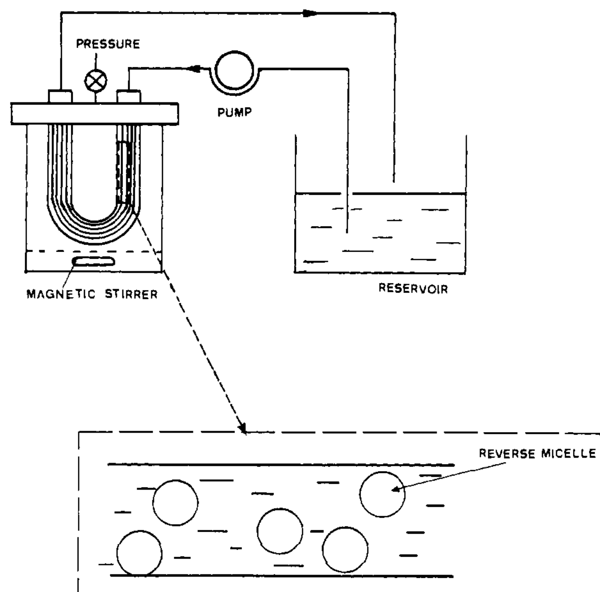
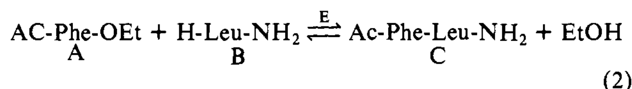


Figure 3. An enzyme reactor for reverse micelles. The enzyme-containing micelles are present only inside the hollow fibers. The hydrocarbon stable, semipermeable hollow fibers (from inert polyamide) are commercially available from Berghof GmbH, Tübingen, Germany ("Miniconcentrator BMS"). The internal volume of the hollow fibers is 0.4 mL, with a surface of 20 cm^2. The volume of the reactor is 6 mL. The pump indicated in the figure has not been used for the experiments described here.

and reagents were first prepared in a stock aqueous solution at the mentioned pH and then added with a microsyringe (typically 10–20 μL) to a 100 mM isooctane solution of AOT. The pH conditions chosen were those that had been optimized in aqueous solution for similar reactions.[7] The product C was found almost exclusively in the exterior hydrocarbon phase. The yield[8] was between 40% and 60% for w_0 values between 5 and 30. Under the typical conditions indicated above, the equilibrium was reached in ca. 25–30 min, with a normal hyperbolic time course of the reaction, after which the compound A was no longer measurable.[8] The reaction products were analyzed by HPLC (Hibar prepacked column EC 250-4, Lichrosorb RP-18 (7 μm), column length 250.0 mm, internal diameter 4.0 mm). The reactants (in this and the following reaction) were commercial preparations from Serva at the highest available purity. Experiments at w_0 below 5, where the enzyme is actually even more active than in water,[3c] were not possible because of the poor solubility of the reagents. This is the first time, to the best of our knowledge, that a successful enzymatic peptide synthesis is reported in a hydrocarbon micellar solution.

Under the same conditions, other enzymatic syntheses of peptide bonds could be achieved. For example, the reaction



$$\underset{A}{\text{AC-Phe-OEt}} + \underset{B}{\text{H-Leu-NH}_2} \underset{}{\overset{E}{\rightleftharpoons}} \underset{C}{\text{Ac-Phe-Leu-NH}_2} + \text{EtOH} \quad (2)$$

gives the product C in about the same yield as in the case of the eq 1. However, whereas the synthesis described by eq 2 takes place also in water[7] (as A and B are highly water soluble), eq 1 represents a case in which enzymes could have not been used in aqueous solution due to the poor solubility of A.

(6) For a compound present in hydrocarbon micellar solutions, and soluble in the water pool, one can define two types of concentration: an overall, C_{ov}, referred to the entire volume (water plus hydrocarbon) or a water pool concentration, c_{wp}, referred only to the volume of water.[3i] For a compound only soluble in the water pools, the two numbers are related by the simple equation $C_{ov} = C_{wp}F_w$, where F_w is the percentage of water in the micellar system.

