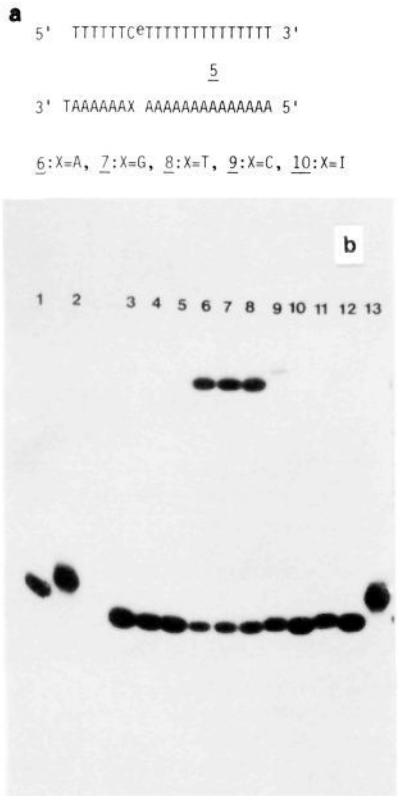

Figure 1. (a) The sequences of the alkylating oligomer **5** and the target oligomers **6**–**10**: A = deoxyadenosine, G = deoxyguanosine, T = thymidine, C = deoxycytidine, I = deoxyinosine. (b) Autoradiogram of 20% denaturing gel. All of these reactions were done in the following buffer: 10 mM NaCl, 10 mM Tris-HCl pH 7.5 2-fold excess of target oligomers (**6**–**10**) at 0 °C for 96 h. An asterisk indicates a ^{32}P label. Lane 1: **9*** [$d(A_{14}CA_6T)$] as size standard. Lane 2: **12*** [d(CATCGTCTAGATCTTTTGCCGC)] standard. Lane 3: **5** [$d(T_6C^eT_{14})$] which was treated with 80% acetic acid/water for 30 min at 24 °C, then concentrated and allowed to hybridize with **9*** [$d(A_{14}CA_6T)$]. Lane 4: **5** [$d(T_6C^eT_{14})$] and **9*** [$d(A_{14}CA_6T)$] in 50% formamide/buffer. Lane 5: **5** and **9*** in presence of 10-fold excess of unlabeled $d(T_{40})$. Lane 6: **5** and **9*** in presence of 10-fold excess **12**. Lane 7: The reaction of **5** and **9*** for 96 h (the cross-linked product **11** is the higher band) followed by 100 °C for 5 min. Lane 8: Reaction of **5** [$d(T_6C^eT_{14})$] with **9*** [$d(A_{14}CA_6T)$]. Lane 9: Reaction of **5** with **8*** [$d(A_{14}TA_6T)$]. Lane 10: Reaction of **5** with **6*** [$d(A_{21}T)$]. Lane 11: Reaction of **5** with **7*** [$d(A_{14}GA_6T)$]. Lane 12: Reaction of **5** with **10*** [$d(A_{14}IA_6T)$]. Lane 13: Reaction of **5** with **12*** {d(CATCGTCTAGATCTTTTGCCGC)}.

conditions of 100 °C for 5 min in 50% formamide (Figure 1b, lane 7), strongly suggesting a cross-linked **5** + **9** adduct. The labeled oligomer **5** was stable in dilute buffer solutions at 24 °C for at least 14 days, as evidenced by the fact that the reaction goes to near completion, giving only **11** after this time (data not shown). Only trace amounts of products of similar mobility were present in the reactions of **5** with the other target oligomers, most notably **8** (Figure 1b, lanes 9–12). The reaction of **5** with **9** has a half-life of ~30 h at 24 °C.

Oligomer **5** did not form a cross-linked product with labeled 22-mer d(CATCGTCTAGATCTTTTGCCGC) **12** under conditions identical with the reaction of **5** and **9** (Figure 1b, lane 13), despite the fact that **12** contains seven potentially reactive cytosines. This result is expected since this sequence cannot form a stable duplex with **5**. The observed formation of **11** is inhibited by the presence of either excess T_{40} (Figure 1b, lane 4) or 50% formamide (Figure 1b, lane 5), while excess 22-mer **12** has no effect on the formation of **11** (Figure 1b, lane 6), further demonstrating the requirement for duplex formation. The formation of **11** is dependent on the ethylenimine moiety in oligomer **5**, as demonstrated by the fact that acid-catalyzed ring opening abolishes the cross-linking reaction (Figure 1b, lane 3). Maxam–Gilbert sequencing of **11** gave a pattern consistent with a cross-link to cytosine on the target strand.[10] Since triple-helix formation was likely in this model system, we investigated conditions (excess **6**–**10**) that should give duplex predominantly.[11] In these experiments the results were qualitatively the same as those observed under conditions that favored triplex formation (data not shown).

