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Pairwise Gibbs Energy Interaction Parameters for \( \alpha \)-Amino Acid–Amide Interactions in Aqueous Solution: A Kinetic Study

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Abstract: In order to establish the way in which the hydration characteristics of \( \alpha \)-amino acids influence their noncovalent interactions with other solutes in aqueous solution, we measured medium effects for the water-catalyzed hydrolysis of an activated amide in aqueous solution in the presence of \( \alpha \)-amino acids and some of their derivatives at 25 °C and \( \mathrm{pH} \) 4. With the exception of phenylalanine and 3-phenylserine, all \( \alpha \)-amino acids induce significant rate enhancements. The kinetic results are analysed using the thermodynamic description of solute–solvent interactions as formulated by Savage and Wood. This procedure allows the evaluation of pairwise Gibbs energy interaction parameters. These parameters neither reflect the hydrophobicity of the \( \alpha \)-amino acid side chain nor the additivity of functional group interactions in the cosolute. The carboxylate group in the zwitterionic \( \alpha \)-amino acid dominates the rate enhancing kinetic medium effect and reduces the availability of nearby methylene groups for interaction with the apolar initial state of the reaction. Some \( \alpha \)-amino acid side chains clearly influence the interaction of the carboxylate group with the reactant, most probably due to destructive hydration shell overlap. Only for phenylalanine and 3-phenylserine are marked rate retardations observed, and presumably the hydrophobic effect of the side chains now dominates over the rate enhancing effect of the carboxylate moiety. The results provide quantitative insights into intermolecular noncovalent interactions between \( \alpha \)-amino acids and the activated amide. It is shown that destructive hydration shell overlap effects play a significant role in noncovalent interactions in aqueous solution.

Introduction

Protein folding is one of nature’s most intriguing processes. Attempts have been made to estimate the contribution of the different noncovalent interactions governing protein folding and stability but only with modest success.\(^1\)–\(^3\) Particularly the interpretation of the thermodynamics of amide–amide hydrogen bonding interactions has been a controversial issue.\(^4\)–\(^6\) Kauzmann\(^7\) was the first to stress the importance of hydrophobic interactions in protein folding and ever since these interactions have been accepted as a major driving force for protein folding. However, the molecular mechanism of hydrophobic interactions is still a matter of debate.\(^8\)

The protein in its folded state is stabilized by only \( 5–20 \) kcal mol\(^{-1}\),\(^9\) a consequence of strong enthalpy–entropy compensation in the interactions. Therefore, small changes in electrostatic, van der Waals, and hydrogen bonding interactions may all contribute significantly to the stability of the protein. The interplay of these noncovalent interactions depends to a large extent on the solvent. Water is unique in mediating protein folding. For an understanding of the hydration of a protein and the role of water in its biological function and structure, it appears a prerequisite to obtain quantitative knowledge on the hydration of \( \alpha \)-amino acids. One approach involves an analysis of the properties of \( \alpha \)-amino acids in terms of their constituent functional groups and their hydration characteristics; another is to consider transfer properties of \( \alpha \)-amino acids from one solvent to the other. The solvation and physical properties of \( \alpha \)-amino acids have been lucidly reviewed by Lilley.\(^10\)

Here, we present quantitative data for noncovalent interactions of a series of \( \alpha \)-amino acids with a hydrolyzable activated amide in aqueous solution based upon kinetic medium effects. Pseudo-first-order kinetics of the neutral (i.e., \( \mathrm{pH} \) independent) hydrolysis of 1-benzoyl-3-phenyl-1,2,4-triazole have been measured in the presence of low concentrations of \( \alpha \)-amino acids. At the experimental \( \mathrm{pH} \) 4, the rate-determining step involves a water-catalyzed reaction via an activated complex containing two water molecules with three protons in flight\(^11\),\(^12\) (Figure 1). Obviously, this activation process is accompanied by a substantial increase in substrate polarity. The medium effects brought about by \( \alpha \)-amino acids on the hydrolysis reaction will therefore reflect their hydrophobicity/hydrophilicity and hydration properties. Solute–solvent interactions can be analyzed by considering additivity of the functional groups present in the solute molecule. This additivity principle was first formulated

