Preferential Formation of Amino Acid Esters in Aqueous Alcohol Solutions: Solvolysis of 6,6′-Bis(aminoacylamino)-2,2′-bipyridine by Metal Coordination

Koji Araki,* Takashi Kuboki, Masaki Yamada and Shinsaku Shiraishi

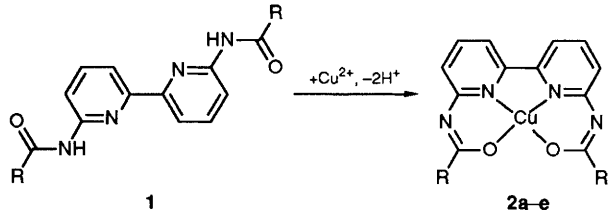
Institute of Industrial Science, The University of Tokyo, 7-22-1 Roppongi, Minato-ku, Tokyo 106, Japan

Exclusive formation of amino acid esters took place at an appreciable rate in the Cu^{II}-catalysed solvolysis of 6,6′-(α-alanylamino or α-phenylalanylamino)-2,2′-bipyridine in alcohol–borate buffer (pH 7.2) solutions at 20 °C *via* formation of an amide-*O*-coordinated complex, even though appreciable amounts of water (0–40% v/v) were present in the solution.

Enzymatic aminoacylation of hydroxy groups is one of the essential reactions in biological systems, but studies on non-enzymatic systems are limited mostly to hydrolysis reactions of amide bonds. Among the hydrolysis of amide bonds, metal-catalysed hydrolysis of peptide bonds has been extensively studied because of its biological importance,[1,2] although most of them require higher temperature or basic conditions to attain an appreciable reaction rate.[1] We report here the unprecedented formation of amino acid esters in aqueous (pH 7.2) alcohol solutions at 20 °C by metal-catalysed alcoholysis of amide bonds between amino acids and 6,6′-diamino-2,2′-bipyridine (dabp). The reaction proceeded at an appreciable rate *via* complex formation with divalent metals, and was quite specific to α-aminoacyl derivatives.

6,6′-Bis(acylamino)-2,2′-bipyridines, where acyl groups are α-alanyl **1a**, phenylalanyl **1b**, β-alanyl **1c**, 4-aminobutanoyl **1d** and propanoyl **1e**, were prepared from dabp and *N*-Z-protected amino acids.† The solvolysis reactions were carried out in alcohol–borate buffer (0.2 mol dm^{-3}, pH 7.2) systems at 20 °C.

In borate buffer, a deprotonated-type complex **2a** was formed quantitatively upon addition of $CuCl_2$ to **1a** (1×10^{-4} mol dm^{-3}, Cu/**1a** molar ratio = 0.05–0.5) (Scheme 1).[3] The electronic spectrum of the solution changed gradually in two



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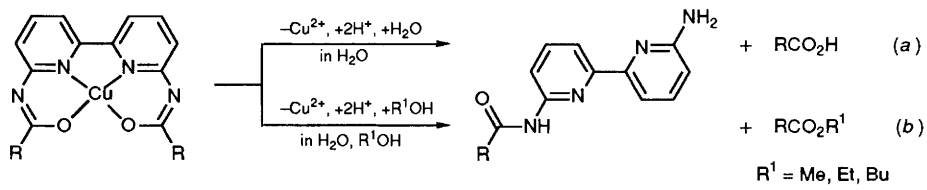
a; R = $NH_2CH(Me)$
b; R = $NH_2CH(CH_2Ph)$
c; R = $NH_2(CH_2)_2$
d; R = $NH_2(CH_2)_3$
e; R = Et

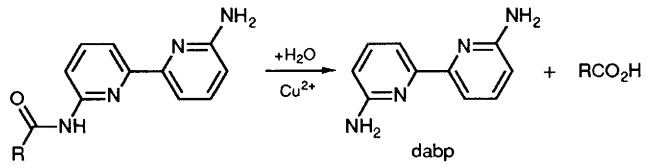
2a–e

Scheme 1

† Compositions and structures of **1a–1e** were confirmed by satisfactory analytical data, IR, and ^1H and ^{13}C NMR spectra.