In summary, we have prepared a synthetic oligodeoxynucleotide **5** which becomes reactive when constrained in a Watson–Crick double helix with **9**. The resulting duplex forms a product (**11**) containing a stable cross-link. The observed selectivity is for cytosine, but so far we have no information on the exact nature of the new bond formed (i.e., N3 vs. N4 linkage). The oligomer **5** is relatively unreactive with amines in solution (e.g., it is not significantly affected by 15 M aqueous ammonia at 22 °C for 30 min) but reactive with a cytosine at the appropriate position of the complementary hybridized oligomer **9**. We hypothesize that this is an example of a significant rate enhancement due to noncovalently bonded (i.e., hydrogen bonded) neighboring group participation.[12] The analogy can be drawn between the model system (**5** and **9**) and the reaction of an enzyme with suitable substrate on an exzyme active site, thus the observed selectivity of **5** for **9** could be explained by sensitive orientation effects or, alternatively, simply by the greater nucleophilicity of cytosine as compared to the other bases. Future experiments will be directed toward answering these questions and to the extension of this system to oligomers containing all four naturally occurring bases.

Acknowledgment. We thank Peter Ng, Parkash Jhurani, and Mark Vasser for their help in the synthesis and purification of the oligodeoxynucleotides used in this study.

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(11) Saenger, W. *Principles of Nucleic Acid Structures*; Springer-Verlag: New York, 1984.
(12) This observation could have some interesting theoretical implications; see: Menger, F. M.; Venkataram, U. V. *J. Am. Chem. Soc.* **1985**, *107*, 4706.

Does Ground State Fe^+ React with H_2?

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While numerous investigations of the gas-phase reactions of iron ions have been conducted in recent years,[1-3] none of these has suggested that the reactivity of the ground state may be over an order of magnitude less than that of the excited states. Previous work in our labs on the reactions of H_2 with several states of V^+[4] and Mn^+[5] led us to believe that the ^6D ($(4s)(3d)^6$) ground state and the ^4F ($(3d)^7$) first excited state of Fe^+ should exhibit very different reactivity with H_2 primarily because they differ in their electronic configuration. In the present study, we find that $Fe^+(^4F)$ reacts efficiently with H_2 but $Fe^+(^6D)$ is about 80 times less reactive. This result obtains despite the fact that these states are separated by only 0.25 eV.[6] This work is the first to assess the individual reactivities of such closely spaced electronic levels of a transition-metal ion.[7]

† NSF Presidential Young Investigator, 1984–1989.
(1) (a) Halle, L. F.; Armentrout, P. B.; Beauchamp, J. L. *Organometallics* **1982**, *1*, 963. (b) Halle, L. F.; Klein, F. S.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1984**, *106*, 2543.
(2) Allison, J.; Ridge, D. P. *J. Organomet. Chem.* **1975**, *99*, C11. Allison, J.; Freas, R. B.; Ridge, D. P. *J. Am. Chem. Soc.* **1979**, *101*, 1332. Babinec, S. J.; Allison, *J. Ibid.* **1984**, *106*, 7718.
(3) Burnier, R. C.; Byrd, G. D.; Freiser, B. S. *J. Am. Chem. Soc.* **1981**, *103*, 4360. Byrd, G. D.; Burnier, R. C.; Freiser, B. S. *Ibid.* **1982**, *104*, 3565. Jacobson, D. B.; Freiser, B. S. *Ibid.* **1983**, *105*, 5197, 7484, 7492.
(4) Elkind, J. L.; Armendtrout, P. B. *J. Phys. Chem.* **1985**, *89*, 5626.
(5) Elkind, J. L.; Armentrout, P. B. *J. Chem. Phys.*, in press.
(6) The ^6D state has an energy of 0.052 eV. The ^4F state has an energy of 0.300 eV. These energies are averaged over statistically weighted J levels from: Corliss, C.; Sugar, J. *J. Phys. Chem. Ref. Data* **1982**, *11*, 135.

