Water Structure and Its Kinetic Effects on the Neutral Hydrolysis of Two Acyl Activated Esters[1]

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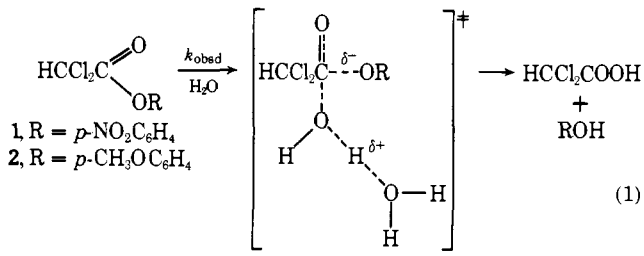
Abstract: The water-catalyzed neutral hydrolysis of *p*-nitrophenyl (**1**) and *p*-methoxyphenyl dichloroacetate (**2**) has been studied in water and in water perturbed by the presence of *t*-BuOH, *n*-Bu_4NBr, KBr, and $NaClO_4$. Except for aqueous $NaClO_4$, extrema in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are observed as a result of large compensatory changes in these quantities of activation as a function of solvent composition. The specific pattern of the $\Delta H^{\ddagger}$-$\Delta S^{\ddagger}$ relationship clearly depends on the nature and concentration of the additive and serves to indicate the overwhelming importance of solvation factors. The combined evidence strongly suggests that effects due to changes in the diffusionally averaged water structure may provide a rationale for understanding these phenomena.

Water is the ubiquitous solvent for the fundamental chemical reactions involved in life processes. There is abundant evidence that chemical reactivity in aqueous media is profoundly influenced by the three-dimensional hydrogen-bonded structure of liquid water.[2] For instance, one of the consequences of this structural property is the unique propensity of water molecules to participate in intermolecular proton transfer processes,[3,4] which constitute such an important feature of enzyme-catalyzed reactions. However, the exact nature of the long-range order in water is a problem of utmost complexity and a topic of considerable controversy as demonstrated by the number of structural models that have been proposed.[5-7] Since it has been recognized that the diffusionally averaged water structure (hereafter referred to as water structure) may be either appreciably decreased or increased (compared with pure water) around the active sites of enzymes,[8,9] it would be of great interest to investigate the effect of perturbation of water structure on rates and energetics of proton transfer reactions in aqueous media. Unfortunately, the desired information is extremely difficult to obtain from kinetic studies of enzymic conversions. Therefore, we have initiated a program directed toward a quantitative elucidation of water-structure effects on mechanistically more simple small solute processes,[10] in the hope that the results may contribute to a better understanding of protein reactions. We have chosen for investigation the neutral (water-catalyzed) hydrolysis of the aryl dichloroacetates **1** and **2**.[11] It is known that the hydrolysis of these esters may be catalyzed by general bases.[12-15] No catalysis by acids has been observed. In the absence of Brønsted bases other than water, **1** and **2** undergo a conveniently fast "water reaction," which, mainly based on the magnitude of the kinetic solvent deuterium isotope effect (k_{H_2O}/k_{D_2O} *ca.* 3), involves a water-catalyzed nucleophilic attack by water. In eq 1, a likely transition state is represented,[14,15] but a kinetic equivalent cannot be excluded. We report here rate constants and activation parameters for hydrolysis of **1**-**2** in water and in water perturbed by the presence of *t*-BuOH and some neutral salts. The choice of these additives was determined by the availability of data concerning their capabilities to alter water structure. For the purpose of our study, the neutral hydrolysis of acyl-activated esters has the desirable features both of a relatively simple mechanism as well as the contribution of at least two (but probably more) water molecules to the transition state, one of which acts as a proton-transferring agent. We anticipated that this type of hydrolysis reaction could be successfully exploited to test kinetic effects due to changes in the long-range order of water induced by small amounts of additives.



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Previous investigations of possible water-structure effects on chemical reactivity in highly aqueous solvents have been mainly limited to SN1 and SN2 reactions (not involving proton transfer).[16-19] Only limited attention has been paid to other processes, including deprotonation of some carbon acids[10,20] and protein denaturation.[21] These studies all demonstrate the intrinsic difficulties in establishing correlations between kinetic effects and changes in microheterogeneity in aqueous media.

Experimental Section

Materials. Compounds **1** and **2** were analytically pure samples prepared according to the literature.[15] The water used in the kinetic measurements was demineralized and distilled twice in an all-quartz distillation unit. D_2O was obtained from Reactor Centrum Nederland (99.94% in D_2O) and was used as such. *t*-BuOH and *t*-BuOD (>98% D) were obtained from Aldrich Chemical Co. Analytical grade KBr and $NaClO_4$ were used without further purification. *n*-Bu_4NBr was obtained from Fluka AG and was crystallized twice from ethyl acetate–ether (3:1). The solvent mixtures were all made up by weight.