(7) Morihara, K.; Oka, T. *Biochem. J.* **1977**, *163*, 531–542.

(8) The fact that the yield is not larger depends mostly on a competitive reaction, namely the hydrolysis of A by α-chymotrypsin. In fact, the corresponding free acids could be detected in the appropriate amount by HPLC.

When one is dealing with the application of enzymes to chemical reactions, one has to deal with the problem of physical separation between enzymes and reagents. This can be achieved with the reactor shown in Figure 3, where the enzyme-containing micelles are entrapped in semipermeable hollow fibers that are hydrocarbon stable.

We have used this reactor in preliminary experiments. The enzyme micellar solution was applied with a microsyringe inside the hollow fiber; in the case of eq 1, compound B and, in the case of eq 2, both A and B were also applied inside the hollow fibers. The products C could be evidenced for both reaction 1 and 2, in the bulk hydrocarbon, but the yield was not satisfactory (below 10%). This is due to the very unfavorable volume ratio between the inside and outside compartments of the commercial reactor[9] and in particular to the exceedingly small volume of the water microphase where the reaction takes place. In principle, however, the problem of an enzyme reactor that is appropriate for micellar hydrocarbon solutions can be considered solved. We are presently working at the optimization of the dimensions of a similar homemade reactor.

Acknowledgment. We are indebted to the Swiss National Foundation, and to the Branco Weiss Fond, for financing part of this research.

(9) Notice that even if the partition coefficient between water and hydrocarbon is about unity, most of the reagents will be localized in the much larger volume of hydrocarbon. In the reactor of Figure 3 with a 10% hydrocarbon micellar solution localized in the 400 μL of hollow fiber, the overall ratio water to hydrocarbon is ca. 1:1000.

Ultrafast Excited-State Proton Transfer in 1-Naphthol[1]

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Proton-transfer reactions provide the fundamental basis for all acid–base chemistry in protic solvents. Literally hundreds of examples of excited-state proton-transfer reactions are known.[2] In nearly all these systems, large changes in pK_a occur upon electronic excitation. Picosecond laser sources can thus be used to effect the sudden introduction of a strong acid or base into an otherwise unchanged solution.[3-5]

In this communication, we present the results of picosecond, time-resolved emission spectroscopy of electronically excited 1-naphthol in aqueous solution. Excited-state proton transfer in 1-naphthol has been studied using both steady-state[6-9] and na-

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(2) Ireland, J. F.; Wyatt, P. A. H. *Adv. Phys. Org. Chem.* **1976**, *12*, 131.
(3) Clark, J. H.; Shapiro, S. L.; Campillo, A. J.; Winn, K. R. *J. Am. Chem. Soc.* **1979**, *101*, 746.
(4) Smith, K. K.; Kaufmann, K. U.; Huppert, D.; Gutman, M. *Chem. Phys. Lett.* **1979**, *65*, 164.
(5) Hou, S.; Hetherington, W. M.; Korenowski, G. M.; Eisenthal, K. B. *Chem. Phys. Lett.* **1979**, *68*, 282.
(6) Förster, T. *Z. Elektrochem.* **1950**, *54*, 42.
(7) Weller, A. *Z. Elektrochem.* **1952**, *56*, 662.

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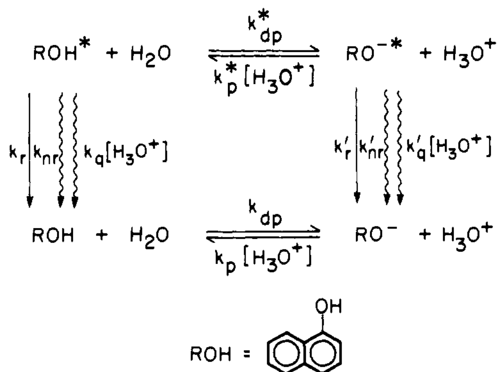

Figure 1. Kinetic scheme for excited-state dynamics of 1-naphthol in aqueous solution: k_{dp}, k^*_{dp}, k_p, and k^*_p represent the ground- and excited-state deprotonation and protonation rate constants, respectively; k_{nr}, k'_{nr}, k_r, k'_r, k_q, and k'_q are the rate constants for nonradiative decay, radiative decay, and proton-induced quenching for the neutral and anionic species, respectively.

