Exploring the Limits of Efficiency of Proton-Transfer Catalysis in Models and Enzymes

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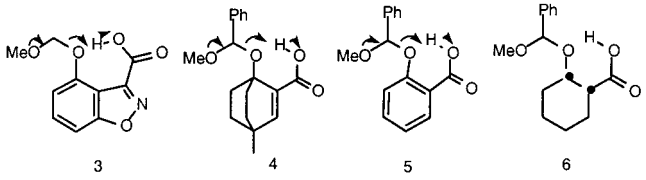
Proton transfer is the most common enzyme-catalyzed reaction, but intramolecular proton transfer (general acid−base) catalysis in model systems is notably inefficient. [1,2] Effective molarities (EM)[3] are generally lower by many orders of magnitude than those for intramolecular nucleophilic (cyclization) reactions, rising above 1−10 M only in special cases. So these special cases are of particular interest. We report the first example (Scheme 1) for which intramolecular general acid catalysis (IGAC) matches typical nucleophilic catalysis efficiencies, and an estimate of a remarkable 14 kcal/mol for the transition state stabilization involved.

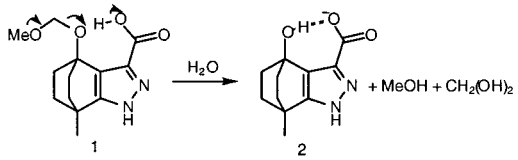
We have developed a handful of systems supporting EMs for IGAC as high as 10^{5-6} M,[1] and identified as a key factor the development of a strong intramolecular hydrogen bond as the reaction proceeds. Most efficient was the benzisoxazole **3**,[4] but more relevant to enzyme mechanism would be systems with alcohol rather than (the much better) phenol leaving groups. A rare example (**4**[5]), patterned on the efficient salicylic acid geometry **5**, showed relatively efficient catalysis, but the reaction was observed only for (activated) acetals of benzaldehyde.



3 4 5 6

We expected a similarly saturated tricyclic system based on the favorable geometry of **3** to show high efficiency also. The corresponding pyrazole proved to be the most readily accessible relevant heterocycle, and we have prepared the methoxymethyl acetal **1** as a test system. We find that **1** is indeed hydrolyzed (Scheme 1) with intramolecular general acid catalysis of unprecedented efficiency (Figure). The half-life of **1** (based on k_0, see the caption for Figure 1 for details) is 3.5 h in water at 39 °C. This corresponds to an acceleration of over 10^{10} compared with a similar system lacking the neighboring general acid: the tertiary alcohol **2** is lost at the rate expected for a leaving group with pK_a around 4.[6,7] In terms of transition state stabilization this acceleration is worth over 14 kcal (58 kJ)/mol: we attribute this primarily to the exceptional stability of the transition state hydrogen bond.[1,8]

Scheme 1



1 2

This figure is mechanism-dependent, and is based on a comparison of the mechanism shown in **1** with the spontaneous, pH-independent hydrolysis of methoxymethyl acetals. In terms of the kinetically equivalent specific acid catalyzed hydrolysis (SAC, Scheme 2) of the anion of **1**, the acceleration is only 107-fold, as indicated by the modest extent of the "plateau" in the pH−rate profile (Figure 1). We have rejected this mechanism for efficient IGAC in the past because linear free energy relationships indicate no significant proton transfer from COOH in the transition state for hydrolysis of salicylic acid derivatives,[14] and our new results reinforce this conclusion. In particular, the SAC mechanism does not explain the exceptional sensitivity of catalytic efficiency to geometry. For example, the benzaldehyde acetal **6** shows no IGAC by the neighboring COOH group:[15] the reaction concerned must therefore be slower than the plateau reaction of **1**, even though benzaldehyde acetals are typically hydrolyzed some 10^5 times faster than their methoxymethyl equivalents.[16]

