Control of Substrate Specificity in Polymer-Catalyzed Solvolysis Reactions of *p*-Nitrophenyl Alkanoates by Changing the Buffer System

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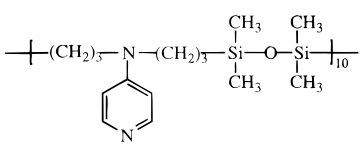
ABSTRACT: The substrate specificity in solvolysis reactions of *p*-nitrophenyl alkanoates **2** ($n = 2-18$) catalyzed by 4-(dialkylamino)pyridine-functionalized polymer **1** was examined in buffered aqueous methanol solution at pH 8.0 and 30 °C. The chemical reactivity and substrate specificity in this catalytic system were found to be controlled by changing the buffer system. In 1:1 (v/v) methanol−aqueous phosphate buffer solution, macromolecule **1** exhibits substrate specificity for **2** ($n = 14$) below 1.0×10^{-5} unit mol L^{-1}, and the substrate specificity changes from **2** ($n = 14$) to **2** ($n = 12$) as the concentration of **1** increases to 2.5×10^{-5} unit mol L^{-1} and changes again from **2** ($n = 12$) to **2** ($n = 10$) when the concentration of **1** increases further to 7.5×10^{-5} unit mol L^{-1}. However, in 1:1 (v/v) methanol−aqueous Tris buffer solution, macromolecule **1** was found to demonstrate the same substrate specificity for **2** ($n = 14$) when the concentration of **1** is increased from 5.0×10^{-6} to 1.0×10^{-4} unit mol L^{-1}. The control of substrate specificity by the change of the buffer system is believed to be unprecedented for catalysis of ester solvolysis.

Introduction

Enzymes can fulfill many functions and catalyze a wide diversity of chemical reactions. Various functions are known to be based on their ability to form highly organized molecular assemblies. Unravelling the principles that underlie the action of enzymes continues to be a challege for chemists and biochemists, and considerable progress toward this goal has been made in recent years. Much work is focused on the enzymes themselves, but also on the synthetic catalysts which can give insight into the details of the catalytic processes involved. The control of substrate specificity in enzymic catalysis by enzyme structures and organic solvents is well-known.[1−6] Numerous studies have shown that many solvent physical properties such as dielectric constant, dipole moment, and hydrophobicity influence enzyme specificity and enantioselectivity.[4−6] However, the origins of substrate specificity in enzymic catalysis are still not well understood at the molecular level.[6−8]

4-(Dialkylamino)pyridine-functionalized polymers have been regarded as useful and simple model systems for obtaining a better understanding of the origins of enzymic efficiency and selectivity.[9−16] We have recently made an attempt to investigate such a model system to gain insight into the dominant control factors in solvolysis of *p*-nitrophenyl esters **2** ($n = 2-18$) catalyzed by 4-(dialkylamino)pyridine-functionalized polymer **1**.[17−21]



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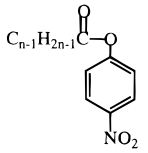

2

(n = 2, 4, 6, 8, 10, 12, 14, 16, 18)

The mechanism of the reaction involves the attack by nucleophile **1** at the carbonyl group of substrates **2** and the formation of an *N*-acylpyridinium intermediate where the breakdown of the intermediate is the rate-determining step in the catalytic reactions.[17] Strikingly, we have found ion-induced substrate specificity in the **1**-catalyzed solvolysis of **2** ($n = 2-18$) in aqueous and methanol−water solutions.[18,19] Salting-in effects of the tris(hydroxymethyl)methylammonium ion in aqueous Tris buffer solution lead to the same substrate specificity for **2** ($n = 6$) that is obtained with the enzyme, cholesterol esterase, for the same hydrolysis reaction.[22] The addition of salting-out agent NaCl leads to a change of substrate specificity from **2** ($n = 14$) to **2** ($n = 12$) in 1:1 (v/v) methanol−aqueous phosphate buffer solution.[19] We have also described the detailed kinetic characterization of the salting-out effects of sodium chloride on the **1**-catalyzed solvolysis of **2** in 1:1 (v/v) methanol−aqueous phosphate buffer solution that identifies the optimum substrate structure.[18,19] Moreover, we have reported an interesting example of electrostatic interactions that contribute to the catalytic activity and substrate specificity for the **1**-catalyzed solvolysis of **2** ($n = 2-18$) in the presence of anionic surfactant sodium dodecyl sulfate (SDS),[20] which provides significant insight into the mechanism of catalytic ester solvolysis. These results may be akin to the observations that many enzymes usually accelerate the hydrolytic reactions at hydrophobic binding sites by electrostatic stabilization of the transition state through interactions with nearby amino acid constituents.[23,24] To our knowledge, the control of substrate specificity by changing the buffer system has not yet been reported for catalysis of solvolysis reactions of *p*-nitrophenyl esters. Both phosphate buffer and Tris buffer are very popular buffer systems that are widely used in biological and chemical catalysis. However, no previous investigations have pinpointed the different effects on catalytic ester solvolysis due to differences in the intrinsic properties of the two buffer salts in the reaction medium.