Kinetic Measurements. The rates of hydrolysis of **1**-**2** were measured by following the appearance of *p*-nitrophenol (at 320 nm) or *p*-methoxyphenol (at 288 nm). The reactions were carried out in 2-cm quartz cells which were placed in the adequately thermostated (±0.04°) cell compartment (equipped with a magnetic stirring device) of a Zeiss PMQ II spectrophotometer. About 5 μl of a concentrated solution of **1**-**2** in acetonitrile was added to the aqueous reaction media in the cuvette (6 ml) by means of a capillary pipet and under vigorous stirring. The conversions were followed to greater than 75% completion, and infinity points were taken after 10 half-lives. Pseudo-first-order rate constants (k_{obsd}) were reproducible to within 2%. All hydrolysis reactions were carried out in the presence of 10^{-2} *M* HCl to suppress catalysis by hydroxide ion. Rate measurements in dilute hydrochloric acid and buffer solutions indicated that k_{obsd} values were constant between at least pH 1.0 and 4.0. Activation parameters were calculated from k_{obsd} values obtained at four different temperatures in the range of 16-30° for **1** and 16-40° for **2**. In the calculation of $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$ the problem arises of the choice of the standard state. Since the "effective water concentration" is unknown for all media we have used, k_{obsd} values have been employed. However, this will not affect our

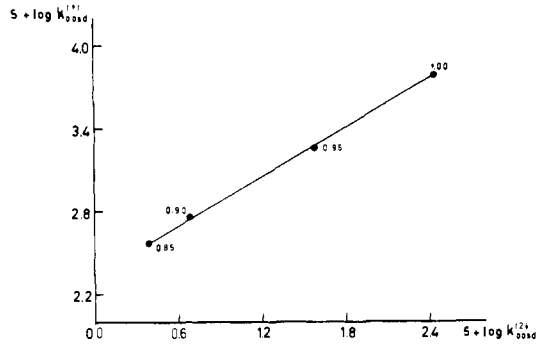

Figure 1. Plot of log k_{obsd} for **1** *vs.* log k_{obsd} for **2** at n_{H_2O} = 0.850–1.000.

Table I. Rate Constants (k_{obsd}) and Activation Parameters for the Neutral Hydrolysis of 1 and 2 at Various Mole Fractions of Water (n_{H_2O}) in *t*-BuOH–H_2O Containing 10^{-2} *N* HCl (25 ± 0.04°)

	1			2		
n_{H_2O}	10^5 k_{obsd}, sec^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, eu	10^5 k_{obsd}, sec^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, eu
1.000	6020	9.2 ± 0.3	− 33 ± 1	278	8.4 ± 0.3	− 42 ± 1
0.980	4160	7.4 ± 0.3	− 40 ± 1			
0.950	1770	5.5 ± 0.3	− 48 ± 1	38.3	3.9 ± 0.3	− 61 ± 1
0.925	829	8.6 ± 0.2	− 39 ± 1			
0.900	561	10.7 ± 0.3	− 33 ± 1	4.83	11.1 ± 0.2	− 41 ± 1
0.850	369	10.9 ± 0.3	− 33 ± 1	2.47	11.0 ± 0.3	− 42 ± 1
0.800	303	10.9 ± 0.2	− 32 ± 1			
0.750	250	9.7 ± 0.4	− 36 ± 1			
0.700	210	10.4 ± 0.2	− 36 ± 1			

discussion of the trends in $\Delta H^{\ddagger}$ *vs.* $\Delta S^{\ddagger}$ behavior. In addition, the solvent compositions at which extrema were found to occur in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are not dependent on the choice of the standard state.

Heats of Transfer. Heats of transfer ($\Delta H_t°$) for *p*-nitrophenyl acetate from water to 0.35 *M* tetra-*n*-butylammonium bromide and to 0.75 *M* potassium bromide were obtained from solubility measurements between 16 and 30° using the method of Jolicoeur and Lacroix.[22]

Results

Hydrolysis in *tert*-Butyl Alcohol–Water. Pseudo-first-order rate constants (k_{obsd}) for the neutral hydrolysis of **1**–**2** in *t*-BuOH–H_2O at various mole fractions of water (n_{H_2O}) are summarized in Table I. Since solvolysis in pure *tert*-butyl alcohol is at least 10^4 times slower than in water, the k_{obsd} values pertain only for water reactions. On lowering n_{H_2O}, k_{obsd} values decrease smoothly for **1** and **2**, the rates for **2** being more sensitive to solvent composition. Rate retardations for the neutral hydrolysis of acyl-activated esters upon addition of organic cosolvents have been noted before.[14] Interestingly, there exists a linear correlation between the log k_{obsd} values for **1** and **2** at the different mole fractions of water[23] (Figure 1 and eq 2). This relationship

$$\log k_{obsd}(\mathbf{1}) = 0.6 \log k_{obsd}(\mathbf{2}) + 0.33 \qquad (2)$$

provides support for the mechanistic similarity of the hydrolysis reactions of **1** and **2** over the solvent composition range studied.

