Apparently the fluorescence lifetime, $\tau = 1/k_f$, has not been directly measured. Integrating the absorption coefficient measured previously,[8,9] in the region 1650–2300 Å, the oscillator strength of 0.067 is obtained which gives a lifetime of 9 nsec. If this value is adopted, eq II may be reduced to

$$I_f^0/I_f = 1 + k_3[Ar]/(k_f + k_d) \qquad \text{(III)}$$

because $k_2[SO_2]$ can be neglected with respect to $k_f + k_d$.

Using $I_f^0/I_f = 7.7$, $k_d = 0$ (Figures 1b and c, band 6), [Ar] = 1.4×10^{19} molecules cm^{-3} and 1.1×10^8 sec^{-1} for k_f, the quenching rate k_3 of the excited state at band 6 is 5×10^{-11} cm^3 molecule^{-1} sec^{-1}. This value is in reasonable agreement with the quenching rate by Ar of 5.2×10^{-11} for the first excited singlet state produced by light of wavelength 2750–3020 Å.[14] Assuming the same quenching rate for the excited state produced at band 7 and from the measured I_f^0/I_f, the lifetime can be calculated to be 3.2 nsec. This reduced lifetime must be due to predissociation; *i.e.*, k_d is no longer zero in band 7. The change in the width of an absorption band introduced by predissociation would be too small to provide evidence for predissociation.

Finally, Figure 1d shows the fluorescence intensity curve of a mixture of 0.3 Torr of SO_2 and 1 atm of air. The fluorescence is still detectable, the radiative life being sufficiently short relative to the quenching rate. The result suggests that a small amount of SO_2 in ambient air can be detected without interference from other pollutants by using an intense light source of appropriate wavelength.

Acknowledgment. This work was partially supported by the Measures for Air Quality Program at the National Bureau of Standards.

(14) H. D. Mettee, *J. Phys. Chem.*, 73, 1071 (1969).

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Intramolecular Bifunctional Catalysis of Ester Hydrolysis by Metal Ion and Carboxylate in a Carboxypeptidase Model

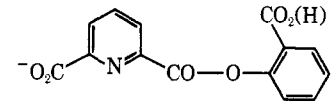
Sir:

Extensive work has clarified the nature of the active site in the enzyme carboxypeptidase A and the functional groups involved in catalysis.[1] In the hydrolysis of peptides there is participation by a zinc ion, a carboxylate ion, and a tyrosine hydroxyl. The enzyme will also hydrolyze esters, but in the latter case apparently only the metal ion and the carboxylate play a role,[2] the former as a Lewis acid coordinated to carbonyl oxygen and the latter as either a general base or nucleophile. This situation naturally raises the question as to how effective such a combination can be in simple chemical models.

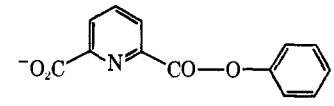
(1) W. N. Lipscomb, J. A. Hartsuck, F. A. Quiocho, and G. N. Reeke, *Proc. Nat. Acad. Sci. U. S.*, **64**, 28 (1969); for a recent review, see J. E. Coleman, *Progr. Bioorg. Chem.*, **1**, 159 (1971).

(2) R. T. Simpson, J. F. Riordan, and B. L. Vallee, *Biochemistry*, 1, 231 (1962).

Of course, there are many examples[3] of metal-promoted hydrolysis of carboxylic acid derivatives, and the effects of metal ions can in some cases be quite high, comparable to enzymatic accelerations.[4] Similarly, neighboring group participation by carboxylate ion in the hydrolysis of compounds such as acetylsalicylic acid has been extensively studied,[5] although in this system catalysis is rather modest. We wish to report the study of a case in which simultaneous catalysis by internally bound metal ion and neighboring carboxylate operates on the hydrolysis of an ester.[6]



1



2

We have studied the hydrolysis of the salicylic acid ester of pyridine-2,6-dicarboxylic acid (**1**) and the corresponding phenyl ester of pyridine-2,6-dicarboxylic acid (**2**) as a function of pH with and without metal ion. For most of the work the metal ion chosen was Ni^{2+} (Ni^{2+} can replace Zn^{2+} in the enzyme[7]) although Zn^{2+} was also examined in two cases. The pyridinecarboxylate group is a good metal ligand[8] and there is no difficulty in binding a metal ion to it in a position to activate the ester group. Kinetic studies as a function of metal concentration showed that under the conditions reported below the substrate was present entirely as a 1:1 metal complex (*i.e.*, the metal concentration is so high that the kinetics are zero order in metal). All hydrolysis studies were carried out at 25.0° in H_2O with $\mu = 0.5$ *M* (NaCl). Substrate concentrations were 5×10^{-4} *M* and metal concentrations were 0.2 *M;* buffers were used at three different concentrations and rates were extrapolated to zero buffer concentration. Runs were monitored at 298 (**1**) or 276 nm (**2**) with a Gilford spectrophotometer, and the computer-processed (least-squares and Guggenheim treatment) kinetic data of at least three runs were averaged (average deviation 3–5%) for each datum point. The results of this study are displayed in Figure 1 and the kinetic constants extracted from these studies are listed in Table I.

