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Kinetics of N-Acylation of Glycine and *L*-Proline with 4-Nitrophenyl 4-Nitrobenzoate in Aqueous-Organic Solutions

L. V. Kuritsyn, A. Yu. Lebedukho, and A. I. Sadovnikov

Ivanovo State University, Ivanovo, Russia

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Abstract—Kinetic characteristics (rate constants and activation energies and entropies) are determined for N-acylation of glycine and *L*-proline with 4-nitrophenyl 4-nitrobenzoate in water–acetonitrile, water–2-propanol, and water–2-methyl-2-propanol systems. With increasing water content in the system, the N-acylation rate constants increase and the activation energy and entropy decrease, which is due to features of solvation of the reagents and activated complex with aqueous-organic solvents.

Previously we studied the kinetics of N-acylation of α-amino acids and dipeptides with benzoyl chloride in aqueous 1,4-dioxane [1–4]. Hirata and Nakasato reported on N-acylation kinetics of α-amino acids with *N*-acetoxysuccinimide in water [5]. Data on N-acylation of α-amino acids with carboxylic acid esters are practically lacking. Evidently, this is due to some experimental difficulties caused by low solubility of α-amino acids in organic solvents, and also by the occurrence of side reactions in aqueous-organic solvents at pH >9. In particular, the rate of hydrolysis of esters is comparable with and sometimes higher than that of N-acylation of α-amino acids. We have developed a method for studying the kinetics of N-acylation of α-amino acids with esters in aqueous-organic solvents, allowing these difficulties to be overcome.

In this work we studied the kinetics of N-acylation of glycine (Gly) and *L*-proline (Pro) with 4-nitrophenyl 4-nitrobenzoate (**I**) in aqueous-organic solvents [water–acetonitrile (**A**), water–2-propanol (**B**), and water–2-methyl-2-propanol (**C**)]. It was demonstrated in a series of works [6–8] that the zwitterionic form of α-amino acids dominates in aqueous and aqueous-organic solutions. Only anionic and neutral forms can be N-acylated with carboxylic acid esters. At various pHs, one or another form of an α-amino acid dominates; for example, at pH 8–9, the equilibrium shifts towards the anionic form, but, in this case, the ester is hydrolyzed more readily. By the essence, the solution of the zwitterionic and anionic forms of α-amino acids is a buffer solution. Therefore, by adjusting the ratio between these forms, one can reduce pH, thus reducing hydrolysis of an ester. The concentrations of the forms of α-amino acids are controlled by the equilibrium constants and solution pH. They can be estimated by the equations derived in [9] on the assumption that the concentration of the neutral form is negligible. We have demonstrated that hydrolysis of an ester in aqueous-organic media can be neglected if the anionic and zwitterionic forms are taken in the ratio of 1/5 and 1/10 for Gly and Pro, respectively. N-Acylation reactions of Gly and Pro can be written as follows.

$$\mathbf{I} + \mathrm{Gly}^- \xrightarrow{\mathrm{OH}^-} \mathrm{Gly\text{-}COC_6H_4NO_2\text{-}4} + \mathrm{4\text{-}NO_2C_6H_4O^-}, \quad (1)$$

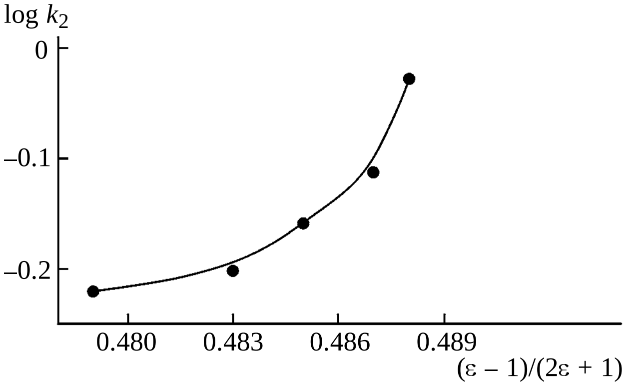
$$\mathbf{I} + \mathrm{Pro}^- \xrightarrow{\mathrm{OH}^-} \mathrm{Pro\text{-}COC_6H_4NO_2\text{-}4} + \mathrm{4\text{-}NO_2C_6H_4O^-}. \quad (2)$$

In studying the kinetics of N-acylation of Gly and Pro, the amino acids were taken in considerable (10^2–10^3) excess relative to the ester. The concentrations of the anionic forms of the amino acids were $(2–4)\times 10^{-3}$ and $(8–9)\times 10^{-4}$ M for Gly and Pro, respectively. The first-order rate constants k_1 were estimated by the Guggenheim method, and the second-order constants k_2, by the equation $k_2 = k_1/\bar{c}$, where $\bar{c}$ is the concentration of the anionic form of an amino acid. Kinetic characteristics of Gly and Pro N-acylation with ester **I** in aqueous-organic solvents are summarized in the table. As seen, with increasing water content, the N-acylation rate constants increase and the activation energy and entropy decrease, suggesting the occurrence of a compensation effect.

