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Factors Controlling Association of Magnesium Ion and Acyl Phosphates[1]

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Abstract: The binding of magnesium ion to acetyl phosphate, acetonylphosphonate, and related compounds was examined by potentiometric and spectrophotometric procedures. The free energy of binding of magnesium ion to these compounds follows a linear correlation with the basicity of the compounds. However, the lack of correlation between the first and second proton dissociation constants (free energies) and the unique deviation of phosphate ion from the correlation of basicity and affinity indicate that factors other than inductive effects are of significance. One major factor suggested is relative solvation of various species. The value obtained for the binding constant of magnesium ion and acetyl phosphate dianion at high ionic strength (30°, pH 8) is 6 M^{-1}. This is in close agreement with a corrected value extrapolated from kinetic results by Oestreich and Jones. The value had been disputed by other workers. Infrared and phosphorus NMR spectroscopic data are presented which indicate that consideration of protonation state simplifies interpretation of carbonyl absorption position in the infrared and phosphorus NMR chemical shift effects due to magnesium ion. It is proposed that the failure of magnesium ion to catalyze many nonenzymic reactions is consistent with the control function of enzymic catalysis. It is suggested that, by minor perturbation of the dominant form of complexation, enzymic binding can bring about observed catalytic patterns.

The role of magnesium ion in the reactions of phosphates in aqueous solution is of particular interest because of the large number of cases of enzymatic catalysis which involve magnesium.[3] Hydrated magnesium ion alone does not compare in effectiveness as a catalyst for phosphate transfer with the combination of an enzyme and magnesium ion and may even hinder reaction.[3,4] It would appear that, if magnesium ion is to participate in enzymic catalysis, it must be involved in a way that differs in some respects from the nonenzymic case. However, since in many cases the magnesium ion becomes associated with the enzyme as a complex of the substrate,[5] one might expect that the catalytically relevant mode of binding between metal ion and substrate can be brought about as a perturbation of the mode in bulk solution. The association of magnesium ion with biologically important phosphate compounds has been studied extensively,[6-11] resulting in uncertainty in conclusions about the details of coordination to complex substrates.[12] Most of the uncertainty arises because there are many possibilities which are difficult to distinguish by conventional physical methods. Similar problems exist for acyl phosphates,[10,13] which are biological phosphate derivatives of much simpler

structure than the nucleotides. To help in clarifying the problem, we have examined the binding of magnesium to a series of acyl phosphates and phosphonate analogs by several independent physical procedures. This series permits the comparison of results to be made between compounds and methods. A consistent pattern of affinity and catalysis appears to be probable.

Experimental Section

Materials. Lithium acetyl phosphate was prepared by the method described by Avison.[14] The material was further purified by dissolving in water, removing suspended material by centrifugation, and precipitating by addition of ethanol. Acetonylphosphonate[15] and sodium methyl acetonylphosphonate[16] were prepared by published procedures. Tris(hydroxymethyl)aminomethane, 8-hydroxyquinoline sulfate, and phenylphosphonic acid were obtained from the Aldrich Chemical Co. Sodium methyl methylphosphonate was prepared by refluxing dimethyl methylphosphonate (Aldrich) in acetone saturated with sodium iodide. The sodium salt was recrystallized from an ethanol–ether mixture. Methanephosphonic acid was prepared from dimethyl methylphosphonate by refluxing in 4 *N* HCl for 12 hr. The product was recrystallized from ethanol–ether. Reflux of trimethyl phosphate (Matheson Coleman and Bell) with sodium iodide in acetone, followed by recrystallization from hot ethanol, yielded the sodium salt of dimethyl phosphate. Passage of sodium dimethyl phosphate in water through a Dowex 50W ion exchange column (acid form) yielded dimethylphosphoric acid on evaporation which was subsequently refluxed with sodium iodide in acetone to yield sodium monomethylphosphoric acid.