In the present paper we report the detailed study of substrate specificity controlled by changing the buffer system in the **1**-catalyzed solvolysis reactions of **2** ($n =$

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Table 1. Summary of the Substrate Specificity of 1-Catalyzed Solvolysis of 2 (n = 2−18) in 1:1 (v/v) Methanol−Aqueous Buffer Solution at pH 8.0 and 30 °C[a]

	substrate specificity[c]	
concn of **1** (unit mol L^{-1})[b]	0.05 M $H_2PO_4^-/HPO_4^{2-}$	0.05 M Tris H^+/Tris
5.0×10^{-6}	**2** (n = 14)	**2** (n = 14)
1.0×10^{-5}	**2** (n = 14)	**2** (n = 14)
2.5×10^{-5}	**2** (n = 12)	**2** (n = 14)
5.0×10^{-5}	**2** (n = 12)	**2** (n = 14)
7.5×10^{-5}	**2** (n = 10)	**2** (n = 14)
1.0×10^{-4}	**2** (n = 10)	**2** (n = 14)

[a] **2**, 5.0×10^{-5} M. [b] In 1:1 (v/v) methanol−aqueous buffer (0.05 M $H_2PO_4^-/HPO_4^{2-}$ or 0.05 M Tris H^+/Tris, pH 8.0) solution. [c] Substrate specificity is defined by the maxima of plots of pseudo-first-order rate constants for the solvolysis of the series of substrates **2** (n = 2−18) as a function of alkanoate chain length (n), see Figure 1.

2−18) in buffered aqueous methanol solution. A more detailed kinetic characterization of the buffer effects on the **1**-catalyzed solvolysis of **2** (n = 10−16) in buffered aqueous methanol solution is also reported.

Experimental Section

Materials and Reagents. Synthesis of the poly(siloxane−bis(trimethylene))-supported 4-(dialkylamino)pyridine (**1**) has been described previously.[15] *p*-Nitrophenyl alkanoates **2** (n = 2−18) and 1,4-dioxane were purchased from Sigma Chemical Co. Tris(hydroxymethyl)aminomethane, hydrochloric acid, methanol, and aqueous buffer solution (0.05 M $H_2PO_4^-/HPO_4^{2-}$, pH 8.0) were used as received from Aldrich and Fisher.

Kinetic Measurements. The fresh catalyst solutions for kinetic experiments were prepared in 1:1 (v/v) methanol−aqueous buffer (0.05 M $H_2PO_4^-/HPO_4^{2-}$ or 0.05 M Tris H^+/Tris, pH 8.0) solution. The cuvette was filled with 2.5 mL of a fresh solution containing catalyst, and the solution was equilibrated for 10 min at 30 °C in the thermostated cell compartment of a Hewlett-Packard Model 8450 spectrophotometer. A fresh stock solution (usually 5 μL) of *p*-nitrophenyl alkanoates **2** (n = 2−18; 2.5×10^{-2} M) in dioxane was added by microsyringe. The reaction mixture was quickly mixed by shaking, and the absorbance at 400 nm was recorded as a function of time. The reactions were performed for 4−5 half-lives and the pseudo-first-order rate constants (k_{obsd}) were obtained as slopes of plots of $\ln[A_\infty/(A_\infty - A_t)]$ vs time, where A_∞ and A_t are the absorbances at infinite time and time t, respectively. The pseudo-first-order rate constants are accurate to within ±5%.