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ary and tertiary amides exhibit a significant deviation from the slope of a linear plot of \( \ln(k_{\text{obs}}) \) versus the molality of the addendum. The theory has been successfully applied to describe solute-solute interactions in aqueous solutions containing, inter alia, alcohols, carbohydrates, amides, and sodium \( n \)-alkylsulfates. These classes of cosolutes all retard the rate of hydrolysis, which has been ascribed to a stabilization of the initial state due to hydrophobic interactions with the additive. In the case of monohydric alcohols satisfactory additivity of functional groups was observed. The methylene moiety gave rise to a negative pairwise group interaction parameter, and the hydroxyl groups contributed positively to the \( G(C) \) value. Primary amides showed additivity, but secondary and tertiary amides exhibit a significant deviation from additivity, presumably due to specific amide–amide interactions. The additivity theory does not allow an analysis of stereospecific interactions as observed in aqueous solutions containing stereoisomeric carbohydrates. A third failure of the additivity theory of functional groups was observed for sodium \( n \)-alkylsulfates, the first series of cosolutes containing an ionic group. Differences in kinetic medium effects were very small when the alkyl chain attached to the sulfate moiety was extended from one to three carbon atoms, indicating that these groups are not available for hydrophobic interactions with the initial state of the hydrolysis reaction. Presumably, this phenomenon is caused by the extensive hydration of the sulfate group. Methylene moieties more remote from the ionic group gave rise to the usual hydrophobic interactions. These studies suggest that the additivity theory is not generally applicable and applies to a number of selected classes of addenda only.

In the present study we observe that for \( \alpha \)-amino acids the pairwise interactions cannot be analyzed in terms of additivity of functional groups either. No simple relationship between side chain hydrophobicity and the \( G(C) \) value was obtained. The results, however, are of immediate relevance for understanding intermolecular noncovalent interactions involving \( \alpha \)-amino acids in aqueous solution.

**Results and Discussion**

**Effects of Aliphatic \( \alpha \)-Amino Acids.** Pseudo-first-order rate constants for the hydrolysis of 1-benzoyl-3-phenyl-1,2,4-triazole were measured at \( 25 ^\circ C \) and \( pH \ 4 \). As demanded by our theory, the natural logarithm of the rate constant varied linearly with the molality of the cosolute up to the maximum molalities measured (usually \( 1 \) molal). At higher concentrations deviation from linearity was observed, most likely due to triplet and higher order interactions. In Figure 2 the effects of glycine, proline, \( \alpha \)-alanine, \( \alpha \)-valine, and \( \alpha \)-serine are visualized. \( G(C) \) values were calculated and are displayed in Table 1. The selection of \( \alpha \)-amino acids used in this study was solely determined by their solubility constraints.

The striking feature reflected by the data in Table 1 is the acceleration of the hydrolysis reaction in aqueous \( \alpha \)-amino acid solutions compared to the reaction in pure water. Interestingly, this is the first time that we have observed substantial rate enhancements for this hydrolysis reaction in the presence of small amounts of cosolutes. An obvious explanation would imply general-base catalysis by the carboxylate group. This could be checked by measuring the rate constants of \( \alpha \)-amino acid solutions with constant concentration over a large \( pH \) range. It was not that the neutral hydrolysis is restricted to \( pH \) values between \( 3.5 \) and \( 5.0 \), where the differences in reaction rates due to changes in ionization of the carboxyl group are outside the kinetic detection limit. If, however, general-base catalysis would interfere with the medium effect, a linear dependence of