All water was redistilled in an all-glass apparatus. The p*K* values were determined by titration in a thermostated apparatus.

Methods. Specific Ion Electrode. An Orion divalent cation electrode Model 92-32 was used with a Radiometer meter PHM 26 and Radiometer K 401 calomel reference electrode. All measurements were made of solutions contained in 250-ml beakers immersed in a jacketed beaker through which circulated water from a constant-temperature bath (Cole Parmer Versitherm controller, Glo Quartz immersion heater, Fisher circulating pump). The pH of the solution was monitored simultaneously with a Radiometer pH meter PHM 28 and GK 2321C combined pH electrode. Readings of the divalent ion electrode were made on the expanded millivolt scale of the meter. It is necessary to assume that the electrode responds only to unbound ions. The electrode is selective but not exclusive; it is most sensitive to Ca^{2+} and Mg^{2+} but also responds to Na^+, K^+, Li^+, H^+, NH_4^+, and other cations. In the 250-ml beaker, 200 ml of a 10^{-3} *M* solution of the sodium or lithium salt of the compound being studied was placed. The solution was brought to a pH greater than 9.5 (so addition and complexation of Mg^{2+} would not lead to sizeable changes in hydrogen ion concentration) but less than 10 (to avoid excessive formation of magnesium hydroxide). The solution was stirred with a magnetic stirrer. Magnesium chloride solution was added from a Radiometer SBU-1 micrometer-driven syringe. Small portions of magnesium chloride were added and readings taken after addition of each portion and a standard wait of 5 min. For comparison, magnesium chloride was added to a solution identical with that of the compound being studied except that sodium chloride or lithium chloride, as appropriate, was substituted for the compound being studied. Readings for this blank on addition of magnesium chloride gave the value of the response of the electrode to "free" magnesium ion. A plot of the reading of the meter vs. the logarithm of the total concentration of magnesium ion added for the standard and the sample was then made. In regions where the curves are parallel, the difference between the curves reflects the amount of complexed magnesium ion present (see Figure 1). At the beginning of the curves, where only small amounts of magnesium are present, nonparallel plots are presumably due to the relatively large amount of interference from sodium ion. Beyond the central region, the ionic strength has become perturbed significantly, and thus linearity is again perturbed.

Spectral Competition. The use of 8-hydroxyquinoline as an indicator for magnesium ion in solution and further use of the indicator to determine indirectly binding constants between magnesium ion and other species has been developed and discussed by Burton.[17] This procedure was followed in detail with the exception

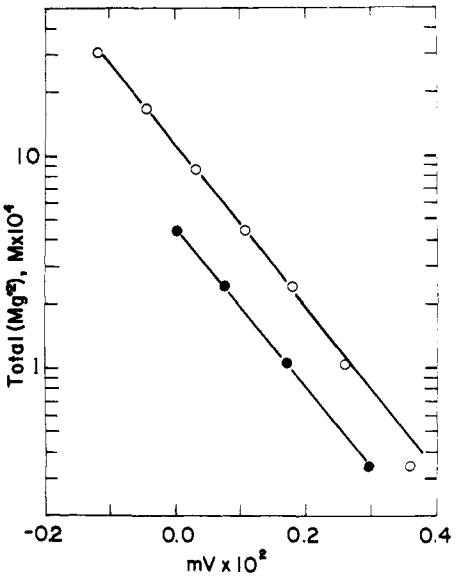


Figure 1. Potential of "divalent ion" electrode (Orion 92-32) with calomel reference in presence of 5×10^{-3} *M* sodium acetonylphosphonate (○) and 5×10^{-3} *M* sodium chloride (●) as a function of magnesium ion concentration.

that the temperature used was 30°. All spectral measurements were performed with a Unicam SP1800A uv-visible spectrophotometer equipped with an AR25 recorder and constant-temperature cell holder.

