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Use of Phosphorus Ligand NMR Probes To Investigate Electronic and Second-Sphere Solvent Effects in Ligand Substitution Reactions at Manganese(II) and Manganese(III)

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Manganese/ligand association dynamics were studied using a series of structurally related anionic phosphorus ester ligand probes [$CH_3OP(O)(X)(Y)^-$, where X = CH_3O, CH_3CH_2, or H and Y = O, S, or BH_3]. Reactions of the probe ions with $Mn(H_2O)_6^{2+}$ and a manganese(III) porphyrin ($Mn^{III}TMPyP^{5+}$) were studied in aqueous solution by paramagnetic ^{31}P NMR line-broadening techniques. A satisfactory linear free energy relationship for reactions of the probe ions with $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$ required consideration of both the basicity and solvent affinity of the probe ligands: $\log(k_{app}) = \log(k_0) + \alpha\, pK_a + \beta \log(K_{ext})$, where k_0, α, and β are metal complex dependent parameters and pK_a and K_{ext} represent the measured Brønsted acidity and water/*n*-butanol extraction constant for the probe anions, respectively. Reactions of $Mn(H_2O)_6^{2+}$ were relatively insensitive to changes in ligand basicity ($\alpha = -0.04$) and favored the more hydrophilic anions ($\beta = -0.54$). These observations are consistent with a dissociative ligand exchange mechanism wherein the outer-sphere complex is stabilized by hydrogen bonding between $Mn(H_2O)_6^{2+}$ and the incoming ligand. In contrast, reactions with $Mn^{III}TMPyP^{5+}$ are accelerated by decreases in both the basicity ($\alpha = -0.43$) and the hydrophilicity ($\beta = +0.97$) of the probe. We conclude that reactions of $Mn^{III}TMPyP^{5+}$ are also dissociative but that the aromatic groups of the porphyrin provide a hydrophobic environment surrounding the ligand binding site in $Mn^{III}TMPyP^{5+}$. Thus, the probe/water solvent interactions must be significantly weakened in order to form the outer-sphere complex that leads to ligand substitution. This work demonstrates the utility of phosphorus relaxation enhancement (PhoRE) techniques for characterizing the second coordination sphere environment of metal complexes leading to ligation and will allow comparison of the second coordination spheres of $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$ to those of other metal complexes.

Introduction

The ^{31}P NMR spectra of certain phosphorus ester anions are remarkably sensitive to the presence of paramagnetic metal ions. The sensitivity of methyl phosphite (MeOPH; Figure 1) toward NMR relaxation by $Mn(H_2O)_6^{2+}$, but not by RNA-bound Mn^{2+}, has recently been exploited to study metal and ligand binding by RNA.[1] We have reported that the extent of paramagnetic broadening of the ^{31}P resonances of MeOPH and methyl ethyl phosphate (MEP) anions by either $Mn(H_2O)_6^{2+}$ ion or a water-soluble manganese(III) porphyrin ($Mn^{III}TMPyP^{5+}$; Figure 1) is determined by the rate at which the probe ions enter the first coordination sphere of Mn.[2] We are building on this observation to develop phosphorus relaxation enhancement (PhoRE) methods to

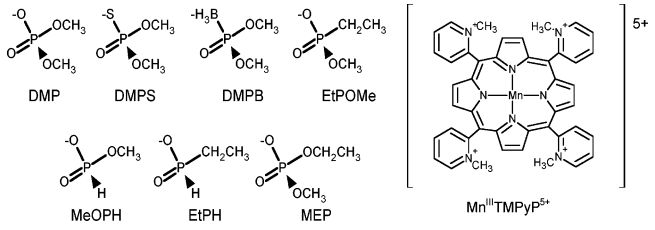

Figure 1. Structural formulas of dimethyl phosphate (DMP), dimethyl thiophosphate (DMPS), dimethyl boranophosphate (DMPB), methyl ethylphosphonate (EtPOMe), methyl phosphite (MeOPH), ethyl phosphonite (EtPH), and $Mn^{III}TMPyP^{5+}$.

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characterize reactivities of metal complexes. Such methods might be used to study other metal complexes, including those of biopolymers and industrial catalysts. Many studies have exploited relaxation enhancement by parmagnetic metals at the active sites of metalloproteins to study the complexes formed between enzymes and their substrates and substrate analogues. Mildvan and co-workers[3] made extensive use of ^{31}P NMR relaxation methods to study the interactions of Mn-containing proteins with natural product phosphates (such as ATP) and analogue molecules. We hypothesize that kinetic behaviors of important metal complexes might be characterized by determining the sensitivities of their ligand exchange kinetics to systematic changes in the structures of the incoming ligand. In this report we present the results of our first such study, aimed at determining how ligand exchange rates respond to systematic changes in the electronic structures of the incoming ligand.