The dramatic changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ associated with the gradual increase in the free energy of activation are also shown in Table I and are plotted as a function of solvent composition in Figures 2 and 3. Table II shows rate constants and thermodynamic quantities of activation for the deuterium oxide reaction of **1** and **2** in *t*-BuOD–D_2O and also solvent kinetic deuterium isotope effects for the range n_{D_2O} = 0.700–1.000. The results are rather similar to those given in Table I, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ exhibiting extrema at $n_{D_2O} \sim$ 0.95, but the variation in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ is clearly less pronounced than that in the protium solvent system (Figure 3). The solvent kinetic deuterium isotope effect tends to increase with the concentration of the organic cosolvent, and its magnitude is primarily determined by the entropy of activation.

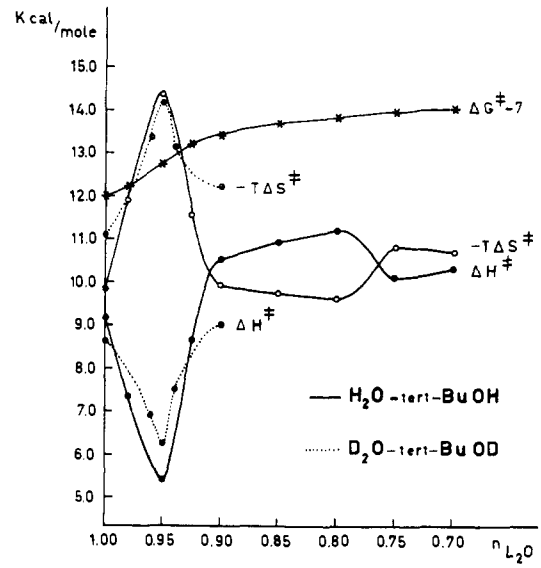

Figure 2. Plot of $\Delta H^{\ddagger}$, $-T\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ *vs.* n_{H_2O} (or n_{D_2O}) for the neutral hydrolysis of **1** in *t*-BuOH–H_2O (——) and in *t*-BuOD–D_2O (····) at 25°.

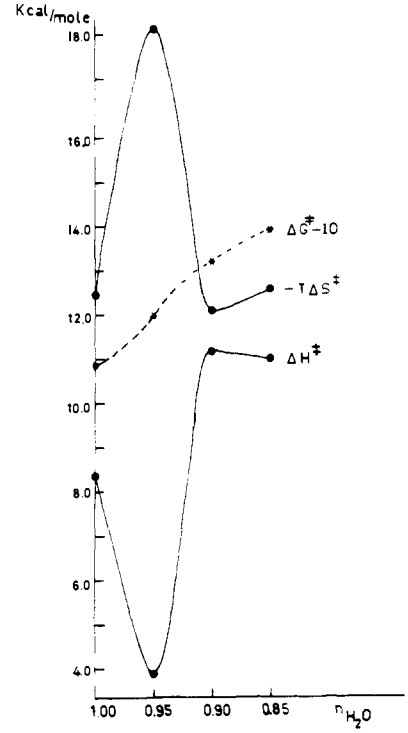

Figure 3. Plot of $\Delta H^{\ddagger}$, $-T\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ *vs.* n_{H_2O} for the neutral hydrolysis of **2** in *t*-BuOH–H_2O.

Hydrolysis in Aqueous Salt Solutions. Table III contains rate constants and activation parameters for the water reaction of **1** in the presence of neutral salts which are "structure making" (tetra-*n*-butylammonium bromide), "structure breaking" (potassium bromide), or rather ineffective in altering the long-range order of water (sodium perchlorate).[24] The data show that the salt effects of the three salts are similar in terms of free energy since they all induce a smooth decrease in rate of hydrolysis. However, for the first two salts, there is a striking difference in the behavior of the activation parameters as a function of salt concentration

Table II. Rate Constants (k_{obsd}), Activation Parameters, and Solvent Kinetic Deuterium Isotope Effects for the Neutral Hydrolysis of 1 and 2 at Various Mole Fractions of D_2O (n_{D_2O}) in *t*-BuOD–D_2O Containing 10^{-2} *N* DCl (25 ± 0.04°)

	1				2			
n_{D_2O}	$10^5 k_{obsd}$, sec^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, eu	$k_{obsd}^{H_2O}/k_{obsd}^{D_2O}$	$10^5 k_{obsd}$, sec^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, eu	$k_{obsd}^{H_2O}/k_{obsd}^{D_2O}$
1.000	1920	8.7 ± 0.4	− 37 ± 1	3.15 ± 0.1	86.0	8.6 ± 0.3	− 43 ± 1	3.24 ± 0.1
0.960	757	6.9 ± 0.3	− 45 ± 1					
0.950	598	6.3 ± 0.3	− 47 ± 1	2.96 ± 0.1	11.7	6.3 ± 0.3	− 55 ± 1	3.27 ± 0.1
0.940	403	7.5 ± 0.3	− 44 ± 1					
0.900	151	9.0 ± 0.3	− 41 ± 1	3.70 ± 0.1				
0.700	56	9.8 ± 0.3	− 40 ± 1	3.70 ± 0.1				

