Pairwise Gibbs Energies of Interaction Involving *N*-Alkyl-2-pyrrolidinones and Related Compounds in Aqueous Solution Obtained from Kinetic Medium Effects

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Kinetic solvent effects of *N*-alkyl-2-pyrrolidinones and structurally related compounds on the water-catalyzed hydrolysis reactions of *p*-methoxyphenyl dichloroacetate (**MPDA**), 1-benzoyl-3-phenyl-1,2,4-triazole (**BPhT**), and 1-benzoyl-1,2,4-triazole (**BT**) in highly dilute aqueous solutions at pH 4 and 298.15 K have been determined by UV/vis spectroscopy. Using a thermodynamic description of solute−solute interactions in aqueous solutions, the kinetic results have been analyzed in terms of pairwise Gibbs energy interaction parameters: G(c) values. These are negative, indicating that hydrophobic interactions in the initial state dominate the medium effects. The interaction parameters increase in the order **MPDA**<**BT**<**BPhT**, suggesting increasing hydrophobic stabilization in the order of **MPDA**>**BT**>**BPhT**. However, when differences in reactivity and transition state effects are taken into account, it appears that **BPhT** is more successful in establishing hydrophobic interactions with the cosolutes than are **MPDA** and **BT**. Using the SWAG-approach for additivity of group interactions, additivity is observed for the first three consecutive CH_2 groups in the cosolute in all three hydrolysis reactions. Larger alkyl substituents cause larger retardations than anticipated on basis of this additivity. The results are explained by intramolecular destructive overlap of the polar hydration shell of the amide functionality and the apolar (hydrophobic) hydration shell of the alkyl group, which extends to the third CH_2 group in the *N*-alkyl group of the cosolute molecule. The inner apolar groups, therefore, have a reduced apparent hydrophobicity. More remote CH_2 groups develop independent hydrophobic hydration shells. The effect of the position of a CH_2 group in the cosolute molecule is also considered. Kinetic solvent effects with structurally related esters show that amide−amide, ester−ester, and amide−ester group interactions affect the transition state in different ways. Finally, the effects of PVP polymers on the three hydrolysis reactions have been examined. The data presented enhance the understanding of pairwise hydrophobic interactions in aqueous solutions. In addition the results provide insights into the interactions between hydrophobic and hydrophilic hydration shells as well as into the energetics of amide hydration and interactions involving amides in aqueous solution, both playing important roles in protein stabilization.

Introduction

Kinetic medium effects on hydrolysis reactions in dilute aqueous solution provide an excellent method for investigating solute hydration and solute−solute interactions in aqueous solution, since the activation parameters are strongly affected by changes in the structural properties of water in the hydration shell of the reactants during the activation process.[1,2] Changes in solvent composition, brought about by addition of small amounts of cosolutes, affect the hydration characteristics of both reactant and activated complex due to interactions with the cosolute via overlap of their hydration cospheres.[3]

Transition-state theory provides a basis of understanding kinetic medium effects. The interacting cosolutes can decrease or increase the chemical potentials of the initial state and the activated complex, depending on whether the interactions are favorable (stabilizing) or not favorable (destabilizing), and consequently affect the Gibbs energy of activation.

About a decade ago, we developed a theory with which kinetic medium effects of solvolysis reactions can be analyzed quantitatively in terms of thermodynamic interaction parameters.[4,5] The Gibbs energy of interaction stems from the nonideal part, the excess Gibbs energy (G^E), of the total solution which is determined by the chemical potentials of the solutes. This nonideal part can be ascribed completely to solute−solute interactions. Usually, G^E is expressed by a molality expansion, using virial coefficients. These coefficients obtain physical significance by the theory of McMillan and Mayer.[6] In sufficiently dilute solutions, G^E is determined by pairwise solute−solute interactions only. The thermodynamics

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(1) Blandamer, M. J.; Burgess, J.; Engberts, J. B. F. N. *Faraday Discuss. Chem. Soc.* **1989**, *85*, 309. For a brief review, see: Engberts, J. B. F. N.; Blandamer, M. J. J. *Phys. Org. Chem.* **1998**, *11*, 841.
(2) (a) Blandamer, M. J.; Burgess, J. *Pure Appl. Chem.* **1983**, *55*, 55. (b) Blandamer, M. J.; Burgess, J. *Pure Appl. Chem.* **1990**, *62*, 9.
(3) Gurney, R. W. *Ionic Processes in Solution*; McGraw-Hill: New York, 1953.
(4) Blokzijl, W.; Engberts, J. B. F. N.; Jager, J.; Blandamer, M. J. *J. Phys. Chem.* **1987**, *91*, 6022.
(5) Blokzijl, W.; Jager, J.; Engberts, J. B. F. N.; Blandamer, M. J. *J. Am. Chem. Soc.* **1986**, *108*, 6411.
(6) McMillan, W. G.; Mayer, J. E. *J. Phys. Chem.* **1945**, *13*, 276.

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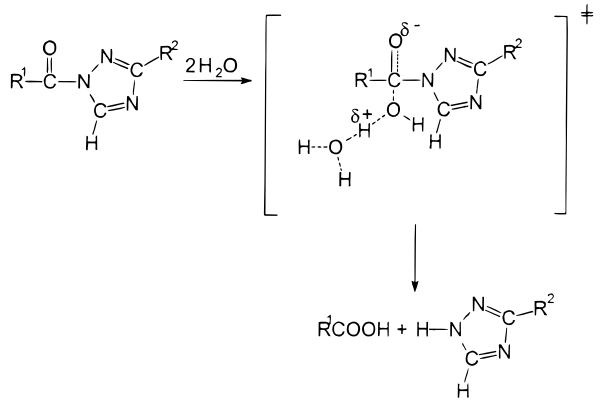
Scheme 1. Reaction Mechanism for the Water-Catalyzed Hydrolysis of 1-Acyl-3-substituted-1,2,4-triazoles[a]



[a] For 1-benzoyl-1,2,4-triazole (BT) $R^1 = C_6H_5$ and $R^2 = H$; for 1-benzoyl-3-phenyl-1,2,4,-triazole (BPhT) $R^1 = R^2 = C_6H_5$. The hydrolysis of MPDA follows the same mechanism.