To study the effects of ligand electronic structure on metal/ligand exchange kinetics, we prepared a series of structurally related probe ions (Figure 1) and measured the rates of their interactions with $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$. These probes are structurally related to phosphate diesters but have groups with varying electronegativity bound to phosphorus. These probes were designed to be as similar to each other as possible in steric profile, and each probe is monoanionic. We anticipated that differences in probe ion reactivities with a given metal complex would reflect the effects of changes in the electronic structure of the probe. We also anticipated that differences in probe ion reactivities toward different metal complexes would correlate with the differences in electronic structures of the metal complexes.

Since our series of ligand probes varies widely in nucleophilicity and solvent affinity, we anticipated this would allow us to characterize the associative/dissociative character and second coordination shell effects of ligand exchange reactions with different metal complexes. Metal/ligand exchange reactions in general proceed by a two-step mechanism involving initial formation of an outer-sphere complex characterized by the equilibrium constant K_{OS},

$$PO^- + (H_2O)ML'_N \overset{K_{OS}}{\rightleftharpoons} \{PO^-\cdot(H_2O)ML'_N\} \xrightarrow{k_1} H_2O + (PO)ML'_N \quad (1)$$

where PO^- represents the probe ligand, $(H_2O)ML'_N$ the metal complex substrate, $\{PO^-\cdot(H_2O)ML'_N\}$ an encounter complex in which the entering ligand is in the second coordination shell of the metal complex, and $(PO)ML'_N$ the first coordination shell ligand-substituted product.[4] While ligand exchange at six coordinate divalent metal ions can proceed via a dissociative or associative mechanism, the activation volume for water exchange at $Mn(H_2O)_6^{2+}$ indicates an associative interchange (I_a) mechanism.[5] If aquo displacement reactions by these ligand probes display associative character, then k_1 in eq 1 should increase with probe nucleophilicity (which can be related to ligand basicity). Thus, a positive correlation between $\log(k_1)$ and ligand pK_a would suggest a mechanism with an associative character while the lack of such a correlation would suggest a more dissociative mechanism.[6]

Measurements of ligand exchange kinetics at paramagnetic metal ions commonly employ NMR relaxation techniques.[7,8] When transverse (T_2) NMR relaxation of the bound ligand is more rapid than dissociation of the metal/probe complex, then the apparent second-order rate constant (k_{app}) for the forward reaction in eq 1 can be determined from the effect of metal concentration on line width of the probe ion resonance, eq 2:[9]

$$\pi(\Delta\nu_{obs} - \Delta\nu_{dia}) = k_{app}[M] = K_{OS}k_1[M] \quad (2)$$

Here $\Delta\nu_{obs}$ and $\Delta\nu_{dia}$ represent the full line widths at half-height of the observed ^{31}P resonance of the probe ion in the presence and absence of the metal complex, [M] is the metal complex concentration, K_{OS} is the outer-sphere association constant, and k_1 is the rate constant for a single ligand exchange between the outer and inner sphere.[10]

In this report we describe how structural variations in phosphorus esters and phosphonates affect the rates of their reactions with $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$ in aqueous solution and how differences in these rates correlate with differences in the probe ion Brønsted basicity and their interactions with aqueous solvent. We discuss how the sensitivities of the reaction rates correlate with differences in the molecular environments surrounding the metal ions in the two complexes and how the environments surrounding the metal ions of other complexes can, in the future, be compared to the environments of $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$ using this set of ligand probes.

Experimental Section

Materials. Salts of DMP, DMPS, DMPB, and MeOPH were prepared from dimethyl phosphite (Aldrich). DMP was prepared by oxidation with aqueous sodium hypochlorite.[2] DMPB[11] and MEP[2] were prepared as described previously. Synthesis of DMPS

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followed the procedure of Seela and Kretschmer.[12] The EtPOMe anion was prepared by treating dichloroethyl phosphite with methanol to give the dimethyl ester which underwent aerobic oxidation to give dimethyl ethylphosphonate, $(CH_3CH_2)P(O)(OCH_3)_2$. The anionic monoester was prepared by hydrolysis of this product with *tert*-butylamine. EtPH was prepared by hydrolysis of ethyldichlorophosphine. Samples were purified by crystallization as their triethylammonium salts. Samples for spectroscopy were prepared as the sodium salts from triethylammonium salts by deprotonation with NaOH followed by evaporation of the resulting solution en vacuo. Probe ion solutions were identified and determined to be pure by FAB-MS and by ^{1}H and ^{31}P NMR. $MnSO_4$ and tetramethylphosphonium chloride (Me_4PCl) were obtained from Aldrich, and $Mn^{III}TMPyP^{5+}$ was obtained as a gift from Irwin Fridovich (Duke University).