These data show fairly large metal ion catalysis in the hydrolysis of either **1** or **2**. Thus, the rate of attack

(3) Reviewed by A. E. Martell, *Pure Appl. Chem.*, 17, 129 (1968).

(4) *E.g.*, an acceleration of 10^9 by Cu^{2+} in a carbonitrile hydration: R. Breslow, R. Fairweather, and J. Keana, *J. Amer. Chem. Soc.*, **89**, 2135 (1967).

(5) *Cf.* W. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969.

(6) Although many apparent examples of intramolecular bifunctional catalysis have been reported, these have been critically examined by T. Maugh II and T. C. Bruice, *J. Amer. Chem. Soc.*, **93**, 3237 (1971), who conclude that there is no authentic case of bifunctional catalysis, as contrasted with monofunctional catalysis accompanied by electrostatic effects associated with protonation. A recent report by T. Higuchi, H. Takechi, I. H. Pitman, and H. L. Fung (*J. Amer. Chem. Soc.*, **93**, 539 (1971)) describes what seems to be an authentic case of bifunctional catalysis of ester hydrolysis. Thus ours may be the second authentic case of intramolecular bifunctional catalysis, and the first involving a metal ion.

(7) J. E. Coleman, P. Pulido and B. L. Vallee, *Biochemistry*, **5**, 2019 (1966).

(8) For Ni^{2+}, log $K_1 = 6.4$ and for Zn^{2+}, log $K_1 = 5.12$, according to L. G. Sillen and A. E. Martell, Ed., *Chem. Soc. Spec. Publ.*, **No. 17** (1964).

Table I. Hydrolysis Rate Constants, 25.0°, $\mu = 0.5\ M$

Compd	k_{H_2O}, M^{-1} sec^{-1}	k_{OH^-}, M^{-1} sec^{-1}	Ref
$1^0 \cdot Ni^{2+}$	2.8×10^{-5}	2.7×10^{7}	*a*
$1^- \cdot Ni^{2+}$	4.8×10^{-5}	2.2×10^{5}	*b*
1^0	5.0×10^{-7}	2.7×10^{5}	*c*
1^-	5.8×10^{-7}	7.1×10^{1}	*d*
$2 \cdot Ni^{2+}$	7.5×10^{-6}	2.8×10^{6}	
2	$<10^{-7}$	3.0×10^{2}	
Acetylsalicylic acid^0	3.4×10^{-7}	6.9×10^{6}	*e*
Acetylsalicylic aci d$^-$	2.8×10^{-6}	2.0×10^{1}	*e*
Phenyl acetate	2.0×10^{-8}	7.6×10^{1}	*e*

[a] $1^0 \cdot Ni^{2+}$ is the nickel complex of **1** in which the salicylate carboxyl is protonated. [b] $1^- \cdot Ni^{2+}$ is the nickel complex of **1** in which the salicylic carboxyl, $pK_a = 4.0$, is ionized. [c] 1^0 is the form of **1** in which the group with apparent $pK_a = 4.0$ is protonated. [d] 1^- is the (set of) species of **1** in which the group with apparent $pK_a = 4.0$ is ionized. [e] Data from T. St. Pierre and W. P. Jencks, *J. Amer. Chem. Soc.*, **90**, 3817 (1968). Acid^0 and acid$^-$ refer to the nonionized and ionized species, respectively.

by hydroxide ion on the anion of **1** (which is thus a dianion) is increased by a factor of 3100 by coordination with Ni^{2+}, while the rate of attack of hydroxide ion on **2** is increased by a factor of 9300. Observations on **1** with Zn^{2+} at pH 4.2 (plateau) and pH 6.2 (slope) indicate that Zn^{2+} is 80% as good a catalyst as Ni^{2+} for the hydrolysis of the anion of **1** in either the H_2O or OH^- term. The data also indicate that the attack by water on the metal complex of **1** is 1.7 times as fast when the carboxyl is ionized as when it is protonated.[9] Since this is in a direction opposite to the expected reactivity of salicylate as a leaving group, it must represent simultaneous catalysis by metal and carboxylate ion. (Because of the complex ionic equilibria involved, with several points of possible protonation, it is not possible to make a meaningful statement about the effect of deprotonation of **1** on the hydrolysis rate in the absence of metal ion.)