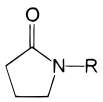
were then linked with kinetics through transition-state theory, yielding the following equation for a water-catalyzed hydrolysis reaction[4,5]

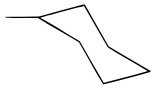
$$\ln \frac{k(m_c)}{k_0(m_c = 0)} = \frac{2}{RTm_0^2}(g_{c-IS} - g_{c-AC})m_c - n\phi M_w m_c \quad (1)$$

where k is the pseudo-first-order rate constant for reaction in the aqueous solution containing the cosolute c, k_0 the pseudo-first-order rate constant for reaction in the absence of cosolute, R the gas constant, T the temperature, m_0 the standard state (1 mol kg^{-1}), m_c the molality of the cosolute, n the number of water molecules involved in the activated complex of the hydrolysis reaction (n = 2 in the hydrolysis reactions investigated in this study), M_w the molar mass of water, and ϕ the practical osmotic coefficient (which equals unity in dilute aqueous solutions). The term ($g_{c-IS} - g_{c-AC}$) is referred to as the G(c) value, the pairwise Gibbs energy interaction parameter, which is the difference in pairwise interactions of the cosolute (c) with the initial state (IS) and the activated complex (AC). The second half of the equation reflects the effect of the cosolute on the reactivity of water, since water is solvent as well as reactant. Thus, G(c) represents the overall effect of the cosolute on the Gibbs energy of activation for the hydrolytic process. G(c) is obtained from the slope of a plot of ln(k/k_0) versus the molality of the cosolute.

Previously, we applied this quantitative treatment of rate constants in the analysis of kinetic medium effects on several hydrolysis reactions in dilute aqueous media. In particular, the neutral (i.e., water-catalyzed) hydrolyses of 1-benzoyl-(3-phenyl)-1,2,4-triazole (**BT** and **BPhT**) and *p*-methoxyphenyl dichloroacetate (**MPDA**) have been investigated in depth. In the pH range where only water acts as a general base (usually between pH 3−5 for these hydrolyses), the water-catalyzed hydrolysis reaction proceeds via a dipolar activated complex in which two water molecules are involved, with three protons "in flight"[7] (Scheme 1). The difference in hydrophobicity between the initial state and the activated complex is responsible for the marked changes in rate constants that are observed when hydrophobic cosolutes are added. In the past, we have studied an extensive range of cosolutes using kinetic medium effects, including mono-, di-, and polyhydric alcohols,[5,8] (alkylated) urea(s),[4,9] mono- and disaccharides,[10,11] carboxamides, sulfonamides, sulfones and sulfoxides,[9,12] sodium *n*-alkyl sulfates,[13] *n*-alkylated ammonium bromides,[14] and α-amino acids.[15,16] The results have given us profound insights into pairwise hydrophobic interactions in aqueous solution.[17] The results did, in most cases, not allow an analysis in terms of additivity of group contributions toward the medium effect using the Savage and Wood additivity of groups (SWAG)[18] approach for solute−solute interactions in aqueous solution, due to the influence of the polar group hydration.

Scheme 2. *N*-Alkyl-2-pyrrolidinones



—R	Abbr.
—CH_3	NMP
—CH_2CH_3	NEP
—$CH(CH_3)_2$	NiPP
—$CH_2CH_2CH_2CH_3$	NnBP
	NCHP

In this study, we have focused on a different class of cosolutes, the *N*-alkyl-2-pyrrolidinones, cyclic amides with alkyl substituents at the nitrogen atom (Scheme 2). Two reasons account for this choice. First, these solutes are highly soluble in water (even *N*-cyclohexyl-2-pyrrolidinone is miscible with water in all proportions). Therefore, they are suitable cosolutes for studying hydrophobic interactions in aqueous solution. Second, we are interested whether the obtained G(c) values can be analyzed in terms of additivity of pairwise group interactions, using the SWAG approach. The results allow a comparison with the results obtained for substituted acyclic amides[9,12] and enhance our understanding of the energetics of amide hydration and amide−amide interactions in aqueous solution, which are important in protein stability and still under debate.[19]

Results and Discussion

N-Alkyl-2-pyrrolidinones. We measured the kinetic solvent effects of five *N*-alkyl-2-pyrrolidinones (Scheme

(7) (a) Karzijn, W.; Engberts, J. B. F. N. *Tetrahedron Lett.* **1978**, 1787. (b) Mooij, H. J.; Engberts, J. B. F. N.; Charton, M. *Recl. Trav. Chim. Pays-Bas* **1988**, *107*, 185.

(8) Blokzijl, W.; Engberts, J. B. F. N.; Blandamer, M. J. *J. Am. Chem. Soc.* **1990**, *112*, 1197.

(9) Kersholt, R. P. V.; Engberts, J. B. F. N.; Blandamer, M. J. *J. Chem. Soc., Perkin Trans. 2* **1993**, 49.

(10) Galema, S. A.; Blandamer, M. J.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1990**, *112*, 9665.

(11) Galema, S. A.; Blandamer, M. J.; Engberts, J. B. F. N. *J. Org. Chem.* **1992**, *57*, 1995.

(12) Engberts, J. B. F. N.; Kersholt, R. P. V.; Blandamer, M. J. *J. Chem. Soc., Chem. Commun.* **1991**, 1230.

(13) Noordman, W. H.; Blokzijl, W.; Blandamer, M. J.; Engberts, J. B. F. N. *J. Org. Chem.* **1993**, *58*, 7111.