Table III. Rate Constants (k_{obsd}) and Activation Parameters for the Hydrolysis of **1** in Some Aqueous Salt Solutions Containing 10^{-2} *N* HCl (25 ± 0.04°)

Salt	Concn, mol $l.^{-1}$	$10^5 k_{obsd}$, sec^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, eu
		6020	9.2 ± 0.3	− 33 ± 1
n-Bu_4NBr	0.10	5320	8.5 ± 0.2	− 36 ± 1
n-Bu_4NBr	0.25	4160	7.6 ± 0.3	− 39 ± 1
n-Bu_4NBr	0.35	3530	7.0 ± 0.3	− 41 ± 1
n-Bu_4NBr	0.50	2720	7.9 ± 0.2	− 39 ± 1
n-Bu_4NBr	0.75	1690	8.6 ± 0.2	− 38 ± 1
n-Bu_4NBr	1.00	1120	8.6 ± 0.2	− 38 ± 1
KBr	0.25	5700	9.8 ± 0.5	− 31 ± 2
KBr	0.50	4650	11.0 ± 0.4	− 27 ± 2
KBr	0.75	4100	12.0 ± 0.4	− 24 ± 2
KBr	1.00	4000	8.8 ± 0.4	− 35 ± 2
KBr	1.25	3850	9.2 ± 0.5	− 34 ± 2
$NaClO_4$	0.34	4890	8.9 ± 0.3	− 34 ± 1
$NaClO_4$	0.75	3470	9.3 ± 0.3	− 34 ± 1
$NaClO_4$	1.19	2500	8.5 ± 0.3	− 37 ± 1

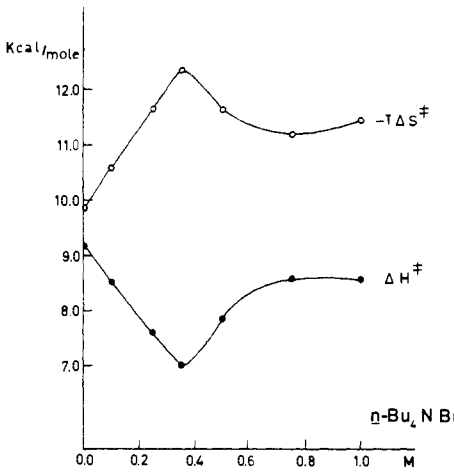

Figure 4. Plot of $\Delta H^{\ddagger}$ and $-T\Delta S^{\ddagger}$ *vs.* concentration of *n*-Bu_4NBr for the neutral hydrolysis of **1**.

(Figures 4 and 5). For *n*-Bu_4NBr, the $\Delta H^{\ddagger}$–$\Delta S^{\ddagger}$ compensation pattern is rather similar to the one found for *t*-BuOH–H_2O, but now there exist extrema at a salt concentration of 0.35 *M*. As compared with those for *n*-Bu_4NBr, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for KBr solutions vary in *opposite* direction upon increasing salt concentration, and the extrema are here at c_{KBr} = 0.75 *M*. The modest salt effect of $NaClO_4$ is the result of only small changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ over a large concentration range. Unfortunately, **1** and **2** are too readily hydrolysed to permit sufficiently accurate measurements of thermodynamic quantities of transfer from water to aqueous salt solutions. Taking the less water sensitive *p*-nitrophenyl acetate as a reasonable model compound for **1**, we have determined the heats of transfer ($\Delta H_t°$) from H_2O to 0.35 *M* *n*-Bu_4NBr ($\Delta H_t° = +3.0 \pm 0.6$ kcal mol^{-1}) and to

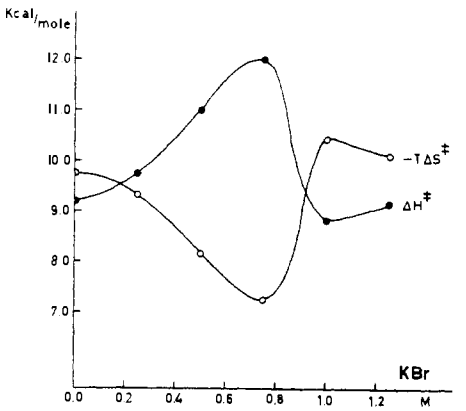

Figure 5. Plot of $\Delta H^{\ddagger}$ and $-T\Delta S^{\ddagger}$ *vs.* concentration of KBr for the neutral hydrolysis of **1**.

