ISSN 1070-3632, Russian Journal of General Chemistry, 2012, Vol. 82, No. 11, pp. 1805–1809. © Pleiades Publishing, Ltd., 2012.
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# The Water–Propanol Solvent Effect on the Reactivity of α-Amino Acids and Dipeptides in the N-Acylation with Picryl Benzoate

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Received October 4, 2011

**Abstract**—The kinetics was studied of the reactions of Gly, L-α-Ala, Gly-Gly, L-α-Ala-L-α-Ala, L-α-Ala-β-Ala and β-Ala-β-Ala with picryl benzoate in the water–2-propanol mixed solvent (58.8–88.6 mol % of water). The linearity between the basicity of the dipeptides and the logarithms of acylation constants was established. The non-monotonic dependence of the rate constants of acylation of amino acid and dipeptides on the composition of binary solvent is shown to originate from the competitive nature of the specific solvation of amino compounds. Quantum-chemical modeling of the anionic form of glycine solvato complexes with the solvent components was performed. We concluded that the reactions proceeded under the orbital control.

**DOI:** 10.1134/S1070363212110114

Studies of kinetics of the acyl transfer involving α-amino acids were reported in a number of papers. It was found that the aqueous-organic solvents affect significantly the rate of N-acylation [1–6]. The kinetics of the reactions of α-amino acids with esters in aqueous-organic media was thoroughly investigated [3–6], but the reactivity of dipeptides in the interaction with the phenyl benzoates so far remained unexplored. However, these data are of interest for understanding the general laws of the formation of the amide (peptide) bond.

The aim of this work was to study the effect of water–2-propanol solvent, when the mole fraction of water ($X_1$) is varied in the range 0.588–0.886, on the kinetics of the reactions of amino acids and dipeptides: Gly (**I**), Gly-Gly (**II**), L-α-Ala (**Ia**), L-α-Ala-L-α-Ala (**IIa**), β-Ala-β-Ala (**IIb**) and L-α-Ala-β-Ala (**IIc**) with 5,4,6-trinitrophenyl benzoate (**III**). The composition range of the binary solvent used in the study is limited, on the one hand, by insufficient solubility of amino compounds and reaction products at a low water content in the system, and, on the other hand, by a rapid hydrolysis of the ester at high content of water.

The reaction was carried out under conditions where the only reactive form of amino acids and dipeptides in solution were the anions [7]. The total concentration of all forms in the amino compound was higher than that of the ester at least 100 times. The observed first-order reaction rate constant $k_{\text{obs.}}$ taking into account the possible hydrolysis of the ester **III** is given by:

$$k_{\text{obs}} = k_{\text{hydrolysis}} + k_{-}c_{-}, \quad (1)$$

where $k_{\text{hydrolysis}}$ is the rate constant of the hydrolysis of ester, $k_{-}$ is the rate constant of N-acylation of the α-amino acid (dipeptide) anion, $c_{-}$ is the concentration of anions in the solution of amino compound.

It is known from [3–6] that under the experimental conditions (pH 8–9) the rate of hydrolysis of ester is negligible compared with the N-acylation of α-amino acid, and its contribution to the overall reaction rate can be ignored. For $k_{\text{hydrolysis}} \approx 0$, Eq. (1) becomes:

$$k_{\text{obs}} = k_{-}c_{-}. \quad (2)$$

Accordingly, the second order rate constants of reactions of anions of the α-amino acids and dipeptides with **III** can be calculated with the equation:

$$k_{-} = k_{\text{obs}}/c_{-}. \quad (3)$$

The results of kinetic studies are listed in Tables 1–3. It follows from the data in Table 1 that in the kinetic experiments the acylation rate constant $k_{-}$ defined by Eq. (3) varies only slightly at varying $c_{-}$. The constant