Scheme 2



Scheme 3

Table 1 Rate constants (k_{obs}) of solvolysis reactions in aqueous alcohol media at 20 °C[a]

Substrate	Solvent: Alcohol	Solvent: % v/v	k_{obs}/s^{-1}	Relative rate[b]
1a	Methanol	0	1.9×10^{-5}	= 1.0
		50	2.0×10^{-4}	11
		70	8.8×10^{-4}	46
		80	1.7×10^{-3}	89
		90	2.8×10^{-3}	150
		100	4.6×10^{-3}	240 = 1.0
	Ethanol	100	9.0×10^{-4}	0.20
	Butanol	100	9.5×10^{-4}	0.21
1b	Methanol	60	9.0×10^{-4}	
		100	3.3×10^{-3}	0.72
	Ethanol	100	4.9×10^{-4}	0.11
	Butanol	100	3.5×10^{-4}	0.076
1c	Methanol	100	1.2×10^{-4}	0.026
1d	Methanol	100	2.5×10^{-4}	0.054
1e	Methanol	100[c]	7.6×10^{-6}	0.0017

[a] *Conditions:* substrate 1.0×10^{-4} mol dm^{-3} and $CuCl_2$, 5.0×10^{-5} mol dm^{-3} in 0.2 mol dm^{-3} borate buffer (pH 7.2)–alcohol at 20 °C. [b] Left hand side values are relative to the rate in buffer solutions, right hand side values are relative to the rate in methanol. [c] Containing 1% v/v of butylamine.

steps on addition of $CuCl_2$. At the first stage of the spectral change, the peak at 304.5 nm due to the free **1a** decreased with a constant rate although the peaks at 357.0 and 373.5 nm due to the complex **2a** remained unchanged, and the rate was dependent linearly on the concentration of **2a** ($-d[substrate]/dt = k_{obs}[complex]$). The concentration of complex **2a** decreased only after almost all the free **1a** was consumed. Equimolar amounts of alanine to the initial amounts of **1a** were found in the solution after this first spectral change ceased. Therefore, the observed spectral change was due to the catalytic hydrolysis of one of the α-alanyl units of **1a** by Cu^{2+} *via* formation of the complex **2a** [Scheme 2(*a*)]. It is worth noting that this hydrolysis rate under mild conditions is considerably higher than those reported for metal-catalysed hydrolysis of peptide bonds.[1] Subsequent to the first spectral change, a slower and smaller change was observed. The amount of α-alanine in the solution after 10 days was 1.5×10^{-4} mol dm^{-3}, and dabp was found to be present in the solution, indicating that the remaining α-alanyl unit was hydrolysed during the time of this spectral change (Scheme 3).

When the reactions of **1a** and **1b** were carried out in methanol–borate buffer systems under the same conditions,