nosecond time-resolved[9-11] emission spectroscopy. These previous results predicted excited-state proton-transfer rates 1–3 orders of magnitude slower than the rate presented here. Analysis of the results reported here shows that previous values for other parameters describing excited-state proton transfer in 1-naphthol are also in error, including the excited-state acid dissociation constant, pK_a^*.[10,12-15]

The experimental method and kinetic analysis used in these measurements can be explained using the generalized excited-state proton-transfer reaction diagram shown in Figure 1. Since 1-naphthol has a pK_a of 9.2,[16] essentially all ground-state 1-naphthol molecules will be fully protonated for a solution pH ≤7. Irradiation of such a solution will produce the substantially more acidic,[2] electronically excited 1-naphthol (ROH*), which can adiabatically transfer a proton to water to produce an electronically excited 1-naphtholate anion (RO^{-*}). As expected from simple thermodynamic arguments such as the Förster cycle,[6] the emission from RO^{-*} is at lower energies than that of ROH*. This spectral difference allows the two species to be distinguished and independently monitored.[13] The reaction scheme of Figure 1 predicts that both the decay time of the ROH* emission and the rise time of the RO^{-*} emission will be equal to the total rate of decay of the ROH* population.

The picosecond, time-resolved emission spectroscopy apparatus will be described in detail elsewhere.[17] Briefly, 20-ps, fourth-harmonic (266 nm) pulses derived from a passively mode-locked Nd:YAG oscillator–amplifier system (Quantel, YG400) were used to excite the sample. The resulting emission was spectrally filtered with appropriate narrow-band (~10-nm fwhm) interference filters and temporally resolved with an ultrafast streak camera (Hadland-Photonics, Imacon 500). The streak records were digitized with an intensified photodiode array (Tracor-Northern, IDARSS) and transferred to a microcomputer (DEC, LSI 11/73) for signal averaging. The limiting time resolution of the detection system was ~3 ps. Because the temporal profile of the excitation pulse was recorded along with that of the emission, rise and fall times substantially shorter than the 20-ps excitation pulse width could be determined using a convolute-and-compare data analysis technique. The data presented here are the sum of the signal from 500 laser pulses. Samples consisted of 10^{-3} to 10^{-4} M aqueous solutions of HPLC-purified 1-naphthol prepared and maintained under oxygen-free conditions.

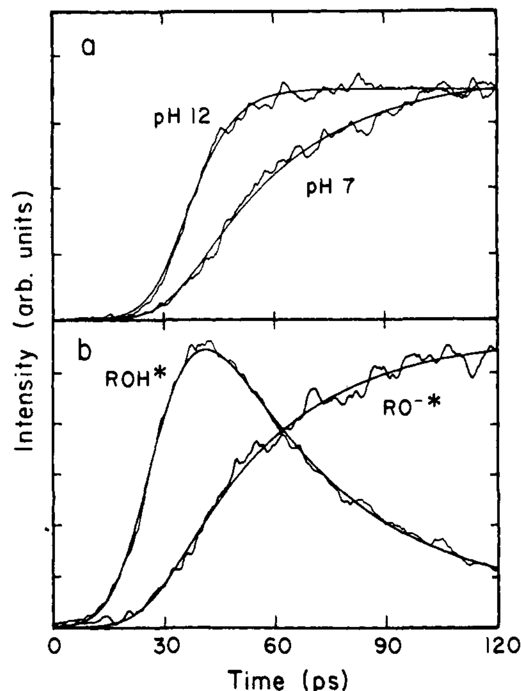

Figure 2. (a) Time-resolved (550 ± 5)-nm emission from electronically excited 1-naphtholate ions in pH 12 and 7 aqueous solutions. The smooth curves are computer-generated fits to the data with rise times of 5.6 ps (pH 12) and 30 ps (pH 7). (b) Time-resolved emission from the protonated (ROH*, 370 ± 6 nm) and anionic (RO^{-*}, 540 ± 5 nm) forms of electronically excited 1-naphthol in pH 7 aqueous solution. Smooth curves show computer-generated fits to the data with a ROH* decay time of 34 ps and a RO^{-*} rise time of 31 ps.