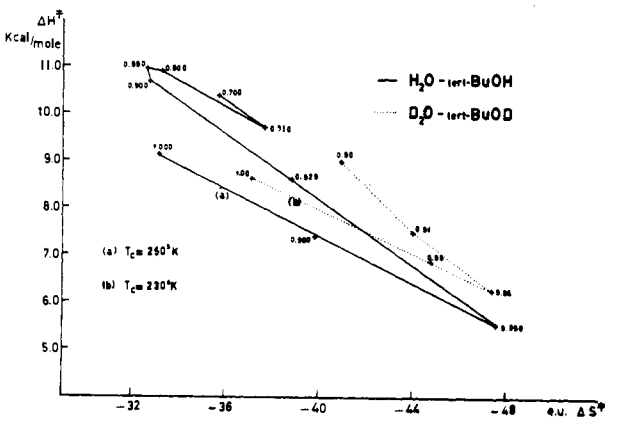

Figure 6. Plot of $\Delta H^{\ddagger}$ *vs.* $\Delta S^{\ddagger}$ for the neutral hydrolysis of **1** in *t*-BuOH–H_2O (——) and in *t*-BuOD–D_2O (····) at various mole fractions of n_{H_2O} (n_{D_2O}).

0.75 *M* KBr ($\Delta H_t° = -0.75 \pm 0.25$ kcal mol^{-1}). The corresponding quantities $\Delta H_t°$ for transfer of one water molecule are assumed to be very small in comparison with the above values.

Isokinetic Relationships. The thermodynamic quantities of activation given in Tables I–III have been analyzed in terms of possible isokinetic relationships. Plots of $\Delta H^{\ddagger}$ *vs.* $\Delta S^{\ddagger}$ for the hydrolysis of **1** are shown in Figure 6 (*t*-BuOH–H_2O and *t*-BuOD–D_2O), Figure 7 (aqueous *n*-Bu_4NBr), and Figure 8 (aqueous KBr). Straight-line plots are obtained over certain ranges of n_{H_2O} (n_{D_2O}) or salt concentrations.

Especially during the last decade it has been realized that such linear plots may not provide reliable evidence for the existence of an isokinetic temperature (T_c). Therefore, following Petersen's suggestion,[25] plots of $\log k_{obsd}T^{-1}$ *vs.* T^{-1} were constructed to examine whether or not the lines intersect at a single point. Using this criterion, real isokinetic temperatures appear to exist for hydrolysis of **1** in 0–0.35 *M*

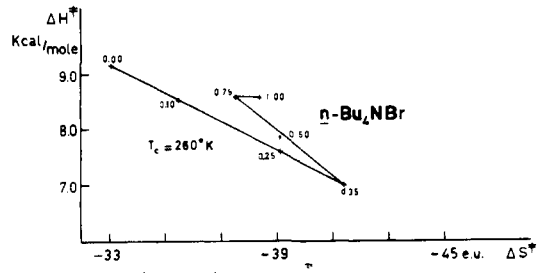

Figure 7. Plot of $\Delta H^{\ddagger}$ *vs.* $\Delta S^{\ddagger}$ for the neutral hydrolysis of **1** at various concentrations of *n*-Bu_4NBr.

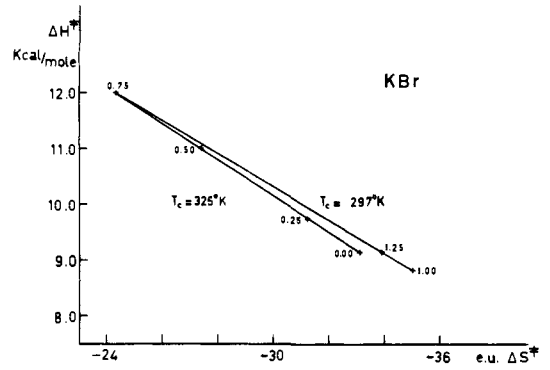

Figure 8. Plot of $\Delta H^{\ddagger}$ *vs.* $\Delta S^{\ddagger}$ for the neutral hydrolysis of **1** at various concentrations of KBr.

aqueous *n*-Bu_4NBr (T_c = 260 ± 5°K, Figure 9) and in 0–0.75 *M* aqueous KBr (T_c = 323 ± 5°K, Figure 10). The data for hydrolysis of **1** in the mixed solvents do not satisfy the criterion with sufficient precision. Nevertheless we feel that the T_c values (Figure 6) are, at least approximately, correct since they are well outside the range where the kinetic measurements have been made.[25] Moreover, the variation in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ is much larger than the experimental error in these quantities.

