enzyme. In support of this, benzoate-bound DAAO (Figure 2) has different spectral properties than oxidized free enzyme (Figure 1). The increase in resolution of the flavin spectra in the presence of benzoate indicates that the flavin is in a more hydrophobic environment.[17] In addition, the p*K* of N(3) of the flavin shifts from 9.6 to 10.7 in the presence of benzoate.[15] Finally, circular dichroism suggests the spectral changes observed on binding of benzoate can be interpreted as a direct interaction between benzoate and the isoalloxazine ring and/or a local conformational change in the protein.[18]

The above data show the net effect of the substrate analogue binding is, in fact, to shift the free energy of the first and second electron transfers such that two-electron transfer is the thermodynamically favored process. DAAO is the first example of a general control mechanism applicable to flavoproteins.

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Relationship between Effective Nucleophilic Catalysis in the Hydrolysis of Esters with Poor Leaving Groups and the Lifetime of the Tetrahedral Intermediate

Jik Chin* and Xiang Zou

Department of Chemistry, McGill University
Montreal, Canada H3A 2K6

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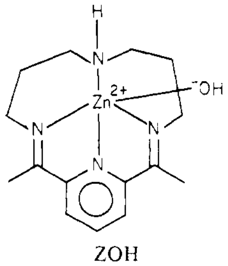
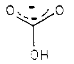
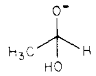
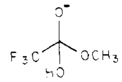
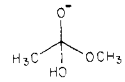
Many enzymes (chymotrypsin,[1] carboxypeptidase,[2] carbonic anhydrase[3]) catalyze the hydrolysis of esters with good leaving groups as well as esters with poor leaving groups. However, simple hydrolytic catalysts are often tested only on esters with good leaving groups. Simple catalysts that are almost as active as hydroxide in cleaving esters with good leaving groups can be millions of times less active than hydroxide in cleaving esters with poor leaving groups.[4]

In this paper we report the catalytic hydrolysis of methyl trifluoroacetate. In a typical run, hydrolysis of 1 μL of the substrate in 10 mL of water at pH 8.0 and 25.0 °C was monitored to completion by the pH stat method. The reaction was monitored in the presence and absence of varying amounts of the zinc hydroxide (ZOH) prepared according to known procedures.[5] As a control, initial hydrolysis of 0.2 mL of methyl acetate was monitored by the method described above in the presence and absence of ZOH. In the presence of 50 mg of ZOH, the rate of hydrolysis of methyl trifluoroacetate is increased sixfold whereas for the hydrolysis of methyl acetate there is no observable increase in the rate. No rate enhancement is observed with 10 mM borate buffer or imidazole buffer. Therefore, any rate increase by general base catalysis must be small.[4] For all of the above reactions, the kinetic data were corrected for a small background rate (rate without substrate).



ZOH

Table I. Catalytic Efficiency of ZOH and the Lifetime (1/*k*) of the Intermediate

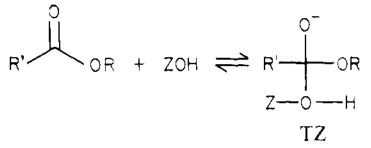
intermediate structure	k_{cat}/k_{OH}	lifetime of C–O bond, s
	6×10^{-2} [a]	10^{4} [b]
	3×10^{-3} [a]	10^{-3} [c]
	6×10^{-3}	10^{-1} [d]
	$<10^{-5}$	10^{-6} [d]

[a] Reference 9. [b] Sirs, J. A. *Trans. Faraday Soc.* **1958**, *54*, 201. [c] Reference 13. [d] Guthrie, J. P.; Cullimore, P. A. *Can. J. Chem.* **1980**, *58*, 1281-1294.