Results and Discussion

We have investigated the solvolysis of **2** (n = 2−18) in the absence and in the presence of **1** as a function of alkanoate chain length in 1:1 (v/v) methanol−aqueous phosphate buffer (0.05 M $H_2PO_4^-/HPO_4^{2-}$, pH 8.0) solution at 30 °C. As reported previously,[21] in the absence of **1** the solvolysis rate of **2** (n = 2−18) is very slow and no substrate specificity is observed in 1:1 (v/v) methanol−aqueous phosphate buffer solution. But, the rates for the **1**-catalyzed solvolysis of **2** (n = 2−18) are very fast and increase remarkably with increasing concentration of **1** in 1:1 (v/v) methanol−aqueous phosphate buffer solution. Significantly, we have found that macromolecule **1** demonstrates different substrate preferences when the concentration of **1** increases from 5.0×10^{-6} to 1.0×10^{-4} unit mol L^{-1} as shown in Table 1. Below 1.0×10^{-5} unit mol L^{-1}, macromolecule **1** exhibits substrate specificity for **2** (n = 14). As the concentration of **1** increases to 2.5×10^{-5} unit mol L^{-1}, the substrate

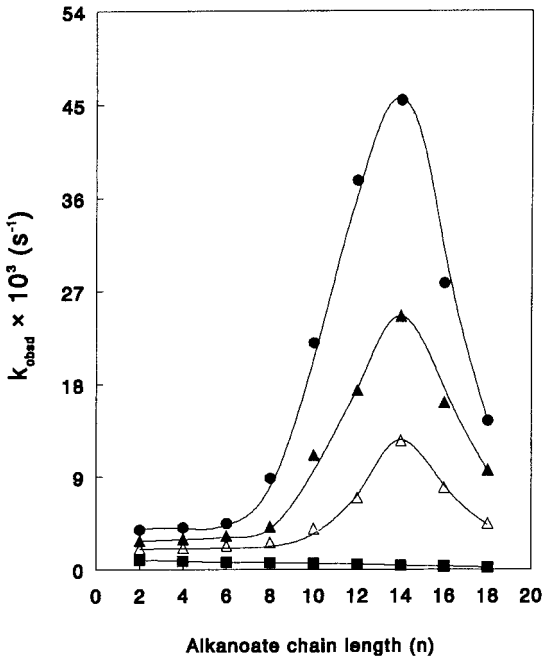

Figure 1. Pseudo-first-order rate constants (k_{obsd}) for the solvolysis of *p*-nitrophenyl alkanoates **2** (n = 2−18, 5.0×10^{-5} M) catalyzed by **1** as a function of alkanoate chain length (n) in 1:1 (v/v) methanol−aqueous Tris buffer (0.05 M Tris H^+/Tris, pH 8.0) solution at 30 °C: (●) 7.5×10^{-5} unit mol L^{-1} **1**; (▲) 2.5×10^{-5} unit mol L^{-1} **1**; (△) 5.0×10^{-6} unit mol L^{-1} **1**; (■) in the absence of **1**.

preference changes from **2** (n = 14) to **2** (n = 12). The substrate specificity changes again from **2** (n = 12) to **2** (n = 10) when the concentration of **1** increases further to 7.5×10^{-5} unit mol L^{-1}. Clearly, the substrate specificity in the solvolysis reactions of **2** (n = 2−18) catalyzed by **1** is modified by the concentration of **1** in 1:1 (v/v) methanol−aqueous phosphate buffer solution (Table 1).

We have also examined the solvolysis of **2** (n = 2−18) in the absence and in the presence of **1** as a function of alkanoate chain length in 1:1 (v/v) methanol−aqueous Tris buffer (0.05 M Tris H^+/Tris, pH 8.0) solution at 30 °C. The results are presented in Table 1 and Figure 1. Without **1**, the solvolysis rate of **2** (n = 2−18) is also very slow, and no substrate specificity is found in 1:1 (v/v) methanol−aqueous Tris buffer solution. As indicated in Figure 1, in the absence of **1** an increase of the alkanoate chain length in **2** causes minor decreases in the solvolysis rates, which is in accordance with previous observations in 1:1 (v/v) methanol−aqueous phosphate buffer solution.[21] However, in the presence of **1** the solvolysis rates of **2** (n = 2−18) are much faster and increase significantly with increasing the concentration of **1**, indicative of catalytic solvolysis reactions. Under conditions where 10-fold excess substrates are used in kinetic study, the catalytic effectiveness is maintained to complete solvolysis reaction. The rates for the **1**-catalyzed solvolysis of **2** depend strongly upon the alkanoate chain length in the substrate esters. Increase of the alkanoate chain length in **2** leads to an increase in the solvolysis rate, which reaches a maximum for **2** (n = 14). The solvolysis rate then decreases with further increase of the alkanoate chain length in the substrate esters. Surprisingly, we have found that macromolecule **1** demonstrates the same substrate specificity for **2** (n = 14) when the concentration of **1** increases from 5.0×10^{-6} to 1.0×10^{-4} unit mol L^{-1} (Table 1). Apparently,