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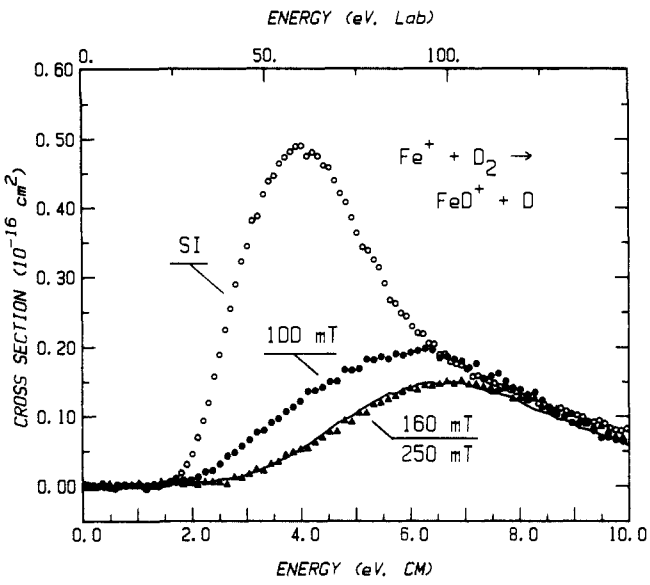

Figure 1. Cross section for reaction of Fe^+ with D_2 as a function of relative kinetic energy (lower scale) and laboratory energy (upper scale). Results are shown for Fe^+ produced in the surface ionization source (open circles) and in the electron impact/drift cell source with Ar pressures of 100 (closed circles), 160 (line), and 250 mtorr (triangles).

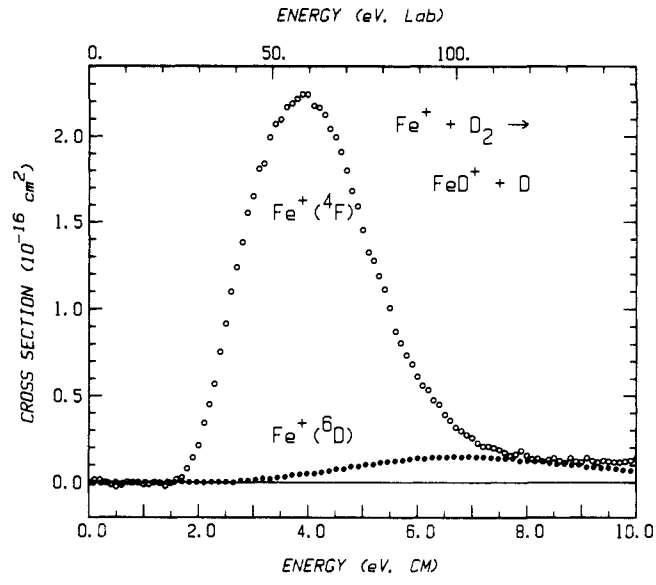

Figure 2. Cross section for reaction of $Fe^+(^6D)$ (closed circles) and $Fe^+(^4F)$ (open circles) with D_2 as a function of relative kinetic energy (lower scale) and laboratory energy (upper scale) as derived from the data in Figure 1; see text.

The guided ion beam apparatus used in these studies and our data reduction procedures have been described in detail elsewhere.[8] Briefly, iron ions emitted from one of two sources (described below) are mass analyzed, decelerated to a particular kinetic energy, and focused into an octopole ion guide. The octopole passes through a gas cell where the ions react with D_2 gas (used to facilitate mass resolution) to form FeD^+ with a probability that depends on the kinetic energy of the ions. The unreacted Fe^+ and product FeD^+ ions are extracted from the octopole, mass analyzed, and detected. The raw ion intensities are converted into an absolute reaction cross section as a function of relative kinetic energy.

Fe^+ is produced by using one of two techniques: surface ionization (SI) or electron impact (EI). In the SI source, vaporized $FeCl_3$ is directed onto a rhenium filament at 2200 ± 100 K. The Fe^+ ions emitted from this filament are believed to have a Maxwell-Boltzmann distribution of electronic energies[9] such that 79.3 ± 1.0% are in the ^6D state manifold, 20.4 ± 1.0% are in the ^4F state manifold, and 0.3 ± 0.1% are in higher states. In the EI source, $Fe(CO)_5$ is ionized by a crossed electron beam with energies of ≈65 eV. These ions are focused into a high-pressure drift cell[10] which is filled with Ar to pressures up to 250 mtorr. Iron ions undergo thousands of collisions with the Ar bath gas while drifting in a weak electric field (1 V/cm) to the exit of the drift cell where they enter the guided ion beam apparatus. As will be seen, the electronically excited states of the Fe^+ are relaxed in the drift cell. That collisions with Ar can induce a spin- and parity-forbidden electronic transition is an interesting result in itself.[11]