(14) Hol, P.; Streefland, L.; Blandamer, M. J.; Engberts, J. B. F. N. *J. Chem. Soc., Perkin Trans. 2* **1997**, 485.

(15) Streefland, L.; Blandamer, M. J.; Engberts, J. B. F. N. *J. Phys. Chem.* **1995**, *99*, 5769.

(16) Streefland, L.; Blandamer, M. J.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1996**, *118*, 9539.

(17) For a review on hydrophobic interactions, see: Blokzijl, W.; Engberts, J. B. F. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545.

(18) Savage, J. J.; Wood, R. H. *J. Solution Chem.* **1976**, *5*, 733.

(19) (a) Williams, D. H. *Aldrichim. Acta* **1991**, *24*, 71. (b) Doig, A. J.; Williams, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 338.

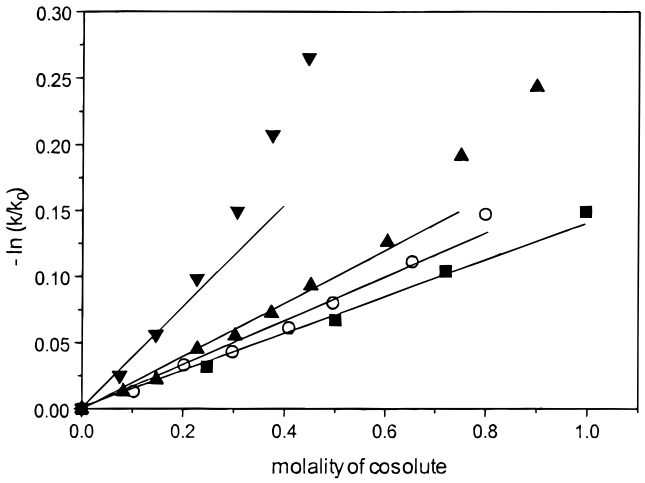


Figure 1. Solvent effects on the hydrolysis of **BPhT** in the presence of NMP (■), NEP (○), NiPP (▲), and NnBP (▼).

Table 1. *G*(c) Values[a] (J kg mol^{-2}) for the Different Cosolute−Probe Combinations

	probe		
cosolute	MPDA	BPhT	BT
NMP	−925(10)	−133(4)	−292(10)
NEP	−1176(18)	−157(10)	−354(11)
NiPP	−1407(10)	−206(15)	−354(4)
NnBP	−1989(16)	−467(21)	−600(15)
NCHP	−2980(23)	−1000(40)	−600(41)

[a] Errors in parentheses.

2) on the hydrolyses of **MPDA**, **BPhT,** and **BT** at different cosolute molalities and up to several molal at 298.15 K and pH 4. *N*-Cyclohexyl-2-pyrrolidinone is in fact not part of the series of homologues shown in Scheme 2 but is interesting in view of its unlimited solubility in water and furthermore can provide information about differences in hydrophobicity of cyclic and acyclic alkyl substituents. As an example, the solvent effects of the series in Scheme 2 on the hydrolysis of **BPhT** are shown in Figure 1 up to 1 *m* of added cosolute. Generally, linear relationships between $\ln(k/k_0)$ and the molality of the cosolute have been obtained up to approximately 0.75 *m*,[20] consistent with pairwise interactions between cosolute and kinetic probe. From the slopes of the correlations in Figure 1, *G*(c) values were obtained, using eq 1. The results, together with the *G*(c) values obtained for the hydrolyses of **BT** and **MPDA**, are shown in Table 1. To illustrate the dependence of *G*(c) on the structure of the cosolute, *G*(c) values have also been plotted versus the number of CH_2 groups in the *N*-alkyl substituent (Figure 2). In all cases, *N*-alkyl-2-pyrrolidinones cause a retardation of the hydrolysis reactions, expressed by negative *G*(c) values. These retardations can be largely attributed to favorable interactions between apolar moieties in the cosolute and the reactant molecule, i.e., an initial state stabilization due to hydrophobic interactions.[21]

(20) With the exception of NCHP as a cosolute. Solvent effects of NCHP on the three hydrolysis reactions show linear behavior up to 0.2−0.3 *m*. Above these concentrations higher-order interactions come into play. Collection of kinetic data up to 3 *m* yielded S-shaped curves, which could be perfectly analyzed with the Menger−Portnoy model for micellar catalysis, indicating the formation of NCHP aggregates. This was confirmed by fluorescence spectrophotometric measurements using pyrene as a probe that is sensitive to the solvent microenvironment.

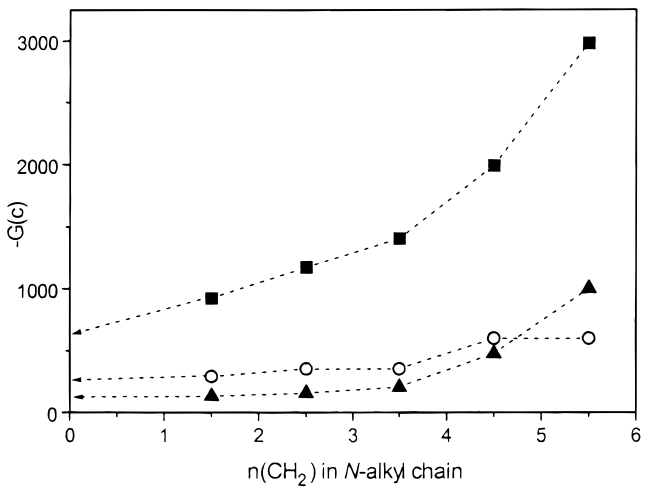


Figure 2. *G*(c) values (J kg mol^{-2}) versus the number of CH_2 groups in the *N*-alkyl chain of *N*-alkyl-2-pyrrolidinones for the kinetic probes **MPDA** (■), **BPhT** (▲), and **BT** (○).