the substrate specificity in the **1**-catalyzed solvolysis of **2** ($n = 2-18$) is controlled by changing the buffer system in buffered aqueous methanol solution. Although enzymes such as elastase, chymotrypsin, and cholesterol esterase and certain synthetic catalysts demonstrate the substrate preference in the solvolysis reactions of **2**,[11,22,25,26] we are not aware of any catalytic systems that show the substrate specificity controlled by the nature of the buffer system.

Macromolecule **1** is an amphiphilic polymer which contains distinct hydrophobic and hydrophilic regions, and it associates to form macromolecular aggregates by self-assembly in aqueous or methanol−water solution.[11−19] The control of aggregate morphology changes of small-molecule and macromolecular amphiphiles from spheres to rods, and to vesicles by increasing the hydrophobic effects in water and water−organic solvent mixtures has already been well-established.[27−35] For many years, the aggregate morphology changes of small-molecule amphiphiles from spherical micelles to micellar rods and vesicles have been known to be controlled by increases of their constituent concentrations in solution.[36−38] The gradual changes of aggregate morphology of polystyrene-*b*-poly(2-vinylpyridine) copolymers from spheres to rods, and to vesicles have also been demonstrated with increasing copolymer concentration.[34] Consistent with the notion that salt-induced hydrophobic effects control aggregate morphology of amphiphiles, the aggregate morphology of these copolymers in water−organic solvent mixtures can also be changed from spheres to rods, and to vesicles by addition of salting-out agents such as NaCl and $CaCl_2$.[35] We have found that the solutions of **1** show appreciable turbidity when its concentration is increased beyond 1.0×10^{-4} unit mol L^{-1} in 1:1 (v/v) methanol−aqueous phosphate buffer solution. These results suggest that changes of the aggregate morphology of **1** from spheres to rods, and to vesicles may accompany the increases of the concentration of **1** from 5.0×10^{-6} to 1.0×10^{-4} unit mol L^{-1} in 1:1 (v/v) methanol−aqueous phosphate buffer solution.[32−34] In line with the observations that the salting-out agent NaCl can change the aggregate morphology of amphiphilic macromolecules from spheres to rods, and to vesicles,[35] we have found that the solution of **1** at 5.0×10^{-6} unit mol L^{-1} becomes opalescent in 1:1 (v/v) methanol−aqueous phosphate buffer solution as the concentration of NaCl is increased to more than 1.00 M due to the salting-out effects of sodium chloride. In contrast, however, we have found that a solution of **1** at 5.0×10^{-4} unit mol L^{-1} in 1:1 (v/v) methanol−aqueous Tris buffer solution still remains clear even after standing for prolonged periods. We believe that the salting-in effects of the tris(hydroxymethyl)methylammonium ion are responsible for the decreased hydrophobic association of **1** in 1:1 (v/v) methanol−aqueous Tris buffer solution,[18] which is in conformity with the observations that the anions and cations of low charge density tend to salt-in organic solutes by decreasing the association of hydrocarbon species while the ions of high charge density tend to salt-out such solutes by increasing the association of hydrocarbon species.[39−45] These results suggest that spherical aggregates of **1** may be maintained when the concentration of **1** is increased from 5.0×10^{-6} to 1.0×10^{-4} unit mol L^{-1} in response to the salting-in effects of the tris(hydroxymethyl)-methylammonium ion in 1:1 (v/v) methanol−aqueous Tris buffer solution.[32−35]