Magnetic Resonance. Phosphorus NMR spectra of acetonylphosphonate at a probe temperature of 30° were obtained by a double resonance method.[18] A Varian HA 100 NMR spectrometer was used to observe the proton resonance signals of the compound. A tuneable 40-MHz crystal oscillator (constructed by C. Arnow, Micro-Now Electronics) was used to generate decoupling signals at resonance frequencies of the phosphorus nucleus. The decoupling frequency was varied by a dc voltage ramp from a Nicolet 1080 computer. A voltage-controlled oscillator was set to the resonance position of a peak in the proton spectrum that was coupled to the phosphorus nuclear resonance and the oscillator frequency range swept. The effect of the frequency sweep on the proton resonance signal was stored in the computer and then plotted on the recorder bed, yielding a phosphorus spectrum of high resolution in a single sweep of a relatively dilute solution. Sample solutions were typically of 0.2 *M* concentration. Those solutions in which magnesium was absent were brought to the same ionic strength, i.e., 0.9 *M*, as those with the metal ion by addition of potassium chloride.

Phosphorus NMR chemical-shift determinations for acetyl phosphate in the presence and absence of magnesium ion in deuterium oxide were determined by pulsed Fourier transform methods at 36 MHz using a Bruker HX90E Fourier transform NMR spectrometer with broad band decoupling. Peak positions were determined relative to an external reference of 85% phosphoric acid.

Infrared spectra were taken using a Beckman IR-10 instrument. Solutions of the various phosphorus compounds were prepared at 1 *M* concentrations in deuterium oxide at differing pD levels, ensuring spectral analysis of both monoanionic and dianionic forms. Wilks minicells (silver chloride) were used for all determinations.

Results

Binding constants of magnesium ion to acetonylphosphonate, acetyl phosphate, their esters, and related compounds were determined by methods described in the Experimental Section. Results obtained using the specific ion electrode were consistently different from those obtained by the method involving observation of spectral perturbations of 8-hydroxyquinoline (see Tables I and II), but the orders of affinity were identical. The values obtained by the specific ion electrode method (see Table II and Figure 1) are much higher (tighter complexation) than those that were obtained

Table I. Association Constants for Magnesium Ion with Phosphates and Phosphonates (Ionic Strength 0.3 M 30°, pH 8.0, Determined by Competitive Complex Formation with 8-Hydroxyquinoline.[17] Values Are Average of at Least Five Trials)

Compd	K (apparent), M^{-1}	pK_2	pK_1	K(corrected),[a] M^{-1}
$C_6H_5PO_3^{2-}$	40	7.5	1.7	53
$CH_3PO_3^{2-}$	30	7.1	2.3	34
$CH_3OPO_3^{2-}$	22	6.3	1.5	22
$CH_3COCH_2PO_3^{2-}$	20	6.3	1.2	20
$HOPO_3^{2-}$	14	7.2	2.1	16
$CH_3CO_2PO_3^{2-}$	6	4.8	1.2	6
$CH_3OPO_2(OCH_3)^-$	6		1.3	6
$CH_3PO_2(OCH_3)^-$	3		1.6	3
$CH_3COCH_2PO_2(OCH_3)^-$	1		0.7	1

[a] Taking account of actual concentration of indicated species based on pK.

Table II. Association Constants for Magnesium Ion with Phosphates and Phosphonates (Ionic Strength 10^{-2} M, 23°, pH 9.7 ± 0.2, Determined by Measurements with Specific Ion Electrode. Values are Reproducible to ±10%)

Compd	K, M^{-1}
$CH_3COCH_2PO_3^{2-}$	345
$HOPO_3^{2-}$	331
$CH_3CO_2PO_3^{2-}$	108
$CH_3COCH_2PO_2^-(OCH_3)$	55

by the specific method (see Table I). Since the specific ion electrode must necessarily operate in solutions of low ionic strength, while the spectral procedure is done under conditions of high ionic strength, the results obtained are expected to be considerably different and are generally consistent with known solution effects.[19] Furthermore, complications introduced by the presence of indicator and the low sensitivity of the spectral competition method for species with low affinity for magnesium make uncertainties greater for the weakly associating species. The overall trend and relative order of affinity of the various anions for magnesium are certain, however.