It is remarkable that the **MPDA** hydrolysis is retarded to a much larger extent than the hydrolyses of both **BT** and **BPhT**. In other words, the ester hydrolysis is more sensitive toward solvent effects than the amide hydrolyses. This is in accordance with earlier findings.[9] This increase in Gibbs energy of activation for the ester hydrolysis is unlikely to be governed by increased stabilization of the initial state, because **MPDA** is less hydrophobic than **BPhT** and **BT**. Stabilization of the initial state by amide−amide or amide−ester H-bonding interactions is out of the question since both reactants (ester and tertiary amides) and cosolutes (tertiary amides) are hydrogen bond acceptors only. Moreover, it has been suggested that amides form stronger H-bonds with water than with other amides;[22,23] i.e., the solute−solvent interactions dominate the solute−solute interactions, and the same is anticipated for the ester functionality. We therefore contend that the larger solvent effects for the ester hydrolysis find their origin in a transition-state effect; i.e., the transition state of the amide hydrolysis is stabilized more than the transition state of the ester hydrolysis in the presence of the *N*-alkyl-2-pyrrolidinones. The transition state of the ester hydrolysis is less polar than that of the amide hydrolyses (i.e., the difference in polarity between IS and TS is smaller for the ester hydrolysis, as is reflected by a larger k_0). There is an increase in polarity of the solvent when the rather polar *N*-alkyl-2-pyrrolidinones are added to the medium, which would explain the larger stabilizing effect on the transition state of the triazole hydrolysis reactions due to polar interactions. Considering these transition-state effects, an interpretation of the results in Table 1 *solely* in terms of hydrophobic effects is inadequate. Although **MPDA** *appears* to be able to interact more strongly with the cosolutes via hydrophobic interactions than **BT** and **BPhT**, this is in fact not true. Table 2, which contains data similar to that in Table 1, but now re-expressed with the NMP retardations as a reference rather than k_0, reflects this fallacy. The pattern emerging from Table 2 is visualized in Figure 3. By using the converted data in

(21) Benak, H.; Engberts, J. B. F. N.; Blandamer, M. J. *J. Chem. Soc., Perkin Trans. 2* **1992**, 2035.
(22) Nilar, S. H.; Pluta, T. S. *J. Am. Chem. Soc.* **1995**, *117*, 12603.
(23) Eberhardt, E. S.; Raines, R. T. *J. Am. Chem. Soc.* **1994**, *116*, 2149.

Table 2. *G*(c)/*G*(NMP) Values for the Different Cosolute−Probe Combinations

	probe		
cosolute	MPDA	BPhT	BT
NMP	1	1	1
NEP	1.27	1.18	1.21
NiPP	1.52	1.55	1.21
NnBP	2.15	3.60	2.05
NCHP	3.22	7.52	2.05

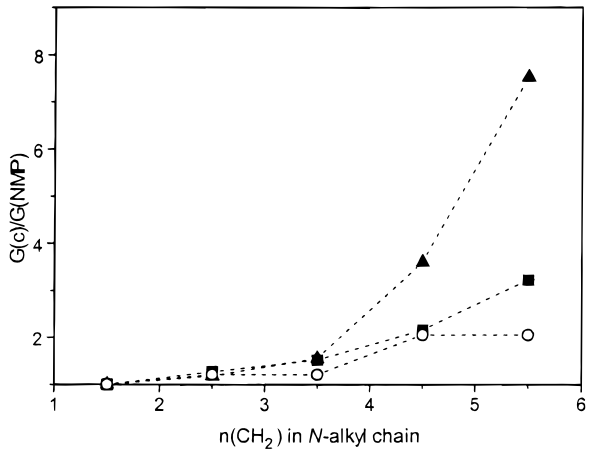


Figure 3. *G*(c)/G(NMP) versus the number of CH_2-groups in the *N*-alkyl chain of *N*-alkyl-2-pyrrolidinones for the hydrolysis of **MPDA** (■), **BPhT** (▲), and **BT** (○).

Table 2, we correct for differences in transition-state stabilization and reactivity, thereby allowing a better comparison of the probes, in which initial state effects (hydrophobic effects) might show up more clearly. As anticipated and as shown in Figure 3, hydrolysis of the more hydrophobic probe (**BPhT**) is then relatively most sensitive to the hydrophobicity of the cosolute. Although the *absolute* values of the medium effects are not solely governed by hydrophobic interactions, the relative values reveal that these interactions play an important role in the recognition process between the cosolutes and the kinetic probes.

The next topic of interest is whether the medium effects, as expressed in *G*(c) values, can be analyzed in terms of additivity of pairwise group interactions, as was described by Savage and Wood.[18] Does each CH_2 group in the cosolute molecule, irrespective of its position, add a constant increment to the medium effect? Figure 2 suggests that this is not the case throughout the whole series of *N*-alkyl-2-pyrrolidinones, since there is no linear relationship between *G*(c) and the number of CH_2 groups. It seems that the longer the alkyl chain, the larger the contribution of a CH_2 unit toward the medium effect. This pattern is particularly pronounced for solutes with apolar groups larger than isopropyl. It appears that for the shorter alkyl chains additivity of CH_2 interactions may be applied. When the *G*(c) values for NMP, NEP, and NiPP are plotted against $n(CH_2)$ on a larger scale (not shown), additivity is reasonably good, with slopes representing the $G(CH_2)$ (Table 3). Thus, there is group additivity for the first 3.5 CH_2 units, but the longer alkyl chains deviate from this additivity pattern. The $G(CH_2)$ value for **MPDA** is more negative than that obtained for a series of acyclic primary, secondary, and tertiary amides for the same kinetic probe.[9] On the contrary, for the hydrolysis of **BPhT** a more negative value is found

Table 3. $G(CH_2)$ Values in J kg Mol^{-2} for Several Cosolute−Probe Combinations

	cosolutes		
probe	cyclic amides[a]	acyclic amides[9]	alcohols[32,8]
MPDA	−241	−142	n.d.[b]
BPhT	−37	−51	−136
BT	−31	n.d.[b]	−90