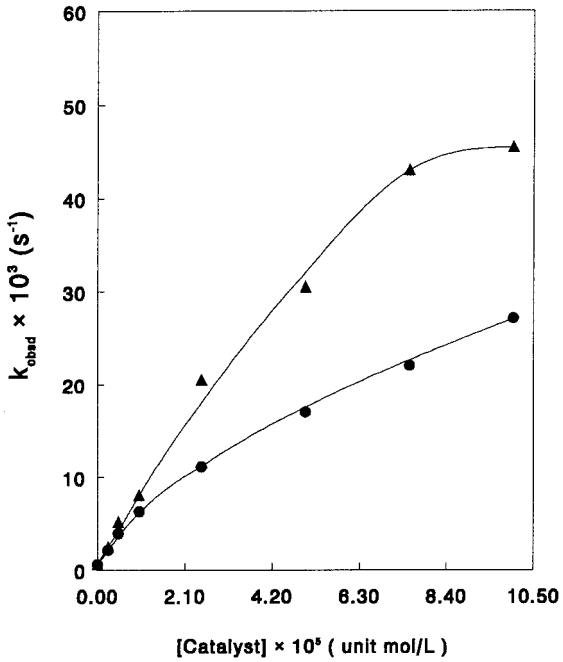

Figure 2. Pseudo-first-order rate constants (k_{obsd}) for the solvolysis of *p*-nitrophenyl alkanoate **2** ($n = 10$, 5.0×10^{-5} M) catalyzed by **1** as a function of catalyst concentration in 1:1 (v/v) methanol−aqueous buffer solution at pH 8.0 and 30 °C: (●) with 0.05 M aqueous Tris buffer solution; (▲) with 0.05 M aqueous phosphate buffer solution.

To probe the relationship between the rate of the model reaction and the change of the buffer system in the reaction medium, we have systematically examined the catalytic activity of the **1**-catalyzed solvolysis of **2** ($n = 10-16$) as a function of catalyst concentration in the two differently buffered aqueous methanol solutions at pH 8.0 and 30 °C. Figure 2 shows the pseudo-first-order rate constants (k_{obsd}) for the solvolysis of **2** ($n = 10$) catalyzed by **1** as a function of the concentration of **1**. In 1:1 (v/v) methanol−aqueous phosphate buffer solution, the solvolysis rates for **2** ($n = 10$) increase strongly with the concentration of **1** up to 7.5×10^{-5} unit mol L^{-1} and then increase slightly with a further increase in the concentration of **1**. In contrast, however, the solvolysis rates of **2** ($n = 10$) increase modestly with an increase in the concentration of **1** from 2.5×10^{-6} to 1.0×10^{-4} unit mol L^{-1} in 1:1 (v/v) methanol−aqueous Tris buffer solution. Significantly, the catalytic efficiency of **1** toward **2** ($n = 10$) is greater in 1:1 (v/v) methanol−aqueous phosphate buffer solution than in 1:1 (v/v) methanol−aqueous Tris buffer solution in the concentration range of **1** investigated.

The dependence of the pseudo-first-order rate constants on the concentrations of **1** for the **1**-catalyzed solvolysis of **2** ($n = 12$) are presented in Figure 3, where macromolecule **1** clearly demonstrates different catalytic behavior for the solvolysis of **2** ($n = 12$) as the concentration of **1** increases in the two differently buffered aqueous methanol solutions. In 1:1 (v/v) methanol−aqueous phosphate buffer solution, the solvolysis reactions for **2** ($n = 12$) show rapid enhancements of the pseudo-first-order rate constants below 2.5×10^{-5} unit mol L^{-1} **1**, followed by gradual leveling off with increasing the concentration of **1**. Finally, the pseudo-first-order rate constants reach plateau values. It is noted that in 1:1 (v/v) methanol−aqueous Tris buffer solution a strong

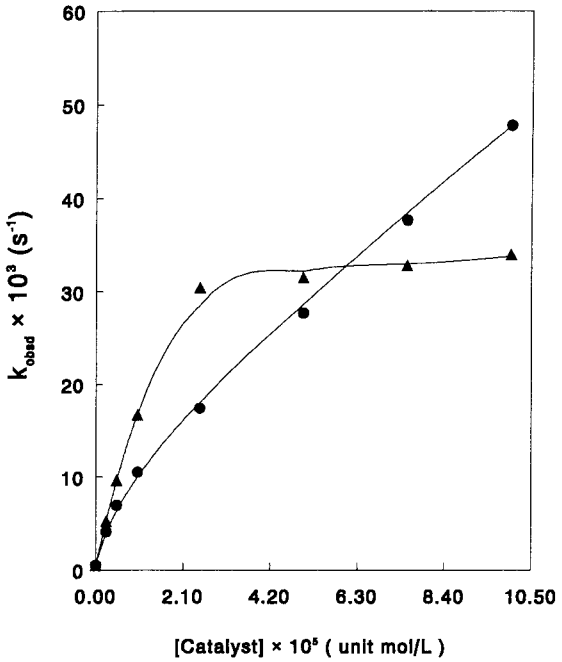

Figure 3. Pseudo-first-order rate constants (k_{obsd}) for the solvolysis of *p*-nitrophenyl alkanoate **2** ($n = 12$, 5.0×10^{-5} M) catalyzed by **1** as a function of catalyst concentration in 1:1 (v/v) methanol−aqueous buffer solution at pH 8.0 and 30 °C: (●) with 0.05 M aqueous Tris buffer solution; (▲) with 0.05 M aqueous phosphate buffer solution.