In order to test the factors controlling association compared with those controlling acidity, a plot of pK_2 vs. the logarithm of the observed binding constant to magnesium ion of that species was made (Figure 2). If the association of magnesium ion is a linear function of the free energy of proton association with the most basic form of the dissociable phosphate compound, then a straight line should be obtained. This is observed, with phosphate ion being the notable exception. A plot of the first dissociation constant, pK_1, vs. the second dissociation constant, pK_2, for the compounds that can undergo two protonic dissociations, however, gave no such correlation (see Figure 3). Thus, by extension, binding of magnesium ion to the phosphate derivatives depends on the factors affecting the second dissociation constants.

Phosphorus NMR was also used to probe the binding of magnesium to acetonylphosphonate and acetyl phosphate. In order to examine solutions of low concentration of magnesium ion and acetonylphosphonate, a double resonance method[18] was employed (see Figure 4). When the chemical shift of the phosphorus atom of acetonylphosphonate was plotted as a function of pH in the presence and absence of magnesium ion, two curves were obtained (see Figure 5). The curves are similar to those obtained by potentiometric titration, using glass and calomel electrodes, under the same conditions. In the region of the curve where the phosphonate exists as a dianion, the shifts in the presence and absence of magnesium ion are identical. In the pK region, the curves are markedly shifted. In the region where monoanion is predominantly present, there is a slight difference in chemical shifts. A further test was performed using acetyl phosphate as a probe molecule. However, since no strongly coupled protons are available ($J_{P\text{-}CH_3}$ = 1 Hz) a pulsed Fourier transform system[20] requiring many accumulations had to be used. Results similar to those obtained for acetonylphosphonate by double resonance were observed.

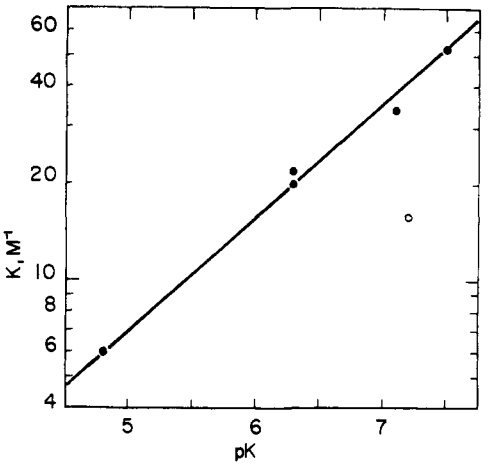


Figure 2. The pK_2 vs. logarithm of magnesium ion association constant (from spectral competition method). See Table I.

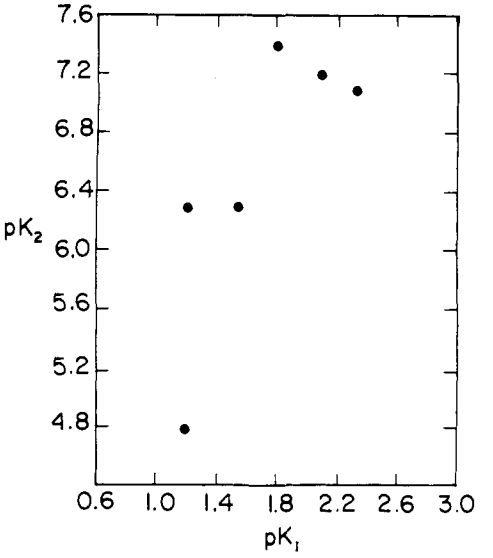


Figure 3. The pK_a for first proton dissociation vs. second pK_a for compounds in Table I.