Table I. Measured Rates, Lifetimes, and Relative Fluorescence Quantum Yields for 1-Naphthol (Present Work) and 2-Naphthol[18] in Aqueous Soltuion

$1/\tau_0' = k'_r + k'_{nr}$

solute	Φ'/Φ'_o	γ_1^{-1}, ps	k^*_{dp}, s^{-1}	τ_o, ps	τ'_o, ns
1-naphthol	0.66	33	2.1×10^{10}	100	8.0
2-naphthol	0.64	5.0×10^3	7.5×10^7	8.0×10^3	9.0

The first time-resolved observation of excited-state proton transfer in 1-naphthol is presented in Figure 2a. At a solution pH of 12, >99.8% of ground state species present is RO^-. Laser excitation produces RO^{-*} emission with an instrument-limited rise time (≤6 ps) and an 8.0 ± 0.4 ns fluorescence lifetime, as expected for direct excitation of RO^{-*}. At a solution pH of 7, >99.4% of the ground-state species present is ROH. Laser excitation produces a RO^{-*} time-resolved emission profile with a distinct, (31 ± 5)-ps rise time and a (7.5 ± 0.4)-ns decay, proof that RO^{-*} is indirectly produced via excited-state proton transfer from ROH*. The validity of the reaction scheme of Figure 1 is confirmed by the results shown in Figure 2b. The average decay time of the fast component of the ROH* emission, measured at wavelengths between 350 and 370 nm, is 35 ± 5 ps. Within experimental error, this decay time is identical with the (31 ± 5)-ps average rise time of the RO^{-*} emission at wavelengths ≥500 nm.

Combination of the results of steady-state measurements with direct, time-resolved measurements yields the most accurate determination of the desired rates. For example, the constants τ_o ($1/\tau_o = k_r + k_{nr}$) and k^*_{dp} (see Figure 1) can be readily determined from

$$\Phi'/\Phi_o = k^*_{dp}/\gamma_1 = k^*_{dp}/(k^*_{dp} + (1/\tau_o)) \quad (1)$$

where Φ'/Φ'_o is the quantum yield of fluorescence of the anion at neutral pH relative to that at high pH, and γ_1 ($\gamma_1 = k_r + k_{nr}$

(8) Henson, R. M. C.; Wyatt, P. A. H. *J. Chem. Soc., Faraday Trans. 2* **1975**, *71*, 669.
(9) Harris, C. M.; Selinger, B. K. *J. Am. Chem. Soc.* **1980**, *84*, 891.
(10) Rosenberg, J. L.; Brinn, I. *J. Phys. Chem.* **1972**, *24*, 3558.
(11) Tsutsumi, K.; Shizuka, H. *Z. Phys. Chem. (Wiesbaden)* **1980**, *122*, 129.
(12) Derkacheva, L. D. *Opt. Spectrosc. (Engl. Transl.)* **1960**, *9*, 110.
(13) Weller, A. *Prog. React. Kinet.* **1961**, *1*, 187.
(14) Schulman, S. G. *Spectrosc. Lett.* **1973**, *6*, 197.
(15) Laws, W. R.; Brand, L. *J. Phys. Chem.* **1979**, *83*, 795.
(16) Weller, A. *Z. Phys. Chem. (Wiesbaden)* **1958**, *17*, 224.
(17) Webb, S. P.; Yeh, S. W.; Clark, J. H., manuscript in preparation.

+ k^*_{dp}) is obtained from the neutral fall time and/or the anion rise time at pH 7. A complete kinetic analysis will be presented in a subsequent paper. A short summary of representative results from this analysis is given in Table I.