The increase in the reaction rate constant with increasing water content in the aqueous-organic solvent is caused by formation of activated complexes

Kinetic characteristics of Gly and Pro N-acylation with ester **I** in systems **A**–**C**

H_2O content, wt%	T, K	Gly			Pro		
		k_2, l mol^{-1} s^{-1}	E_a, kJ mol^{-1}	$-\Delta S^{\neq}_{298}$, J mol^{-1} K^{-1}	k_2, l mol^{-1} s^{-1}	E_a, kJ mol^{-1}	$-\Delta S^{\neq}_{298}$, J mol^{-1} K^{-1}
A							
40	298	0.19	55.1 ± 1.3	82.3 ± 4.4	1.12	48.9 ± 2.1	88.7 ± 6.7
	308	0.41			2.02		
	318	0.78			3.86		
50	298	0.22	51.1 ± 0.5	95.0 ± 1.7	1.24	45.9 ± 1.4	96.6 ± 4.5
	308	0.42			2.20		
	318	0.79			4.01		
60	298	0.29	48.5 ± 1.5	101.2 ± 5.1	1.29	43.5 ± 2.4	102.6 ± 7.6
	308	0.55			2.15		
	318	0.98			3.86		
70	298	0.37	43.1 ± 1.3	117.1 ± 4.4	2.02	42.3 ± 0.8	105.7 ± 2.7
	308	0.66			3.46		
	318	1.09			5.92		
80	298	0.38	39.7 ± 0.5	134.3 ± 1.7	4.33	40.2 ± 2.5	108.9 ± 2.5
	308	0.64			7.20		
	318	1.19			11.99		
B							
40	298	0.61	45.2 ± 1.8	124.3 ± 1.6	4.73	37.1 ± 0.6	116.1 ± 2.0
	308	1.03			7.28		
	318	1.64			12.12		
50	298	0.63	40.4 ± 0.3	130.9 ± 1.2	4.99	36.0 ± 1.4	119.3 ± 4.6
	308	1.06			7.87		
	318	1.73			13.37		
60	298	0.70	38.6 ± 1.4	135.4 ± 2.2	5.21	35.0 ± 1.7	122.3 ± 5.5
	308	1.12			8.02		
	318	1.85			13.60		
70	298	0.77	36.1 ± 1.7	142.9 ± 4.0	6.00	34.2 ± 0.8	123.9 ± 2.4
	308	1.18			9.36		
	318	1.93			15.19		
80	298	0.94	34.4 ± 1.1	146.2 ± 3.3	6.94	32.9 ± 0.3	127.0 ± 1.0
	308	1.55			10.61		
	318	2.26			16.00		
C							
40	298	0.60	44.8 ± 2.7	107.3 ± 8.8	4.67	38.3 ± 1.8	111.9 ± 5.9
	308	1.16			8.06		
	318	1.86			12.40		
50	298	0.62	41.9 ± 1.9	116.9 ± 6.3	4.84	36.6 ± 0.6	117.3 ± 2.0
	308	1.09			8.08		
	318	1.76			12.51		
60	298	0.64	37.9 ± 1.2	130.1 ± 3.9	5.02	35.5 ± 1.7	119.2 ± 5.7
	308	1.03			9.50		
	318	1.61			14.24		
70	298	0.67	36.4 ± 1.4	134.9 ± 4.6	5.86	34.9 ± 1.5	121.1 ± 5.0
	308	1.03			9.94		
	318	1.65			14.76		
80	298	0.81	33.9 ± 0.4	141.6 ± 1.2	6.58	32.8 ± 0.6	127.8 ± 2.1
	308	1.25			10.24		
	318	1.92			15.11		



Rate constant (log k_2) of Gly N-acylation with ester **I** in system **B** at 298 K vs. Kirkwood function.

of the α-amino acids and ester **I** with water molecules through hydrogen bonding, thus decreasing the activation energy. In its turn, H bonding makes the structure of the solvated activated complex more regular, resulting in increased negative activation entropy.

The table shows that the activation energy is higher in system **A**, which can be attributed to the features of solvation of the reagents with the nonaqueous component of a binary solvent. It should be pointed out also that the Pro N-acylation rate constants in all the solvents are considerably higher as compared to Gly. This can be explained by higher basicity of Pro (pK_a^{Gly} 9.6, pK_a^{Pro} 10.6) [9]. The Gly and Pro N-acylation rate constants in system **A** are considerably lower than those in systems **B** and **C**, the latter two being practically equal. This is probably due to the presence of hydroxy groups in the alcohol molecules, which are capable of forming hydrogen bonds with the reagents, thus enhancing the amino acid reactivity.

The Gly acylation rate constant in system **B** is plotted in the figure vs. the Kirkwood function. Similar dependences were observed in all the solvents studied. The observed nonlinearity of the log k_2–(ε – 1)/(2ε + 1) plot (ε is the dielectric constant of the solvent [10]) suggests the effect of other solvent parameters, such as polarizability, donor–acceptor characteristics, etc., on the rate constant [11].

EXPERIMENTAL

In the work we used glycine and *L*-proline of analytical grade. The Gly and Pro N-acylation rates were determined spectrophotometrically from the variation in the 4-nitrophenolate concentration (λ = 400 nm). In measurements we used a KFK-2UKhL 4.2 photoelectric colorimeter equipped with a thermostatically controlled cell holder and a Shch-300 digital voltmeter. Acetonitrile (analytical grade) was distilled from P_2O_5. 2-Propanol (chemically pure grade) was distilled on a column. 2-Methyl-2-propanol (chemically pure grade) was purified according to the procedure described in [12]. Reaction mixtures were prepared from saturated NaOH (analytical grade), Gly, and Pro. The apparent rate constants (k_1) were estimated by the Guggenheim method to within 1–2% (at a 0.95 confidence level).

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