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(14) Blokzijl, W.; Jager, J.; Engberts, J. B. F. N.; Blandamer, M. J.
(15) Blokzijl, W.; Engberts, J. B. F. N.; Jager, J.; Blandamer, M. J.
(18) Kershott, R. F. P.; Engberts, J. B. F. N.; Blandamer, M. J.
(19) Noordman, W.; Blokzijl, W.; Engberts, J. B. F. N.; Blandamer, M.
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the logarithm of the second-order catalytic rate constant on the pK values of the α-amino acid is expected. Such a Brønsted relationship was not found. Solvent deuterium kinetic isotope effects (see Experimental Section) showed that the mechanism of hydrolysis is unchanged upon replacing water for an aqueous solution containing Gly. Finally, in case of general-base catalysis, plots of kobs versus the molality of cosolute should yield a linear relationship with a slope equal to kcat. Such a relationship was not found either. We contend that the observed kinetic effects are not governed by general-base catalysis of the amino acid carboxylate group.

In view of the zwitterionic character of the α-amino acids it seems likely that a stabilization of the activated complex, which also note that Gly is always found in solvent-exposed regions. Wolfenden et al. pointed out that the loss of internal mobility of the ring system when entering the secondary structures. Analogue of proline. This is explained by the relatively small transfer of a Pro residue from the vapor to the aqueous phase. 

Proline also induces a large rate acceleration. Comparison of Pro with other common α-amino acids is not fully justified, because of its cyclic structure and modified hydrogen bonding capabilities. The high affinity for water of this imino acid is likewise reflected by its position in protein structures; it is often found in solvent-exposed regions. Proline does not fit into the dielectric constants of aqueous α-amino acid solutions are higher than that of pure water. The dielectric constants of aqueous solutions are higher than that of pure water. 22

Despite the lack of straightforward correlation, it is possible to identify the structural features of the α-amino acid which dominate the G(C) value. As can be seen from Table 1, glycine is outstanding in accelerating the rate of the hydrolysis. Most likely, the central methylene group is not available for participating in hydrophobic interactions with the initial state of the reaction. Due to the presence of the extensive hydration shells of both ionic groups, the hydrophobic hydration shell of the methylene group is badly developed. This was also concluded from a Monte Carlo computer simulation of the glycine zwitterion26 in which the hydration number of the central methylene group was found to be far less than anticipated on the basis of studies on compounds modeling this group. We also note that Gly is always found in solvent-exposed regions in proteins, consistent with its hydrophilicity. Moreover an ultrasound velocity and density study on α,ω-amino acids yielding partial molar volumes, expansibilities, and compressibilities showed that hydration shell overlap of the two ionic groups takes place up to five methylene groups, and it is assumed that the hydration of these solutes is mainly determined by electrostatic solute–solvent interactions.

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| Table 1. G(C) Values (J·kg mol⁻¹) for α-Amino Acids and Derivatives at 298.15 K and pH 4 |
|-----------------|-----------------|-----------------|-----------------|
| α-amino acid    | α-C substituent | pK (α-CO₂H)     | G(C)            |
| Glycine         | hydrogen        | 2.35            | 875 (21)        |
| Alanine         | methyl          | 2.35            | 558 (16)        |
| α-aminoisobutyric acid | 2x methyl       | 2.36            | 429 (17)        |
| Proline         | (CH₂)₂⁻         | 2.00            | 632 (15)        |
| α-amino-n-butyl acid | ethyl          | 2.55            | 565 (15)        |
| α-aminoaleric acid (norvaline) | n-propyl      | 2.30            | 556 (15)        |
| Valine          | isopropyl       | 2.29            | 467 (9)         |
| Leucine         | isobutyl        | 2.33            | 518 (21)        |
| Isoleucine      | sec-butyl       | 2.32            | 499 (20)        |
| Lysine          | (CH₂)₃NH⁺       | 2.16            | 642 (29)        |
| Threonine       | CH(CH₂)OH⁺      | 2.09            | 445 (9)         |
| Serine          | CH₂OH⁻         | 2.19            | 383 (6)         |
| Asparagine      | CH₂CONH₂⁺       | 2.02            | 436 (8)         |
| 3-Phenylserine  | CH(OH)CH₂H₃⁺    | n.d.            | −479 (25)       |
| Phenylalanine   | CH₂CH₂H₃⁺       | 2.20            | −709 (6)        |

*a Standard deviations between brackets.