The most striking result revealed by this work is the contrast between the excited-state dynamics of 1-naphthol and those of 2-naphthol[18] (see Table I). For 2-naphthol, nanosecond time resolution is sufficient for direct observation of the excited-state dynamics. In 1-naphthol, excited-state proton transfer (k^*_{dp}) is ~280 times faster, and solvent quenching (as manifested by τ_0) is ~80 times faster than in 2-naphthol. Proton-induced quenching of RO^{-*} is found to be substantial in 1-naphthol, direct confirmation of the results of previous indirect studies.[7,9,18] These effects can be explained by postulating that the charge-transfer character of the lowest excited singlet state of 1-naphthol is stronger than that of 2-naphthol, due, for example, to increased ^1L_a character in the 1-naphthol excited state.[11] If this results in a decrease in the relative electron density on the oxygen, it would cause an increase in the driving force for the excited-state proton-transfer reaction, consistent with the observed increases in both the excited-state proton-transfer rate and the pK^*_a of 1-naphthol relative to 2-naphthol. The measurements reported here are currently being extended by the systematic study of excited-state dynamics in naphthols and substituted naphthols as a function of solvent, temperature, pressure, and excitation wavelength.

Registry No. 1-Naphthol, 90-15-3.

(18) Harris, C. M.; Selinger, B. K. *J. Phys. Chem.* **1980**, *84*, 891.

Fragmentation of Oligopeptide Ions Using Ultraviolet Laser Radiation and Fourier Transform Mass Spectrometry[1]

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Collisionally activated dissociation (CAD) has become a powerful and widely used method for the structural characterization of ions by mass spectrometry.[2] The cross section for collisional fragmentation is large, typically 10–100 Å^2 at ion energies less than 100 eV, when the mass of the parent ion is comparable to the mass of the collision gas.[3,4] But higher molecular weight ions are not efficiently fragmented because only a small fraction of the translational energy of the ion is available for internal excitation.[5] For example, in a collision between argon and a 30-eV ion of mass 1000 u, only 3.8% of the initial ion energy (1.2 eV) is available in the center of mass frame for internal excitation and subsequent fragmentation of the ion. An alternative method is to use laser radiation to fragment the ions. With an excimer laser, radiation at 193 nm (6.42 eV) can be deposited specifically into internal electronic excitation of the ion, and absorption of even one UV photon is usually sufficient to cause fragmentation.

The effective use of pulsed lasers to fragment ions in mass spectrometers has proven to be difficult. One problem is that with quadrupole and magnetic sector mass spectrometers the interaction region between the laser and the ion beam is very small. Even with a 1-nA ion beam current fewer than 1000 ions are irradiated during the 20-ns pulse of an excimer laser. Another difficulty is that a complete mass spectrum cannot be obtained for each laser pulse because the scan speed is far slower than the duration of the laser pulse.

These limitations are alleviated in ion cyclotron resonance spectrometers (ICR) because the ions are stored in an analyzer cell for up to several seconds and can be irradiated with many laser pulses.[6-9] In this paper we demonstrate that oligopeptide ions stored in an ICR analyzer cell are fragmented efficiently by ultraviolet laser radiation. In addition, a complete mass spectrum of the photofragment ions is obtained for each pulse of the laser by Fourier transform detection.[10-14]

The experiments were performed with a Fourier transform mass spectrometer (FT-MS) that has been described previously.[15] The peptides (Sigma Chemical Co.) were dissolved in methanol and then evaporated to dryness on the tip of a direct insertion probe. Since the vapor pressure of the peptide is typically less than 10^{-8} torr, low-pressure chemical ionization[16] was used to generate the protonated oligopeptide ions. In a typical FT-MS photodissociation experiment an electron beam is fired through the analyzer cell to produce gaseous reagent ions. With dimethylamine as the reagent gas, dimethylammonium ions form rapidly and are stored in the analyzer cell of the FT-MS instrument. Protonated peptide ions are generated continuously by reaction of the gaseous peptide molecules with dimethylammonium ions. At the end of a 3-s reaction period an excimer laser (Tachisto Model 800XR) is triggered a predetermined number of times to fragment the ions. Fragment ions produced by the laser radiation are trapped in the FT-MS analyzer cell and after a 2-ms delay time are accelerated by a radiofrequency (rf) pulse. Ion image current signals induced on the plates of the analyzer cell by the coherent cyclotron motion of the ions are amplified, digitized, and stored in a 16K word buffer memory. Finally, the data are transferred over a parallel interface to an IBM 9001 computer having a Sky Computer floating point array processor which calculates the fast Fourier transform to yield a mass spectrum.

