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Effect of pH on the Reactivity of Dipeptides and α-Amino Acids in the N-Acylation

L. V. Kuritsyn, N. V. Kalinina, and L. B. Kochetova

Ivanovo State University, ul. Ermaka 39, Ivanovo, 153025 Russia
e-mail: kochetova_lb@mail.ru

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Abstract—The reaction kinetics of Gly, L-α-Ala, Gly-Gly, L-α-Ala-L-α-Ala and β-Ala-β-Ala with picryl benzoate in water (40 wt %)–2-propanol was investigated. At pH = 4–8 the rate constants of N-acylation of the anionic form of dipeptides are less than those of the corresponding amino acid anions, in agreement with their basicity, whereas the relative effective rate constants of reactions depend on pH: in acidic, neutral and slightly alkaline media the k_{ef} values are higher for the dipeptides, and in a strongly alkaline medium, for the amino acids. These differences are due to the changes in the concentrations of reactive forms of amino acids and dipeptides in the system at varying the medium pH.

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Over the years we have studied the kinetics of N-acylation of α-amino acid with the derivatives of aromatic carboxylic acids in aqueous-organic solvents [1–10]. In continuation of these studies we aimed to study the kinetics of reactions Gly (**I**), Gly–Gly (**II**), L-α-Ala (**Ia**), L-α-Ala–L-α-Ala (**IIa**) and β-Ala–β-Ala (**IIb**) with picryl benzoate in the solvent water (40 wt %) – 2-propanol.

The studied reaction proceeds according to Eq. (1):

$$C_6H_5COOC_6H_2(NO_2)_3 + NH_2RCOO^- \xrightarrow{k_-} C_6H_5CONHRCOO^- + HOC_6H_2(NO_2)_3, \quad (1)$$

where R is the substituent in compounds **I**, **II**, **Ia**–**IIb**; k_- is the rate constant of the N-acylation of α-amino acid (dipeptide) anion.

The reaction rate was monitored spectrophotometrically (λ = 400 nm). The conditions of kinetic experiments were chosen in order to provide only one reactive form of α-amino acid or dipeptide in solution: the anionic form. We have previously shown that at the concentration ratio of zwitter-ions and anions of α-amino acid $c_\pm/c_-$ in the range of 4–10 the conditions are provided under which the rate of hydrolysis of the ester can be neglected [2–4]. Therefore, a known amount of alkali was added to the amino acid (dipeptide) solution. The reaction was performed in the first-order conditions with 10^2–10^3 times excess of amino compound concentration over the ester. The observed first-order reaction rate constant k_{obs} was calculated by the method of Guggenheim. The second order reaction rate constants k_- were calculated according to Eq. (2):

$$k_- = k_{obs}/k_- \quad (2)$$

The equations of the relationship between the rate constants k_{obs} and k_- of the α-amino acid reactions with esters are presented in [2–4].

The k_{obs} and k_- values of the studied reactions, as well as the activation energy and entropy E_- and $\Delta S^{\neq}$, respectively, of the picryl benzoate reaction with anionic forms **IIa** and **IIb**, calculated from the temperature dependence of the rate constants k_-, are presented in Table 1. The data in Table 1 indicate the relationship of basicity of the compounds studied to their reactivity in the N-acylation, which is manifested in the decrease in the acylation rate constant k_- of dipeptides in comparison with the corresponding amino acids. A similar pattern is observed in the interaction of α-amino acids and dipeptides with benzoyl chloride in binary mixtures of water with dioxane [1, 3, 6]. However, the basicity of the amino group of dipeptides is not the only factor governing their reactivity in the N-acylation.

