Micelles

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Unusual Rate Enhancement of Bimolecular Dehydrocondensation To Form Amides at the Interface of Micelles of Fatty Acid Salts**

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Nature has long succeeded in utilizing the interface of membranes as a reaction field for a variety of biological chemical transformations in which bimolecular reactions between reactants of very low concentrations can be effec-

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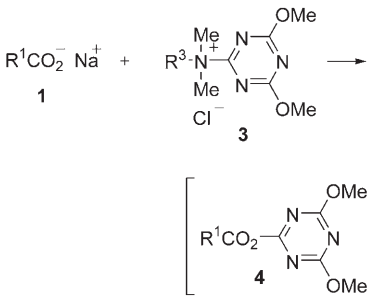
tively accelerated. In contrast, mankind has not yet succeeded in the practical application of either membrane–water or micelle–water interfaces as a reaction field. To enhance the rate of reactions in the micellar phase, the reactions must proceed in water, and all the compounds (reactants and intermediates) involved in the rate-determining step must be incorporated in the micelles to increase their local concentration. Furthermore, the molecules must be oriented appropriately for the reaction to take place. The fact that very few organic reactions (e.g. hydrolysis) meet these requirements makes it difficult to utilize micelles as a reaction field.

We believe that dehydrocondensation is most ideally suited for such rate enhancement in micelles, because all reactants that bear long hydrophobic alkyl chains, whether carboxylic acids, amines, or alcohols, are capable of forming micelles by themselves or can be incorporated in micelles formed by surfactants. Furthermore, the reacting moieties involved in the condensation (carboxy, ammonium, and hydroxy groups) are polar, and thus are located in close proximity to one another at the micellar interface. However, it has been very difficult to verify such a rate enhancement because of two major problems: 1) dehydrocondensations generally require dry conditions because many reagents and activated intermediates derived from carboxylic acids are susceptible to hydrolysis, and 2) hydrolysis reactions, the reverse reaction of dehydrocondensation, are originally promoted at the interface of micelles.[1]

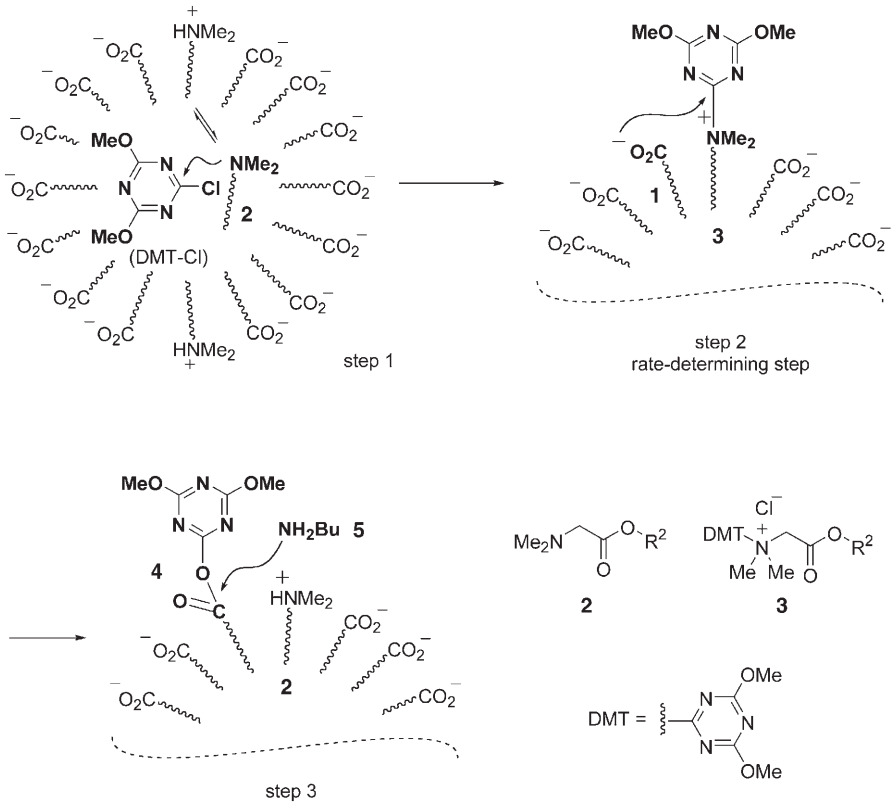
In contrast to the numerous studies of unimolecular hydrolysis reactions with micelles,[1–3] successful studies on bimolecular dehydrocondensation in micelles, which involves the activation of carboxylic acids in micelles formed in an aqueous medium, are very limited. Both lactonization with a carbodiimide[4] and lactamization in the presence of an amphiphilic Mukaiyama reagent[5] at a reverse micelle proceeded in low yields under dry conditions. Peptide synthesis at a reverse micellar interface in the presence of water proceeded in moderate yields.[6,7] As no significant rate acceleration was observed with these reactions, the micellar effect mentioned above would seem to be inadequate. More recently, Kobayashi and co-workers realized a 60-fold rate enhancement in acid-catalyzed esterification, which proceeded in the hydrophobic interior of emulsion droplets formed in water.[8,9]

In the course of our studies on 1,3,5-triazine-based dehydrocondensing agents that are available in an aqueous solvent,[10–12] we found that the reaction of the carboxylate **1** with the dehydrocondensing agent **3** to form acyloxytriazine **4** was the rate-determining step, because the reaction rate depended on the concentration of **1** [Eq. (1)]. Since both the reactants **1** and **3** have ionic structures, they should be aligned at the interface of micelles when a hydrophobic alkyl group is introduced into their structure. In the studies discussed herein, we employed amphiphilic dehydrocondensing agents based on 1,3,5-triazine to demonstrate that a large rate enhancement of bimolecular dehydrocondensation occurs in micelles as a result of both the proximity and the preorientational effects.