**Table 1.** Values of $k_{obs}$ and $k_-$ at different initial concentrations of glycine and dipeptides anions $c_-$ in the water–2-propanol solvent[a]

| Compound | $T$, K | $X_1$ | $c_- \times 10^3$, M | $k_{obs} \times 10^2$, $s^{-1}$ | $k_-$, $l\ mol^{-1}\ s^{-1}$ | α | $r$ |
|---|---|---|---|---|---|---|---|
| Gly | 298 | 0.833 | 7.25 | 9.06±0.14 | 12.5±0.2 | 0.887 | 0.9999 |
| | | | 3.63 | 4.27±0.04 | 11.8±0.1 | 0.850 | 0.9997 |
| Gly-Gly | 298 | 0.886 | 7.25 | 2.63±0.06 | 3.62±0.08 | 0.818 | 0.9997 |
| | | | 3.63 | 1.26±0.03 | 3.49±0.09 | 0.841 | 0.9988 |
| α-Ala-α-Ala | 318 | 0.769 | 7.25 | 1.88±0.06 | 2.59±0.08 | 0.913 | 0.9998 |
| | | | 1.45 | 0.38±0.01 | 2.6±0.1 | 0.921 | 0.9985 |

[a] $X_1$ is mole fraction of water in the solvent; α is degree of conversion; $r$ is linear correlation coefficient in the Guggenheim equation.

**Table 2.** Kinetic data of acylation of amino acids and dipeptides with picryl benzoate in the solvent water–2-propanol, 298 K

| Comp. no. | Rate constants | $X_1$ | | | | |
|---|---|---|---|---|---|---|
| | | 0.588 | 0.690 | 0.769 | 0.833 | 0.886 |
| **I** | $k_{obs} \times 10^2$, $s^{-1}$ | 2.25±0.05 | 2.19±0.04 | 1.93±0.03 | 1.82±0.03 | 2.32±0.04 |
| | $k_-$, $l\ mol^{-1}\ s^{-1}$ | 15.5±0.4 | 15.1±0.3 | 13.3±0.2 | 12.6±0.2 | 16.0±0.3 |
| **Ia** | $k_{obs} \times 10^2$, $s^{-1}$ | 2.72±0.08 | 1.69±0.05 | 1.45±0.04 | 1.87±0.05 | 2.31±0.04 |
| | $k_-$, $l\ mol^{-1}\ s^{-1}$ | 7.5±0.2 | 4.7±0.1 | 4.0±0.1 | 5.2±0.1 | 6.4±0.1 |
| **II** | $k_{obs} \times 10^3$, $s^{-1}$ | 11.7±0.3 | 10.7±0.9 | 8.25±0.07 | 8.71±0.06 | 13.1±0.3 |
| | $k_-$, $l\ mol^{-1}\ s^{-1}$ | 3.23±0.09 | 3.0±0.2 | 2.27±0.02 | 2.41±0.02 | 3.62±0.08 |
| **IIa** | $k_{obs} \times 10^3$, $s^{-1}$ | 3.91±0.01 | 3.66±0.07 | 7.4±0.2 | 3.85±0.05 | 10.7±0.1 |
| | $k_-$, $l\ mol^{-1}\ s^{-1}$ | 1.07±0.04 | 1.01±0.02 | 1.02±0.03 | 1.06±0.01 | 2.96±0.04 |
| **IIb** | $k_{obs} \times 10^2$, $s^{-1}$ | 2.25±0.03 | 1.85±0.03 | 1.69±0.01 | 2.06±0.02 | 2.79±0.09 |
| | $k_-$, $l\ mol^{-1}\ s^{-1}$ | 6.21±0.09 | 5.10±0.08 | 4.68±0.04 | 5.71±0.06 | 7.7±0.3 |
| **IIc** | $k_{obs} \times 10^3$, $s^{-1}$ | 3.75±0.06 | 3.42±0.03 | 3.45±0.07 | 3.44±0.05 | 4.94±0.07 |
| | $k_-$, $l\ mol^{-1}\ s^{-1}$ | 1.04±0.02 | 0.94±0.01 | 0.95±0.02 | 0.95±0.02 | 1.36±0.02 |

values $k_-$ evidence the non-essential contribution of the ester hydrolysis to the overall reaction rate.