[a] This study (first three CH_2 groups included only). [b] Not determined.

for the acyclic amides. This pattern is not easily explainable in terms of pairwise group interactions. The different noncovalent interactions playing a role in both the initial state and the transition state are difficult to unravel. Moreover, since the *N*-alkyl-2-pyrrolidinones are tertiary amides, a comparison is probably not justified. The $G(CH_2)$ values for the cyclic amides are about one-third of the values obtained for short-chain alcohols for the hydrolyses of **BPhT** and **BT** (Table 3). This indicates that the amide group is more extensively hydrated than the alcohol functionality (presumably in terms of hydrogen bonding), and the apparent hydrophobicity is lower for CH_2 groups directly attached to an amide functionality. Or, differently formulated, the hydration shells of the polar and apolar parts of the cosolutes are more incompatible in the case of the cyclic amides.

The intercepts in Figure 2 represent the contribution of the pyrrolidinone unit (p) toward the medium effect. The *G*(p) values are −567 (**MPDA**), −74 (**BPhT**), and −25 (**BT**) J kg mol^{-2}. The fact that these values are substantially different for the different kinetic probes suggests again that there are specific cosolute-probe interactions cooperative, most likely in the initial as well as in the transition state. An extrapolation toward a contribution for the amide (CONH) unit only (i.e., $n(CH_2)_{total} = 0$) is not so straightforward either, since the contribution to the *G*(c) of the three CH_2 units located in a ring system is not obvious in this case. It was shown previously that CH_2 units in a ring can either reduce the apparent hydrophobicity[5] or increase the apparent hydrophobicity[9] relative to CH_2 groups that are not joined in a ring system. In addition, a modified *G*(CONH) would be obtained in this case; its solvent effect is influenced by the attached alkyl groups. A rough estimate, however, would imply a positive contribution toward *G*(c) for the amide functionality. This is not in agreement with previous findings, which suggest a negative contribution.[9]

The above discussion is based on the CH_2 group additivity that is observed for the short-chained *N*-alkyl-2-pyrrolidinones. Upon extending the chain to *n*-butyl and cyclohexyl, deviation from additivity develops. These cosolutes cause a solvent effect much larger than expected when the observed additivity for the short-chained solutes is extrapolated. A similar pattern is observed for other cosolutes bearing hydrophilic and hydrophobic groups.[14,24]

The explanation for this phenomenon is that the smaller alkyl substituents are entirely located within that part of the hydration sphere of the amide functionality where the hydration water is strongly affected by hydrogen bonding with the amide group. This position in the hydration sphere prevents complete development of hydrophobic hydration shells for these alkyl moieties that

(24) Streefland, L.; Blandamer, M. J.; Engberts, J. B. F. N. *J. Chem. Soc., Perkin Trans. 2* **1997**, 769.

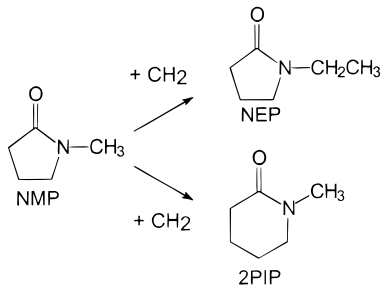
are incompatible with the hydrophilic amide hydration shell. Consequently, their availability to interact with hydrophobic groups in the substrate via hydrophobic interactions is reduced. The effect of the cosolute amide group on the hydrogen-bonding interactions is sensed particularly in the first hydration shell. Since a break in additivity is observed after three consecutive CH_2 groups, it seems that this intramolecular hydration shell overlap stretches over one layer of water molecules in the hydration shell.

Outside the influencing effect of the amide hydration sphere, CH_2-group additivity is likely to occur as well,[25] but with a more negative value for $G(CH_2)$, since these CH_2 groups are less influenced in their interactions with the kinetic probe. Unfortunately, insufficient data are available to support this view.

At first sight it is rather surprising that additivity is found for NMP, NEP, and NiPP. A gradually decreasing influence of the amide functionality would be expected. But when elaborating on the assumption that the part of the amide hydration shell which affects the hydrophobic hydration of attached alkyl groups contains only one layer of water molecules, the Me, Et, and *i*-Pr groups are indeed situated in this layer because the effective diameter of a water molecule[26] is ca. 2.75 Å and the distance between the nitrogen atom and the β carbon atom in the N-alkyl substituent is 2.39 Å. This assumption is supported by calculations[27] on the interaction between >CH− and >NH in water, which spans 3 Å. Therefore, deviation from additivity is likely to occur already for *N*-(*n*-propyl)-2-pyrrolidinone, which contains a γ-C atom (G(c) not determined), and the deviation is clear-cut in the case of NnBP. For these cosolutes, the outer CH_2 moieties are more available for hydrophobic interactions with the kinetic probes, i.e., for the kinetic probe they have larger apparent hydrophobicities. NCHP has one more CH_2 moiety than NnBP, but they have similar distances between the N-atom and the most remote C-atom. For both the **MPDA** and **BPhT** hydrolyses, $|G(c)|$ doubles with an enormous decrease of 1000 and 500 J kg mol^{-2}, respectively, but, interestingly, for **BT** there is no difference in the G(c) value for NnBP and NCHP. In this case, interactions do not seem to be of a hydrophobic nature but instead may be purely determined by effects caused by interactions involving the amide functionality which are modified by the size of the substituent.