(1)

To simplify the reaction system, we employed the sodium salt **1** of a fatty acid which can serve both as a substrate and a surfactant. The reaction is illustrated in Scheme 1. Both hydrophobic 2-chloro-4,6-dimethoxy-1,3,5-triazine (DMT-Cl) and an amphiphilic tertiary amine **2** are incorporated in the micelles consisting of **1**. Nonionized amine **2** is buried in the micelle and then couples with DMT-Cl (step 1). This step can occur either in the outer core or in the palisade layer of the micelle[3] and can be accelerated by increasing the local concentration of the reactants. Amine **2** acts as a catalyst for the activation of DMT-Cl toward **1** through the formation of **3** (step 2). The resulting quaternary ammonium group of the amphiphilic agent **3** will be located at the interface of the



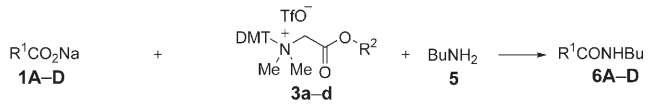
Scheme 1. Micellar effects on the catalytic dehydrocondensation.

micelle, thus forming an ion pair with **1**. Therefore, as **1** is closely concentrated around **3**, it can readily attack the triazinyl group of **3** with concomitant liberation of **2**, which can be recycled to step 1. Step 2 is accelerated by the preorientational effect as well as the local concentration effect of the reactants at the micellar interface. The resulting activated triazinyl ester **4** undergoes aminolysis with butylamine (**5**), which is expected to be partitioned mainly in the aqueous phase, to form the amide **6** (step 3). As this step is known to be much faster than hydrolysis in water,[10] it is not rate-limiting and thus it appeared to be unimportant to ensure that it occurs in the micelle rather than at the aqueous phase.

We employed four sodium carboxylates **1A–D** with alkyl chains of different lengths. On the basis of the critical micelle concentration and Kraft points of these compounds, **1A** and **1B** should form a simple molecular dispersion phase in which these electrolytes are homogeneously dissolved in a dissociated form, whereas **1C** and **1D** should form a micellar phase at a concentration of 15 mM (25 °C).[13,14] If the bimolecular reaction rate constants between carboxylates **1** and **3** are assumed to be independent of their chain length, the observed change in reaction rate can be attributed to the micellar effect.

First, we examined the reaction of carboxylates **1A–D** with condensing agents **3a–d**[15] to estimate the acceleration of the rate-determining step (step 2) in the micelle. The reaction should be close to first-order with respect to **3**. The reaction was conducted using **1** (15 mM), **5** (as hydrochloride, 20 mM), and **3** (1.5 mM) in phosphate buffer (pH 8, 20 mM) containing MeOH (3 %)[16] at 25 °C (Table 1). The pseudo-first-order rate

Table 1: Relative rates for the stoichiometric reaction of **1** and **3**.[a]



A: $R^1 = C_3H_9$ **a**: $R^2 = C_2H_5$
B: $R^1 = C_7H_{15}$ **b**: $R^2 = C_8H_{17}$
C: $R^1 = C_{11}H_{23}$ **c**: $R^2 = C_{12}H_{25}$
D: $R^1 = C_8H_{17}CH{=}CHC_7H_{14}$ **d**: $R^2 = C_{16}H_{33}$

3	1A	1B	1C	1D
3a	1.0[a]	1.1	56	63
3b	0.7	3.0	1200	830
3c	21	280	860	840
3d	30	340	1400	690

[a] Pseudo-first-order rate constant: $k = 1.0 \times 10^{-3}$ min^{-1}.

constants for the reaction with respect to **3** were calculated based on the amount of the amide **6** produced. The relative rates were normalized to the reaction rate of **3a** with **1A** (rate defined as 1). In the reactions of **3a**, which has a short alkyl chain (ethyl group), no rate acceleration was observed in the reaction with octanoate **1B**, which has an alkyl chain that is four carbon atoms longer than that of butyrate **1A**, whereas the reaction with laurate **1C**, whose alkyl chain is elongated by an additional four carbon atoms, was accelerated by a factor of 56. In a series of reactions with **1C**, the reaction rate increased up to 1400 times by elongation of the ester alkyl chain of **3**. Interestingly, the reaction rates appeared to plateau with the **1C** series; reactions were not accelerated further when a longer acyl chain was used (see series **1D**).

