are available,[1,2] carbanion stabilization is smaller[10] (lower K_1, higher k_2), and the intrinsic rate constants are higher[6,9c] (higher k_2).

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Hammett Analysis of Enzyme Action in Organic Solvents

Liisa T. Kanerva† and Alexander M. Klibanov*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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Among the means available in physical organic chemistry to probe transition-state structure, linear free energy correlations of structure and reactivity have been the most valuable.[1] This methodology, specifically Hammett analysis, has also been profitably used in mechanistic enzymology.[2] A major recent development in biochemistry is enzymatic catalysis in anhydrous organic solvents.[3] The ability of enzymes to function as catalysts in water-free media poses a challenging fundamental question of whether enzymatic reaction mechanisms in such media are the same as in aqueous solution. This issue is directly addressed in this study using Hammett analysis.

We selected a protease from *Bacillus licheniformis*[4] (subtilisin Carlsberg)[5] as a model for our investigation. This enzyme, whose physiological role is to hydrolyze water-soluble proteins in aqueous solutions,[5] is nevertheless catalytically active in a number of anhydrous organic solvents[6] (allowing for useful preparative transformations[7]); furthermore, substrate[8] and enantiomeric[9] specificities of subtilisin in organic media are radically distinct from those in water.[8,9] In the present work, we kinetically investigated subtilisin-catalyzed cleavage of para-substituted phenyl acetates (nitro-, acetyl-, chloro-, methyl-, and methoxy-) in water (hydrolysis) and in five anhydrous organic solvents (transesterification with 1-hexanol). Figure 1 depicts the dependencies