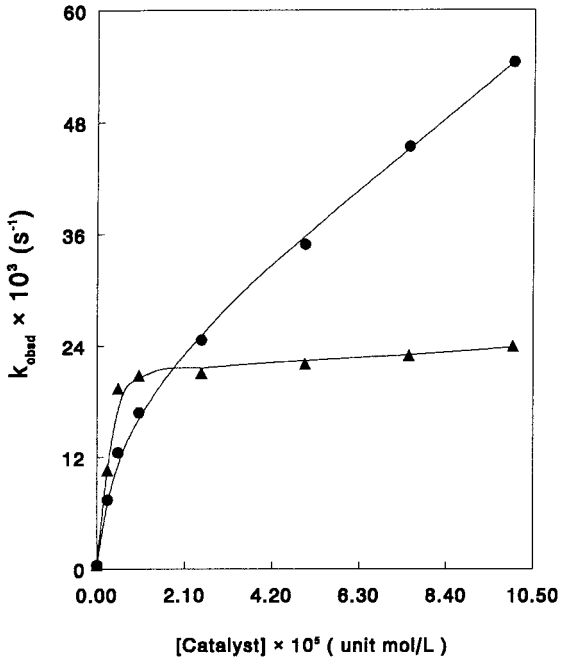

Figure 4. Pseudo-first-order rate constants (k_{obsd}) for the solvolysis of *p*-nitrophenyl alkanoate **2** ($n = 14$, 5.0×10^{-5} M) catalyzed by **1** as a function of catalyst concentration in 1:1 (v/v) methanol−aqueous buffer solution at pH 8.0 and 30 °C: (●) with 0.05 M aqueous Tris buffer solution; (▲) with 0.05 M aqueous phosphate buffer solution.

increase in the solvolysis rate is observed when the concentration of **1** is increased from 2.5×10^{-6} to 1.0×10^{-4} unit mol L^{-1}. Interestingly, the reactivity for **2** ($n = 12$) is greater below 5.0×10^{-5} unit mol L^{-1} **1** but less above 7.5×10^{-5} unit mol L^{-1} **1** in 1:1 (v/v) methanol−aqueous phosphate buffer solution than in 1:1 (v/v) methanol−aqueous Tris buffer solution.

The pseudo-first-order rate constants for the **1**-catalyzed solvolysis of **2** ($n = 14$) as a function of the concentration of **1** are graphically shown in Figure 4. The results indicate that the solvolysis rates for **2** ($n = 14$) appear to be proportional with the concentration of **1** up to 1.0×10^{-5} unit mol L^{-1} and then stay at plateau values to 1.0×10^{-4} unit mol L^{-1} in 1:1 (v/v) methanol−aqueous phosphate buffer solution. Under the same experimental conditions, in 1:1 (v/v) methanol−aqueous Tris buffer solution the solvolysis rates of **2** ($n = 14$) increase very rapidly with an increase in the concentration of **1** from 2.5×10^{-6} to 1.0×10^{-4} unit mol L^{-1}. Below 1.0×10^{-5} unit mol L^{-1} **1**, the solvolysis of **2** ($n = 14$) shows greater reactivity in 1:1 (v/v) methanol−aqueous phosphate buffer solution than in 1:1 (v/v) methanol−aqueous Tris buffer solution. However, above 2.5×10^{-5} unit mol L^{-1} **1**, the reactivity of **2** ($n = 14$) is less in 1:1 (v/v) methanol−aqueous phosphate buffer solution than in 1:1 (v/v) methanol−aqueous Tris buffer solution.