Paramagnetic Line Broadening of Probe Ion ^{31}P Resonances. The effects of $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$ on probe ion ^{31}P NMR line widths were determined for samples containing from two to four probe ions and a Me_4PCl reference in sodium acetate/acetic acid buffered D_2O. By measurement of the effects of the metal complexes on multiple probes in the same solution, kinetic parameters for the probe ions in each experiment were determined under identical conditions (pH, temperature, metal complex concentration, etc). Line broadening was independent of probe concentration (and, hence, ionic strength) over the entire range of concentrations used (~5−300 mM).[13] We note that ionic strengths of solutions used in our line-broadening measurements fall well within this range. The reference was added to ensure that line width data were not influenced by inconsistent shimming. NMR spectra were recorded using a Varian Inova 400 MHz NMR spectrometer. With the exception of DMPB, probe concentrations were 5−10 mM. Because of its inherently broad ^{31}P NMR lines, DMPB concentrations of 20−50 mM were required for satisfactory signal-to-noise. Total probe ion concentrations were maintained below 70 mM to ensure that the major metal containing species was not complexed by the probe ion.[13] Manganese complex concentrations were chosen to give line broadenings of between 4 and 100 Hz and were typically in the range of between 5 and 250 μM. We employed line broadening instead of pulse echo methods because the former can be made in a much shorter time. Although the CPMG technique is unquestionably more accurate for measuring T_2 values in diamagnetic systems with sharp lines, this is not necessarily the case for the systems reported here, where the T_2 of the observed nucleus can be adjusted to a range of maximum accuracy by addition of the proper amount of paramagnetic agent. A minimum of three metal concentrations was used to determine slopes of the $\Delta\nu$ versus [M] plots. Samples of $Mn(H_2O)_6^{2+}$ were prepared by dilution of an atomic absorption standard. The $Mn^{III}TMPyP^{5+}$ concentrations were measured spectrophotometrically.

Probe Ion Basicity. The relative Brønsted basicity of the probe ions were determined in D_2O solution by studying the effects of acid on their ^{31}P NMR resonant frequencies. While the values of acid dissociation constants for the probes measured in D_2O are expected to be different from values measured in H_2O, each D_2O pK_a is expected to shift from its value in H_2O by the same amount.[14] Thus, differences between pK_a values of the various probe ions are expected to be independent of whether they were measured in D_2O or in H_2O. Samples of selected probe ions and the reference, Me_4PCl, were prepared in 0.2 N NaOD, 6 N DCl, and neutral D_2O solutions. These samples were used to prepare solutions with varying pH, and their ^{31}P NMR spectra were recorded. Equilibrium positions were calculated from observed chemical shifts (δ_{obs}) according to

$$[A^-]/[HA] = (\delta_{obs} - \delta_{HA})/(\delta_A - \delta_{obs}) \quad (3)$$

where A^- and HA represent the phosphorus anion and conjugate acid, and δ_A and δ_{HA} the chemical shifts of the phosphorus anion and conjugate acid. For the DMP, MeOPH, MEP, and DMPB ions, pH measurements were made at two different probe ion concentrations; in the two experiments analyte concentrations either ranged from 0.14 to 0.49 mM or from 2.1 to 6.0 mM. Plots of $[HA]/[A^-]$ (eq 3) for DMP versus the same function for each of the analogues were linear with a slope equal to the ratio of the two protonation constants.[15] Protonation constants for the DMPS, DMPB, and MeOPH anions were calculated from the slopes of these plots, using the literature value (1.29) for the pK_a of DMP.[16] For the less acidic probes, samples of EtPOMe (7 mM), EtPH (7 mM), and DMPB (36 mM) were prepared and the pH of the solutions were calculated from shifts in the resonance frequency of DMPB and its pK_a value, which was determined as described above.

Extraction Coefficients. Equilibrium constants for partitioning the probe ions between D_2O and *n*-butanol solutions (defined as $K_{ext} = [A]_{buOH}/[A]_{D_2O}$, where $[A]_{buOH}$ and $[A]_{D_2O}$ represent the concentration of the anion probe in butanol and aqueous phases, respectively) were determined as follows. Stock aqueous and butanol solutions were equilibrated prior to addition of the probe ion(s) by shaking an acetate-buffered D_2O solution of tetraethylammonium chloride (45 mL) with 1.5 L of D_2O-saturated butanol solution at 29 C. After 24 h, the aqueous phase contained 225 mM Et_4N^+ and 30 mM acetate, pH 5.3. The pH was determined from the chemical shift of the acetate ^{1}H resonance. In a typical measurement, a weighed sample of a probe ion sodium salt was dissolved in 5 mL of the stock D_2O solution and the pH was adjusted to 5.3. For selected experiments, a single probe ion (DMP, DMPS, or EtPOMe) was used. In others, mixtures of probes (either DMPB and DMPS or DMP, EtPH, and EtPOMe) were extracted. Values from the two types of experiments are in good agreement. In each experiment, the total initial probe ion concentration was approximately 50 mM. Aliquots of the probe containing solution (1 mL) were shaken for 24 h at 29 °C with differing amounts of the butanol solution (ranging from 2 to 64 mL) before they were removed for analysis. Changes in probe concentration were determined by comparison of the ^{31}P NMR peak integrations to that of an external reference in a concentric NMR tube.