Discussion

Both the studies using the specific ion electrode and those utilizing the spectral competition procedure were done under conditions where the predominant form of the compounds analyzed was anionic. The fact that the methyl ester of acetonylphosphonate has a much lower affinity for magnesium ion than the corresponding dianion and that the dianion of methylphosphonate has a greater affinity for magnesium than does acetonylphosphonate indicates that binding energy depends primarily on the nature of the phosphonate functional group, without significant secondary effects due to the presence of a carbonyl group. Whether this condition is true at hydrogen ion concentrations at which the predominant form of the nonesterified compounds is mo-

Figure 4. Phosphorus NMR spectrum at nominal frequency of 40.48 MHz by double resonance procedure of acetonylphosphonate in water. Chemical shift is in parts per million relative to trimethyl phosphate (J = 22 Hz).

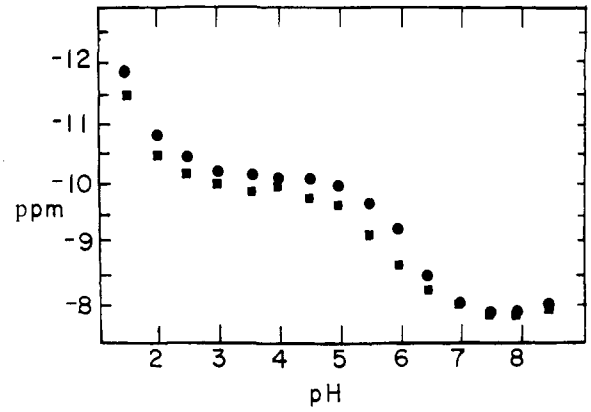


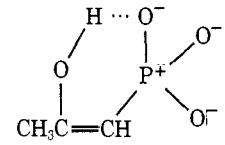
Figure 5. Chemical shift of phosphorus NMR triplet (central peak) of acetonylphosphonate (0.2 M) as a function of pH in presence (■) and absence (●) of 0.3 M magnesium chloride.

noanionic cannot be ascertained from these data; one might expect bidentate coordination to become significant at lower pH since binding to the phosphonate monoanion ester is weaker than to the dianion, permitting perturbations to be more noticeable. Unfortunately, both procedures are limited to the higher pH regions.

The plot of the logarithm of the binding constants, K, vs. pK_2 (corresponding to the equilibrium between monoanionic and dianionic species) (Figure 2) is linear with phosphate deviating significantly. The values of K are corrected to reflect the concentration of dianion in view of the result that the metal ion binds relatively poorly to the monoanionic esters. This linear correlation indicates that the compounds containing carbonyl groups do not acquire a significantly stronger basicity through hydrogen bonding between the carbonyl oxygen and the P-OH group. The binding constant studies indicate magnesium does not bind predominantly to the carbonyl group; if the proton did, then log K vs. pK_2 would probably not be linear.

Infrared spectroscopy of acetyl phosphate and acetonylphosphonate is consistent with this finding. (We have confirmed earlier results.) For acetyl phosphate, a pD change from 6.9 to 3.8, corresponding to a change from the dianionic to monoanionic form, leads to a shift toward higher energy for the C-O stretching vibration (1707 to 1732 cm^{-1}).[10] Similarly, changing the pD of a solution of acetonylphosphonate from 9.0 to 4.0 (dianionic to monoanionic forms) leads to stretching frequencies being observed at 1671 and 1685 cm^{-1}, respectively. One would predict that, if anything, hydrogen bonding between the phosphoryl proton and carbonyl group would lead to the requirement of lower energies for the carbonyl stretch, not the opposite.[21] However, the effect of hydrogen bonding to the solvent complicates matters[22] and may be the predominant factor. Therefore, the criterion of carbonyl frequency shifts in the infrared region cannot positively answer the question of whether or not hydrogen bonding as depicted above does in fact occur. For example, there have been a number of instances where hydrogen bonding exists but where the carbonyl peak shift is very small,[23] less than 10 cm^{-1}, or not even detectable.[24] At least, however, shifts that did occur were in the predicted direction, i.e., toward lower energy.[24] It thus appears reasonable that, because solution effects are unknown, the conclusion that metal ion coordination does not occur at the carbonyl group because no large shift in frequency is observed in the infrared spectrum is unjustified.[10]