In general, nucleophiles do not catalyze the hydrolysis of esters efficiently if the basicity of the nucleophile is significantly less than that of the leaving group.[4] If the base strength of the nucleophile is significantly greater than the base strength of the leaving group, transacylation to the nucleophile is fast but the subsequent hydrolysis is slow.[6] Nucleophilic catalysis for hydrolysis of amides or esters involving strongly basic leaving groups and weakly basic nucleophiles had not been observed except when the nucleophile is bound to the substrate.[7,8]

The conjugate acid of ZOH (ZOH_2) has a pK_a of 8.6 and the metal hydroxide has been shown to catalyze the hydration of acetaldehyde and carbon dioxide, and the hydrolysis of propionic anhydride by a nucleophilic mechanism.[9,10]

Equation 1 shows the addition of ZOH to an ester. The adduct



$$R'C(=O)OR + ZOH \rightleftharpoons R'C(O^-)(OR)(O{-}Z{-}H) \qquad (1)$$

TZ

(TZ) has three bonds that can be cleaved rapidly around a single oxygen. In general, adducts between esters with poor leaving group and weakly basic nucleophiles revert back to starting material rapidly.[4] Reversion of TZ to starting material can be significantly reduced if the metal–oxygen bond or the hydrogen–oxygen bond is cleaved more rapidly than the carbon–oxygen (metal hydrate oxygen) bond. The lifetimes (1/*k*) of the three bonds can be estimated since metal hydrate exchange rates,[11] proton transfer rates,[12] and lifetimes of tetrahedral intermediates[13] can be measured or estimated.

The lifetime of the C–O bond in TZ is more difficult to estimate than the lifetime of the C–O bond in T (eq 2). The lifetime of

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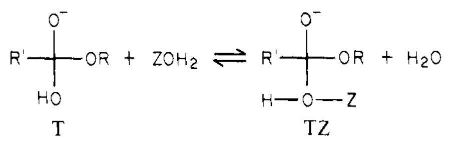
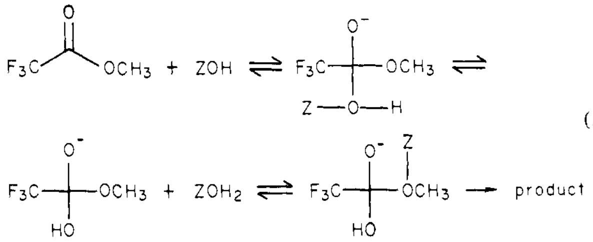

(2)

the C–O bond in T can also be used to predict the efficiency of the catalyst. In accordance with the principle of microscopic reversibility, if the metal hydroxide can catalyze the formation of T from the substrate, it should also catalyze the formation of the substrate from T. There will be no catalysis if T in eq 2 is so short lived that the metal–oxygen exchange cannot take place. At 10 mM catalyst concentration, the formation of TZ from T should have a pseudo-first-order rate constant of about 10^4 s^{-1}. In order to have any chance at catalysis, T must be long lived (lifetime > 10^{-4} s).

Table I shows the relationship between the lifetime of the anionic intermediate in various reactions and the catalytic efficiency of ZOH with respect to hydroxide. All of the anions live long enough to bind with the catalyst except the anion formed from methyl acetate. Knowing the tetrahedral intermediate stability is important in understanding the mechanism of these reactions, and there has been much progress in measuring and estimating the lifetimes of these species.[13-15]

At equilibrium, the ratio TZ/T (eq 2) is equal to ZOH_2/H_2O if it is assumed that the Z–O bond strengths are the same.[16-18] At 10 mM ZOH, TZ/T is about 10^{-4}. In order to account for the observed ZOH-catalyzed hydrolysis of methyl trifluoroacetate (sixfold rate enhancement under these conditions) the lifetime of T must be longer than the lifetime of TZ by a factor of 10^4–10^5. Since the lifetime of T is 10^{-1} s (Table I), the lifetime of the C–O bond in TZ is about 10^{-5} to 10^{-6} s. The basicity of the leaving group in T is higher than that in TZ by a factor of 10^7. The metal hydroxide not only catalyzes the formation T but also the breakdown of T to form the products (eq 3) since the expulsion of methoxide should be at least partially rate determining for the uncatalyzed process.[19,20]


(3)

In conclusion, a weakly basic nucleophile has been shown, for the first time, to be highly effective in catalyzing the hydrolysis of an ester with a poor leaving group. This catalysis is effected by rapid metal–oxygen ligand exchange processes. The effectiveness of the catalyst is related to the lifetime of the tetrahedral intermediate involved and can be estimated. We are currently investigating the reactions of other esters and metal hydrates.

