intermediate A can be explained by two different pathways[7] (see Scheme I).

The predominant formation of the α,β-ethylenic ester when ether is used can be explained by assuming that the metal atom in intermediate A is better solvated than in pentane. This solvatation results in a weakening of the the carbon–zinc bond, and halocyclopropanone acetal C is formed by internal electrophilic substitution. In pentane (or benzene), species A loses $XSiMe_3$ giving rise to carbenoid B, which undergoes insertion into a carbon–hydrogen bond. This same intermediate can undergo intramolecular cyclopropanation if a CC double bond is available. Carbenoid formation seems to be without precedent. It is well-known that carbenes and carbenoids insert into carbon–hydrogen bond.[8] The exclusive insertion into the β-position is probably due to the presence of the ester functionality.

The usefulness of this reaction was illustrated by a new eight-step (±) synthesis of sabinene (**4**) (Scheme II).

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Enzymatic Synthesis of Hydrocarbon-Soluble Peptides with Reverse Micelles

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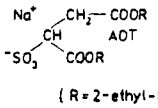
Reverse micelles have been investigated also because of their capability to host several types of molecules.[1,2] Also enzymes can be solubilized in reverse micelles (Figure 1) without loss of activity.[3] Reverse micelles formed by the anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT), swell by increasing the amount of water in the system (generally expressed by the parameter $w_0 = [H_2O]/[AOT]$), and at the same time the physical properties of the water of the water pool become closer to those of bulk water.[4] Reverse micelles can then be viewed as microreactors, whose dimensions can be easily changed and with a milieu whose physical properties (e.g., dielectric constant, microviscosity, acidity) can be continuously modulated and possibly tailored to the characteristics of the reaction taking place in the water pool. Another important aspect of reverse micelles is the difference between the polar core and the outside (essentially hydrocarbon). This difference in environment can be utilized advantageously for mass transport and separation in chemical reactions.

It is the aim of this communication to show for the first time the use of reverse micelles for the enzymatic synthesis of peptides



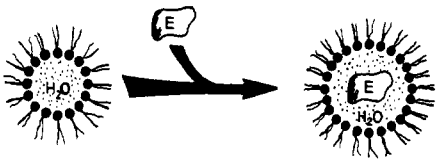


Figure 1. Schematic representation of the solubilization of enzymes in reverse micelles (cross section) and of the structure of the surfactant AOT used in our studies. Particularly at small w_0 values ($w_0 = [H_2O]/[AOT]$) the uptake of the protein will bring about a reequilibration of the material present in the micelles.[3i]

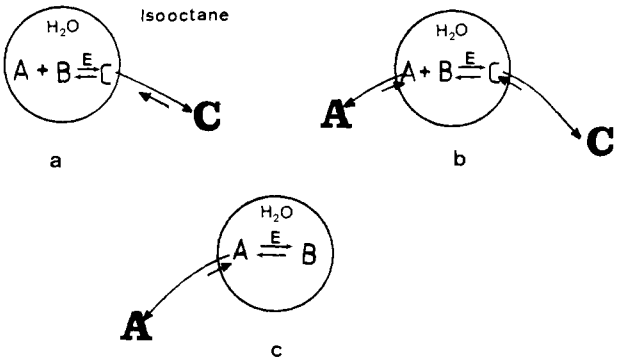


Figure 2. Three examples of compartimentalization of reactants in reverse micelles in enzymatic reactions. In the first case (a) the two reagents A and B are preferentially soluble in water and the product C in hydrocarbon. The second case (b) represents a different reaction, where one of the two reagents (A) is also soluble in hydrocarbon. Finally (c), the case of a quite different reaction is schematized, where an overwhelming hydrocarbon-soluble compound A yields, upon enzymatic cleavage, a water soluble product B which would remain entrapped in the water pools.

and to point out the potentialities of reverse micelles in enzymatic reactions involving lipophylic (and in particular water insoluble) reagents. It has been already shown that water-soluble enzymes solubilized in reverse micelles can accept highly lipophylic substrates.[3g,h] On the basis of this, and on the distribution of reagents as dictated by their relative solubilities, several possibilities can be envisaged, as shown in Figure 2. In the first and second case (Figure 2a, b) the product C, once formed in the water pool, will be expelled into the bulk hydrocarbon.

In this communication, we will illustrate this, using as an example the α-chymotrypsin-induced synthesis of a hydrocarbon-soluble (water insoluble) protected tripeptide. In the past, we have investigated the enzymatic synthesis of peptide bonds in aqueous solution,[5] and the extension to reverse micelles appeared an obvious thing to do, also in view of the small amount of water present in the hydrocarbon micellar system. The reaction chosen is

$$\underset{A}{\text{Z-Ala-Phe-OMe}} + \underset{B}{\text{H-Leu-NH}_2} \overset{E}{\rightleftharpoons} \underset{C}{\text{Z-Ala-Phe-Leu-NH}_2} + \text{MeOH} \quad (1)$$

catalyzed by α-chymotrypsin, where Z is the benzyloxycarbonyl protecting group. Both A and C are practically insoluble in water and soluble in isooctane, and therefore the situation can be depicted as in Figure 2b. Typically, the reaction conditions were room temperature, pH 10, 0.1 M borate buffer, and overall concentration[6] of A and B 1 mM and of the enzyme 5 μM. Enzyme

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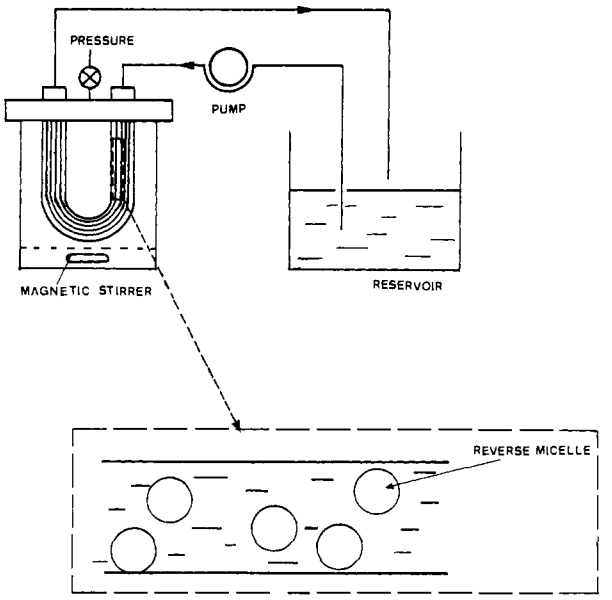

Figure 3. An enzyme reactor for reverse micelles. The enzyme-containing micelles are present only inside the hollow fibers. The hydrocarbon stable, semipermeable hollow fibers (from inert polyamide) are commercially available from Berghof GmbH, Tübingen, Germany ("Miniconcentrator BMS"). The internal 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

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

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.

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.

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(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.

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(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.

(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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