By the reactivity in the water–2-propanol system the studied compounds fall to the following order:

$$\mathbf{I} > \mathbf{IIb} > \mathbf{Ia} > \mathbf{II} > \mathbf{IIa} > \mathbf{IIc}. \qquad (4)$$

This sequence is observed for all systems studied except for a solvent with $X_1 = 0.588$, in which the compounds **Ia** and **IIb** are interchanged, which may be due to their solvation at the high content of the organic component.

The position of amino acids and dipeptides in the series (4) corresponds to the sequence of their basicity. When $X_1 = 0.690–0.866$, log ($k_-$) and p$K_a$ values of protonated amino groups of dipeptides show a linear relationship (Fig. 1). A similar pattern is observed in the interaction of α-amino acids and dipeptides with benzoyl chloride in water–dioxane binary mixtures [1–3].

The energy ($E$) and activation entropy ($\Delta S^{\neq}$) of the reaction of picryl benzoate with **IIa** and **IIb** in aqueous 2-propanol calculated from the temperature dependence of $k_-$ are listed in Table 3. Within the error of determination, they are independent of the solvent composition. These values are consistent with the activation parameters of the reactions of α-amino acids with substituted phenyl benzoates [3–6], which indicates the generality of the mechanisms of interaction of esters with α-amino acids and dipeptides.

Note the complexity of relationships of the acylation rate constants $k_-$ and the water–2-propanol solvent composition, which has common features for all the studied reactions. With increasing mole fraction of water in the binary solvent from 0.6 to 0.7 the $k_-$ decreases. At further increase in $X_1$ there occurs some independence of the rate constants on the solvent composition, and at $X_1 > 0.85$ the value of $k_-$ increases. The dependence of this kind of the constants of acylation on the water fraction in the system cannot be understood in terms of nonspecific solvation. When the dielectric constant ε > 15, the specific solvation plays the major role, that is, the formation of molecular complexes of the reagents with components of the solvent is the most important.

It is known [3] that the rate of the N-acylation reaction depends primarily on the amine solvation, while the solvation of the acylating agent plays a secondary role. It can be assumed that the interaction of amino acids and dipeptides with picryl benzoate occurs in parallel processes with involvement of amino compound solvato complexes with different reactivity and different stability depending on the ratio of the components of the solvent. If we assume for simplicity that in a certain range of compositions of binary systems the main reacting forms are two solvato complexes with the stability constants $\beta_1$ and $\beta_2$, whose acylation rate constants are $k_1$ and $k_2$, we can show that $k_{-}$ is given by:

$$k_{-} = [k_2\beta_2 + (k_1\beta_1 - k_2\beta_2)X_1]/[(1 + \beta_2) + (\beta_1 - \beta_2)X_1], \quad (5)$$

where $X_1$ is the mole fraction of water in the solvent.

Equation (5) provides us the understanding of the dependence of $k_{-}$ on the water–2-propanol solvent composition obtained in the kinetic experiments: when $k_2\beta_2 > k_1\beta_1$, the $k_{-}$ decreases with increasing $X_1$; at $k_2\beta_2 = k_1\beta_1$, the $k_{-}$ is independent of $X_1$; when $k_2\beta_2 < k_1\beta_1$, the $k_{-}$ increases with $X_1$.