The properties of α-amino acids and dipeptides in solution largely depend on their acid–base interactions with the components of the medium. The protonation

Table 1. Kinetic parameters of N-acylation of anionic forms of amino acids and dipeptides with picryl benzoate in the water–2-propanol solvent at 298 K[a]

Parameter	Gly	α-Ala	Gly-Gly	α-Ala-α-Ala	β-Ala-β-Ala
$k_{obs}\times10^2$, s^{-1}	2.19±0.04	1.69±0.05	1.07±0.09	0.366±0.007	1.85±0.03
k_-, l mol^{-1} s^{-1}	15.1±0.3	4.7±0.1	3.0±0.2	1.01±0.02	5.10±0.08
	α-Ala-α-Ala			β-Ala-β-Ala	
k_{-318}, l mol^{-1} s^{-1}	2.57		k_{-318}, l mol^{-1} s^{-1}	13.1	
E_-, kJ mol^{-1}	37		E_-, kJ mol^{-1}	37	
$-\Delta S^{\neq}$, J mol^{-1} K^{-1}	129		$-\Delta S^{\neq}$, J mol^{-1} K^{-1}	116	

[a] c_{total} = 0.022 M, c_- = 0.00362 mol l^{-1}, $c_{picryl\ benzoate}$ = 2×10^{-5} M, the mole fraction of water in solvent $X(H_2O)$ = 0.690.

and deprotonation of functional groups most strongly affect the reactivity. It is known [1–9] that the protonated amino group of α-amino acids, as well as other aliphatic and aromatic amines, is inert in the N-acylation, therefore in reactions with acylating agents only two of the four forms of α-amino acids in the solution can be involved: uncharged and anionic. Zwitterions and cations are not active in these reactions. It was also established that in aqueous-organic media the rate of reaction of acylating agents with anions of α-amino acids is significantly higher than with non-ionized molecules.

The main factor determining the concentrations of various ionic forms of amino acids and dipeptides in solution is the acidity. By varying the pH, the concentration of reactive species that determine the effective rate of acylation [3, 5, 10] can be changed by several orders of magnitude, and therefore it is of interest to establish the dependence of the effective kinetic parameters of the interaction of amino acids and dipeptides with picryl benzoate on the pH of the solution.

The equation of reaction rate constant [Eq. (1)] can be written as [Eq. (3)]:

$$-dc/d\tau = k_{ef}c_{total}c_{picryl\ benzoate} = k_{obs}c_{picryl\ benzoate}, \quad (3)$$

where c_{total} is the total concentration of all forms of amino acid (dipeptide) in solution, $c_{total} = c_\pm + c_0 + c_- + c_+$; $c_\pm$, c_0, c_- and c_+ are the concentrations of a zwitterionic, uncharged, anionic, and cationic forms, respectively; $c_{picryl\ benzoate}$ is the concentration of picryl benzoate; k_{ef} is the effective rate constant.

It follows from Eqs. (2) and (3) that the relationship between k_{ef} and k_- is defined by the Eq. (4):

$$k_{obs} = k_-\, c_- = k_{ef}c_{total}, \quad (4)$$

which shows that the k_{ef} is the observed rate constant divided by the total concentration of amino acid (dipeptide) [Eq. (5)]:

$$k_{ef} = (c_-/c_{total})k_- = \alpha_- k_-. \quad (5)$$

Here $\alpha = c_-/c_{total}$ is the fraction of anions in the total concentration of amino acid (dipeptide) in solution.

According to [3], at the change of pH in the range of 4–12 the fraction of the neutral molecules and cations of α-amino acid in water–isopropanol solvent containing more than 10 wt % of water is negligible, so we may assume that $c_0 + c_+ = 0$, then $c_{total} = c_\pm + c_-$. It can be shown [2–5] that under these conditions the k_{ef} is related to pH in accordance with Eq. (6):

$$k_{ef} = k_- K_a/(K_a + c_{H^+}), \quad (6)$$

where K_a is the acid dissociation constant of the protonated amino acids (dipeptide).