Specific acid catalysis of the hydrolysis of the anion of **1** would formally involve rapid, preequilibrium protonation followed by rate-determining cleavage of the conjugate acid **7** (Scheme 2). **7** would be stabilized by a strong intramolecular hydrogen bond to the neighboring carboxylate group, providing a low-energy (probably barrierless) pathway to the thermodynamically favored **1**. *Formation* of **7** must therefore involve the same pathway, via the COOH group of **1**. Furthermore,[16] a strong intramolecular hydrogen bond will inhibit the rapid proton-transfer equilibrium which defines SAC. Thus the proton that catalyzes the cleavage of the acetal group of **1** must be supplied by the COOH group. This defines the mechanism formally as general acid catalysis. This mechanism is observed in systems such as **1**, with the correct geometry, because it supports a faster reaction than the SAC

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(3) The EM is defined as the concentration of a catalytic group, in a separate molecule, required to make the corresponding intermolecular reaction go as fast as the intramolecular reaction of interest. Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183−278.
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(6) It is not possible to estimate an EM convincingly for the hydrolysis of an alcohol methoxymethyl acetal because the comparison intermolecular reaction is too slow to measure. Our estimate of the efficiency of catalysis is based on the rate acceleration. A reasonable linear free energy relationship is available for the spontaneous hydrolysis of reactive methoxymethyl acetals.[8,9] Using the measured rate constant (3.15×10^{-6} s^{-1} at 39 °C) for the hydrolysis of methoxymethyl 3,4-dinitrophenol,[10] Cordes' $\beta_{LG} = 0.82$,[9] and a conservative estimate of 16.8 for the pK_a of the tertiary alcohol leaving group we arrive at a figure of 1.5×10^{-15} s^{-1} for the rate constant for hydrolysis at 39 °C of **1** lacking the carboxyl group.
(7) Such a large ratio inevitably depends on a long extrapolation, the reliability of which demands careful scrutiny: especially since recent work of Wolfenden et al.[11] suggests that a similar extrapolation based on data for aryl esters may underestimate the rate of hydrolysis of the methyl phosphate dianion substantially. This raises the possibility that methoxide may behave significantly differently as a leaving group in comparison with aryloxide anions. This does not appear to be the case for glucosides: the rate constant for the spontaneous hydrolysis of methyl β-D-glucoside, estimated from a β_{LG} extrapolation from the data of Nath and Rydon,[12] is about the same (10^{-12} s^{-1} at 60 °C) as that obtained from the measurements of Wolfenden et al.[11]
(8) Craze, G.-A.; Kirby, A. J. *J. Chem. Soc., Perkin Trans. 2* **1978**, 354−6.
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(13) 10 mL of a stock solution of **4** in methanol (5 mg/mL) was added to 0.01 M aqueous buffer (190 μL) of ionic strength 0.05 M (KCl), containing 4-nitrobenzenesulfonate as integration standard. Two 70 μL aliquots were sealed in ampules and incubated in a water bath at 39 °C, and the remaining solution retained as a control. Sealed ampules were removed at intervals, cooled, and opened and 20 μL aliquots were added to 0.2 M KOH (5 μL) to stop the reaction. Both samples and the control were analyzed by capillary electrophoresis using a Beckmann-Coulter PACE MDQ instrument (at 264 nm, 25 °C, run buffer 0.1M phosphate, pH 6.20).
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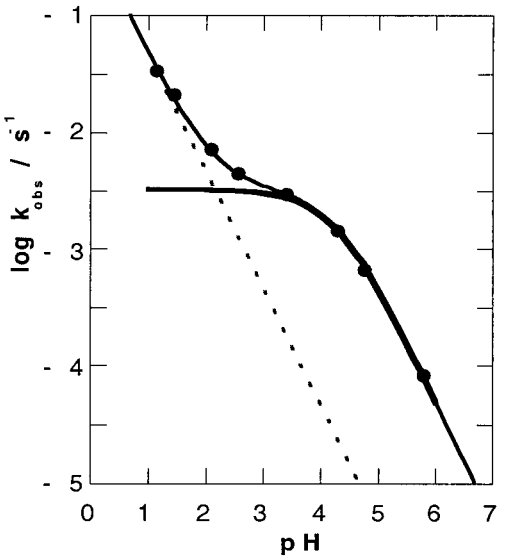
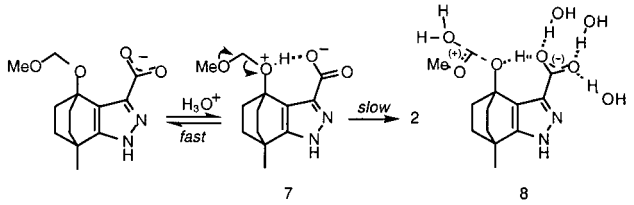