When leucylalanine (Leu-Ala) is protonated by dimethylammonium ion, only the protonated molecular ion at m/z 203 is observed in the FT-MS spectrum. Dimethylamine is a good reagent gas for the peptides because it has a proton affinity comparable to that of the amino acids.[17] Previous studies of oligopeptides by chemical ionization mass spectrometry have utilized low proton affinity reagents, such as isobutane and methane, which cause such extensive fragmentation that the protonated molecular ion is low in abundance.[18,19]

When the unfocused beam of an excimer laser (one pulse at 193 nm and an energy of 42 mJ) is crossed with protonated Leu-Ala ions stored in the analyzer cell, fragment ions are produced by photodissociation. The fragmentation pattern is

(1) Bowers, W. D.; Delbert, S.-S.; Hunter, R. L.; McIver, R. T., Jr., paper presented in part at the 32nd Annual Conference on Mass Spectrometry and Allied Topics, San Antonio, TX, May 27–June 1, 1984.
(2) McLafferty, F. W. "Tandem Mass Spectrometry"; McLafferty, F. W., Ed.; Wiley: New York, 1983; pp 1–10.
(3) Yost, R. A.; Enke, C. G.; McGilvery, D. C.; Smith, D.; Morrison, J. D. *Int. J. Mass Spectrom. Ion Phys.* **1979**, *30*, 127.
(4) Dawson, P. H. *Int. J. Mass Spectrom. Ion Phys.* **1982**, *43*, 195.
(5) Dawson, P. H.; Douglas, D. J. "Tandem Mass Spectrometry"; McLafferty, F. W., Ed.; Wiley: New York, 1983; pp 126–128.
(6) Dunbar, R. C. "Gas Phase Ion Chemistry"; Bowers, M. T., Ed.; Academic Press: New York, 1979; Vol. 2, pp 182–220.
(7) Dunbar, R. C. "Ion Cyclotron Resonance II"; Hartmann, H., Wanczek, K. P., Eds.; Springer-Verlag: Berlin, 1982; pp 1–26.
(8) Thorne, L. R.; Wright, C. A.; Beauchamp, J. L. "Ion Cyclotron Resonance II"; Hartmann, H., Wanczek, K. P., Eds.; Springer-Verlag: Berlin, 1982; pp 43–97.
(9) Jasinski, J. M.; Rosenfeld, R. N.; Meyer, F. K.; Brauman, J. L. *J. Am. Chem. Soc.* **1982**, *104*, 652–658.
(10) Comisarow, M. B.; Marshall, A. G. *Chem. Phys. Lett.* **1974**, *25*, 282.
(11) McIver, R. T., Jr. *Am. Lab.* (*Fairfield, Conn.*) **1980**, *12* (11), 18.
(12) Wilkins, C. L.; Gross, M. L. *Anal. Chem.* **1981**, *53*, 1661A.
(13) Bowers, W. D.; Hunter, R. L.; McIver, R. T., Jr. *Ind. Res./Dev.* **1983**, *25* (11), 124.
(14) Carlin, T. J.; Freiser, B. S. *Anal. Chem.* **1983**, *55*, 955.
(15) Irion, M. P.; Bowers, W. D.; Hunter, R. L.; Rowland, F. S.; McIver, R. T., Jr. *Chem. Phys. Lett.* **1982**, *93*, 375.
(16) Hunter, R. L.; McIver, R. T., Jr. *Anal. Chem.* **1979**, *51*, 699.
(17) Locke, M. J.; McIver, R. T., Jr. *J. Am. Chem. Soc.* **1983**, *105*, 4226.
(18) Mudgett, M.; Bowen, D. V.; Kindt, T. J.; Field, F. H. *Biomed. Mass Spectrom.* **1975**, *2*, 254.
(19) Hunt, D. F.; Buko, A. M.; Ballard, J. M.; Shabanowitz, J.; Giordani, A. B. *Biomed. Mass Spectrom.* **1981**, *8*, 397.

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