While accounting for Eq. (6) and using Eqs. (7), (8), we can calculate the effective values of the energy and changes in the activation entropy, E_{ef} and $\Delta S_{ef}^{\neq}$ for different pH:

$$E_{ef} = E_- + \Delta H_a c_{H^+}/(K_a + c_{H^+}), \quad (7)$$

$$\Delta S_{ef}^{\neq} = R\ln(k_{ef}h/k_BT) + E_{ef}/T. \quad (8)$$

Here ΔH_a is the enthalpy of dissociation of the protonated amine, h is the Planck constant, k_B is the Boltzmann constant.

Analysis of Eq. (6) shows that the dependence of log k_{ef} on pH is different for different intervals of acidity:

(a) at pH = 4–8, where $K_a << c_{H^+}$, the relationship is linear with the slope equal to 1:

$$\log k_{ef} = \log(k_- K_a) + \mathrm{pH}; \quad (9)$$

(b) at pH = 8–11, the values K_a and c_{H^+} are comparable, and Eq. (6) has the form:

Table 2. Effective kinetic parameters of the reactions of dipeptides with picryl benzoate in the water (40 wt %)–2-propanol solvent at 298 K

pH	4	5	6	7	8	9	10	11
				α-Ala-α-Ala				
$-\log k_{ef}$	4.45	3.45	2.45	1.46	0.578	0.104	0.008	0.003
E_{ef}	84	84	84	82	71	47	38	37
$-\Delta S^{\neq}_{ef}$	48	30	11	–2.9	16	89	118	121
				β-Ala-β-Ala				
$-\log k_{ef}$	5.15	4.15	3.15	2.15	1.16	0.209	–0.471	–0.677
E_{ef}	74.5	74.5	74.5	74.5	74.0	69.9	52.5	39.1
$-\Delta S^{\neq}_{ef}$	94	74	55	36	19	14	60	101

Table 3. The ratio of k_{ef} of dipeptides and the corresponding amino acids

$\gamma_{dip/am\text{-}acid}$	pH									γ_k	γ_t
	4	5	6	7	8	9	10	11	12		
$\gamma_{II/I}$	9.8	9.8	9.5	9.2, 8.8[a]	6.6, 1.7[b]	1.8	0.39	0.22	0.20	0.195, 0.176[a], 0.063[b]	50, 50[a], 27[b]
$\gamma_{IIa/Ia}$	24	24	24	23, 16[a]	18	5.4	0.86	0.28	0.22	0.22, 0.03[a]	110, 16[a]

[a] γ, γ_k and γ_t were calculated from data in [3, 6]. [b] For the calculation the data of [11] were used.

$$\log k_{ef} = \log (k_{-}K_a) - \log (K_a + c^{+}_{H}); \quad (10)$$

(c) at pH = 11–13, $K_a >> c^{+}_{H}$, thus:

$$\log k_{ef} = \log k_{-}. \quad (11)$$

The results of calculation of the effective values of the rate constants k_{ef}, the activation energy E_{ef}, and effective changes in the entropy of activation $\Delta S^{\neq}_{ef}$ for the reactions of picryl benzoate with **IIa** and **IIb** by Eqs. (6)–(8) are presented in Table 2. These data show that at pH < pK_a the k_{ef} grows with increasing pH value, and in an alkaline medium at pH > pK_a it tends to become constant. The values of E_{ef} decrease with increasing pH, and in the region close to the pK_a a sharp decrease occurs in E_{ef} , from ~ 80 kJ mol^{-1} to ~40 kJ mol^{-1}, $\Delta S^{\neq}_{ef}$ dependence on pH is rather complex, and passes through a maximum.

It must be borne in mind that all these changes of effective kinetic parameters are associated not with the changes in the acylation mechanism but rather with the acid–base equilibria in the solutions of dipeptides determining the concentration of their reactive forms.