Results and Discussion

The ^{31}P NMR line width of each probe ion (but not that of the Me_4P^+ reference ion) was very sensitive to the presence of both $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$. These

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(13) Line widths were independent of probe ion concentrations below 300 mM. The independence of line broadening on probe ion concentration indicates that a negligible fraction of the ligand probe is bound to the metal complex and that the major metal-containing species in these solutions were not complexed by the probe ions under the conditions of the study. Equilibrium binding constants were found to be less than 3 M^{-1} for any combination of metal complex and probe ions employed. These data will be published elsewhere.

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Table 1. Protonation, Ligand Exchange Kinetics, and Partitioning Data for the PhoRE Probes

species	param	DMPB	MEP[a]	DMP	DMPS	MeOPH[a]	EtPOMe	EtPH	H_2O[b]
H^+	pK_a[c]	1.71	1.40	1.29	0.80	0.86	2.44	2.71	
$Mn(H_2O)_6^{2+}$	$10^{-6}k_{app}$ ($M^{-1}s^{-1}$)[d]	1.3	3.9	3.9	2.8	5.8	3.0	3.9	21[e]
	ΔH^* (kcal/mol)[d]	10.5		9.4	10.7	11.3	9.7	9.9	7.9[e]
$Mn^{III}TMPyP^{5+}$	$10^{-6}k_{app}$ ($M^{-1}s^{-1}$)[d]	5.8	1.2	1.4	9.1	2.1	0.41	0.98	
	ΔH^* (kcal/mol)[d]	9.0		7.6	6.5	8.7	10.0	9.1	
butanol	K_{ext}[f]	0.305		0.035	0.124	0.043	0.050	0.074	

[a] Data from ref 2. [b] Rate parameters for water exchange. [c] Equilibrium constants were determined from the pH dependence of ^{31}P chemical shifts referenced to the literature value for the pK_a of dimethyl phosphate;[16] see Experimental Section. [d] Experimetally determined rate constant and enthalpy of activation for aquo ligand substitution (eq 1). [e] Data from ref 5. [f] Equilibrium constant for extraction of probe ion from water into *n*-butanol; see Experimental Section.

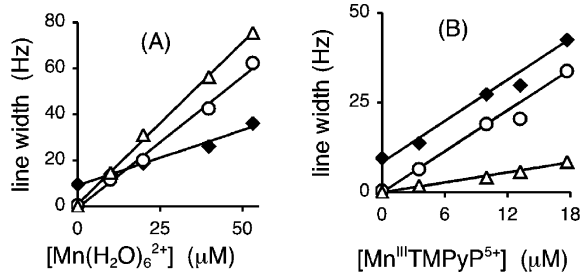

Figure 2. Effects of metal complexes on ^{31}P NMR line widths dependent on the identities of both the anion and manganese complex: (A) influence of $[Mn(H_2O)_6^{2+}]$ concentration on probe ^{31}P line width; (B) influence of $[Mn^{III}TMPyP^{5+}]$ concentration on probe ^{31}P line width. Key: DMPB (filled diamonds); DMPS (open circles); DMP (open triangles).

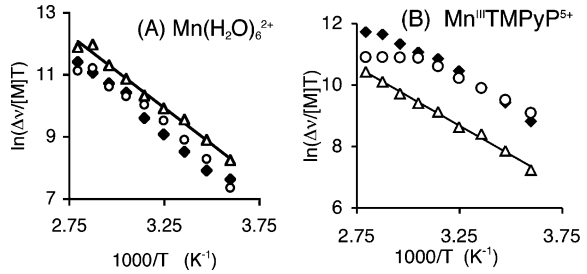

Figure 3. Temperature dependence of probe ion ^{31}P NMR line broadening by (A) $Mn(H_2O)_6^{2+}$ and (B) $Mn^{III}TMPyP^{5+}$. Filled diamonds, open circles, and open triangles represent the resonances of DMPB, DMPS, and DMP, respectively. The lines represent the least-squares fits to the DMP data.

results are indicative of a case where efficient relaxation requires coordination of the probe ion to the metal center (eq 1). A solution of DMPB, DMPS, and DMP in 40 μM $Mn(H_2O)_6^{2+}$ at pH 5.3 and 25 °C gave data that are representative of these experiments (data not shown). Under these conditions, the ^{31}P resonances of DMPB, DMPS, Me_4P^+, and DMP are broadened by 16, 42, 0, and 56 Hz, respectively, when compared to their line widths in diamagnetic solutions. The extent of paramagnetic broadening was proportional to the manganese complex concentration (Figure 2A,B) and independent of ligand concentration under the conditions of this study.