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Registry No. ZOH, 90065-64-8; methyl trifluoroacetate, 431-47-0; methyl acetate, 79-20-9.

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Resonance Raman Spectra of the First Three Reduction Products of Tris(bipyridyl)ruthenium(II)

S. M. Angel, M. K. DeArmond,* R. J. Donohoe,
K. W. Hanck, and D. W. Wertz*

Department of Chemistry
North Carolina State University
Raleigh, North Carolina 27695-8204

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The extent of localization in the emitting state of the tris(bipyridyl)ruthenium(II) ion, $Ru(bpy)_3^{2+}$, has been of considerable interest. Photoselection,[1] absorption,[2] and resonance Raman[3,4] studies have all been interpreted in terms of a metal ligand charge-transfer (MLCT) excited state in which the promoted electron is localized on one of the bipyridines rather than delocalized over all three. Electrochemical[5] and absorption spectra[6] investigations of the reduced species have been interpreted in a similar manner. We have initiated a study of the resonance Raman spectra (RRS) of various reduction products of a series of metal di- and triimmine complexes. We report here our preliminary results for complexes, $Ru(bpy)_3^n$, where $n = 2, 1, 0, -1$. Our results are consistant with a localized redox orbital which appears to be similar in nature to the optical orbital.

As an electron is added to the redox orbital, one of two possibilities exists: the electron will be delocalized over all three bipyridine rings with a net increase of $^1/_3$ of an electron per bipyridine or the electron will be localized on one ring thus forming one bpy^- anion and two neutral bipyridines on the resulting +1 cation. The addition of a second electron will then result in a neutral molecule in which there are either three bipyridines with $-^2/_3$ charge each or two bipyridine anions and one neutral bipyridine. In the case of the three-electron reduction product both models predict three equivalent bipyridine anions on the resulting −1 anion. Since these electrons are being added to π^* orbitals of the bipyridine, one would expect a decrease in energy of most of the bipyridine skeletal modes as the number of electrons in these orbitals increases. Thus in the delocalized model, one would expect to see only one set of bipyridine vibrations whose frequencies would decrease with the addition of each electron. In the localized model, both bpy and bpy^- modes would be expected in the cases of $[Ru(bpy)_3]^{1+}$ and $Ru(bpy)_3$ depending on the nature of the excited state in resonance with the resonance Raman excitation. The frequencies of the bipyridine vibrations should be essentially the same as in the $[Ru(bpy)_3]^{2+}$ species and the bipyridine anion frequencies should be those observed for the $[Ru(bpy)_3]^{1-}$ species. Thus the resonance Raman spectra of this series of complexes should allow for a clear distinction between these two models.

Figure 1 shows the Raman spectra of the four complexes in the region of 980 to 1630 cm^{-1} and Table I gives the frequencies of these complexes as well as those for the MLCT excited state and the bipyridine anion reported by Woodruff et al.[3] and by Forster and Hester.[4] The resonance Raman spectrum of the ground state of the +2 ion has been reported previously[3,4] but has been included for comparison. The spectrum of the −1 species is predicted to be that of a bipyridine anion in either a localized or delocalized model. That this is the case can readily be seen by referring to Table I. With the exception of the bipyridine peaks at 1090 and 1429 cm^{-1}, which are covered by solvent peaks in the −1 species, there is a one to one correlation of every peak in the region studied. Thus, the top spectrum ($n = 2$) is the bipyridine limit whereas the bottom spectrum ($n = -1$) is the bipyridine anion limit. The peaks at 1174 and 1273 cm^{-1} in the +2 species are

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