The pseudo-first-order rate constants for the **1**-catalyzed solvolysis of **2** ($n = 16$) are plotted as a function of the concentration of **1** in Figure 5. In 1:1 (v/v) methanol−aqueous phosphate buffer solution, the solvolysis rates for **2** ($n = 16$) increase rapidly upon an increase of the concentration of **1** up to 1.0×10^{-5} unit mol L^{-1} and then level off with a further increase in the concentration of **1** and finally reach plateau values. However, under the same experimental conditions, the solvolysis rates of **2** ($n = 16$) increase strongly with

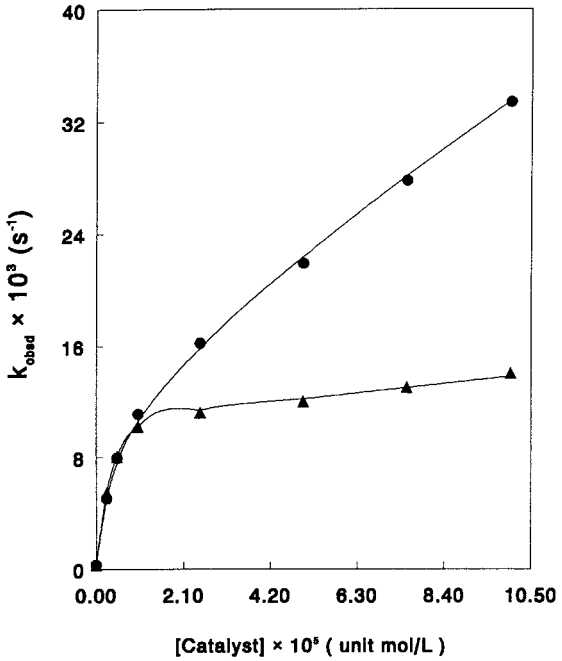

Figure 5. Pseudo-first-order rate constants (k_{obsd}) for the solvolysis of *p*-nitrophenyl alkanoate **2** ($n = 16$, 5.0×10^{-5} M) catalyzed by **1** as a function of catalyst concentration in 1:1 (v/v) methanol−aqueous buffer solution at pH 8.0 and 30 °C: (●) with 0.05 M aqueous Tris buffer solution; (▲) with 0.05 M aqueous phosphate buffer solution.

increasing concentration of **1** in 1:1 (v/v) methanol−aqueous Tris buffer solution in the concentration region of **1** studied. Importantly, the catalytic efficiency for **2** ($n = 16$) is found to be greater in 1:1 (v/v) methanol−aqueous Tris buffer solution than in 1:1 (v/v) methanol−aqueous phosphate buffer solution, in particular at high concentrations of **1**. It is clear that the chemical reactivity for the **1**-catalyzed solvolysis of **2** ($n = 10-16$) is

controlled by changing the buffer system. We suggest that the aggregate morphology of **1** and **1·2** complexes and the solvation of substrates in the reaction medium may play a pivotal role in the substrate specificity of catalytic solvolysis reactions of **2**. The distribution of both catalyst **1** and substrates **2** among solution and aggregate phases controlled by the salting-in effects of the tris(hydroxymethyl)methylammonium ion may also affect the rates of the solvolysis reactions of **2** in 1:1 (v/v) methanol−aqueous Tris buffer solution.