The ratio of effective rate constants of acylation of dipeptides $k_{ef\ dip}$ and the corresponding amino acids $k_{ef\ am\text{-}acid}$ can be described using a coefficient γ:

$$\gamma = k_{ef\ dip}/k_{ef\ am\text{-}acid}. \quad (12)$$

Table 3 shows the values of γ for the reaction of **I**, **II**, **Ia**, and **IIa** with picryl benzoate at different pH calculated according to Eq. (12). As seen, the value of γ depends on the acidity: in acidic and nearly neutral media $\gamma > 1$, that is, dipeptides are more reactive; in the alkaline medium the amino acids are more active in the acylation ($\gamma < 1$). The pH value at which the inversion of this ratio occurs (pH_{inv}) is given by Eq. (13):

$$pH_{inv} = pK_{a\ dip} - \log (\gamma - 1) \quad (13)$$

At pH = 4–8, in accordance with Eqs. (9), (12), the parameter γ can be represented as follows:

$$\gamma = (k_{-dip}/k_{-am\text{-}acid})\cdot(K_{a\ dip}/K_{a\ am\text{-}acid}) = \gamma_k\gamma_t. \quad (14)$$

The value of γ_k in the expression (14) characterizes the contribution of kinetic factors in the reactivity of the amino acids and dipeptides, and the parameter γ_t can be regarded as the thermodynamic factor. The values of γ_k and γ_t for the reactions of **I**, **II**, **Ia**, and **IIa** with picryl benzoate, as well as with benzoyl chloride in aqueous dioxane [6] and *N*-acetyloxysuccinimide in water [11] are presented in Table 3. Analysis of the data in Table 3 shows that the kinetic factor is less than unity in all the reactions, while the thermodynamic factor contributes significantly more to the effective rate of acylation. We can assume that γ_t is much larger than γ_k also in the reactions of amino acids and

oligopeptides with other acylating agents, which under certain conditions may cause an increase in the effective rate of acylation with increasing length of the peptide chain. The k_{ef} may growth due to the growth of γ_t which occurs due to the decrease in pK_a of terminal amino groups. In particular, in the series (15) the pK_a values decrease as follows: 9.6, 8.13, 7.91, and 7.75, respectively [12–14], which leads to an increase in γ_t. Therewith the values of k_- and γ_k should decrease due to diminishing basicity, but, owing to the predominance of the thermodynamic factor over the kinetic, the effective rate constant k_{ef} may increase. Similar considerations are applicable in the case of α-alanine and tri- and tetrapeptides.

Gly, Gly–Gly, Gly–Gly–Gly (**III**),
Gly–Gly–Gly–Gly (**IV**). (15)

We performed quantum-chemical simulation of the anionic forms of compounds **I–IV**, **Ia**, **IIa**, as well as L-α-Ala-L-α-Ala-L-α-Ala (**IIIa**) and L-α-Ala-L-α-Ala-L-α-Ala-L-α-Ala (**VIa**).The calculations were carried out with the software package Firefly 7.1.G [15], by the method of UHF/6-31++G** with full geometry optimization of the system. Some simulation results are shown in Table 4.

Comparison of the results for **I–IV** and **Ia–IVa** with published data on the dissociation constants of protonated amino groups of these compounds in water [12–14] indicates the existence of linear dependence between the values of pK_a and charges on the amino groups and $q(NH_2)$ (Fig. 1) . The charges on nitrogen atoms q_N of terminal amino groups in **I–IV**, **Ia–IVa** also correlate with the pK_a values. The dependence shows that the thermodynamic component of the effective rate of acylation γ_t is related to the charge characteristics of amino groups.

Therewith, the effect of the charge characteristics on the rate of reaction (1) cannot be completely excluded, as seen from the influence of basicity on the k_-. However, in several studies [7–9] it has been shown that the reactions of α-amino acids with esters [3] proceed predominantly under the orbital control. Table 4 shows calculated energies of frontier orbitals of the nucleophiles **I–IV**, **Ia–IVa** and the acylating agent picryl benzoate. The comparison of these values suggests a possibility of orbital control also in the reactions of dipeptides with picryl benzoate. A decrease in the energy of HOMO of the dipeptides **II** and **IIa** compared with the E_{HOMO} of amino acids **I** and **Ia** is consistent with the experimentally established decrease in k_- in going from the amino acids to the corresponding dipeptides (Table 1).