Figure 1. pH−rate profile for the hydrolysis of **1**, in 95:5 water−methanol at 39 °C and ionic strength 0.05 M.[13] The experimental points define the main calculated curve (based on $k_{obs} = (k_H \cdot a_H + k_0)[AH]$) and least-squares values for k_H, k_0, and the pK_a of $7.7 \pm 0.4 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}, $5.5 \pm 0.3 \times 10^{-5}$ s^{-1}, and 4.18 ± 0.04, respectively. The dashed line indicates rates expected for the specific acid catalyzed hydrolysis of **1** in the absence of the COOH group, based on k_H: the bold curve shows the reaction catalyzed by the COOH group.

Scheme 2



mechanism. Furthermore, it is available to COOH groups shielded from bulk solvent in enzyme active sites: in terms of the curves drawn in Figure 1, the plateau (bold curve describing the rate of the COOH catalyzed reaction) can in principle be extended to higher effective pH, depending only on the local microenvironment. (Linear free energy relationships for the hydrolysis of salicylic acid acetals[14] showed reactivity was independent of the pK_a of the catalytic COOH group.)

There has been much recent discussion[17−20,21] about the potential contribution of hydrogen bonding to the catalytic efficiency of enzymes, conveniently summarized as a question:[21] "Is it possible that some hydrogen bonds...in the active sites of enzymes have energies in the range 10−20 kcal/mol?" The evidence from model system **1** suggests strongly that the answer is positive, at least for transition state hydrogen bonds. (The distinction is important, because of the extra covalent contribution to the strength of the transient H-bond (**8**)—and thus to dynamic binding.[22]) It has been clear for many years that the mechanism of efficient IGAC differs in detail from "classical" GAC, where proton transfer and heavy atom motion are considered to be closely coupled or concerted, and a more detailed study of the reaction of **1** and related systems, including a calculational investigation, is under way. Very recently we have obtained a crystal structure of the product anion **2** (as the tetra-*n*-butylammonium salt), in which the intramolecular H-bond is indeed close to linear (angle O—H···O 175.1°, O···O interatomic distance 2.71 Å). The geometry provides a well-defined starting point for calculations. So far we can say that the evidence is consistent with a transition state involving more or less barrierless proton transfer[23] at a point where the basicities of the donor and acceptor oxygens have become more or less equal, with partial covalent bonding to both. (The kinetic isotope effects k_H/k_D for similar reactions are of the order of 1.5 ± 0.2.[1]) There seems little doubt that the key to understanding this mechanism lies in the properties of the intramolecular hydrogen bond.[24]

The strongest ground-state hydrogen bonds are ionic (which they must be in a transition state for proton transfer) and linear, and involve donor and acceptor centers of equal basicities an optimal distance apart: system **1** was designed specifically to provide such a favorable geometry. They are also typically observed, experimentally or calculationally, in apolar solvents or in the gas phase (whence much discussion of how far such conditions might apply to certain enzyme active sites). Our experiments were done in water, so we can interpret apparent pK_a values with some confidence. The effective pK_a of the tertiary alcohol leaving group of **1** equals that of the COOH group (4.18 ± 0.04, Figure 1) of the reactant, within the accuracy of our estimate. This pK_a value tells us also that there is no strong hydrogen bonding in the reactant ground state.

The data for the hydrolysis of **1** are consistent with a transition state involving strong intramolecular hydrogen bonding, in which the pK_a values of the leaving group oxygen and the carboxyl group are matched. These are properties likely to characterize the in-flight proton central to general acid−base catalysis in an enzyme active site. We have no reason to suppose that our model system marks maximum efficiency, so conclude that *at least* 14 kcal mol^{-1} of transition state stabilization is available from this source to enzymes catalyzing intrinsically slow reactions.

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Supporting Information Available: Synthetic scheme and details of the preparation of compound **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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