(21) A Brønsted α value of 0.35, together with the rate constant in pure water (1.26 × 10⁻³ s⁻¹), can be used to estimate the catalytic rate constants. This Brønsted value is reasonable when compared to other, structurally closely related 1,3-substituted acrylamides, which show values between 0.34 and 0.36 for sodium alkane carboxylates (see ref 11). The rate equations thus obtained yield theoretical values for kcat which are indisputably different from the experimental values for kobs.


aqueous medium as compared to the flexible linear chain. The loss of enthalpy due to reduced hydrogen bonding ability at the amino end is probably more than compensated by this effect. In our study another possible explanation for the hydrophilicity of proline could be the reduced accessibility of the inside region of the apolar ring to achieve hydrophobic interactions. A similar effect is observed when the G(C) value of cyclopentanol is compared to that of linear alcohols with the same number of CH₂ groups.¹⁴

We now turn attention to a comparison of α-alanine, α-amino-n-butric acid, and norvaline, with methyl, ethyl, and n-propyl side chains, respectively. The G(C) values are 558, 565, and 556 J·kg⁻¹·mol⁻², respectively, and equal within experimental error. Even side chains up to four carbon atoms, though branched (Leu and Ile), show only a minor effect when compared to the shorter chains. This pattern shows that hydrophobic interactions (at least up to four carbon atoms) play no significant role because they are overwhelmed by interactions mediated by the ionic groups. A similar conclusion was drawn from results obtained for sodium n-alkylsulfates as cosolutes.¹⁹

The importance of the ionic carboxylate in governing the medium effect is also obvious from the results of some α-amino acid derivatives (vide infra). The functionalization of the ionic carboxylate to a neutral ester or amide functionality leads to a decrease in reaction rate, whereas methylation of the ammonium group only results in a slight decrease of the rate acceleration (see Table 1). Electrostatic interactions between the carboxylate and the positive pole of the dipole in the transition state (either direct or via polarization of solvation water) are hence considered as the dominant interactions leading to the observed rate accelerations.

Effects of α-Amino Acids Containing Aromatic Side Chains. In contrast to the α-amino acids with short aliphatic side chains, phenylalanine and 3-phenylserine, which contain the strongly hydrophobic benzylic group, appear to exert their kinetic medium effect mainly through hydrophobic interactions. In fact, these cosolutes now retard the hydrolysis, and the large negative G(C) values (−709 J·kg⁻¹·mol⁻² and −479 J·kg⁻¹·mol⁻², respectively) are striking. There is evidence against aromatic stacking, because retardations are also observed for the hydrolysis of 1-ethanoyl-3-tert-butyl-1,2,4-triazole, lacking the benzoyl substituent.²⁰ Moreover the hydrolysis of the less hydrophobic 1-benzoyl-1,2,4-triazole is accompanied by a less negative G(C) value which is in accord with hydrophobic interactions stabilizing the initial state of the reaction. These negative G(C) values therefore appear to reflect the anomalous hydrophobicities of phenylalanine and 3-phenylserine. The kinetic results for these cosolutes and some α-amino acid amides which retard the hydrolysis as well are visualized in Figure 3.

Comparison of α-Amino Acid Isomers. α-Aminoisobutyric acid (2-methylalanine) (G(C) = 429 J·kg⁻¹·mol⁻²) and α-amino-n-butric acid (G(C) = 565 J·kg⁻¹·mol⁻²) are isomeric cosolutes, and a comparison of their rate enhancing medium effects (see Table 1) is worthwhile. The G(C) values indicate that when the α-carbon atom is substituted twice, the effect on the rate of hydrolysis is larger compared to that of the monosubstituted isomer. The most acceptable explanation consists of a combination of two effects. One is the reduction of the favorable interactions of the ionic groups with the transition state, caused by steric restraints due to enforced ionic hydration shell overlap between the ammonium and carboxylate group. At the same time, the hydrophobic hydration shell of the additional methyl group may develop at the expense of the hydrophilic hydration shell of the carboxylate group, favoring increased hydrophobic interactions with the initial state.