The overall bifunctional catalysis by a combination of metal and carboxylate is relatively modest. The two function simultaneously, but their interaction is only *semicooperative*.[10] This must partly be because the catalytic effect of a neighboring carboxylate in compounds related to aspirin is small, the increase in k_{H_2O} for aspirin on deprotonating the neighboring carboxyl being only a factor of 8.2. In addition, there are a number of other respects in which this simple compound **1** as its metal complex does not contain factors involved in carboxypeptidase A. It is likely that in our system the carboxylate acts as a general base,[11] while there is some evidence[12] that in carboxypeptidase it acts as a nucleophile. The catalytic effects of our metal ion and our carboxylate are presumably strongly diminished by solvation with water, and in the protein itself the groups are protected from the leveling effect of the solvent by enclosure in a hydrophobic region of the protein. Furthermore, the conformation of $1 \cdot Ni^{2+}$ is not well defined. Indeed a principal function of the protein must be as a clamp, freezing out rotations and stiffening vibrations so as to decrease the residual entropy of the bound reactants. Thus, a number of other features must be built into such models before they can be expected to approach enzymatic rates even when, as in this case, the correct functional groups are involved in the catalysis.

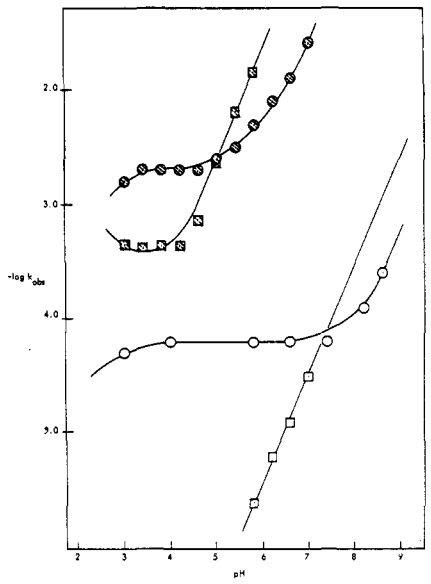


Figure 1. Rate of hydrolysis *vs.* pH for compounds **1** and **2**: ●, **1** + Ni^{2+}; ■, **2** + Ni^{2+}; ○, **1**; □, **2**. The curves are theoretical, drawn from the expression $k_{obsd} = (1 - \alpha)k_{H_2O \cdot CO_2H}[H_2O] + \alpha k_{H_2O \cdot CO_2^-}[H_2O] + \alpha k_{OH^- \cdot CO_2^-}[OH^-]$, using the rate constants of Table I, in which α is the degree of ionization of the salicylate carboxyl group (or in the case of simple **1**, whichever group has apparent $pK_a = 4.0$).

Acknowledgment. Support of this work by the National Institutes of Health is gratefully acknowledged.

(9) Of course this comparison involves the assumption that the protonated carboxyl group is not catalytic. T. St. Pierre and W. P. Jencks (*J. Amer. Chem. Soc.*, **90**, 3817 (1968)) describe the evidence that such hydrolyses are more weakly catalyzed by carboxyl than carboxylate, but if there is in fact any catalysis by carboxyl our comparison gives an underestimate of the carboxylate effect.

(10) In principle, cooperativity (synergism) in a polyfunctional catalytic system can take several forms, for which we propose the terms "semicooperative," "cooperative," and "supercooperative." The interaction of one catalytic group with an ester function, for instance, would in general change the character of the transition state for the ester hydrolysis and thereby change the extent to which this new transiton state can be stabilized by interaction with a second catalytic group. If the new transiton state be now less susceptible to stabilization by a second catalytic function, but still show some stabilization, we refer to the case as "semicooperative." The most interesting possibility is that the new transition state be *more* susceptible to stabilization by a second catalytic group because of its interaction with the first catalytic group, the case we refer to as "supercooperative." These possibilities are summarized by eq 1, in which the aggregate effect of a set of catalytic

$$(k_{cat}/k_{uncat})_{polycat} = c_{cat}(k_{cat}/k_{uncat})_I(k_{cat}/k_{uncat})_{II} \quad (1)$$

groups is the product of the individual rate factors multiplied by c_{cat}, the catalytic cooperativity constant for the particular polycatalyzed system. Alternatively, eq 2 puts it in the form of a linear free-energy

$$(\Delta\Delta G^{\ddagger})_{polycat} = (\Delta\Delta G^{\ddagger})_I + (\Delta\Delta G^{\ddagger})_{II} + \ldots + C_{cat} \quad (2)$$

relation, in which C_{cat} is now the catalytic cooperativity factor. The switch from semicooperativity to supercooperativity occurs when c_{cat} goes from less than 1 to greater than 1, or C_{cat} goes from a positive energy to a negative one. Of course, there is also the possibility that catalysis would be completely "noncooperative," in the sense that only one catalytic group at a time would be able to function to the exclusion of the others; or indeed, even "anticooperative" in the sense that the resultant would be less than for any individual group. These cases are also covered by appropriate values of c_{cat} or C_{cat}. Since in the present case the salicyl carboxylate group contributes less than the "normal" catalytic factor it contributes for esters which do not have simultaneous metal catalysis, the present bifunctional catalysis is semicooperative.

(11) Based on the extensive studies of salicylate derivatives reviewed by A. J. Kirby and J. A. R. Fersht, *Progr. Bioorg. Chem.*, 1, 1 (1971).

(12) T. Kaiser, private communication.

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Received August 30, 1971