Discussion

The most striking features of the data summarized in Table I are the very pronounced compensatory changes in the relative contributions of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ to the free energy of activation upon variation of n_{H_2O} between 1.000 and 0.900. Upon addition of the first 5 mol % of *t*-BuOH, the rate slows down due to the dominating *entropy* term, then from 5–10 mol % *t*-BuOH, the increase in free energy of activation is governed by the *enthalpy* term. This mirror image behavior of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ (Figures 2 and 3) can be reconciled neither with the decrease in water concentration[26] nor with the decrease in solvent polarity. Bulk dielectric constant and empirical solvent polarity parameters (for instance solvatochromism scales like *Z* and E_T values) all lie on a smooth curve when plotted *vs.* n_{H_2O} in *t*-BuOH–H_2O. It is most significant to note that we find extrema[27] in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ in the narrow concentration range (3–6 mol % of *t*-BuOH) at which a maximum in solvent heterogeneity has been found.[28] Therefore, we are led to conclude that the neutral hydrolysis of **1** and **2** responds kinetically to water structure perturbation. A possible mechanistic explanation is the following. The strongly negative $\Delta S^{\ddagger}$ values for hydrolysis of **1** and **2** in pure water imply that the transition state is highly hydrated relative to the ground state. This will primarily be the result of hydrogen bonding of water molecules to the water molecule attacking the carbonyl moiety and to the phenolic oxygen atom of the para-substituted phenolate leaving group. The importance of the latter process is probably reflected in the more negative $\Delta S^{\ddagger}$ value for hydrolysis of **2** compared with the value for **1**. It may be

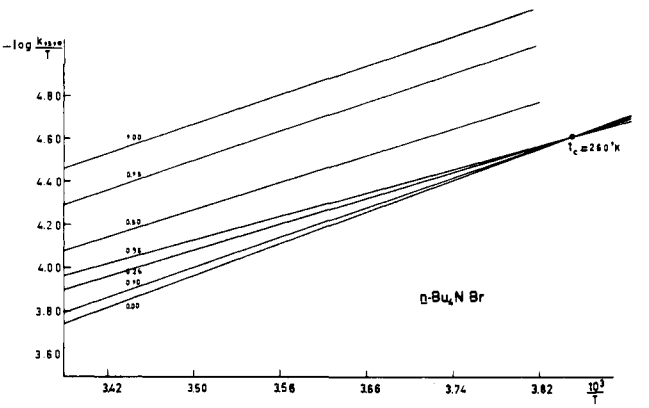

Figure 9. Plot of log $k_{obsd}T^{-1}$ as a function of the reciprocal absolute temperature (T) for the neutral hydrolysis of **1** in the presence of various concentrations of *n*-Bu_4NBr.

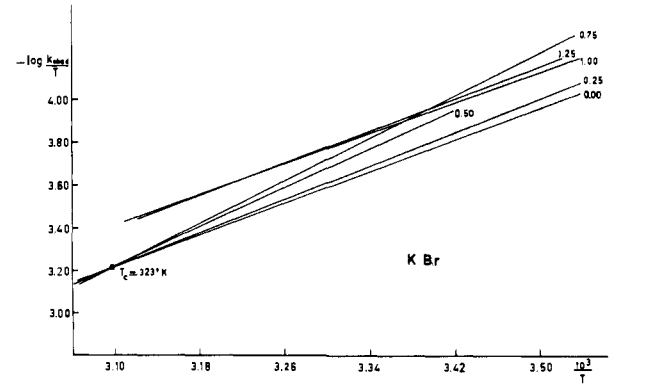

Figure 10. Plot of log $k_{obsd}T^{-1}$ as a function of the reciprocal absolute temperature (T) for the neutral hydrolysis of **1** in the presence of various concentrations of KBr.

anticipated that interaction with water molecules in the transition state will be more extensive when this species is accommodated in voids in the water structure surrounded by water molecules of enhanced structuredness at n_{H_2O} = 0.95. The increased hydrogen-bonding interaction in the hydration sphere of the transition state (eq 1) will then logically explain the decrease in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$. This qualitative rationale,[29] of course, does not take into account possible ground-state destabilization (increase in enthalpy and a possible increase in entropy) at n_{H_2O} = 0.95. However, it seems most likely that water-structure effects in *t*-BuOH–H_2O will predominantly operate on those species that will interact most strongly with water, *i.e.*, the transition state (eq 1).[29] The data in Table II show that for hydrolysis in *t*-BuOD–D_2O, the general behavior of $\Delta S^{\ddagger}$ control between n_{D_2O} = 1.000–0.950 and $\Delta H^{\ddagger}$ control between n_{D_2O} = 0.950–0.900 is retained. Since structuring effects have been proposed to be manifested more strongly in D_2O than in H_2O,[30] the smaller changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ in the *t*-BuOD–D_2O solvent were rather unexpected. It is evident that the situation is complicated, and that the overall change in $\Delta H^{\ddagger}$ upon going to the deuterium solvent system is the result of at least two partially counteracting influences: (i) a primary kinetic deuterium isotope effect (increase in $\Delta H^{\ddagger}$) due to zero-point energy differences for the O–H and O–D bonds which are stretched in the transition state and (ii) a "non-specific solvent deuterium isotope effect" mainly due to the greater structuredness of the *t*-BuOD–D_2O medium[31] (decrease in $\Delta H^{\ddagger}$). The relative contributions of i and ii may vary with the solvent composition. In view of the complexity of this situation, we choose not to pursue a detailed interpretation of the modest change in the overall solvent deuterium isotope effect in the range n_{H_2O} = 1.000–0.900.

