000)			

[a] Association constants determined under the same conditions except that [ATP] = [metal dichloride] = 0.0110 M.

Ba^{2+}, and Zn^{2+} to ATP^{3-} and ATP^{4-}, respectively. Note that the standard deviations are maximal (eq 5). For ATP^{4-}, the *K* values are not precise, but both *K* and $\Delta\delta_{max}$ are of interest. For ATP^{3-}, *K* values show good precision except for the results from the chemical shifts for the α-^{31}P.

The data presented in Tables II and III were analyzed on the assumption of a one-to-one interaction of cation and nucleotide because the concentrations of nucleotide never exceeded 0.1 M and the cation concentrations were less than 10% of the nucleotide concentration for the $\Delta\delta_{max}$ determinations and the association constants for a second coordination of a dication to NTP's are more than 2 orders of magnitude less than the first association constants.[6] Self-stacking may have some effect on the results. The equilibrium constant for the ATP^{4-} self-stacking is 1.3 ± 0.2 M^{-1}.[12] At most there might have been 15% stacking at 0.1 M ATP, but for most runs the ATP concentrations were lower so self-stacking was less than 10%. The association constants were determined when both nucleotide and cation concentrations were 0.01 M; at this concentration self-stacking is less than 1.5%. The results for ATP are in fair agreement with literature values (Tables II and III), indicating that the neglect of multiple associations and self-stacking is reasonable.

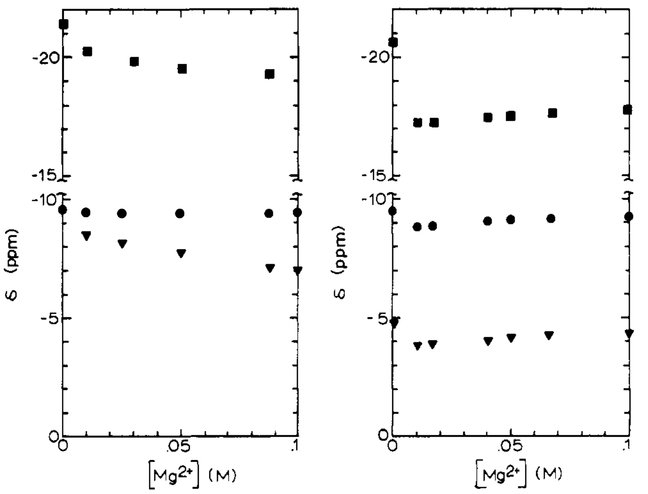

Figure 3. Plots of ATP ^{31}P NMR chemical shift (ppm) against [Mg^{2+}] (M). The circles, squares, and triangles are the α, β, and γ resonances, respectively. In plot A, pH is 4.1 and [Cl^-] = 0.200 M (held constant by addition of $(CH_3)_4NCl$). In plot B, pH is 8.0 and [Cl^-] = 0.400 M (held constant by addition of $(CH_3)_4NCl$). In both A and B the concentrations of ATP and trimethyl phosphate were 0.010 M, [EGTA] = 0.1 mM, and *T* = 22 °C. The chemical shifts are referenced internally to trimethyl phosphate, 4.933 and 4.942 ppm in A and B, respectively. See Experimental Section for further details.

In Figure 3 we report the results of changes in chemical shifts on adding enough Mg^{2+} to give association of a second Mg^{2+}; [ATP] was 0.01 M and [Mg^{2+}] ranged from 0.01 to 0.1 M in these experiments.

Discussion

One sharp ^{31}P resonance was observed for each phosphate moiety. This is consistent with the kinetics of association of phosphate anions to metal cations that have been studied and have been found to fit a two-step process: first, diffusion-controlled formation of an outer-sphere complex and, second, rate-limiting dissociation of water enabling inner-sphere coordination.[16] Both steps are very fast for the ions we have studied; therefore, observed association constants for these metal ions represent time averages of all possible coordination states in fast equilibrium.