Another way in which hydrogen bonding might perturb the pK of the phosphonate involves stabilization of the enol:



This possibility, however, would probably not affect the linear relationship for the plot of binding constant vs. pK since the perturbation by the hydrogen bonding to the phosphonate group is in the same direction for proton binding as for magnesium binding. In this case, hydrogen bonding would tend to make the acetonylphosphonate molecule a better acid (and lead to poorer magnesium binding) by stabilization of the negative charge. The infrared spectra indicate no major contribution from this structure since the carbonyl peak is evident and there is no large frequency shift in the P-O stretching vibration or enol absorption. (The infrared spectrum of acetonylphosphonate was compared with that of monomethyl methylphosphonate, which contains no carbonyl group so a hydrogen bond cannot be formed; the P-O peaks were in the same position as in the carbonyl compound, 1200 cm^{-1}.) Hydrogen bonding of the phosphoryl can be expected to lead to shifts of 50 to 80 cm^{-1}.[25-27]

We have observed that a plot of the pK of a series of phosphates and phosphonates against the logarithms of the binding constants of the conjugate bases to magnesium ion gives a linear relationship with a slope of 0.35 (see Figure 2). The linearity indicates that factors controlling the acidity of the compounds are similar to those controlling the dissociation of magnesium ion from a complex with the conjugate base, but that the sensitivity is lower. In addition, other modes of complexation such as dimers may exist. The parent phosphate ion does not fall on the correlation line, indicating that these factors are not uniform, especially when the ion in question is of different symmetry and therefore solvated quite differently than the compounds containing hydrophobic substituents. The fact that there is no correlation between the first and second pK's of the compounds capable of undergoing two dissociations in the solutions we examined (Figure 3) confirms the fact that pK depends on factors besides the inductive effect of substituents, and attempts to predict metal ion affinity based only on inductive effects necessarily are inadequate. Some other factors of obvious importance include overall charge changes, steric bulk, and relative effectiveness of solvation of the various species involved in the equilibrium.

Examination of Table I reveals that the binding constant, K, for the magnesium ion-acetyl phosphate dianion complex is approximately 6 M^{-1} at 30° and ionic strength 0.3 M. Kinetic studies by Oestreich and Jones[28] on the effect of magnesium ion on the rate of hydrolysis of acetyl phosphate dianion led those authors to calculate an apparent binding constant of 5.7 M^{-1} (39° and ionic strength 0.6 M). The higher ionic strength of that system compared with ours would tend to decrease the value obtained for the formation constant, but the higher temperature would tend to increase that value so the factors are partially compensating. These values agree with the values we have determined directly. Satchell and coworkers[29] pointed out that Oestreich and Jones[28] made an error in the analysis of the kinetic plots used to determine K; redetermination of K by Satchell et al.,[29] utilizing the same spectral competition method of Burton[17] as we have used in the present study, yielded a value of 140 M^{-1} at 0.6 M ionic strength and extrapolated to 39° (which is larger by a factor of over 20 than the value we determined). Recalculation of the data of Oestreich and Jones by Klinman and Samuel[13] led to a value of K of 8.2 M^{-1}, a value close to the originally determined value of Oestreich and Jones.[28] Our work clearly indicates that an error occurred in the earlier spectral study of Satchell et al.[29] As pointed out by Klinman and Samuel, one consequence of the lower K value is the conclusion that cleavage of the acetyl phosphate-magnesium complex by OH^- apparently occurs by CO cleavage rather than by PO cleavage of the anhydride.[13]

Studies on the magnesium ion catalyzed deuteration of the 2-position of acetonylphosphonate indicated the presence of a kinetic term which is dependent on the concentration of metal ion, acetonylphosphonate monoanion, and acetonylphosphonate dianion.[15] Based on our finding that acetonylphosphonate dianion has a very high affinity relative to the monoanionic ester, we would reformulate that kinetic term as resulting from abstraction of a proton from the monoanion of acetonylphosphonate by the dianion-magnesium ion complex. A more complete study of that system is in progress.