For a more detailed understanding of the influence of specific solvation on the reactivity of amino acids and dipeptides in the N-acylation, we carried out the quantum-chemical modeling of the solvation shell of the glycine anion in aqueous 2-propanol. The calculations were performed using a software package Firefly 7.1.G [15], method UHF/6-311++G** with full optimization of the system geometry. We calculated structural and energy characteristics of H-complexes of the anionic form of glycine with the components of a binary solvent: $(Gly^{-})\cdot(H_2O)_{10}$ (**IV**), $(Gly^{-})\cdot(H_2O)_{10}\cdot(i\text{-}PrOH)$ (**V**), $(Gly^{-})\cdot(H_2O)_8\cdot(i\text{-}PrOH)_2$ (**VI**), $(Gly^{-})\cdot(H_2O)_5\cdot(i\text{-}PrOH)_2$ (**VII**), $(Gly^{-})\cdot(H_2O)_3\cdot(i\text{-}PrOH)_3$ (**VIII**), and not solvated glycinate ion (**IX**).

Some simulation results are shown in Table 4. Figure 2 shows an example of the solvato complex **V**. The data in Table 4 indicate that all simulated structures are very stable, as evidenced by the energy of the complex formation ($\Delta E_c$), calculated as the difference between the total energy of a complex **IV–VIII** and the total energy of isolated molecules composing it. The strength of the complexes increases with the number of solvent molecules in the solvation shell, as indicated by the decrease of $\Delta E_c$ value.

**Table 3.** Kinetic parameters of reactions of **IIa** and **IIb** with picryl benzoate in the water-2-propanol solvent

| Dipeptide | Parameter | $X_1$ | | | | |
|---|---|---|---|---|---|---|
| | | 0.588 | 0.690 | 0.769 | 0.833 | 0.886 |
| **IIa** | $k_{-}^{a}$, l mol$^{-1}$ s$^{-1}$ | 2.88 | 2.57 | 2.59 | 2.67 | 4.14 |
| | $E$, kJ mol$^{-1}$ | 39 | 37 | 37 | 37 | 41 |
| | $-\Delta S^{\neq}$, J mol$^{-1}$ K$^{-1}$ | 122 | 129 | 129 | 128 | 115 |
| **IIb** | $k_{-}^{a}$, l mol$^{-1}$ s$^{-1}$ | 16.4 | 13.1 | 7.8 | 8.7 | – |
| | $E$, kJ mol$^{-1}$ | 38 | 37 | 30 | 39 | – |
| | $-\Delta S^{\neq}$, J mol$^{-1}$ K$^{-1}$ | 110 | 116 | 138 | 107 | – |

[a] At 318 K.

Effect of solvent on the kinetics of N-acylation is determined mainly by the amino group specific solvation. According to calculations, the formation of hydrogen bonds does not change the length of the covalent bonds in the N–H amino group, which are 1.002–1.003 Å for in all simulated compounds **IV–IX**. The amino group in solvato complexes **IV–VIII** can act both as H-acceptor, forming a hydrogen bond N···H with components of a binary solvent by an unshared electron pair of the nitrogen atom, and as H-donor, interacting with the oxygen atoms of water or alcohol (O···H). It should be noted that in all complexes the length of the hydrogen bond O···H significantly exceeds that of the N···H bond (Table 4), indicating the greater strength of the latter.

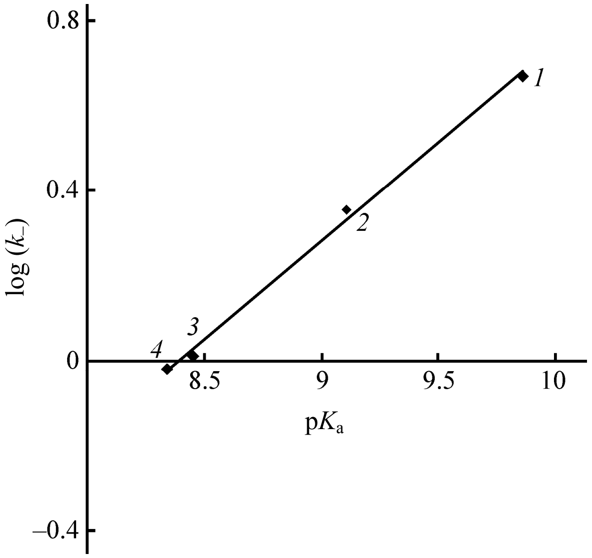

**Fig. 1.** Dependence of reaction rate constants of picryl benzoate with dipeptides on the basicity of the latter. (*1*) **IIb**, (*2*) **II**, (*3*) **IIa**, (*4*) **IIc**. Solvent: water ($X_1$ = 0.769)–2-propanol, 298 K.