α-Valine and norvaline, with isopropyl and n-propyl side chains, respectively, are isomers as well, but norvaline is more effective in accelerating the hydrolysis reaction than valine. Again this difference might be due to intramolecular overlap of hydrophobic and ionic hydration shells which is more pronounced in the case of valine. Whether or not its lower G(C) value is caused by increased hydrophobic interactions with the initial state or decreased stabilization of the transition state is not clear.

Effects of Serine and Threonine. At first sight the results obtained for serine and threonine appear unexpected because a hydroxyl group can interact more favorably with the activated complex than with the initial state and a positive group contribution to the G(C) is anticipated (as is the case for alcohols¹⁴b). In contrast, serine and threonine, with a methanol- and ethanol-like side chain, respectively, are the weakest accelerators among the α-amino acids examined. This effect is the most pronounced for serine. It is plausible that the overlap of the rather extensive hydration shell of the hydroxyl group with the ionic hydration shells is larger for serine than for threonine, leading to a reduced rate acceleration. Comparison between Ala and Ser (substitution of H for OH) shows the importance of a well-developed ionic hydration shell in the acceleration in the reaction.

α-Amino Acid Derivatives. From the above results it is obvious that the additivity principle¹⁴b is not applicable for the side chains in common α-amino acids and that this can be rationalized by a disturbing effect of the hydrated charges present in the cosolute.

To obtain more insight into the effects exerted by the ammonium and carboxylate groups several α-amino acid derivatives have been studied. As noted above, the primary amide and ethyl ester derivatives of Gly illustrate the importance of the carboxylate group in governing the medium effect. The sign of G(C) even changes to a negative value, and a particularly large rate retardation is observed for phenylalaninamide (Table 1). On the other hand, methylation of the amino group does not induce such striking effects. One methyl group reduces the G(C) value from 875 to 555 J·kg⁻¹·mol⁻², an effect which is the same for the change from Gly to α-Ala. Apparently the position of the methyl group in this case is unimportant; the reactant does not distinguish between these isomers. However, when a second and a third methyl group are introduced on the nitrogen atom, the observed rate of hydrolysis of the amide is in contradiction with the notion that an additional apolar group encourages hydrophobic interactions. Even the carboxylate basicity predicts the reverse effect. Again we refer to work by Chalikian et al.²⁷ who showed that destructive overlap of ionic...
hydration shells in α,ω-amino acids takes place up to a separation of four carbon atoms, and indeed this may well explain our results. Alkylation of the nitrogen atom dramatically changes its hydration and subsequently its effect on the ionic hydration shell of the carboxylate group. These mutual interactions of hydration shells complicate the interpretation of the present results. Measurements of G(C) values for a series of α-amino acids where the carboxylate is functionalized, for example to α-amino acid amides, will probably reflect the side chain hydrophobicity in a more decent way.

In an attempt to avoid the mutual interactions of the ionic groups in the zwitterionic α-amino acids, we finally measured the medium effect of aqueous solutions containing ammonium cyanoacetate as a cosolute. The cyano group was chosen in order to obtain a carboxylate group basicity that approximates the pK of Gly. This solute is still able to speed up the hydrolysis reaction, but its G(C) value is much lower than that of Gly. Though the carboxylate group is now able to interact without being disturbed by the hydration of the ammonium group, the cyano group is not masked for interaction either, and therefore a comparison between the two cosolutes is not completely justified. Sodium instead of ammonium as the counterion reduces the G(C) value only slightly, in accord with the view that the carboxylate group dominates the medium effect.