Comparison with Association Constants from the Literature. In our previous paper,[1d] we reported association constants for monophosphates. Some had not been previously determined, but there was reasonable agreement between our association constants for dianions and those that had been done by titration and reported in the literature. Smith and Alberty reported an association constant of 28 ± 4 M^{-1} for Mg^{++} and ADP determined by titration.[17] Although this value and ours in Table I are close to agreement when one considers the standard deviations, this comparison and those for monophosphates[1d] support the expectation that our method generally gives lower values than those from titration because ^{31}P chemical shifts are sensitive only to direct-contact association whereas association constants determined by titration will include a blend of effects from direct-contact and solvent-separated association.

The last lines of Tables II and III give comparison values from the literature for ATP. Our values are in reasonable agreement

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(16) (a) Frey, C. M.; Stuehr, J. "Metal Ions in Biological Systems"; Sigel, H., Ed.; Marcel Dekker: New York, 1974; Vol. 1, pp 51–116. (b) Eigen, M.; Tamm, K. *Z. Elektrochem.* **1962,** *66,* 107–121.

(17) Smith, R. M.; Alberty, R. A. *J. Am. Chem. Soc.* **1956,** *78,* 2376–2380.

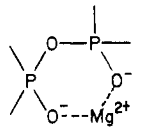
although the precision in our values is not as good as for diphosphates and monophosphates because of the special method that had to be used for ATP. These comparisons suggest that our ^{31}P NMR method is more accurate for lower values of association constants but not for determination of the large association constants for dications and ATP. However, by our method, one gets data for α-, β-, and γ-phosphates (Tables II and III), and this has useful consequences (see below).

Previous ^{31}P NMR Studies. Cohn and Hughes investigated ATP and its metal complexes by utilizing ^{31}P NMR and concluded, from qualitative data, that Mg^{2+}, Ca^{2+}, and Zn^{2+} bind to the β and γ positions of ATP.[10] Similar studies have subsequently concluded from ^{31}P NMR data that Mg^{2+} binds to the β-phosphate while others suggest α, β, and γ coordination.[18] Several papers have suggested that ^{31}P NMR is not sufficiently sensitive to determine the site of Mg^{2+} binding;[10-12] Gerlt et al. have recently summarized problems with ^{31}P chemical shifts.[19] However, Tables I–III, our previous paper,[1d] and the above discussion, demonstrate that our method can be used to determine quite accurate association constants. Chemical shift changes should be overwhelmingly due to direct interactions with the cations, whereas potentiometric methods[1a] would be expected to be affected by water-separated ionic interactions. Our method also is capable of quantifying very weak interactions: we were able to determine very low stability constants for monoanion–monocation association.[1d]

Cation-engendered ^{31}P chemical shifts have been rationalized by Van Wazer and Gorenstein, and both consider the effects of changes in O–P–O bond angles.[20,21] This approach contributed to our understanding of the chemical shifts of monophosphate mono- and dianions,[1d] and it appears to be supported by crystallographic data.[22-24] The contrasting effects of Na^+ and Mg^{2+} on NAD (Table I) could be explained in terms of the difference in effects on bond angles. But most of the $\Delta\delta$ values that we have observed are downfield shifts consistent with a major contribution to chemical shifts from polarization of electron density away from the phosphorus atom when phosphates associate with cations.

Evidence for Predominant Coordination Structures. Our studies on monophosphates demonstrated consistent upfield shifts upon metal dication coordination to both mono- and dianions.[1d] This observation was related to coordination to more than one oxygen atom on a given phosphate.[22-24] In contrast, in these studies on di- and triphosphates, the $\Delta\delta_{max}$ values (Tables I–III) are always downfield shifts with the exception of Mg^{2+} association with NAD.

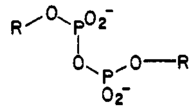
Since there is rapid ligand exchange for the cations we have studied, these chemical shift changes reflect a time average of the predominant coordination states. The downfield shifts for ADP and ATP indicate that each phosphate probably contributes only one oxygen atom to coordination in chelate rings.



It is extremely interesting that the association constants for the α-phosphate are roughly the same as for the β- and γ-phosphates of ATP (Tables II and III). Coordination to the α-phosphates could occur either by equilibrium between α,β and β,γ chelation or by α,β,γ coordination, which appears structurally possible.[10,18] It is also possible that the $\Delta\delta_{max}$ values for the α position are a secondary effect from β,γ coordination (Table II). However, the data in Table III for ATP tetraanion show that the $\Delta\delta_{max}$ values for the α position are similar to those for the γ position, so the tetraanion data indicate direct coordination to the α position.