The temperature dependence of the paramagnetic relaxation enhancement indicates that relaxation was governed by kinetic factors (eq 2) and not by thermodynamics or ligand/metal complex geometry. The ^{31}P line broadening of each anion by either $Mn(H_2O)_6^{2+}$ or $Mn^{III}TMPyP^{5+}$ increased with increasing temperature (Figure 3). For the majority of the probe ion/metal complex pairs, the temperature dependence of the line broadening obeyed Arrhenius behavior over most of the observed range. Arrhenius temperature dependence is widely accepted as indicating that transverse relaxation is limited by chemical association kinetics and that line broadening is governed by eq 2.[7] For three of the probes (DMPS, DMPB, and MeOPH), plots of $\ln(\Delta\nu/T)$ versus $1/T$ with $Mn^{III}TMPyP^{5+}$ show significant deviation from linearity at the higher temperatures. This deviation was most pronounced for line broadening of DMPS (open circles in Figure 3) and is indicative of a switch to an intermediate kinetic regime where relaxation is governed by a combination of thermodynamic and kinetic terms.[7] Consistent with the results of our earlier study,[2] Eyring plots for broadening of the probe ^{31}P lines by $Mn(H_2O)_6^{2+}$ were linear over a greater temperature range than broadening by $Mn^{III}TMPyP^{5+}$.

Rate constants for aquo ligand displacement by the probe ions (eq 1) at 25 °C and activation enthalpies (proportional to the slopes of linear portions of Eyring plots; Figure 3) are similar to those we reported for MeOPH and MEP[2] (Table 1). Reactivity at $Mn(H_2O)_6^{2+}$ varies by a factor of ~4.5 and at $Mn^{III}TMPyP^{5+}$ by a factor of ~22. While the differences in rate constants are relatively small, the sensitivity of the PhoRE technique to these differences is substantial. To illustrate, consider the data presented in Figure 2, which show a change in the order of reactivity of the probe ions at the two metal complexes. $Mn(H_2O)_6^{2+}$ selectively affects the resonances of DMP (open triangles) and DMPS (open circles) over that of DMPB (filled diamonds). In contrast, $Mn^{III}TMPyP^{5+}$ selectively affects DMPB and DMPS over DMP. At $Mn(H_2O)_6^{2+}$, the rate of water displacement by DMPB is 0.33 times the rate of displacement by DMP; at $Mn^{III}TMPyP^{5+}$, the reactivity of DMPB is 4.1-fold greater than that of DMP. While these differences are small, they are none the less real, and the sensitivity of the technique is sufficient to measure them.

Activation enthalpies for probe ligand reaction with $Mn(H_2O)_6^{2+}$ averaged 10.1 kcal/mol with a standard deviation (σ = 0.6 kcal/mol) that reflects the precision of the measurement (Table 1). Excluding the DMPS data,[17] activation enthalpies for reaction with $Mn^{III}TMPyP^{5+}$ were 8.9 kcal/mol (σ = 0.9 kcal/mol) (Table 1). This agreement further supports the contention that phosphorus T_2 relaxation rates

(17) While the activation enthalpy for DMPS/$Mn^{III}TMPyP^{5+}$ line broadening (6.5 kcal/mol) is well below what we would expect on the basis of the other data, the temperature-dependent behavior of the data was reproducible. The anomalously low result could arise from a difference in the ground state or transition state energetics or could be an artifact of the measurement. It is possible that the system is in the intermediate kinetic regime over a greater temperature range than expected but that curvature of the Eyring plot is masked by an unforeseen effect (such as an unexpected temperature dependence of the electronic relaxation time). If the latter case holds, then the value of the 25 °C rate constant we report would be low.

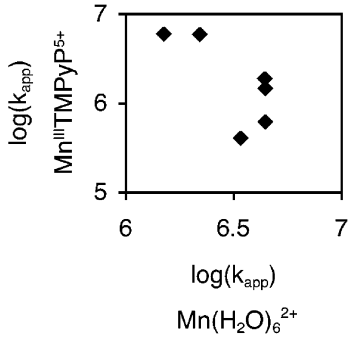

Figure 4. Rate constant data for displacement of water by different probe ions at $Mn^{III}TMPyP^{5+}$ and $Mn(H_2O)_6^{2+}$.

for each of the ions are equal to their respective rates of metal association. While rates of water exchange at manganese(III) porphyrins have been reported,[18] the interpretation of these data has been called into question.[19]