Finally, ammonium methanesulfonate was examined as a cosolvent. Although the sulfonate anion is a very weak base and general-base catalysis by this cosolvent is clearly out of the question, a positive G(C) was recorded. This result supports the hypothesis of polarization of the water molecules in the activated complex and provides further evidence against general-base catalysis (vide supra). Unfortunately, the solubility of ammonium methanesulfonate in water is low and only measurements at low concentrations could be performed, providing a less accurate G(C) value.

**Activation Parameters.** To obtain additional information about the type of interactions responsible for the observed accelerations in terms of charge stabilization in the transition state, isobaric activation parameters have been measured (Table 2). It appears that the rate enhancements observed for Gly, α-Ala, and α-Val are entropy driven. The large and unfavorable negative entropy of activation becomes less negative when Gly, α-Ala, or α-Val are present in the reaction medium. By contrast, the enthalpy of activation becomes more unfavorable. Glycine can be considered as a dipolar ion and hydrophobic interactions with the initial state can be neglected for reasons mentioned earlier. The change in activation parameters upon adding glycine to the aqueous solution therefore purely reflect the polar interactions of glycine with the activated complex. It is striking that in this case the acceleration is not enthalpy driven. Presumably part of the hydrogen bonding interactions in the carboxylate hydration shell of the zwitterionic molecule are broken upon activation and are exchanged for dipole–dipole interactions with the polarized transition state leading to an overall loss of pairwise interaction enthalpy.

For α-phenylalanine, one of the cosolutes that was found to retard the rate of the hydrolysis, the change in Gibbs energy of activation is enthalpy driven. The entropy of activation becomes more favorable here as well but cannot fully compensate the unfavorable change in enthalpy of activation. As no trend is observed in the entropies and enthalpies of activation (the entropy (and enthalpy) gain decreases and subsequently increases upon going from Gly to Phe, see Table 2) it appears that the nature of the dominant interactions in these series is variable. When hydrophobic interactions come into play between the larger side chains and the initial state, it is anticipated that the entropy of activation becomes more negative going from Ala → Val → Phe, because the entropy of the initial state is raised even more in relation to the transition state. However the opposite trend is observed. At present it is not feasible to unravel the details of the thermodynamic interplay of the different interactions between zwitterionic amino acids and amides in aqueous solution. Obviously, a description of pairwise solute–solvent interactions in terms of one single interaction mechanism is not warranted.

Effects of small peptides on this reaction may yield further information on the noncovalent amide–amide interactions. In addition, it is of considerable interest to examine the possibility of diastereomeric noncovalent interactions in the case of a chiral activated amide in the presence of the α-amino acid enantiomers.

**Conclusions**

Kinetic medium effects have been measured for α-amino acids on a water-catalyzed hydrolysis of an activated amide. All α-amino acids, except α-phenylalanine and 3-phenylserine, increase the reaction rate. Strong evidence was obtained that general-base catalysis by the carboxylate group is not the origin of the observed effects. The kinetic results were analysed in terms of a pairwise Gibb’s energy interaction parameter G(C), which represents the overall effect of the addendum on the activation process. The hydrophilic α-amino acids glycine and proline accelerate the hydrolysis considerably. Generally, factors that play a role in the medium effect are thought to be steric in origin. Bulky side chains with large hydration shells seem to weaken the favorable effect of the carboxylate moiety. From measurements using several α-amino acid derivatives it appears that the carboxylate group is the main determinant in the observed rate enhancing medium effects. The difference between a methyl, ethyl, or n-propyl side chain is not distinguished by the amide. However, a quaternary α-carbon atom has a distinctive effect. Additivity of the different group interactions was not observed. These results may be consequences of the large and masking effect of the hydrated ionic groups which prevents the effect of the side chains to show up. The present study suggests that intramolecular destructive hydration shell overlap effects should be taken into account in understanding intermolecular interactions and conformational preferences of biomolecules, including protein folding.