The results for Fe^+ produced by SI, Figure 1, are in good qualitative agreement with previously published data.[1b] The other data in Figure 1 make it clear that in going from the SI source to increasing pressures in the drift cell the cross section below a kinetic energy of 6 eV decreases. At drift cell pressures of 160 and 250 mtorr, identical results are obtained. Thus, these curves represent the coldest Fe^+ beam that we can produce. We presume this reaction is due predominantly to $Fe^+(^6D)$. The cross section for nearly pure $Fe^+(^4F)$ can be obtained by subtracting 79.3% of the $Fe^+(^6D)$ cross section from the SI curve and scaling the remainder by the population of the ^4F state in the SI beam, 20.4%. This cross section is shown in Figure 2 along with that for the ^6D state. It is obvious that these two states have dramatically different reactivities. This can be quantified by comparing these results with phase space calculations for these reactions.[12] Such calculations reproduce the absolute magnitude of the ^4F state reaction within experimental error while overestimating that of the ^6D by about a factor of 80. Thus, the answer to the question posed in the title is yes, but not very efficiently.

Simple molecular orbital arguments based on previous work in our laboratories[4,5] suggest that the inertness of the $Fe^+(^6D)$ state is a consequent of the electron configuration. Essentially, occupation of the 4s atomic orbital on Fe^+ leads to largely repulsive interactions with the σ_g molecular orbital of D_2 which are reinforced by the high-spin coupling of the 4s with the 3d electrons. The $Fe^+(^4F, 3d^7)$ state avoids this repulsion and thus reacts efficiently. Interestingly, the evolution of the molecular orbitals during the reaction of $Fe^+(^4F)$ leads naturally to the $^5\Delta$ ground state of FeH^+ in which the binding orbital on the metal is largely 4s in character.[13,14]

An analysis of the threshold behavior of these curves (after correction for zero point energy effects) yields an FeH^+ bond energy at 0 K of 2.06 eV (47.6 kcal/mol). Until a more comprehensive investigation is completed, we estimate this has an uncertainty of 0.2 eV. This bond energy is substantially less than previous ion beam measurements, 2.55 ± 0.2 eV,[1b] primarily because the differences in electronic state reactiivty were not accounted for in the earlier work. The present value is in ex-

(7) A number of studies have been performed on the reactions of Cr^+ states which are separated by about 2.5 eV. Halle, L. F.; Armentrout, P. B.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1981**, *103*, 962. Reents, W. D.; Strobel, F.; Freas, R. B.; Wronka, J.; Ridge, D. P. *J. Phys. Chem.* **1985**, *89*, 5671. Elkind, J. L.; Armentrout, P. B., work in progress.

(8) Ervin, K. M.; Armentrout, P. B. *J. Chem. Phys.* **1985**, *83*, 166.

(9) Previous work in our laboratories indicates that this is a very good approximation. See ref 5 and: Aristov, N.; Armentrout, P. B. *J. Am. Chem. Soc.*, in press.

(10) Van Koppen, P. A. M.; Kemper, P. R.; Illies, A. J.; Bowers, M. T. *Int. J. Mass Spectrom. Ion Processes* **1980**, *54*, 263. Ervin, K. M.; Armentrout, P. B. *J. Chem. Phys.*, in press.

(11) The cooling process can conceivably be either a direct collisional cooling by the rare gas, radiative cooling induced by collisions, or simply a consequence of holding the ions long enough for them to radiate at their natural lifetime. Estimates by Garstang (Garstang, R. H. *Mon. Notice R. Astron. Soc.* **1962**, *124*, 321; private communication) suggest, however, that this parity- and spin-forbidden transition has a lifetime in excess of 1 s. We estimate the ion residence time in the drift cell to be only ≈100 μs. Studies designed to better characterize the electronic cooling are in progress.

(12) Details of these calculations will be provided in a full paper of this system. See Ref 4 for a similar application. The programs used are outlined in: Chesnavich, W. J.; Bowers, M. T. *J. Chem. Phys.* **1977**, *66*, 2306; **1978**, *68*, 901. Webb, D. A.; Chesnavich, W. J. *J. Phys. Chem.* **1983**, *87*, 3791.