Figure 3A,B presents the trend in ^{31}P chemical shifts when a second Mg^{2+} cation binds ATP^{3-} and ATP^{4-}, respectively. For ATP^{3-}, the α chemical shift remains unchanged, but the β and γ chemicals shifts continue their downfield progression. At 0.10 M Mg^{2+}, approximately 40–50% of ATP^{3-} is in the dication complex. This indicates that the site of coordination of the second Mg^{2+} cation is primarily at β and γ. In contrast to the ATP^{3-} data, the chemical shifts of the α-, β-, and γ-phosphates of ATP^{4-} (Figure 3B) change little—they are at slightly more upfield frequencies upon coordination to a second Mg^{2+}. Upfield perturbations of chemical shift are indicative of coordination to one phosphate moiety,[1d] so the simplest way to explain the chemical shifts (Figure 3B) would be a mixture of coordination geometries including some coordination to single phosphates of ATP by the two cations.

The upfield chemical shifts for NAD, in contrast to ADP, engendered by Mg^{2+} association lead to the conclusion that the Mg^{2+} cations must associate to only one phosphate moiety at a time. In the 1:1 crystalline complex of Rb^+ and ADP, each Rb^+ cation is chelated by two oxygens from each phosphate moiety of one ADP, and it is hydrogen bonded to the 2′-ribose hydroxyl and N_1 adenine nitrogen of an adjacent ADP molecule,[25] but in the 1:1 Li/NAD crystalline complex, Li^+ cations are associated with only one phosphate per NAD molecule.[26] This difference in the mode of binding between ADP and NAD might be explained by the following energetic contributions to the time average of structures: (1) an energy advantage for placing the two nucleosides and the two negative charges as far apart as possible, which would also separate the coordinating oxygens on the two phosphates (2) an entropy factor that favors the adenine and nicotinamide moieties to be as far apart as possible in order to allow all possible rotamers to be populated (the essence of increased entropy is that a molecule be able to populate many states of low energy; in a chelate in which both phosphates coordinate to a metal ion, although one can see that a conformer exists with low steric repulsions, other conformers may not be populated, thereby lowering the entropy and raising the free energy of that state); (3) coordination of the amide oxygen of the nicotinamide moiety and the phosphate alpha to the adenosine.



Our results may be useful in biochemical and intracellular observations of ^{31}P chemical shifts.[27] The structural evidence for Mg^{2+}–ATP and Mg^{2+}–ADP has revelance to kinases that have two domains separated by a cleft in which substrates bind.[28] Upon

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binding substrates, the two domains move toward each other. During these conformational events, the coordination site of Mg^{2+} could easily change. Spectroscopic and crystallographic results support such changes and differences in coordination as part of the catalytic action of kinase.[29-33]

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Registry No. NAD, 53-84-9; ADP, 58-64-0; ATP, 56-65-5; Na, 7440-23-5; Mg, 7439-95-4; Ca, 7440-70-2; Ba, 7440-39-3; Zn, 7440-66-6.

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Multinuclear Magnetic Resonance Study of Tris(cyclopentadienyl)scandium: A Fluxional Dimer with Bridging Cyclopentadienyl Groups

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$(C_5H_5)_3Sc$ is known to be associated via bridging cyclopentadienyl groups in the solid state, but hitherto nothing was known as to the nature of the compound in solution. ^1H NMR studies of the compound in THF show that it exhibits two fluxional processes. The first process observed on cooling is an exchange between the π- and σ-bonded rings. The second process, which can only be slowed and not completely stopped on the NMR time scale, is a metallotropic 1,5-shift of the Cp_2Sc moiety around the σ-bonded cyclopentadienyl ligand. The existence of only one species in THF, over the temperature range +30 to -80 °C is shown by ^{45}Sc NMR. In toluene, however, the exchange between σ- and π-bonded cyclopentadienyl ligands is complicated by the onset of dimer formation via bridging cyclopentadienyl groups at low temperature. The presence of two different scandium environments at low temperature is shown by variable-temperature ^{45}Sc NMR spectra.

