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An Altered Mechanism of Hydrolysis for a Metal-Complexed Phosphate Diester

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Previous studies of methyl aryl phosphodiesters coordinated to a dinuclear Co(III) complex (**1**) reported that the methyl 4-nitrophenyl phosphate moiety undergoes hydrolysis about 10^{11} times faster than the corresponding uncomplexed diester.[1,2] We have measured kinetic isotope effects (KIEs) which show that this rate acceleration is accompanied by a change in mechanism as compared to uncomplexed phosphate diesters.

Linear free energy relationships and KIEs indicate that phosphoryl transfer reactions of uncomplexed diesters with good (aryl) leaving groups proceed by concerted mechanisms.[3,4] Both the dependency of the rate on the leaving group pK_a (β_{lg}) and the KIEs in the leaving group are much smaller than those for reactions of the dianions of phosphate monoesters. The latter reactions are generally agreed to have very loose transition states and exhibit much larger β_{lg} (-1.23)[5] and larger leaving group KIEs[6] (Table 1). These data indicate that fission of the bond to the leaving group is much less advanced in the transition states of diester reactions than in monoester reactions.

Reaction of **1** involves equilibrium deprotonation of a bridging hydroxide followed by nucleophilic attack at the phosphorus.[1,2] The β_{lg} of -1.38 is much larger than that for the alkaline hydrolysis of uncoordinated phosphodiesters ($\beta_{lg} = -0.64$). An estimation[1] of β_{eq} suggests leaving group bond fission in the hydrolysis of **1** is about twice as advanced in the transition state as that for the uncomplexed diester. While only an approximation, the large difference in β_{lg} makes it likely that leaving group bond fission has significantly changed.

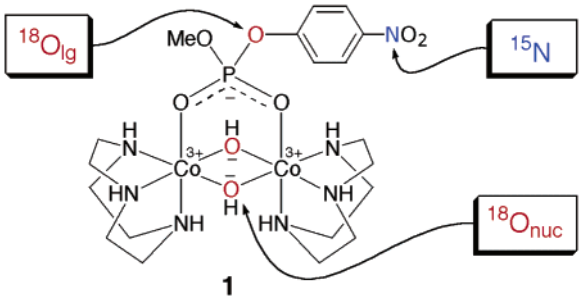
We have measured the nucleophile ^{18}O KIE for this reaction to ascertain the degree of nucleophilic participation in the transition state of the rate-limiting step. We have also measured KIEs in the leaving group for comparison with data for reactions of uncomplexed monoesters and diesters. Figure 1 shows the positions at which kinetic isotope effects were measured. Both ^{18}O KIEs were measured via the remote-label method using the nitrogen atom in the nitro group as the reporter and using isotope ratio mass spectrometry;[6] experimental details are given in Supporting Information.

Table 1 shows the KIE results for **1** and previously reported data for the hydrolyses of the *p*-nitrophenyl phosphate dianion and several phosphate diesters in which *p*-nitrophenol is the leaving group.[6] The KIEs for the uncatalyzed reactions were measured at 95 °C as compared to 25 °C for **1**. The higher temperature reduces the magnitudes of these KIEs by ~20%[7] (i.e., corrected KIE = 1 + 1.2 × (observed KIE − 1)), a small correction that does not affect the following discussion.

Table 1. Kinetic Isotope Effect Results for Complex **1** and for Phosphate Monoester and Diester Hydrolysis in Alkaline Solution (Standard Errors in the Last Decimal Place Are in Parentheses)

isotope effect	complex **1**	4-nitrophenyl phosphate dianion (*p*NPP)[a]	4-nitrophenyl phosphate diesters
^{15}N	1.0026 (2)	1.0028(2)	1.0007−1.0016
$^{18}O_{lg}$	1.029 (2)	1.0189(5)	1.0042−1.0063
$^{18}O_{nuc}$	0.937 (2)	not measured	1.027[18]

[a] In the *p*NPP dianion reaction, the close proximity in the late transition state of the departing phenolate anion and the negatively charged metaphosphate-like phosphoryl group enhances negative charge delocalization. This causes an increase in the ^{15}N KIE[7] and a decrease in the $^{18}O_{lg}$ KIE (due to increased C−O bond order from the quinonoid resonance form).[8]



1

Figure 1. The positions at which the isotope effects were measured.

The magnitude of the ^{15}N KIE depends on the amount of negative charge developed on the leaving group in the transition state, while the $^{18}O_{lg}$ KIE is a measure of P−O bond fission.[6] The data indicate much greater P−O bond fission and more negative charge on the leaving group in the transition state of the hydrolysis of diester complex **1** than in the hydrolysis of uncomplexed alkyl-*p*-nitrophenyl phosphodiesters, consistent with the larger β_{lg}.

The value for $^{18}O_{nuc}$ is obtained from experiments in which both bridging hydroxides are labeled; assuming the contribution from the spectator atom is negligible, the value of 0.937 is then solely due to the nucleophilic atom.