The results of our present NMR study indicated that there is a small difference in the chemical shift for the phosphorus resonance of acetonylphosphonate (Figure 5) in the presence and absence of magnesium from pH 2 to 5, a larger perturbation from pH 5 to approximately 6.5 (the pK_2 region of acetonylphosphonate), and almost no perturbation from pH 7 to 8.5. From the binding constant studies, we conclude that magnesium binds much more strongly to the dianion than it does to the monoanion of acetonylphosphonate, perturbing the pK. By affecting the protonation state of the phosphonate, magnesium ion causes a shift in the apparent absorption resonance in the vicinity of the pK. In the higher pH region, the dianionic form of acetonylphosphonate is the predominant form. In the presence and absence of magnesium ion, the same resonance frequency is observed, although coordination must occur. (It has previously been shown that the absence of a perturbation cannot be taken as proof of lack of binding of magnesium ion to a phosphate.[30] This further confirms that observation.) The dominating effect of the state of protonation on the chemical shift of other systems has been noted[31,32] and appears to apply in this case. The small perturbation in the pH 2-5 region may be due to some magnesium in bidentate coordination with acetonylphosphonate (carbonyl to phosphonate monoanion); since the dominating effect of the dianion is no longer present, the carbonyl becomes relatively more important, but the concentration is low. This may give rise to special contact shift effects. However, in essence, the curves in Figure 5 fit two titration curves which differ only in the pK region.

These studies, in summary, indicate that, consistent with earlier considerations,[10] the association of magnesium ion with acetyl phosphate involves the phosphate portion of the anhydride. This type of coordination should convert the phosphate into a superior leaving group since the pK of that group will be lowered.[33] The finding that magnesium ion assists the C-O cleavage of acetyl phosphate[13] is consistent with this view since it has been shown that charge neutralization via esterification leads to a markedly enhanced hydrolysis rate involving attack at the acyl carbon atom.[33] The relatively low affinity for magnesium ion shown by the acetyl phosphate dianion and the involvement of higher order kinetic terms in the metal ion promoted hydrolysis[13,28,29] suggest that enzymic systems must have special binding modes to enhance P-O cleavage if such binding is to be a catalytic component of the reaction. Charge neutralization should not promote attack by neutral water. The encounter of the species is not facilitated except by dipole interactions, although stabilization of the polar transition state may occur. It would be reasonable to expect that the catalytically useful mode of magnesium binding would not be radically different from the thermodynamically favorable mode since this would minimize formation of high-energy species. For the case of acetyl phosphate, the rapid nonenzymatic hydrolysis rate[34] is the result of the availability of a favorable pathway for the expulsion of metaphosphate ion.[35] Any metal ion assisted reaction then must facilitate metaphosphate expulsion or promote a favorable alternate mechanism. The expulsion of metaphosphate would be assisted if the metal ion coordinated at the carboxyl moiety enhanced the leaving ability of acetate; however, as long as the metal ion remains coordinated to phosphate, it will hinder elimination and favor C-O cleavage. Thus, for the elimination of metaphosphate to be facilitated by the metal ion, this barrier must be overcome. An alternative mode of catalysis of phosphate transfer has been suggested by Farrell et al.[36] and by Mildvan.[37] This involves coordination of the metal to phosphate in a way that promotes formation of the pentacoordinate state of phosphorus by converting binding energy to strain energy which provides a new, energetically favorable catalytic pathway. However, this requires creating an unfavorable energetic situation to favor a route that overcomes the newly created barrier. It is unlikely that the binding energy is sufficient to produce enough steric strain to accomplish this. Another model proposed by Steffens et al.[38] is that the role of the catalytic metal ion involves stabilizing charges in unfavorable positions of a trigonal bipyramidal hydrolysis intermediate. This model does not require creation of new barriers by binding of magnesium but rather causes catalysis by stabilization of transition states essentially along the original path.