**Table 4.** Electronic and energy characteristics of the glycinate ion solvato complexes calculated by UHF/6-31++G** method

| Complex, ($X_2$)[a] | $-\Delta E_c$, kJ mol$^{-1}$ | $-E_{\mathrm{HOMO}}$, eV | $\varphi p_z$(N) | $Cp_z$(N) | $-q(\mathrm{NH_2})$, au | $-q$(N), au | $r_{\mathrm{N\cdots H}}$, Å | $r_{\mathrm{O\cdots H}}$, Å |
|---|---|---|---|---|---|---|---|---|
| **IV**<br>(1.00) | 441 | 8.02 | 1.382 | 0.552 | 0.124 | 0.751 | 2.050 | 2.722;<br>3.001 |
| **V**<br>(0.909) | 456 | 8.21 | 1.251 | 0.595 | 0.184 | 0.778 | 1.971[б] | 2.867;<br>2.685 |
| **VI**<br>(0.800) | 414 | 7.83 | 1.304 | 0.583 | 0.278 | 0.773 | 1.975[б] | 2.818[b];<br>2.686 |
| **VII**<br>(0.714) | 317 | 7.126 | 1.262 | 0.528 | 0.310 | 0.774 | 1.989[б] | 2.788[b];<br>2.668 |
| **VIII**<br>(0.500) | 215 | 6.64 | 1.443 | 0.538 | 0.357 | 0.815 | 1.961[б] | 2.269[b];<br>2.820[b] |
| **IX** (0) | – | 5.01 | 1.249 | 0.453 | 0.050 | 0.514 | – | – |

[a] Mole fraction of water in the solvation shell. [b] H-bond with alcohol molecule.

The formation of H-complexes **IV–VIII** leads to a significant decrease in the charge on the nitrogen atom $q$(N) and the whole amino group $q(\mathrm{NH_2})$ compared with non-solvated glycine **IX** anion. The decrease in the water content in the composition of the solvation shell significantly lowers $q$(N) and monotonically decreases $q(\mathrm{NH_2})$ (Table 4), which can increase the reactivity of the amino group in the acylation. However, this change in charge characteristics cannot explain the complicated dependence of the acylation constants on the solvent composition.

Specific solvation lowers the energy of the HOMO of glycinate ion (Table 4). The dependence of $E_{\mathrm{HOMO}}$ of the solvato complexes on the mole fraction of water in the solvation shell is similar to the dependence of the rate constants of acylation of glycine $k_-$ on the solvent composition (Fig. 3). The dependence of the contributions of $2p_z$-AO of the nitrogen atom $Cp_z$(N) in the HOMO on $X_2$ is also complicated, but the $Cp_z$(N) values vary oppositely to $k_-$. The established facts suggest that the reactivity of solvato complexes of the glycinate ion is largely determined by their orbital characteristics and confirm our previous [8, 9] assumption about the orbital control of the reactions of α-amino acids with esters in aqueous-organic media.