(13) Armentrout, P. B.; Halle, L. F.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1981**, *103*, 6501–6502. Elkind, J. L.; Armentrout, P. B. *Inorg. Chem.*, in press.

(14) Schilling, J. B.; Goddard, W. A.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1986**, *108*, 582.

ceptionally good agreement with a recent ab initio calculation, 2.04 eV (47.0 kcal/mol).[14]

The results obtained here raise questions concerning the relative reactivity of the ^6D and ^4F states of Fe^+ with other molecules. In previous studies, Fe^+ has been formed by surface ionization,[1] electron impact,[2] and laser desorption ionization,[3] all of which probably produce significant populations of both of these states. Further investigations in our laboratories will seek to determine whether other systems exhibit the substantial difference in reactivity observed here. Preliminary studies indicate that exothermic reactions with larger molecules may not be as sensitive to the state of the ion.

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Substrate Specificity of Enzymes in Organic Solvents vs. Water Is Reversed

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The ultimate challenge in the area of biocatalysis is the alteration of substrate specificity of enzymes at will. Redesigning enzymes by site-directed mutagenesis is emerging as a powerful strategy toward that goal.[1] Substrate specificity of enzymes stems from their ability to utilize the free energy of bonding with substrates to facilitate the reaction.[2,3] Since the net binding energy is the difference between the binding energies of the substrate with the enzyme and with water,[4] an alternative approach to changing substrate specificity (in addition to modification of the enzyme's active center) would be replacing water with another reaction medium.

As a result of our recent studies, it is becoming clear that enzymes can vigorously function as catalysts in organic solvents, provided that some basic rules are followed.[5] In the present work we have discovered that substrate specificities of several enzymes in organic solvents are radically different from, and sometimes opposite to, those in water.

α-Chymotrypsin[7] has been selected as the initial target because the nature of its substrate specificity is well established.[8] We have found that the enzyme can catalyze the transesterification reaction between *N*-acetyl-L-phenylalanine ethyl ester[9] (**1**) and primary alcohols in a variety of anhydrous organic solvents, with paraffins affording the highest reactivities.[10] For example, when a 1 mg/mL suspension of chymotrypsin in octane containing 5 mM **1** and 1 M propanol was shaken for 10 h,[10] 50% of **1** was converted into the corresponding propyl ester. The enzyme inactivated by the active-site-directed inhibitor phenylmethanesulfonyl fluoride[12] exhibited no activity in octane, thus confirming the nonartifactual origin of the transesterification reaction.

Table I. Substrate Specificity of Chymotrypsin and Subtilisin in the Reactions of Hydrolysis in Water and Transesterification in Octane

	k_{cat}/K_M, M^{-1} s^{-1}			
	chymotrypsin		subtilisin	
substrate	hydrolysis[a]	trans-esterification[b]	hydrolysis[a]	trans-esterification[b]
N-Ac-L-Phe-OEt	4.0×10^4	0.72	1.3×10^4	1.7
N-Ac-L-His-OMe	2.0×10^2	15.0	5.5×10^2	3.1
N-Ac-L-Ser-OMe	0.87	2.5	1.6×10^2	4.5

[a] Enzymatic hydrolysis of the three esters in water was followed potentiometrically with a Radiometer pH-stat. Conditions: 0.1 M KCl, pH 7.8, 20 °C, 10^{-7} to 10^{-4} M enzymes, and 0.3-5.0 mM esters. [b] Initial rates of the enzymatic reactions were measured as described in ref 10 at the concentration of the enzymes and *n*-propyl alcohol of 1 mg/mL and 1 M, respectively; the ester concentrations were varied in the range from 2 to 30 mM. The kinetics of the enzymatic transesterification strictly followed the compulsory order mechanism with no ternary complexes whose conventional treatment has been used to calculate kinetic parameters (Dixon, M.; Webb, E. C. *Enzymes*, 3rd ed.; Academic Press: New York, 1979; pp 92-93). Both chymotrypsin and subtilisin were lyophilized prior to use in the same manner[10] except that for the latter 0.25% phosphate buffer (pH 7.8) was used instead of *N*-Ac-L-Phe. Octane (99+% purity) contained[11] less than 0.02% water.