The catalytic importance of hydrophobic interactions between enzymes and substrates has long been realized. In many enzyme-catalyzed hydrolysis reactions occurring on hydrophobic active sites, the substrate reactivity can be modified by changing the hydrophobic interactions between enzymes and substrates.[23,24] Since the homologues of **2** differ only in the chain length of their alkanoate component, the substrate specificity obtained may originate, at least in part, from a consequence of hydrophobic−lipophilic interactions between **1** and **2**. The stretching of hydrophobic chains of macromolecular amphiphiles is known to be greatest when they are located within spherical aggregates, and the stretching decreases as the aggregate morphology changes from spheres to rods and decreases further as vesicles are formed.[32,33] Thus, the spherical aggregates of amphiphilic macromolecules would tend to provide the strongest hydrophobic binding to lipophilic substrates among common aggregate morphologies in the reaction medium. The changes of aggregate morphology of amphiphilic macromolecules from spheres to rods and then to vesicles would lead to decreased hydrophobic binding to the lipophilic substrates in the reaction medium. Our calculations[21] suggest that a parallel and equivalent decrease in hydrophobic effects is involved in both processes for the substrate specificity changes from **2** ($n = 14$) to **2** ($n = 12$) and **2** ($n = 10$) and the aggregate morphology changes from spheres to rods and vesicles.[32−35] The migration and binding of the lipophilic substrates to hydrophobic microdomains of polymers in aqueous solution have been well-known in catalytic ester hydrolysis reactions.[46] The rate enhancements for the **1**-catalyzed solvolysis of **2** have been attributed to hydrophobic association between catalyst and substrate in the reaction medium.[9−13,16−20] Therefore, the substrate specificity changes that accompany an increase in the concentration of **1** in 1:1 (v/v) methanol−aqueous phosphate buffer solution may be caused by an energetically favorable matching of hydrophobicities of substrate **2** and aggregates of **1** leading to enhanced stabilization of **1·2** complexes. We suggest that in 1:1 (v/v) methanol−aqueous phosphate buffer solution the substrate specificity change from **2** ($n = 14$) to **2** ($n = 12$) may result from a change of aggregate morphology of **1** from spheres to rods leading to decreased hydrophobic binding and increased access of **1·2** complexes to the nucleophilic medium that is optimum for **2** ($n = 12$), and the specificity change from **2** ($n = 12$) to **2** ($n = 10$) may be attributed to further change of aggregate morphology of **1** from rods to vesicles leading to further decreased hydrophobic binding and further increased access of **1·2** complexes to the nucleophilic medium that is optimum for **2** ($n = 10$). The same substrate specificity for **2** ($n = 14$) with increasing the concentration of **1** in 1:1 (v/v) methanol−aqueous Tris buffer solution may be ascribed to the spherical aggregates of **1** formed due to the salting-in effects of the tris(hydroxymethyl)methylammonium ion that contribute to optimum hydrophobic binding for **2** ($n = 14$). We suggest that the matching of the hydrophobicities of substrate and aggregate of catalyst may be an important contributor to the molecular discrimination described by the term hydrophobic interactions at hydrophobic active sites of enzymes and catalysts in controlling substrate specificity for biological and chemical catalysis. The increases of polymer and NaCl concentrations can change the aggregate morphology of amphiphilic macromolecules from spheres to rods, and to vesicles in the same way.[34,35] We have found that increasing the concentration of **1** has a parallel effect on the substrate specificity changes, as does increasing the concentrations of NaCl in 1:1 (v/v) methanol−aqueous phosphate buffer solution.[19] This consistency supports the notion that the matching of the hydrophobicities of substrate and aggregate of **1** may control the substrate specificity for the **1**-catalyzed solvolysis of **2** ($n = 2-18$) in the reaction medium.

The present results provide the first example that substrate specificity in catalytic ester solvolysis can be controlled by changing the buffer system in the reaction medium. They bring a new approach to the control of chemical reactivity that can be useful in understanding the origins of catalytic efficiency and selectivity in biological and chemical catalysis. These studies also identify a potential fundamental problem in the definition of many enzyme-catalyzed reactions. Much remains to be learned about the effects of buffer upon the reaction rates obtained in enzymic catalysis.

Moreover, these results may offer a reasonable explanation to the observations that the hydrolysis rates of **2** ($n = 2-12$) in the presence of cetyltrimethylammonium bromide (CTAB) increase significantly in aqueous phosphate buffer solution[47,48] but decrease markedly in aqueous Tris buffer solution.[49,50] Gitler et al. have explained that in aqueous Tris buffer solution the substrate esters are incorporated into or on CTAB micelles, and thus they are protected from the hydrolysis leading to decreased hydrolysis rates.[49,50] However, the binding of the substrates to CTAB micelles can also be invoked in CTAB micelle-catalyzed hydrolysis reactions in aqueous phosphate buffer solution, and would account for the increases in the hydrolysis rates observed.[47,48] An alternative explanation would be that the salting-in effects of the tris(hydroxymethyl)methylammonium ion may serve as a major factor in the CTAB micelle-catalyzed hydrolysis of **2** in aqueous Tris buffer solution leading to decreased hydrolysis rates. Salting-in effects of urea are known to destroy the hydrophobic microdomains of polymers in aqueous solution and to modify significantly the hydrolysis rates in catalytic ester hydrolysis.[46] Similarly, the salting-in agents such as guanidinium chloride have been known to reduce the rates of the Diels−Alder reactions and benzoin condensations by decreasing the hydrophobic effects of the reactants in water.[45] Furthermore, this interpretation of our results may also provide a better explanation for the different catalytic behavior of *N*-acylhistidine derivatives[49−52] and *N*-alkylimidazoles[53,54] in ester hydrolysis in aqueous phosphate or carbonate buffer solution[51−53] vs aqueous Tris buffer solution.[49,50,54]

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References and Notes

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