**Experimental Section**

**Materials.** All α-amino acids were used as supplied by Janssen Chimica, Fluka, or Sigma. Solutions were made up by weight using demineralized water which was distilled twice in an all-quartz distillation unit. The pH was carefully adjusted with a HCl solution and measured using an Orion pH-meter.

Ammonium cyanacetate was prepared by leading gaseous ammonia through a solution of cyanacetate acid in dry ether. The white solid that was formed was purified by extraction in a Soxlet apparatus with dry ether and dried afterwards in vacuo. Sodium cyanacetate was prepared by adding an equivalent amount of sodium ethanoate to a solution of cyanacetate acid in absolute ethanol. The solution was heated to dissolve the salt and subsequently filtered. The white solid that crystallized upon cooling was dried in vacuo.

**Table 2.** Isobaric Activation Parameters for the Hydrolysis of 1 in Aqueous Mixtures Containing Gly, Ala, Val, and Phe

<table>
<thead>
<tr>
<th>solvent composition</th>
<th>ΔG(^circ) (kJ·mol⁻¹)</th>
<th>ΔH(^circ) (kJ·mol⁻¹)</th>
<th>ΔS(^circ) (J·mol⁻¹ K⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>89.65 (+0.02)</td>
<td>45.9 (+0.5)</td>
<td>-147 (+2)</td>
</tr>
<tr>
<td>H₂O/Gly (0.5 M)</td>
<td>88.94 (+0.02)</td>
<td>51.6 (+0.6)</td>
<td>-125 (+2)</td>
</tr>
<tr>
<td>H₂O/Ala (0.15 M)</td>
<td>89.40 (+0.01)</td>
<td>47.4 (+0.3)</td>
<td>-141 (+1)</td>
</tr>
<tr>
<td>H₂O/Val (0.5 M)</td>
<td>89.04 (+0.03)</td>
<td>49.7 (+0.3)</td>
<td>-132 (+1)</td>
</tr>
<tr>
<td>H₂O/Phe (0.15 M)</td>
<td>89.78 (+0.01)</td>
<td>52.0 (+0.7)</td>
<td>-127 (+2)</td>
</tr>
</tbody>
</table>
Kinetic Measurements. Pseudo-first-order rate constants for the neutral hydrolysis of 1-benzoyl-3-phenyl-1,2,4-triazole (mp 79–80 °C) were determined by following the change in absorbance at 273 nm. About 5 µL of a stock solution of 1 in acetonitrile was added to the reaction medium in a quartz cell, placed in a thermostated cell compartment (25.0 °C) of a Perkin Elmer λ5 or a λ2 spectrophotometer. Care was taken that the absorption did not exceed 0.5 because of the low solubility of 1. The reaction was followed for about 5 half-life times, and perfect first-order kinetics were observed. Rate constants were calculated using a data station connected to the λ5 spectrophotometer and using a fitting program on the λ2 spectrophotometer. Rate constants were determined in triplicate and found to be reproducible to within 2%. All cosolvents used were measured at at least four molalities and up to the solubility limit. The $G(C)$ values and the errors in $G(C)$ values were calculated using a linear regression program.

For the determination of isobaric activation parameters, rate constants were measured at eight temperatures over a range of 20 °C. Plots of $\ln(k/T)$ versus $1/T$ were perfectly linear, indicating that in the temperature range $\Delta^\theta H^0$ is independent of temperature. The $\Delta^\theta S^0$ was calculated from $\Delta^\theta G^0$ and $\Delta^\theta H^0$ at 25 °C.

For the solvent kinetic isotope effects the measurements in D2O were performed in solutions which were adjusted to pD 4 with DCl. The values for $k(H_2O)/k(D_2O)$ in pure water and water containing 0.5 molal glycine were 2.69 and 2.46 ($\pm0.05$), respectively. No deuterated glycine was used which might explain the slightly lower value for the latter.

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