It is clear that enzymes should be capable of directing the binding of the metal ion to provide assistance in catalysis by small geometric perturbations. We feel that it is reasonable to consider that the binding of magnesium ion to substrates which undergo reactions as magnesium complexes occurs in solution in a manner which does not necessarily promote nonenzymic catalysis. However, upon binding to an enzyme, the position of coordination is readily perturbed to promote reaction. Thus, control and catalytic efficiency are maximized. Furthermore, the ready dissociability of the complexes we have studied indicates that, in the enzymic systems, rapid turnover can be achieved. This advantage is shared by magnesium ion and hydronium ion. Since hydronium ion concentration is controlled mainly by the bulk pH of the solution, metal ions can serve to provide mobile elec-

trophilic catalysis without pH limitations.

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References and Notes

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The Role of Reversible Hydrogen Abstraction in the Mechanism of the Bromination of Cyclohexane. A Comparison of the Differences between the Liquid and Vapor Phase Reactions

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Abstract: The role of reversible hydrogen abstraction in the bromination of cyclohexane has been investigated by a study of the kinetics of the bromination of perdeuteriocyclohexane in the presence of large amounts of hydrogen bromide and molecular bromine. By a determination of the relative rate constants for transfer of the radical with the two transfer agents, bromine (k_2) and hydrogen bromide (k_{-1}), a ratio of rate constants k_2/k_{-1} could be obtained. In solution at 30° k_2/k_{-1} varied depending upon the concentration of molecular bromine and hydrogen bromide, while in the vapor phase, 20–28°, $(k_2/k_{-1}) = 2.81 \pm 0.06$ at all concentrations. The change in the ratio of transfer rates in solution and their difference from the vapor phase value is attributed to a complex formation between hydrogen bromide and molecular bromine, the complex acting as a transfer agent at a faster rate (k'_{-1}) than hydrogen bromide itself. The ratio of transfer rates for the solution reaction of bromine relative to HBr_3, k_2/k'_{-1}, is approximated, $(k_2/k_{-1})^{vp}/(k'_{-1}/k_{-1}) = 0.13$, and found to be in good agreement with the values obtained at high bromine concentration. Cage return of the radical with hydrogen bromide as a kinetically masked process in the bromination reaction is also discussed.

The effect of reversible hydrogen abstraction on the kinetics of bromination of alkanes and substituted alkanes with a number of brominating agents (e.g., bromine,[2] *N*-bromosuccinimide,[2] bromotrichloromethane[3]) has been a topic of considerable concern. For any understanding of the details of the mechanism of bromination, it is necessary to know the relative rates of transfer of alkyl radicals with bromine and hydrogen bromide. As the reaction appeared to be more complicated than previously reported,[4] a comparison of the kinetics of the liquid and vapor phase brominations of perdeuteriocyclohexane has been undertaken.

In the simplest mechanism involving reversible abstraction, the alkyl radicals generated by abstraction of a hydrogen atom from an alkane by bromine atoms have two fates: transfer with molecular bromine to give substitution product, or transfer with hydrogen bromide to regenerate the substrate.

$$RH + Br\cdot \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R\cdot + HBr \xrightarrow[k_2]{Br_2} RBr + Br\cdot \quad (1)$$

The importance of the reversal reaction relative to product formation is related to the ratio k_2/k_{-1}. This ratio may be