The simulation results are consistent with the conclusion based on the kinetic data that in the binary solvent of a certain composition there are the solvato complexes of different structure, for example, **IV–VIII**, differing in the reactivity in the N-acylation. The increase in the water content in the solvent displaces the existing balance in the system, leading to increased

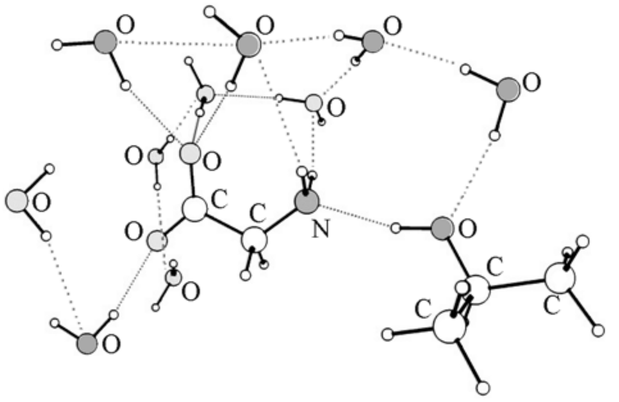

**Fig. 2.** Structure of solvato complex **V**.

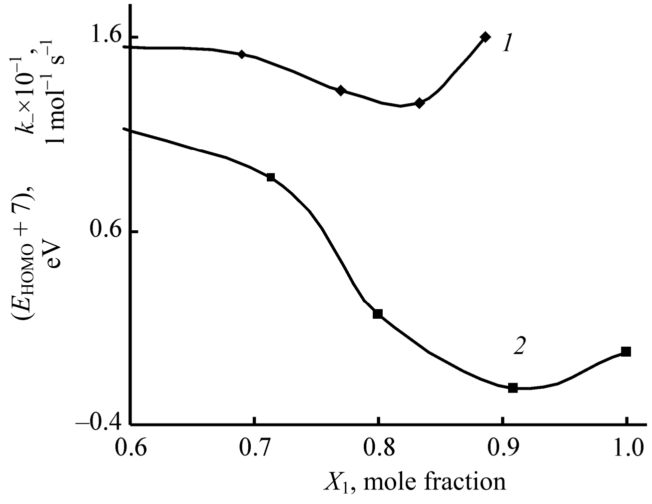

**Fig. 3.** Dependence of the acylation rate constant $k_-$ of glycinate ion (*1*) and $E_{\mathrm{HOMO}}$ of the solvato complexes (*2*) on the mole fraction of water in the solvent $X_1$.

concentration of solvates with high water content. In the mixed complexes the energy of the HOMO diminishes, the population of $2p_z$-AO of nitrogen $\varphi p_z$(N) decreases, and their contribution to the HOMO $Cp_z$(N) increases (Table 4).

These changes reduce the nucleophilicity of the nitrogen atom and the reactivity of solvato complexes, which is reflected in the reduction of $k_{-}$ with increasing concentration of water in the solvent $X_1$ in the range 0.588–0.833. Further increase in the water content in isopropanol leads to an increase in the proportion of hydrates, for example, **IV**. The increase in $E_{\mathrm{HOMO}}$ and $\varphi p_z$(N) occurring under these conditions is consistent with an increase in the rate constant of acylation of glycinate ion with increasing mole fraction of water in the system to 0.886 (Table 2).

Thus, we can conclude that the experimental rate constants of the reaction of amino acids and dipeptides with picryl benzoate $k_{-}$ are apparent values, which include the acylation constants of the solvato complexes of different structure. The nonmonotonic dependence of $k_{-}$ on the composition of binary solvent water–2-propanol is due to the competitive nature of the solvation of amino compounds. The data of quantum-chemical modeling suggest the decisive contribution of the orbital interactions in the reactivity of the amino acid and dipeptide solvato complexes in the N-acylation.

## EXPERIMENTAL

The rate of interaction was measured by the spectrophotometric method observing the changes in the transmission of the light flux through the colored solution (λ = 400 nm) using photoelectrocolorimeter KFK-2 equipped with a digital voltmeter V7-38. For measurements a thermally controlled cell was used, where the temperature was maintained within ±0.1 K. The observed rate constants $k_{\mathrm{obs.}}$ were calculated with the Guggenheim equation.

All physicochemical constants of the reagents and solvents used in the study are consistent with published data [10].

## ACKNOWLEDGMENTS

This work was supported by the Ministry of Education and Science (project RNP.2.2.1.1/11465).

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