Effect of Adding a CH_2 Moiety Either to the *N*-Alkyl Chain or to the Pyrrolidinone Ring. One of the assumptions of the SWAG approach is that group interactions are independent of the group's position in the molecule. To check the validity of this assumption, we measured the kinetic solvent effect of *N*-methyl-2-piperidone (2PIP), a six-membered cyclic amide. The results can be compared to the data already available for NMP and NEP (Scheme 3). In the case of perfect pairwise group additivity, NEP and 2PIP should cause the same solvent effect. Due to overlap of the UV absorption spectra, medium effects on the hydrolysis of **BPhT** and **BT** could not be determined, and G(c) values have only been obtained for the hydrolysis of **MPDA**. The G(c) value for 2PIP for the hydrolysis of MPDA is −1095 J kg mol^{-2}. G(c) values for NMP and NEP are −925 and −1176 J kg mol^{-2}, respectively (Table 2). In other words, a CH_2 group in the ring system adds −170 J kg mol^{-2} to the medium effect, while a CH_2 group in the *N*-alkyl chain contributes −251 J kg mol^{-2}. The SWAG approach is clearly not applicable to these cases. The obvious explanation for the lower apparent hydrophobicity of the CH_2 group in the ring system is the reduced hydrophobic surface area. The part of the apolar surface inside the ring is not exposed to the solvent, and interactions of that part of the molecule with (the hydration shell of) the kinetic probe do not take place. In addition, the conformational differences between a five- and six-membered ring may have an impact on their hydrophobicity. This would be particularly important when there is a preferential site of interaction with the kinetic probe.

Scheme 3. Addition of a CH_2 Group To, Respectively, the *N*-Alkyl Chain and the Ring System of NMP



Changing the Functional Group: Structurally Related Ester Cosolutes. It was noted above that hydrophobic interactions do not solely determine the medium effects of *N*-alkyl-2-pyrolidinones on the hydrolysis reactions of **MPDA**, **BPhT**, and **BT**. Since these reactions involve the hydrolysis of an ester and two amides, it would be particularly relevant to study the effects of ester cosolutes of similar structure as the *N*-alkyl-2-pyrrolidinones. In this way, information about amide−amide, amide−ester, and ester−ester interactions in the activated complex can be obtained, providing a more complete picture of the interplay of the several noncovalent interactions governing the medium effect. In view of this discussion, the kinetic results pertinent to the structurally related esters and amides shown in Scheme 4 have been compared. The G(c) values are compiled in Table 4. For a valuable comparison, the results for the ester/amide cosolute pairs shown above are also given relative to G(c) for amide cosolutes (Table 5). From Table 5, it appears that the Gibbs energies of pairwise interactions of **MPDA** are rather similar for both amide and ester cosolutes, whereas for **BPhT** and **BT**, pairwise Gibbs energy interaction parameters for ester cosolutes are, respectively, 3.5 and 1.4 times more negative than those for the amide cosolutes.

Clearly, the ester probe does not "distinguish" between either ester or amide solutes, whereas the amide probes do. This pattern is illustrated in Figure 4, where the solvent effects caused by the amide cosolutes have been plotted versus those caused by ester cosolutes. The data points for **BPhT** in particular seem to deviate from equal cosolute effects. How can these results be explained? The differences in retardation caused by amide and ester cosolutes for the hydrolysis of **BPhT** and **BT** do not seem to reflect the hydrophobicity of the cosolute. In terms of number of CH_2 moieties, the amide cosolutes are more

(25) We observed additivity of the CH_2 group contribution outside the influence of the polar ammonium hydration shell in the case of alkylated ammonium bromides (see ref 14).
(26) Pierotti, R. J. *J. Phys. Chem.* **1965**, *69*, 281.
(27) Pettitt, B. M.; Karplus, M. *J. Phys. Chem.* **1988**, *92*, 3994.

Scheme 4. *N*-Alkyl-2-pyrrolidinones and Structurally Similar Ester Cosolutes

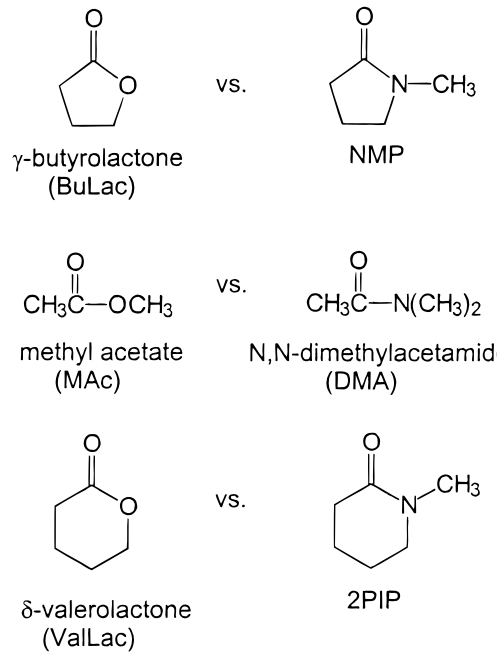


Table 4. *G*(c) Values[a] (J kg mol^{-2}) for the Three Pairs of Ester/Amide Cosolutes (See Scheme 4) for the Hydrolysis of MPDA, BPhT, and BT

	probe		
cosolute	MPDA	BPhT	BT
BuLac	−836(10)	−435(9)	−395(11)
NMP	−925(10)	−133(4)	−292(10)
MAc	−857(15)	−498(13)	−408(14)
DMA	−841(13)	−139(15)	−301(14)
ValLac	−964(18)	n.d.[b]	n.d.
2PIP	−1095(12)	n.d.	n.d.

[a] Errors in parentheses. [b] Not possible to determine.

Table 5. *G*(c) Values (J kg mol^{-2}) for Each Ester/Amide Cosolute Combination Relative to the Amide Cosolute

	probe		
cosolute	MPDA	BPhT	BT
NMP/BuLac	1:0.9	1:3.3	1:1.4
DMA/MAc	1:1	1:3.6	1:1.4
2PIP/ValLac	1:0.9	n.d.[a]	n.d.