Our results require that at least two independent properties of the ligand contribute to differences in their reactivities toward these two manganese complexes. This condition leads to a linear free energy relationship (LFER) of the form shown as

$$\log(k_{app}) = \log(k_0) + \alpha f(\mathrm{PO}) + \beta g(\mathrm{PO}) \qquad (4)$$

where k_{app} is the experimentally measured rate constant (eq 2), k_0 is a standard rate constant (calculated for the hypothetical case where f(PO) and g(PO) are both zero), f(PO) and g(PO) are properties of the anion probe, and α and β are properties of the metal complexes that indicate the sensitivities of their reaction rates to changes in f(PO) and g(PO). If ligand exchange at the two metal complexes were governed by a single probe ion dependent variable (as in eq 5), then a plot of $\log(k_{app})$ for $Mn(H_2O)_6^{2+}$ versus $\log(k_{app})$ for $Mn^{III}TMPyP^{5+}$ would necessarily be linear.

$$\log(k_{app}) = \log(k_0) + \alpha f(\mathrm{PO}) \qquad (5)$$

The nonlinearity of such a plot (shown in Figure 4) requires the more complex LFER (eq 4) to account for differences in metal association kinetics.

Given the multifactorial influences on the reaction rates, the problem becomes (1) identifying properties of the probe ions that might reasonably influence association kinetics, (2) measuring these properties for each of the probe ions, and (3) demonstrating the correlation. In addition, all our assertions must be consistent with the known and expected chemistries of the systems. We find that a combination of ligand probe pK_a and hydrophilicity fulfill these criteria.

The Brønsted basicities of the probe ions in aqueous solution were compared by monitoring the effects of acid concentration on their ^{31}P NMR spectra. Each probe is monoanionic at neutral pH and is protonated in acid. Figure 5 shows the effects of pH on the resonance frequencies of

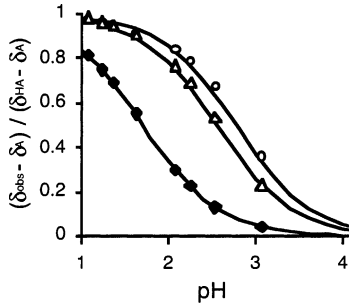

Figure 5. Relative Brønsted basicities of DMPB (filled diamonds), EtPH (open circles), and EtPOMe (open triangles) determined from the effects of acid on their ^{31}P resonance frequencies. The symbols δ_{obs}, δ_A, and δ_{HA} represent the observed chemical shift and those of the phosphorus anion and its conjugate acid, respectively. Solution pH was determined from the chemical shift of the DMPB resonance using the pK_a value in Table 1 (determined by the same procedure using the literature value, 1.29 for the pK_a of DMP[16]). The solid lines represent behaviors predicted using the pK_a values in Table 1.

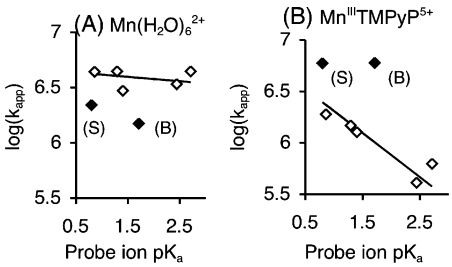

Figure 6. Plot of observed metal/probe ion association rate constants as a function of probe ion pK_A for (A) $Mn(H_2O)_6^{2+}$ and (B) for $Mn^{III}TMPyP^{5+}$. Filled symbols labeled S and B represent data for DMPS and DMPB, respectively. Slopes of solid lines are equal to pK_a sensitivity parameters (α) from Table 2. Data for MEP from ref 2 were included.

EtPOMe, EtPH, and DMPB, the three most basic probes in this study. The basicity of the probes (Table 1) follows the order EtPH > EtPOMe > DMPB > MEP > DMP > MeOPH > DMPS. In agreement with an earlier comparison, the boranophosphate (DMPB) was more basic than the corresponding phosphate (DMP).[20] While our value for the pK_a of DMPS is lower than that from the literature (0.80 versus 1.18 [21]), both values indicate that this sulfur analogue is less basic than the oxygen parent, DMP (given by different sources as either 1.25 or 1.29).[22] The lack of a positive correlation between $\log(k_{app})$ and probe ligand pK_a in Figure 6 suggests that metal/probe ligand bond formation does not play a dominant role in stabilizing the transition state in aquo substitution reactions of $Mn(H_2O)_6^{2+}$ or $MnTMPyP^{5+}$. If incoming ligand bond formation were a strong factor in determining reactivity, then $\log(k_{app})$ would tend to increase with ligand pK_a, as σ donor strength is a common measure of nucleophilicity. In our experiments, the DMPS and DMPB data (filled symbols in Figure 6A,B) are displaced from the rest of the data by as much as a log unit. If these outlying data are excluded from consideration, the data roughly conform to the two trend lines, neither of which display the pronounced upward trend predicted by an associative (or