As **1C** and **1D** form micelles under these reaction conditions, the large rate enhancements observed with these two carboxylates are attributable to the micellar effect (Scheme 1, step 2). Dehydrocondensing agents **3b–d** have alkyl chains with eight carbon atoms or more, which puts them on the borderline in their ability to be incorporated in micelles. Moderate accelerations in the reactions of **1B** with **3c** or **3d** do not arise from micelle formation but rather from a disordered aggregation owing to the hydrophobic effect. This explanation is based on the fact that **3c**, which is poorly soluble in water, does not form micelles under the reaction conditions. In fact, a white turbidity appeared upon addition of **3c** or **3d** to initiate the reaction with **1B**, whereas no turbidity was observed with **1C** and **1D**, presumably as a result of mixed micelle formation.

The substrate concentration dependence of the reaction rate in the micellar system when using **3b** was also examined. The reaction rate was found to be independent of the concentration of **1C** (15, 30, and 60 mM) whereas the reaction rate showed a linear relationship to the concentration of butylamine **5** (5, 10, 15, and 20 mM). The results indicate that step 3 becomes the rate-determining step in the micellar system instead of step 2.[17] The rate of aminolysis of the triazinyl ester with **5** dissolved in the aqueous phase is independent of the length of the acyl chain. Thus, in the micellar system, there was no significant difference between **1C** and **1D**, despite the difference in chain length (six carbon atoms).

Competitive reactions between **1A** and **1B** with either **3a** or **3b** afforded amides **6A** and **6B** (42:58 or 25:75, respectively) after the reaction mixture was stirred for 4 h at room temperature (Table 2). In contrast, the competitive reaction

Table 2: Substrate selectivity in the competitive reaction between two carboxylates in the stoichiometric system.

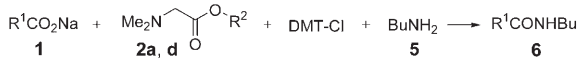
Carboxylates	3	*t* [h]	Yield [%]	Ratio
1A vs. **1B**	**3a**	4	16	**6A**/**6B** 42:58
1A vs. **1B**	**3b**	4	24	**6A**/**6B** 25:75
1A vs. **1C**	**3b**	1	88	**6A**/**6C** 0.4:99.6

between **1A** and **1C** with **3b** proceeded within 1 h in both good yield (88 %) and high selectivity (0.4:99.6). These selectivities are in good agreement with the relative reaction rates shown in Table 1.

Finally, the catalytic reaction of *N*,*N*-dimethylglycine alkyl esters **2** and DMT-Cl, which generate condensing agent **3** in situ, was also found to be accelerated by a factor of 140 in micelles (Table 3). The moderate acceleration in the catalytic system relative to the stoichiometric system may be attributed to the generation of **3** (step 1) which may become the rate-determining step after acceleration of step 2. This catalytic system can be considered as an acyl transferase model for preparing lipid molecules.

The amide-forming reaction in the cyclodextrin-based artificial enzyme was accelerated by a factor of 13 because of

Table 3: Relative rates for the catalytic reaction of tertiary amines **2** with carboxylates **1B** and **1C**.



2	1B	1C
2a ($R^2 = C_2H_5$)	1.0[a]	33
2d ($R^2 = C_{16}H_{33}$)	11	140

[a] Pseudo-first-order rate constant: $k = 8.5 \times 10^{-5}$ min^{-1}.

a proximity effect resulting from the formation of an inclusion complex.[12] By comparison, the large rate enhancement of the present system should be ascribed to the micellar effect (preorientational effect and local concentration effect) illustrated in Scheme 1. Micelles of quaternary ammonium surfactants are known to promote the hydrolysis of both activated esters and condensing agents because the hydroxide ion with a negative charge opposite to that of the surfactant is concentrated at the interface.[1–7] As the present system employs carboxylate anions as surfactants, the hydroxide ion (with the same charge) does not concentrate at the micellar interface, and the hydrolysis of **3** and **4** does not become a serious side reaction. Thus, we have succeeded in demonstrating that amphiphilic dehydrocondensing agents based on 1,3,5-triazine offer the micellar interface as a superior reaction field for dehydrocondensation.

Experimental Section

General procedure: **3** (3 μmol) in aqueous methanol (40%; 0.15 mL) was added to a stirred aqueous solution (1.85 mL) of **1** (30 μmol) and **5**·HCl (40 μmol) in sodium phosphate buffer (pH 8) at 25°C. The initial concentration of reactants in the resulting solution were as follows: **1**: 15 mM; **5**: 20 mM; **3**: 1.5 mM; NaPi: 20 mM; and MeOH: 3%. The mixture was stirred at 25°C, and HCl (5M; 0.3 mL) was added at a specific time. The resulting mixture was applied to Extrelut NT (Merck, 2 g) and eluted with AcOEt. The product **6** was quantified by GC (silicone SE-30 for **6A**, silicone OV-17 for **6B**–**D**). The pseudo-first-order rate constants were determined from the slopes of liner plots of $\ln([\mathbf{3}]_t/[\mathbf{3}]_0)$ versus time (min).

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