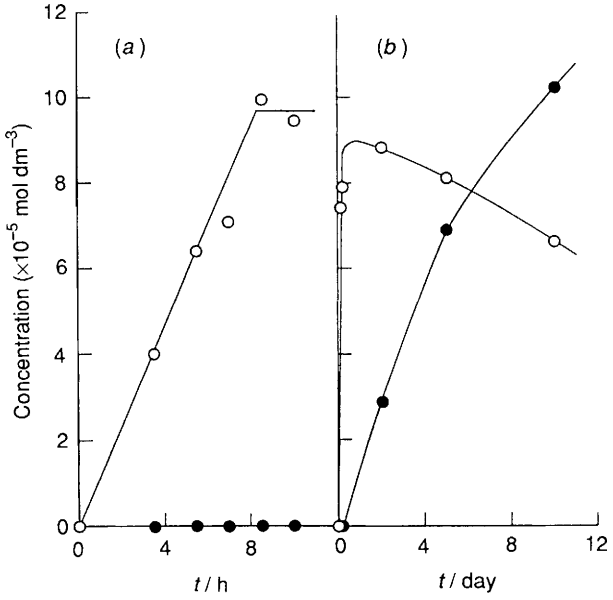


Fig. 1 Time course of the concentrations of phenylalanine methyl ester (○) and phenylalanine (●) in the solvolysis of **1b** (1×10^{-4} mol dm^{-3}) in 0.2 mol dm^{-3} borate buffer (pH 7.2, 40% v/v)–methanol (60% v/v) solution at 20 °C; (*a*) in the presence of 5.0×10^{-6} mol dm^{-3} of $CuCl_2$ showing only the initial stage of the reaction, and (*b*) in the presence of 5.0×10^{-5} mol dm^{-3} of $CuCl_2$ showing the fast initial and subsequent slow stages of the reaction

similar two-stage spectral changes were observed.‡ However, the rate constant for the first stage of the reaction, k_{obs}, was dramatically increased as methanol content increased (Table 1), and reaction of half of the initial substrates **1a** and **1b** in methanol (Cu/substrate = 0.5) at 20 °C required only 220 and 376 s, respectively. Furthermore, the product of this stage was found to be exclusively the methyl ester of amino acid by HPLC and GC–MS§ analysis even in the presence of appreciable amounts of water [Fig. 1(*a*), Scheme 2(*b*)]. Free amino acid was formed during the subsequent slow stage reaction [Fig. 1(*b*), Scheme 3]. The small decrease in the concentration of the ester in this stage might be due to the gradual hydrolysis of the ester. Thus, the reaction in aqueous methanol solution can be explained by the preferential methanolysis over hydrolysis in the first cleavage of the aminoacyl unit and subsequent slow hydrolysis of the remaining aminoacyl unit. Ester formation in the presence of appreciable amounts of water (0–40% v/v) at 20 °C and neutral pH by amide solvolysis is an unprecedented finding.

Nickel(II), which could form the deprotonated-type amide-*O*-coordinated complex[3], was also effective for this reaction, but Mg^{II} and Ca^{II}, which showed no sign of complex formation, were ineffective, indicating that the ester formation took place only from the complex **2**. Solvolysis of **1a** and **1b** in various alcohols under the same conditions also yielded exclusively esters of the corresponding alcohols during the

‡ **1b** was not soluble when the methanol content was below 60% v/v.

§ GC–MS analysis after trifluoroacetylation, m/z = 275 for phenylalanine methyl ester, m/z = 289 for phenylalanine ethyl ester and m/z = 317 for phenylalanine butyl ester.

first stage of the reaction,§ although the rate constants were smaller than those in methanol (Table 1). Solvolysis of the β-aminoacyl (**1c**) and γ-aminoacyl (**1d**) derivatives was more than an order of magnitude slower than that of **1a**, although they formed quantitatively the complexes **2c** and **2d**, respectively, under the same conditions. The propanoyl derivative **1e** in the presence of 1% v/v butylamine reacted much slower. The results demonstrated that the presence of an α-amino group played an important role in the ester formation.

Thus, formation of the amide-*O*-coordinated complex **2** in this reaction was shown to be essential for the formation of amino acid ester in aqueous alcohol solutions, and the presence of an α-amino group on the acyl side-chain was important for the subsequent alcoholysis of the amide bonds of the complex. Non-enzymatic hydrolysis of amide bonds under mild conditions has been the subject of continuing interest, and enhanced hydrolysis rate of distorted amide groups has been discussed actively.[4] It is noteworthy that the formation of the N_2O_2-type square-planar complex requires considerable distortion of the amide bond of the substrates.[3] As esterification of the 3′-hydroxy end of tRNA with α-amino acid is an important step of protein synthesis in living systems, formation of amino acid esters in aqueous media under mild conditions might have considerable importance.

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