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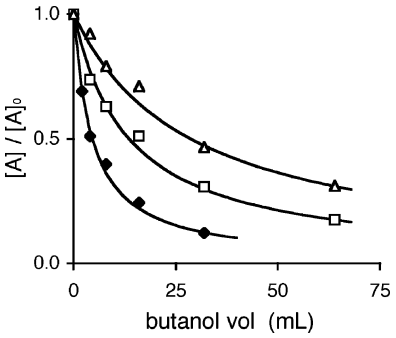

Figure 7. Fraction of probe ion remaining in 1.0 mL of D_2O solution after extraction with *n*-butanol solution ($[A]/[A]_0$) plotted as a function of butanol volume. Concentrations of DMPB (filled diamonds), EtPH (open squares), or DMP (open triangles) in 30 mM acetate buffer (pH 5.3) and Et_4NBr (0.225 M) were determined from ^{31}P NMR integrations relative to the Me_4P^+ reference in an external concentric tube. Solid lines represent behavior predicted by the extraction coefficients in Table 1.

associative interchange) model.[23] We conclude that bond formation does not significantly stabilize the transition state in ligand exchange reactions in these systems.

If differences in k_{app} are not due entirely to differences in probe ion nucleophilicity, then it is likely that they arise from differences in the outer-sphere association constant, K_{OS} (eq 1). We reasoned that differences in the environments surrounding the metal binding sites of $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$ (i.e. the second coordination shell) might also influence the observed change in entering ligand selectivity. If the hydrophilic/hydrophobic character of the metal complex coordination site influences reactivity, then the solvation energies of the probe ions should parallel outer-sphere association of the probes with the metal complexes. To determine whether changes in the solvation energetics could account for differences in probe ligand reactivities, we measured the equilibrium constants for extraction of each of the probe ions from water into *n*-butanol and examined whether these extraction coefficients correlated with probe ion reactivity. Representative plots of the concentrations of three probe ions remaining in the aqueous phase after extraction with different volumes of *n*-butanol are presented in Figure 7 (along with the behavior predicted using the extraction coefficients in Table 1). While the extraction coefficients presumably represent a product of ion pairing (with tetraethylammonium cation) and partitioning coefficients, we expect that their differences reflect changes in the strengths of the probe ion interactions with the aqueous solvent. We therefore interpret the extraction coefficient (K_{ext}) as a quantitative reflection of ligand probe hydrophilicity (or, more properly, a measure of the abilities of the probes to form hydrogen bonds with water). Each of the probe ions was more soluble in the aqueous phase than in *n*-butanol (Table 1). For ions requiring solvent cavities of similar size, the order of hydrophilicities is DMPB < DMPS < EtPOMe < DMP. For the smaller ions, the order was EtPH < MeOPH. These relative hydrophilicity series are chemically reasonable: substitution of a phosphoryl oxygen of DMP for either a sulfur or BH_3 group or substitution of a CH_2 group for a bridging oxygen is expected to decrease the ability of the probe ion to form hydrogen bonds with the solvent.

(23) Slopes of the trend lines in Figure 6A,B are pK_a sensitivity parameters (α) determined by linear regression for the two-parameter model described in eq 6.

Table 2. Standard Rate Constants (k_0) and Sensitivities of Reaction Rates toward Changes in Nucleophile pK_a (α) and K_{ext} (β)[a]

complex	log(k_0)	α	β
$Mn(H_2O)_6^{2+}$	5.96(20)	−0.04(7)	−0.54(16)
$Mn^{III}TMPyP^{5+}$	8.08(28)	−0.43(8)	+0.97(20)

[a] Parameters defined as in eq 6; standard errors in parentheses.

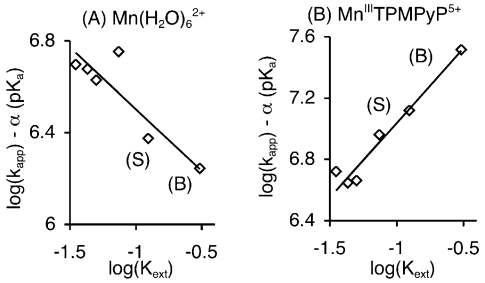

Figure 8. Plot of observed metal/probe ion association rate constants corrected for probe ion basicity ($\log(k_{app}) - \alpha(pK_a)$) as a function of probe ion extraction coefficients ($\log(K_{ext})$ for (A) $Mn(H_2O)_6^{2+}$ and (B) $Mn^{III}TMPyP^{5+}$. The solid line represents behavior predicted by eq 6 ($\log(k_{app}) - \alpha(pK_a) = \log(k_0) + \beta \log(K_{ext})$). Values of probe ion p$K_a$ and K_{ext} are from Table 1. Values of α, β, and $\log(k_0)$ parameters are from Table 2.