The main driving force of the enzyme-substrate binding in the case of chymotrypsin is hydrophobic interactions between the side chain of the amino acid substrate and the binding pocket of the enzyme.[4,8] This is illustrated by the data in the first column of Table I: upon transition from hydrophobic Phe to hydrophilic Ser in the series of esters of *N*-acetyl-L-amino acids (specific model substrates for chymotrypsin[4,8]), k_{cat}/K_M for the enzymatic hydrolysis drops nearly 5×10^4 fold. Since hydrophobic interactions owe their existence to water as the reaction medium,[13] one might expect that the substrate preference of chymotrypsin in organic solvents should be entirely different because hydrophobic bonding will play no part. The results in the second column of Table I confirm that prediction: in the transesterification reaction in octane, the ester of *N*-Ac-L-Ser is 3 times more reactive than that of *N*-Ac-L-Phe. Furthermore, *N*-Ac-L-His-OMe, whose reactivity in water was 0.5% of that of **1**, in octane becomes a 20-fold more reactive substrate.[14]

(1) Ulmer, K. *Science* (*Washington, D.C.*) **1983**, *219*, 666-671. Fersht, A. R.; et al. *Nature* (*London*) **1985**, *314*, 235-238. Craik, C. S.; et al. *Science* (*Washington, D.C.*) **1985**, *228*, 291-297.

(2) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; Chapter 5. Jencks, W. P. *Adv. Enzymol.* **1975**, *43*, 219-410.

(3) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman: New York, 1985; Chapters 12 and 13.

(4) Reference 3, Chapter 11.

(5) These rules include:[6] (i) a proper choice of the solvent (with hydrophobic ones being the best as they do not strip the essential layer of water from the enzyme molecule); (ii) the use of an enzyme recovered from an aqueous solution of the pH optimal for enzymatic activity; (iii) elimination of diffusional limitations by vigorous agitation and fine dispersion of the enzyme powder in an organic solvent.

(6) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192-3196. Kazandjian, R. Z.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 5448-5450. Klibanov, A. M. *CHEMTECH* **1986**, *16*, No. 6.

(7) Bovine pancreatic α-chymotrypsin (EC 3.4.21.1) was purchased from Sigma Chemical Co. as a lyophilized powder with a specific activity of 58 BTEE units per mg of protein.

(8) Bender, M. L.; Kezdy, F. *Annu. Rev. Biochem.* **1965**, *34*, 49-76. Hess, G. P. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1970; Vol. 3, pp 213-248. Blow, D. M. *Acc. Chem. Res.* **1976**, *9*, 145-152.

(9) One of the best classical substrates for α-chymotrypsin.[8]

(10) The optimal mode of preparation of chymotrypsin for these experiments (used throughout this study) was lyophilization of 5 mg/mL enzyme from an aqueous solution, pH 7.8, containing the ligand *N*-Ac-L-Phe (0.25%) (Zaks, A.; Klibanov, A. M., unpublished results). The lyophilized sample contained[11] about 2.5% (w/w) water, i.e., no more than 50 molecules of water per enzyme molecule. An enzyme suspension in a solution of substrates in an organic solvent was placed in a stoppered flask and shaken on an orbit shaker at 250 rpm and 20 °C. Periodically, aliquots were withdrawn and assayed by gas chromatography using a 530-μm fused silica capillary column (Hewlett-Packard). The rates of chymotrypsin-catalyzed transesterification were not controlled by diffusion of the substrate through the enzyme bead: ultrasonication of a suspension of the enzyme in octane (resulting in a decrease of an average enzyme particle from 270 to 5 μm) had no appreciable effect on the reaction rate.

(11) The water concentration both in the organic media and in the enzymes was measured by the optimized Fischer method (Laitinen, H. A.; Harris, W. E. *Chemical Analysis*, 2nd ed.; McGraw-Hill: New York, 1975; pp 361-363).

(12) Fahrney, D. E.; Gold, A. M. *J. Am. Chem. Soc.* **1963**, *85*, 997-1000.

(13) Kauzmann, W. *Adv. Protein Chem.* **1959**, *14*, 1-63. Tanford, C. *The Hydrophobic Effect*; Wiley: New York, 1980.

(14) It should be stressed that although in water the values of k_{cal}/K_M in most instances are much greater than in octane, this does not necessarily mean that chymotrypsin and subtilisin are less efficient catalysts in octane than in water. This is because two entirely different processes are being compared, hydrolysis in water and transesterification in octane, for which the rate constants of nonenzymatic reactions are also totally different.

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