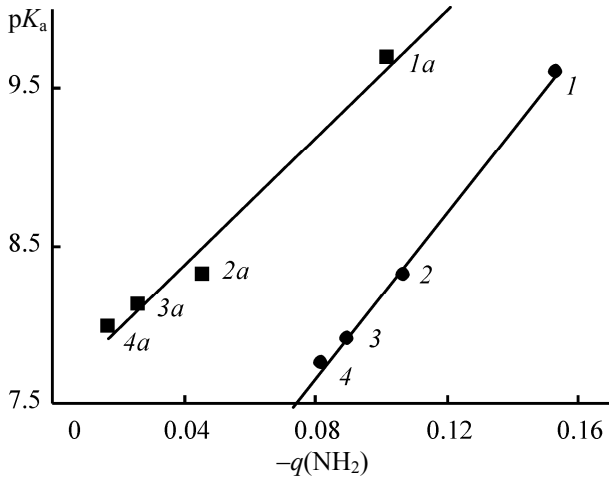
Table 4. Electronic and energy characteristics of the amino acids and oligopeptides anions calculated by the UHF/6-31++G** method

Compound	$-q_N$, au	$-q(NH_2)$, au	$-E_{HOMO}$, eV	E_{LUMO}, eV
I	0.735	0.153	5.034	5.524
II	0.707	0.107	6.013	4.435
III	0.696	0.090	6.231	3.782
IV	0.690	0.082	6.340	3.401
Ia	0.685	0.102	5.034	5.279
IIa	0.646	0.046	6.068	4.490
IIIa	0.631	0.026	6.313	3.837
IVa	0.624	0.017	6.286	3.428
Picryl benzoate	–0.785[a]	–	0.354	0.271

[a] The charge on the carbonyl C atom.

In this regard, we can assume that the kinetic component of the effective rate of acylation of dipeptides γ_k characterizes both the interaction of molecular orbitals and the charge on the amino group. In this case, as suggested above, the values of k_- will decrease in going from **I** to **IV** and from **Ia** to **IVa** due to the decrease in E_{HOMO} and also as a result of reducing negative charge on the amino group (Table 4).

The results of our study give some idea concerning the acylation rate. The rate constant k_- of the acylation



The plots of pK_a of the protonated amino groups of amino acids and peptides [12–14] vs. the charge on the amino group $q(NH_2)$: (*1*) I, (*2*) **II**, (*3*) III, (*4*) **IV**, r = 0.999, (*1a*) **Ia**, (*2a*) **IIa**, (*3a*) IIIa, (*4a*) **IVa**, r = 0.985.

of reactive forms of amino acid with esters is much higher than the k_- of the corresponding dipeptides, which is consistent with the higher basicity of amino acids and charge characteristics of their amino groups. The decrease in the basicity of the peptides in the series (15) due to reduced negative charge on the amino group and a decrease in E_{HOMO} suggests that the rate constant k_- of the acylation of peptide anionic forms **II–IV**, **IIa–IVa** should decrease with increasing length of the peptide.

Effective kinetic parameters of reactions of the amide (peptide) bond are defined by the medium acidity: increase in pH in the range 4–9 reduces by 4–5 orders of magnitude the effective rate constants k_{ef} of dipeptide acylation. The ratio of effective rate constants of amino acids acylation and of the corresponding dipeptides also is pH-dependent: in acidic, neutral and slightly alkaline media the k_{ef} of dipeptides are higher compared with amino acids, while in strong alkaline media the situation is reversed.

Note that all the above mentioned changes in the effective kinetic parameters of acylation are due to the changes in the concentration of reactive forms of amino acids and dipeptides in the system at varying the pH of the medium.

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