Isotope effects are comprised of two factors, the temperature-independent factor (TIF) and the temperature-dependent factor (TDF).[9] The TIF reflects differences in the imaginary frequencies in the transition state and is always normal because the imaginary frequency is always larger for the lighter isotope. The TDF can be either normal or inverse and arises from changes to the vibrational energy as the reactant is converted to the transition state. A KIE contains contributions from both factors, while an equilibrium isotope effect has no TIF contribution. For nucleophiles, even though new vibrational modes are introduced that make the TDF contribution inverse, the normal TIF contribution results in observed KIEs that are nearly always normal.[10−14] Inverse nucleophile KIEs

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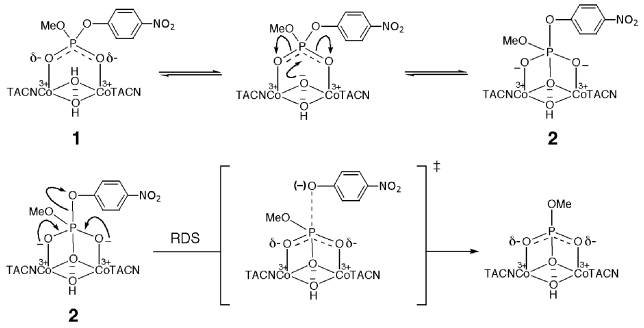
Scheme 1



are rare, their magnitudes are small (<1%), and they have been attributed to special factors such as rate-determining solvation changes.[15,16] However, theoretical calculations predict large inverse nucleophile KIEs[13,17] in very late transition states, in the case of ^{18}O, 0.966 for a nucleophile bond order of 0.9 for hydroxide attack on acetaldehyde at 25 °C.[17]

The very large inverse value for $^{18}O_{nuc}$ in **1** demands that there be essentially no contribution from the TIF. This implies that $^{18}O_{nuc}$ is an equilibrium isotope effect (EIE), which would be the case if nucleophilic attack occurs before the rate-determining step. This conclusion is accommodated by formation of a phosphorane intermediate followed by rate-determining expulsion of the leaving group (Scheme 1). In view of the theoretical calculations, a concerted reaction in which the bond to the nucleophile is nearly fully formed in the transition state cannot be ruled out. However, to accommodate all of the KIE data would require an exceedingly productlike transition state with both bond formation and leaving group bond fission very far advanced (≥90%). The large inverse value for $^{18}O_{nuc}$ reported here also sharply contrasts with the value of 1.027 reported for $^{18}O_{nuc}$ in the concerted reaction of hydroxide with thymidine-5′-*p*-nitrophenyl phosphate.[18]

It has been pointed out that Co(III) coordination of phosphates may result in effects similar to alkylation,[19] in effect making a complexed diester behave more like a triester. Linear free energy relationships indicate acyclic triesters with good leaving groups react by a concerted mechanism, while stereochemical data imply an intermediate forms in reactions of six-membered ring cyclic triesters with aryl leaving groups.[20] Despite this difference, the alkaline hydrolyses both of uncomplexed cyclic[21] and of acyclic[22] aryl triesters exhibit β_{lg} of −0.4, indicative of modest bond fission in the transition states of their rate-limiting steps.

Because the pK_a of the bridging hydroxide in **1** is estimated to be >14, the dominant species present under the reaction conditions is the hydroxide form. Previous data indicate a specific base mechanism in which the oxide is the nucleophile.[1] Thus, in the mechanism shown in Scheme 1, $^{18}O_{nuc}$ will in effect be the fractionation factor between species **1** and **2** because the nucleophilic oxygen is not directly involved in the rate-determining step.

While no large experimental inverse nucleophile KIEs are known, large inverse EIEs are common. The fractionation factor between water and the alcohol of malate is 0.968 in the direction from water to malate.[23] Similarly, the calculated ^{18}O fractionation factors from water to methanol, and from methanol to dimethyl ether, are ∼2.2% inverse.[24] In each of these cases, as in the reaction of **1**, hydrogen is replaced by a larger group introducing new vibrational modes. In the reaction of **1**, some of these modes are very constrained, presumably accounting for an isotope effect that is even more inverse than these examples. Such factors would apply to both the stepwise and the concerted mechanisms.

In conclusion, the mechanism in Scheme 1 accounts for all of the KIE data. Following deprotonation of the bridging hydroxide, nucleophilic attack occurs to form a coordinated phosphorane intermediate **2**. The leaving group is then expelled in the rate-limiting breakdown of this intermediate. In this transition state, the P−O bond is substantially broken, and there is essentially a full negative charge on the departing nitrophenolate. We favor this mechanism, although the alternative of a concerted reaction with an extremely productlike transition state is not excluded by these data. In either case, this represents a substantially different mechanism/transition state than the uncatalyzed hydrolysis of an uncomplexed aryl phosphate diester.

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Supporting Information Available: Experimental information (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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