We next consider the kinetic salt effects listed in Table III. The $\Delta H^{\ddagger}$–$\Delta S^{\ddagger}$ profiles for hydrolysis of **1** as a function of the concentration of *n*-Bu_4NBr and KBr (Figures 4 and 5) again reinforce the idea that an electrostatic picture is inadequate to account for the kinetic response of the system. It is important to note that the variation of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ in 0.00–0.35 *M* *n*-Bu_4NBr resembles the $\Delta H^{\ddagger}$–$\Delta S^{\ddagger}$ plot for *t*-BuOH–H_2O in the range n_{H_2O} = 1.000–0.950 (decrease in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$). This is in accord with the structure-making properties of *n*-Bu_4NBr which are attributed to the hydrophobic alkyl chains in the cation.[24,32] In addition, the isokinetic temperatures for hydrolysis of **1** in both media are identical within the limits of accuracy (T_c *ca.* 255°K). However, the somewhat dubious existence of a real T_c for hydrolysis in *t*-BuOH–H_2O has been discussed earlier (*vide supra*). Above 0.35 *M* *n*-Bu_4NBr, the hydration spheres of the ions begin to overlap significantly,[33] resulting in a gradual decrease in cooperative hydrogen bonding between the water molecules. As a consequence, both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ increase again as shown in Figure 4. Turning now to the salt effect of the structure-breaking KBr, we also observe rate retardation but contrary to *n*-Bu_4NBr, this is at low salt concentration (0.00–0.75 *M*) now due to an *increase* in $\Delta H^{\ddagger}$ which is not totally compensated by an accompanying increase in $\Delta S^{\ddagger}$ (Figure 5). The isokinetic temperature associated with this compensation phenomenon (T_c *ca.* 323°K) is much higher than the one found for hydrolysis in media of increased structuredness. Extrema in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ again occur in the concentration range (*ca.* 0.75 *M*) where the hydration spheres of the ions are no longer independent of each other.[34] Additional support for the contention that the salt effect on the neutral hydrolysis of **1** operates predominantly through the mechanism of water-structure modification is found in the small changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ induced by $NaClO_4$. In the concentration range examined, this salt is known to alter the water structure only slightly.[24]

Since many salts are known to have large effects on the activity coefficients of uncharged solutes, also here the question should be raised whether ground-state or transition-state solvation effects predominate in determining the specific $\Delta H^{\ddagger}$–$\Delta S^{\ddagger}$ compensation pattern. Upon acceptance of *p*-nitrophenyl acetate as a reasonable model compound for salt effects on the ground state of **1**, the small $\Delta H_t°$ of -0.75 ± 0.25 kcal mol^{-1} for transfer from water to 0.75 *M* KBr indicates the major importance of a transition state effect (for **1**, $\Delta\Delta H^{\ddagger} = 2.8 \pm 0.7$ kcal mol^{-1}, see Table III). In contrast, the salt effect of 0.35 *M* *n*-Bu_4NBr ($\Delta\Delta H^{\ddagger} = -2.2 \pm 0.6$ kcal mol^{-1}, see Table III) may well be explained by a change in ground-state solvation since $\Delta H_t°$ for *p*-nitrophenyl acetate is $+3.0 \pm 0.6$ kcal mol^{-1} for transfer from water to 0.35 *M* *n*-Bu_4NBr.

Conclusion

The neutral hydrolysis of the acyl-activated esters **1** and **2** represents a simple protolytic reaction which is very sensitive to solvation changes in highly aqueous media. This is demonstrated by the strongly negative and highly solvent-dependent entropies of activation. The rationale advanced for the observed solvent effects implies the dominant role of changes in the diffusionally averaged water structure in determining the kinetic response in the presence of structure-making or structure-breaking additives. This conclusion is mainly based on an analysis of the delicate balance of large, mutually compensatory changes in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ as a function of solvent composition. The process by which $\Delta H^{\ddagger}$–$\Delta S^{\ddagger}$ compensation is attained is clearly a consequence of the peculiar properties of liquid water. It should be stressed that the dominance of either the enthalpy or the entropy term in the free energy of activation as a function of solvent microheterogeneity will depend on the mechanistic details of the proton-transfer process. In some cases, even extrema in the free energy of activation may occur.[10,20] In the present study, the effect of enhanced cooperative water–water hydrogen bonding (relative to pure water at 25°) is reflected in a decrease in $\Delta S^{\ddagger}$ which is only partially offset by a decrease in $\Delta H^{\ddagger}$. In media of diminished water structure, the free energy of activation is also increased, but this is now governed by an unfavorable change in $\Delta H^{\ddagger}$.