While neither probe ion pK_a (Figure 6) nor K_{ext} (comparison not shown) correlate with the rate data when used alone, an expression that considers both terms described the data well. Using ligand pK_a and $\log(K_{ext})$ for the probe dependent terms in eq 4 (f(PO) and g(PO)) gives

$$\log(k_{app}) = \log(k_0) + \alpha\, pK_a + \beta \log(K_{ext}) \qquad (6)$$

Using linear regression, we fitted the parameters in eq 6 ($\log(k_0)$, α, and β) to the pK_a, K_{ext}, and kinetic data in Table 1. The fitted values are presented in Table 2. Equation 6 predicts that rate data treated to compensate for the effects of differences in probe ion basicity ($\log(k_{app}) - \alpha\, pK_a$) should correlate with $\log(K_{ext})$. This correlation is presented graphically for $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$ in Figure 8A,B, respectively. The solid lines in these figures show the behaviors predicted by eq 6 using the parameters in Table 2. The fit of the basicity compensated rate data (open diamonds in the figures) to the solid lines predicted by eq 6 supports the hypothesis that both the probe ion basicity and ligand solvation energetics affect the kinetics of aquo ligand substitution.

Our results indicate that differences in the reactivities of our probe ions toward the two manganese complexes are mediated by differences in how the probes interact with solvent and reflect differences in the extent of ligand solvation in the outer-sphere complexes that lead to inner-sphere complex formation. We note that consideration of probe hydrophilicity (as done in eq 6) can be used to explain the deviation of the DMPB and DMPS rates from those of the other probes (Figure 6A,B). While extraction constants for DMP, MeOPH EtPOMe, and EtPH are all similar

(average = 0.051, $\sigma = 0.017$, Table 1), the measured values for DMPB and DMPS (0.305 and 0.124, respectively) are much greater. Thus, DMPB and DMPS are much less hydrophilic than the other probes, presumably due to the decreased ability of the BH_3 and S moieties to hydrogen bond with the solvent. Fitting the constants in eq 6 to the ligand exchange data allows us to quantify (for each complex) the sensitivities of the reaction rates to changes in probe ion extraction constants (K_{ext}). This sensitivity (the term β in eq 6) is expected to reflect the solvation character of the complex in close proximity to the metal. Fitting the parameters in eq 6 to $Mn(H_2O)_6^{2+}$ ligand exchange data gives a value of $\beta = -0.54$. The negative sign of β indicates that association kinetics with this complex are enhanced by increasing probe hydrophilicity. This suggests that, for reactions of $Mn(H_2O)_6^{2+}$, hydrogen bonding between the probe ion and coordinated water molecules significantly stabilizes the outer-sphere complex leading to ligand exchange. Consequently, the reactivity of DMPB and DMPS toward $Mn(H_2O)_6^{2+}$ is decreased relative to the other probes (Figure 6A). In contrast, fitting eq 6 to the $Mn^{III}TMPyP^{5+}$ ligand exchange data indicates that $Mn^{III}TMPyP^{5+}$ associates preferably with the less hydrophilic ligand probes ($\beta = +0.97$; Table 2). Thus, the rates of reaction with DMPB and DMPS are increased relative to the other probes (Figure 6B). For $Mn^{III}TMPyP^{5+}$ the free energy required to "desolvate" the interface between the probe ion and the metal complex appears to govern differences in the reactivities of the probes. We expect that the aryl groups of the porphyrin provide a more hydrophobic environment close to the metal ion, and steric interactions may require deformation of the hydration shell of the probe before physical contact between the reacting partners is possible.

Summary and Conclusions

In conclusion, this study shows that ^{31}P line broadening in each of the phosphorus ester probe ions; DMP, DMPB, EtPH, EtPOMe, and DMPS (like that of MEP and MeOPH) by either $Mn(H_2O)_6^{2+}$ or $Mn^{III}TMPyP^{5+}$ are governed by aquo ligand replacement reaction kinetics at temperatures below 40 °C. The LFER developed for these studies suggests that differences in the reactivities of the phosphorus probe ligands stem from differences in their outer-sphere association with the metal complexes, caused primarily by hydrophobic/hydrophilic effects in the second coordination shell of Mn. Examination of the data suggests that this set of probes might be used to determine the extent to which outer-sphere association constants for specific metal complexes (or metalloenzymes) are influenced by the hydrophobic/hydrophilic nature of the metal binding environment. Future investigations that employ this set of ligand probes will allow direct comparison of the reactivity profiles of other metal complexes to those of $Mn(H_2O)_6^{2+}$ and $Mn^{III}TMPyP^{5+}$.

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