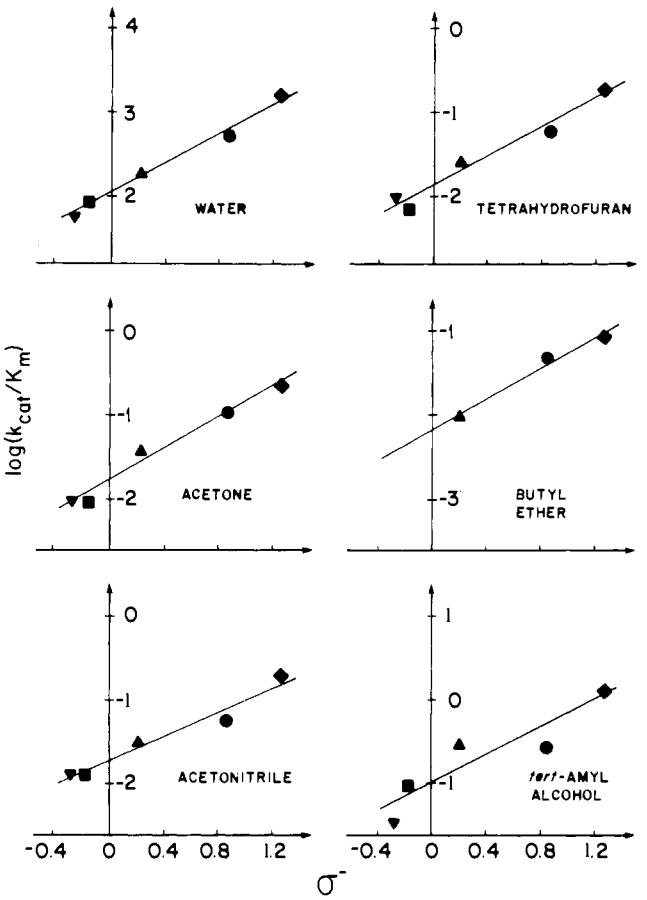

Figure 1. Hammett correlations for k_{cat}/K_m of subtilisin-catalyzed cleavage (hydrolysis in water and hexanolysis in organic solvents)[10] of para-substituted phenyl acetates. Symbols for the substituents are as follows: (♦), NO_2; (●), CH_3CO; (▲), Cl; (■), CH_3; and (▼), CH_3O. Conditions: (i) For enzymatic hydrolysis in water, substrate concentrations were varied from 0.2 to 1.3 mM, concentrations of the enzyme were in the range from 5.5 to 16.5 mg/L, pH 7.75 (20 mM phosphate buffer containing 2% acetonitrile), 30 °C; all reactions were followed spectrophotometrically as described in the literature.[17] (ii) For enzymatic transesterifications with 1-hexanol in anhydrous organic solvents, phenyl ester concentrations were varied from 10 to 100 mM, hexanol concentration was 1 M, and the concentration of the enzyme (lyophilized from the phosphate buffer, pH 7.75, as previously described[6]) was 1 mg/mL. All reactions were carried out at 30 °C with shaking at 300 rpm and were followed by capillary gas chromatography as described earlier.[6] Organic solvents were of analytical grade and were dried by shaking overnight with 4 Å molecular sieves prior to use. In the case of butyl ether as a solvent, enzymatic reactions with *p*-methyl- and *p*-methoxyphenyl acetates were too slow to measure accurately. The units of k_{cat}/K_m are $M^{-1}\cdot s^{-1}$.

of k_{cat}/K_m[10] on the substituent constant σ^- for both aqueous and nonaqueous reaction media. One can see that in each instance a satisfactory linear dependence is obtained (all correlation coefficients greater than 0.9), thereby allowing for the determination of the reaction constant ρ[11] (Table I).

Inspection of the subtilisin data in Table I reveals that in all solvents the ρ values are between 0.72 and 0.93, and the ρ value for the enzymatic reaction in water is near the middle of this range. The organic solvents employed in these experiments are both water-immiscible (butyl ether and *tert*-amyl alcohol) and

† On leave from Departments of Chemistry and Biomedicine, University of Turku, SF-20500 Turku 50, Finland.

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Table I. Values for the Enzymatic Acylation of Subtilisin and α-Chymotrypsin by a Series of Para-Substituted Phenyl Acetates in Various Solvents[a]

enzyme[b]	solvent[c]	ρ[d]	ref
subtilisin	water	0.87	this work
subtilisin	tetrahydrofuran	0.86	this work
subtilisin	acetone	0.93	this work
subtilisin	butyl ether	0.91	this work
subtilisin	acetonitrile	0.72	this work
subtilisin	*tert*-amyl alcohol	0.83	this work
α-chymotrypsin	water	1.8	*e*
α-chymotrypsin	water	1.8	ref 17
α-chymotrypsin	water	2.1	*f*
α-chymotrypsin	water	1.9	*g*

[a] For experimental conditions, see the legend to Figure 1. [b] Subtilisin was obtained, prepared, and used as previously described (ref 6). We established that the enzyme irreversibly inactivated by the active center-directed inhibitor phenylmethanesulfonyl fluoride (Fahrney, D. E.; Gold, A. M. *J. Am. Chem. Soc.* **1963**, *85*, 997-1000) was completely inactive in organic solvents; hence the transesterification reactions observed and their ρ values correspond to the molecular events occurring in subtilisin's active center. [c] All nonaqueous solvents were dried to bring the water content below 0.01% (measured as described in ref 6). [d] All ρ values are determined using σ^- substituent constants, as had been done in the four studies of chymotryptic hydrolysis in water depicted at the bottom of the table. [e] Bender, M. L.; Nakamura, K. *J. Am. Chem. Soc.* **1962**, *84*, 2577-2582. [f] Hubbard, C. D.; Shoupe, T. S. *J. Biol. Chem.* **1977**, *252*, 1633-1638. [g] Ikeda, K.; Kunugi, S.; Ise, N. *Arch. Biochem. Biophys.* **1982**, *217*, 37-46.

water-miscible (acetone, tetrahydrofuran, and acetonitrile) and differ widely in terms of their hydrophobicity[12] and polarity;[13] and yet, all the ρ values fall within less than a ±15% range (Table I). Therefore, given the substantial error inherent in the experimental determination of this reaction constant,[11] it can be concluded that the ρ value for the subtilisin-catalyzed cleavage of phenyl acetates is essentially independent of the nature of the solvent.