Our observations may have substantial bearing on our understanding of protolytic reactions occurring in the hydrophobic pocket of enzymes. We infer that kinetic control of water reactions involving proton transfer may be exercised through water-structure perturbation, the magnitude of the effect being determined by the specific $\Delta H^{\ddagger}$–$\Delta S^{\ddagger}$ compensatory pattern. Caution is therefore necessary in extrapolating substituent effects derived from kinetic studies on simple model systems *in pure water* to mechanistically related enzymic conversions.

Finally, we like to emphasize that we are left with two major problems: the unique structural properties of water and the specific linear relationship between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for both chemical and biochemical processes in water.[35] Further refined studies will be necessary to elucidate more quantitatively the importance of these highly interrelated factors in determining the reactivity of biological systems.

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There are indications that the water association around n_{H_2O} = 0.95

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Proton-Exchange Reactions of Acetone and Butanone. Resolution of Steps in Catalysis by Acetoacetate Decarboxylase

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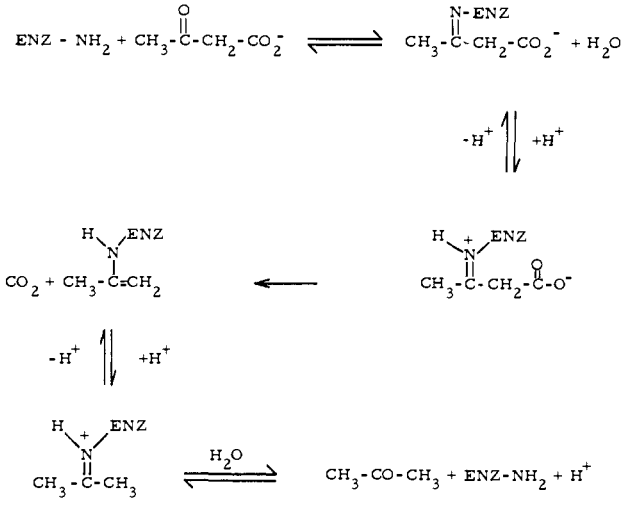
Abstract: The conversion of acetoacetate to acetone and carbon dioxide, catalyzed by acetoacetate decarboxylase (AAD), involves the enamine of acetone and the enzyme as a compulsory intermediate. The enzyme catalyzes the protonation of this enamine to the corresponding iminium ion. Previous investigations had shown that, consistent with this activity, AAD catalyzes the proton-exchange reactions of acetone. In this study, evidence is presented that AAD will catalyze a stereospecific exchange at the 3 position of butanone, and that the exchange of protons with those on the methyl group of acetone occurs in steps, with the rate constants for exchange of successive protons identical except for statistical factors. The hydrolysis of acetone imine must therefore be rapid compared with proton exchange at carbon.

The decarboxylation of acetoacetate, catalyzed by acetoacetate decarboxylase, occurs by way of imines as intermediates. According to the mechanism proposed for the process, acetoacetate reacts with the ϵ-amino group of an active-site lysine residue to yield an imine of acetoacetate, which then undergoes decarboxylation to form the enamine of acetone; subsequently, protonation of this enamine yields the cation of a second imine, that of acetone.[2-5] A review of the experiments upon which this mechanism is based has recently appeared.[6] The overall mechanism for the decarboxylation is presented in Scheme I.

Scheme I



Tagaki et al.[5] showed that the enzyme will catalyze exchange of the deuterons of acetone-d_6 with the protons of water and similarly exchange the protons of acetone with the deuterons of deuterium oxide. Since these exchange reactions almost certainly proceed by way of the imine of acetone and the enzyme, they are relevant to the enzymic reaction. The present study is concerned with the mechanism of the exchange process.

We have determined by NMR spectroscopy and by mass spectrometry that the AAD catalyzed proton-exchange reactions of acetone occur in a stepwise manner. This leads to the conclusion that hydrolysis of the acetone-derived imine of AAD occurs rapidly compared with tautomerization of the imine to the enamine. Furthermore, the AAD-catalyzed deuteration of butanone at the 3 position is stereospecific, confirming that the proton-exchange reaction itself is enzymic and defining some of the geometric requirements of the active site.

Experimental Section

All reagent grade materials were used as purchased. All nonreagent organic chemicals were purified by distillation or recrystallization. Mr. Jerome V. Connors extracted acetoacetate decarboxylase from *Clostridium acetobutylicum* and purified it by published procedures.[4] The spectroscopic assay method described by Fridovich[7] was used to determine the activity of solutions of the enzyme. All enzyme used in these studies had been crystallized and stored at 5° as a suspension in 50% saturated ammonium sulfate solution.

The deuteration of acetone was followed by NMR spectrometry (Varian HA 100 spectrometer) using 0.15 *M*, pD 5.9, 2-picoline buffer and 0.5 *M* acetone in a manner similar to published proce-