[a] Not possible to determine.

hydrophobic than the ester cosolutes, since they contain an additional CH_3 group. On the other hand, the hydration of the polar functional group can influence the hydrophobicity of the apolar groups, so it might be erroneous to assume that the amide cosolutes are more hydrophobic. Probably their hydrophobicities are similar and thus do not explain the observed effects. We note that amides are more polar than esters. For example, IR wavenumbers for C═O stretching vibrations are 1775 and 1700 cm^{-1} for ValLac and NMP, respectively.[28] Also, the strengths of H-bond interactions with phenol as an H-bond donor show that amides are more polar than esters.[29] Since the amide cosolutes are better H-bond

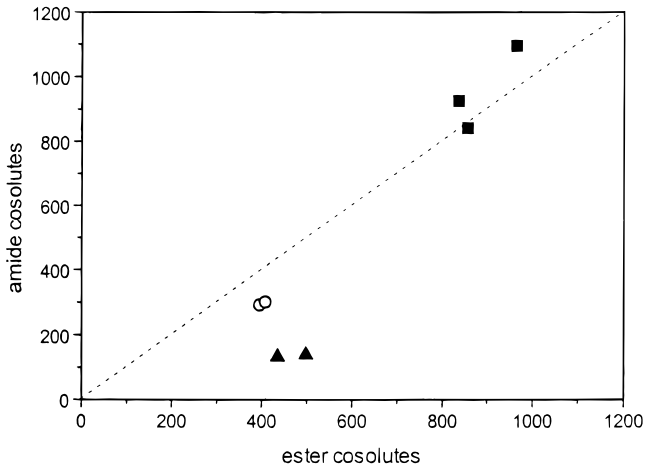


Figure 4. |*G*(c)| values of amide cosolutes plotted versus the |*G*(c)| values of ester cosolutes for the hydrolysis of **MPDA** (■), **BPhT** (▲), and **BT** (○). Dotted line represents equal solvent effects for both amide and ester cosolutes (*G*(c) values in J kg mol^{-2}).

acceptors, they can stabilize the polarized transition state of the hydrolysis reactions to a greater extent than the ester cosolutes can. This larger stabilization of the transition state would explain the smaller kinetic medium effects caused by the amide cosolutes for the hydrolysis of **BT** and **BPhT**, but not for **MPDA**. On the whole, the *G*(c) values for the effects of the ester and amide cosolutes on the hydrolysis of **MPDA** are remarkably similar (see Table 4). Despite the fact that the **MPDA** hydrolysis is the most sensitive to changes in the medium, it is obviously not particularly sensitive to changes in functional groups (i.e., amide vs ester functionality) or to the exact structure of the cosolute. This observation could lead to the conclusion that medium effects on the hydrolysis of **MPDA** do reflect the hydrophobicity of the cosolute better than those for **BT** and **BPhT**. However, a clear correlation between the *G*(c) and $n(CH_2)$ does not appear to exist. Presumably, the insensitivity to the polar group is fortuitous and caused by a number of counteracting contributions to the Gibbs energy of interaction, leading to comparable retardations for ester and amide cosolutes.

PVP as a Cosolute. We also investigated the effects of poly(vinylpyrrolidinone) (PVP) on the hydrolysis of **MPDA**, **BPhT**, and **BT**. In this way, the kinetic effects of a concentrated number (cluster) of amide bonds can be studied as a simple model for a protein backbone. PVP has a broad variety of applications,[30] due to its nontoxic nature and the fact that it is very soluble in both water and a large number of organic solvents. PVP interacts both with hydrophobic and with hydrophilic groups.[30] The solvent effects of PVP have been investigated for two molecular weights: MW 8100 and 57 500. The results have been analyzed in terms of retardation caused per monomer molality of PVP, to make a comparison with NMP, which is equivalent to the PVP monomer in terms of number of CH groups. Clearly, a *pairwise* Gibbs energy interaction parameter cannot be determined, since there are many kinetic probe molecules interacting with one polymer molecule. In Figure 5 the solvent effects of the high molecular weight polymer have been plotted as a

(28) Silverstein, M.; Clayton Bussler, G.; Morrill, T. C. *Spectroscopic Identification of Organic Compounds*, 3rd ed.; Wiley: New York, 1974; p 102.

(29) Murthy, A. S. N.; Rao, C. N. R. *Appl. Spectrosc. Rev.* **1968**, *part 2*, 69.

(30) *Encyclopedia of Polymer Science and Technology*; Mark, H. F., Gaylord, N. G., Eds.; Wiley: New York, 1971; Vol. 14, p 243.

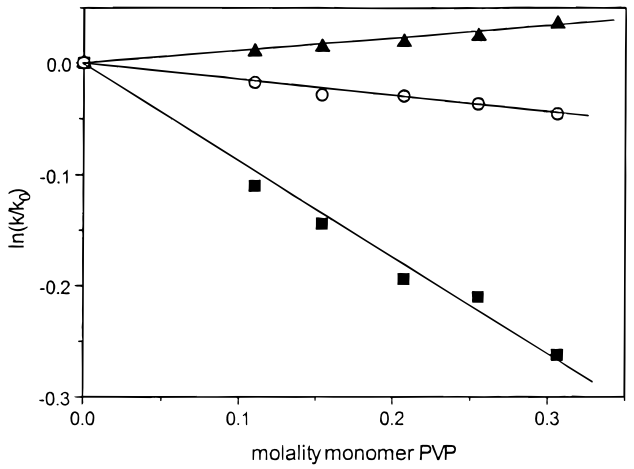


Figure 5. Rate effects caused by PVP (MW 57 500) on the hydrolyses of M**PDA** (■), **BPhT** (▲), and **BT** (○), relative to the rate constant in water (k_0).

function of monomer molality for the three kinetic probes. Within the experimental error, the results for the low molecular PVP are similar to those shown in Figure 5. The retardations of the hydrolyses of **MPDA** and **BT** caused by PVP are slightly less than those caused by similar concentrations of NMP. Since the retardations of NMP and PVP are similar, we contend that the polymer monomeric units behave similarly compared with NMP in their interactions with the two probes. Remarkably, PVP slightly *increases* the reaction rate of the hydrolysis of (the most hydrophobic probe) **BPhT**. Note that the effect of NMP on the hydrolysis of **BPhT** was also small, although retarding. Thus, for **BPhT** at least, NMP has only little hydrophobic character.