It should be stressed that the value of ρ, which reflects the charge distribution in the transition state, is very sensitive to even subtle changes in the mechanism of chemical processes in general,[1,11] and subtilisin catalysis in particular. With respect to the latter, two examples are particularly indicative. First, the ρ value for subtilisin-catalyzed hydrolysis (in aqueous solutions) drops almost 4-fold when substituted phenyl hippurates are used as substrates instead of substituted phenyl acetates.[14] Second, when subtilisin is replaced with the closely related[15] serine protease α-chymotrypsin as a catalyst, the ρ value for the hydrolysis of phenyl acetates in water more than doubles (Table I). Comparison of these results with our data suggests that the structure of the transition state formed upon acylation of subtilisin with phenyl acetates is substantially the same regardless of the solvent.

The ρ values for the (nonenzymatic) alkaline hydrolysis of substituted phenyl acetates (as well as for other chemical processes[1,11]) strongly depend on the reaction medium, e.g., upon a transition from water to 62% aqueous acetone the reaction constant increases from 0.66-0.71 to 1.14;[16] presumably, an even greater change would be observed for more concentrated acetone solutions. In contrast, the ρ values for subtilisin-catalyzed cleavage of phenyl acetates in water and in neat tetrahydrofuran and *tert*-amyl alcohol are virtually identical (Table I). These data suggest that the microenvironment of the transition state of the enzymatic reaction is the same for the three solvents, i.e., that the enzyme's active center is shielded from the bulk solvent. Overall, the results of this study indicate profound mechanistic similarities of subtilisin catalysis in water and various organic solvents.

(12) Log *P* (where *P* is the logarithm of the partition coefficient between 1-octanol and water) for the solvents used varies from −0.33 for acetonitrile to +2.9 for butyl ether (Laane, C.; Boeren, S.; Vos, K.; Veeger, C. *Biotechnol. Bioeng.* **1987**, *30*, 81-87).

(13) For example, the dielectric constant and dipole moment, respectively, vary from 5.8 and 1.7 for *tert*-amyl alcohol to 36 and 3.9 for acetonitrile (Gordon, A. L.; Ford, R. A. *The Chemist's Companion*; Wiley: New York, 1972; pp 2-13).

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Metalloporphyrin π-Cation Radicals: Intrinsically Ruffled or Planar Core Conformations? Molecular Structure of (Mesitylporphinato)copper(II) Hexachloroantimonate

Hungsun Song,[2] Christopher A. Reed,*[,1] and W. Robert Scheidt*[,2]

Departments of Chemistry, University of Notre Dame Notre Dame, Indiana 46556 University of Southern California Los Angeles, California 90089-0744

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Metalloporphyrin π-cation radicals are important in the redox chemistry of a variety of biological systems including heme catalysis and photosynthesis. Our groups have recently been concerned with their structural characterization with special emphasis on understanding their magnetic properties.[3,4] Spin coupling mechanisms between the porphyrin radical and unpaired electrons at the metal are exquisitely sensitive to structure. Ruffled porphinato cores lead to antiferromagnetic coupling, whereas planar cores show ferromagnetic coupling, a phenomenon that can be understood from inspection of the symmetries of the magnetic orbitals. Although all structurally characterized species follow this correlation, the full characterization of a pair of π-cation derivatives that have the same metal and ligation state but which individually display magnetic properties characteristic of the two coupling modes has remained elusive.

One key structural question that has arisen from the studies to date is whether the S_4-ruffled porphinato core observed in all previously structurally characterized four- and five-coordinate π-cation complexes is an intrinsic structural property of these molecules. We have suggested that the unusual saddle-shaped S_4-ruffled porphinato core[5] observed in the π-cation radical species is a necessary molecular distortion required to allow dimerization and not an intrinsic property resulting from some distinctive electronic state of the π-cation. This argument is supported by the observation of the same type of conformation in a number of neutral tetraphenylporphyrin derivatives, all of which exhibit a π–π dimer interaction in the solid state.[6] In this communication and the following one, we present the structural characterization of two metalloporphyrin π-cation radicals that we believe conclusively resolve this issue. The present paper reports the solid-state structure of [Cu(TMP•)]$SbCl_6$,[7] the long sought ferromagnetically

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(2) University of Notre Dame.

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