In recent years the organometallic chemistry of the early transition metals has received considerable attention.[1] However, data in certain areas are still rather fragmentary. In the case of scandium, the widest group of compounds characterized are those in the $(C_5H_5)_2ScX$ series, where X is a monovalent ligand. In solution, when strongly coordinating solvents are present, monomeric species are formed.[2] However, in nonpolar solvents dimeric species are observed.[2a]

Crystallographic investigation of $(C_5H_5)_2ScCl$ and $(C_5H_5)_3Sc$ by Atwood has shown not only that $(C_5H_5)_2ScCl$ is dimeric[3] but also that$(C_5H_5)_3Sc$ is associated via bridging cyclopentadienyls in the solid state.[4] Indeed, the structure of $(C_5H_5)_3Sc$ is particularly interesting and can be interpreted in several ways. One possible description would be to regard the bridging cyclopentadienyl ligands as three-electron donors, i.e., σ bonding to one scandium and π bonding to the other as in the purported dimer (Figure 1a). An alternative, and somewhat extreme, view would be to take the dimer as a zwitterionic system (Figure 1b) comprised of $(C_5H_5)_2Sc^+$ and $(C_5H_5)_4Sc^-$ fragments. This unrealistically extreme view does, however, provide a formal analogy between the isoelectronic $(C_5H_5)_4Sc^-$ and $(C_5H_5)_4Ti$ systems; the relevance of such a comparison will shortly become apparent.

We now present some ^1H and ^{45}Sc NMR data on $(C_5H_5)_3Sc$ that give us some insight into the molecular dynamics of this interesting system.

Experimental Section

^1H NMR spectra were obtained on Bruker WM 400 and WM 250 spectrometers operating at 400 and 250 MHz, respectively. ^{45}Sc NMR spectra were obtained on Bruker WM 250 and WH 90 spectrometers operating at 60.735 and 21.964 MHz, respectively. Mass spectrometry was perfomed on a VG micromass 7070 F spectrometer equipped with a VG 2035 data system; the electron energy was 70 eV, and the ion source temperature was 200 °C.

The synthesis and manipulation of Cp_3Sc was carried out in an atmosphere of prepurified nitrogen with rigorous exclusion of air and moisture. Tetrahydrofuran was freshly distilled before use from sodium benzophenone ketyl.

Preparations. Anhydrous Scandium Trichloride. Anhydrous scandium trichloride was prepared by heating Sc_2O_3 (Nucor, Phoenix, AZ) with NH_4Cl in a furnace at 200 °C for 6 h. The excess NH_4Cl was removed by vacuum sublimation at 300 °C.

Cp_3Sc. To a suspension of anhydrous scandium trichloride (0.53 g, 3.5 mmol) in dry tetrahydrofuran (50 cm^3) was added freshly sublimed cyclopentadienylthallium (3.1 g, 11.5 mmol). The resulting mixture was refluxed for 3 h, allowed to cool, and filtered. The solvent was removed under vacuum and the resulting white solid pumped for several hours, to ensure complete removal of solvent. Data: ^1H NMR (C_6D_6) δ 6.3 (Cp); ^{45}Sc NMR (C_6D_6) δ 13.5 ($w_{1/2}$ = 280 Hz); mass spectrum (m/e (%)) 175 (Cp_2Sc^+, (100), 110 ($CpSc^+$, (20), 65 (Cp^+, 15).

Results and Discussion

In the solid state tris(cyclopentadienyl)scandium is associated via bridging cyclopentadienyl groups.[4] Comparison of the X-ray crystallographic data for $(C_5H_5)_3Sc$ and $(C_5H_5)_2Nb(C_2H_5)(C_2H_4)$[5] reveals structural parallels and lends credence to the proposition that in the solid-state structure the bridging cyclopentadienyl ligands can be regarded as three-electron donors.

In solution, however, the degree of association in $(C_5H_5)_3Sc$ had not been established. One might, therefore, envisage that, in a coordinating solvent such as tetrahydrofuran, the monomeric species $(C_5H_5)_3Sc(THF)$ would predominate whereas in a non-

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