The differences observed for the solvent effects caused by PVP and NMP find their origin in the differing hydration properties of PVP and NMP. First, PVP presumably adopts a random coil configuration in aqueous solution, whereby inner residues are less hydrated and also less accessible for interactions with the kinetic probes. The size of the probes, and therefore steric aspects, may be of importance as well. Second, in the PVP molecule, the amide groups are sufficiently close for the hydration shells to overlap and it is unknown how this overlap affects the polar and apolar hydration within the molecule. A space-filling model of PVP shows that the nitrogen atom of the amide bond is buried between the CH-groups of both the backbone and ring system. Hence, polar stabilizing interactions with the activated complex take place via a carbonyl rather than via an amide group. Considering these differences, it is clear that the relative accelerations caused by PVP cannot be ascribed to a single structural or hydration property. Overall, PVP does not significantly disturb the 3-D structure of water; the hydrophobic and hydrophilic parts reduce each other's effects on water−water interactions, and its presence is only just "noticed" by the kinetic probes. The kinetic solvent effects clearly reflect the Gibbs interaction energies of both the initial state and the transition state with PVP.

Conclusions

At low molalities, kinetic medium effects of *N*-alkyl-2-pyrrolidinones on the water-catalyzed hydrolyses of **MPDA**, **BPhT**, and **BT** can be analyzed in terms of pairwise Gibbs energy interaction parameters. These are negative, reflecting the dominant stabilization of the initial state by hydrophobic interactions. However, hydrophobic interactions do not solely govern the solvent effects, as was particularly revealed by comparison of the results for the different kinetic probes. Noncovalent interactions involving the polar amide and ester functional groups in kinetic probe and cosolute affect the medium effects as well and play a role in the stabilization of the activated complexes.

Regarding additivity of functional group interactions: there is no unique rate-retarding effect by a CH_2 group, but its effect in the molecule depends on the distance to the amide functionality. As a result of the intramolecular destructive overlap of the amide (hydrophilic) and alkyl (hydrophobic) hydration shells of the cosolute molecules, reduced contributions of CH_2 interactions to the medium effects are observed for the first three consecutive carbon atoms on the amide nitrogen atom. These contributions are additive. However, the hydrophobic effect becomes increasingly more pronounced for hydrophobic moieties which are more than three consecutive carbon atoms away from the amide functionality. Additivity of these CH_2 groups is anticipated but was not investigated for the *N*-alkyl-2-pyrrolidinones.

A kinetic solvent effect study involving cyclic esters that are structurally related to the *N*-alkyl-2-pyrrolidinones showed how subtle the balance of noncovalent interactions can be.

The effects of PVP polymers on the three hydrolysis reactions are small and suggest that the hydrophilic and hydrophobic interactions nearly cancel. For **BPhT**, a slight rate acceleration is observed in the presence of PVP, indicating the more hydrophilic character of the cosolute.

The results contribute to a better understanding of noncovalent interactions in dilute aqueous media. In particular, the energetics of hydrophobic interactions and interactions involving the amide functionality, which are of crucial importance for the rather marginal stability of the folded protein, have been considered in detail.

Experimental Section

Materials. *p*-Methoxyphenyl dichloroacetate (**MPDA**), 1-benzoyl-1,2,4-triazole (**BT**), and 1-benzoyl-3-phenyl-1,2,4-triazole (**BPhT**) were prepared according to literature procedures.[7,31] Cosolutes were commercially available (DMA, MAc from Janssen, NiPP, 2PIP from Aldrich, NEP, ValLac from Fluka, NMP, NCHP, BuLac, PVP from GAF, NnBP from Tokyo Kassei, Japan). All cosolutes, except for PVP, were purified by distillation in vacuo prior to immediate use.

Kinetic Measurements. Aqueous solutions for the kinetic measurements were prepared by weight immediately before use. Water was distilled twice in an all-quartz distillation unit. The pH of the solution was carefully adjusted with an aqueous HCl solution with an Orion pH-meter. Between 5 and 8 μL of a stock solution containing the kinetic probe (ca. 10^{-4} M) in acetonitrile (P.A. quality) were injected into 2.5 mL of reaction medium in a quartz cuvette and placed in a thermostated cell compartment (25.0 ± 0.05 °C) of a Perkin-Elmer λ5 or λ2 spectrophotometer. Pseudo-first-order rate constants were determined by following changes in absorbance at 288 nm

(31) Engbersen, J. F. J.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1975**, *97*, 1563.
(32) Blokzijl, W.; Blandamer, M. J.; Engberts, J. B. F. N. *J. Org. Chem.* **1991**, *56*,1832.

(**MPDA**), 273 nm (**BPhT**), or 250 nm (**BT**) using a Perkin-Elmer $\lambda 2$ or $\lambda 5$ UV/vis spectrophotometer. Rate constants for the reaction in the absence of cosolute were 3.0×10^{-3} s^{-1}, 1.24×10^{-3} s^{-1}, and 2.07×10^{-3} s^{-1}, respectively, in good agreement with literature values.[4,5] The half-lives of these hydrolysis reactions are such that any possible hydrolysis of cosolutes can be safely neglected.

In general, the absorbance did not exceed 0.7. The reactions were followed for about 10 half-lives, and excellent first-order kinetics were observed. Data were converted to rate constants using a commercially available data station. Rate constants were obtained in triplicate and generally reproducible to within 1%. Rate constants for PVP were reproducible to within 3%. Rate constants were determined for at least five different molalities. G(c) values were obtained by a linear regression program. Errors in G(c) values are standard deviations.

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