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The Peptide Formation Mediated by Cyanate Revisited. *N*-Carboxyanhydrides as Accessible Intermediates in the Decomposition of *N*-Carbamoylamino Acids

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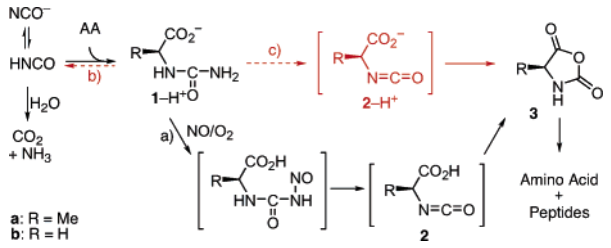
Cyanate-promoted peptide bond formation has been observed by heating α-amino acids (AAs) in phosphate-buffered aqueous solutions,[1a] but it gave low peptide yields and its mechanism has remained unclear; peptide formation in molten urea (140 °C) has also been reported.[1b] On the other hand, AAs are easily converted by cyanate into *N*-carbamoylamino acids (CAAs),[2] which are usually considered to be poorly reactive species that can only be hydrolyzed under alkaline conditions[2] or by heating at moderate pH.[3] However, CAAs **1** can be cleanly activated into amino acid *N*-carboxyanhydrides (NCAs, **3**) by nitrosating the urea group (Scheme 1, pathway a), then releasing N_2 and water as only byproducts.[4] Reconsidering this process, we inferred that, even without activation, CAAs could partly, but spontaneously, be transformed into NCAs provided that the decomposition proceeds through elimination (Scheme 1, pathways b and c), potentially explaining the observation of cyanate-mediated peptide formation. This hypothesis is supported by elimination mechanisms that account for urea decomposition at moderate pH.[5] The addition−elimination mechanism, involving HO^- attack, only prevails at high pH. Then, CAAs, as nonsymmetrical ureines, must undergo elimination either through the release of AA or that of ammonia, giving NCO^- or isocyanate **2**−H^+, respectively. Finally, the isocyanate **2**−H^+ is likely to be converted into NCA by cyclization involving the carboxylate group. We show here that the decomposition of CAA in water at 80 °C actually produces peptides under favorable conditions and gives rise to a complex behavior that could not have been expected from a simple hydrolytic process.

Scheme 1. Pathways for the Conversion of *N*-Carbamoylamino Acids into Amino Acid *N*-Carboxyanhydrides



The decomposition of Ala CAA **1a** was studied by NMR analysis of samples of the reaction medium. In CO_2/HCO_3^- buffers, the addition of alanine (Ala) to the reaction medium strongly depressed the initial decomposition rate (Figure 1). A similar kinetic effect was not induced by the addition of glycine (Gly) (Supporting Information). Nevertheless, in the latter experiment, Gly was partly converted into carbamoylated derivative **1b**. All together, these results support the elimination pathway in which cyanic acid (as the reactive form of cyanate, Scheme 1) is trapped by the nucleophilic attacks of water, Gly, or Ala, but they are incompatible

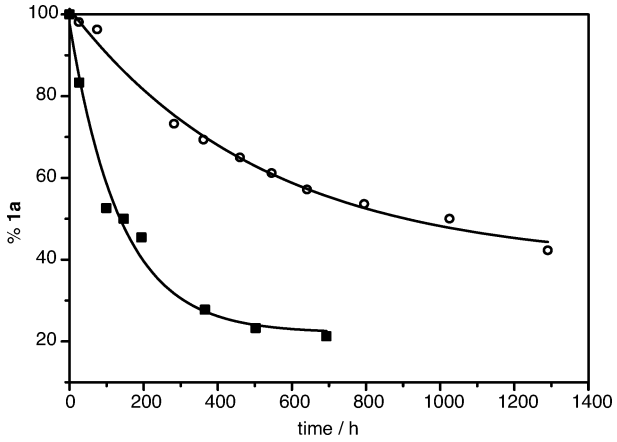
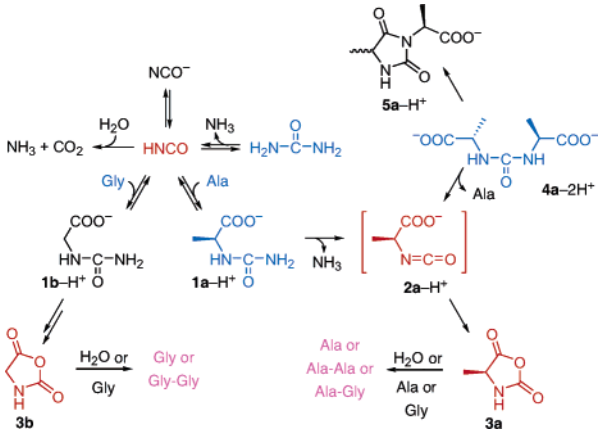


Figure 1. Decomposition of 20 mM Ala CAA **1a** in buffered aqueous solution (50 mM $NaHCO_3$/saturated CO_2) at 80 °C, without added AA (■) and in the presence of 100 mM Ala (○).

Scheme 2. Activation of α-Amino Acids by Cyanate or Related Reagents



with an addition−elimination process. Indeed, a process reverting cyanate into CAA **1a** can account for the decrease in rate observed in the presence of added Ala, whereas an addition/elimination mechanism is unable to explain this observation. From these experiments, we can conclude that the elimination pathway is largely predominating for CAAs, at least at the temperature of the experiment (80 °C), as already observed from other ureas.

The decomposition of Ala CAA **1a** predominantly takes place through the elimination of Ala (pK_a of Ala zwitterion = 9.87), a better leaving group than ammonia (pK_a of NH_4^+ = 9.3). This is confirmed by the ca. 5-fold slower rates observed for the decomposition of symmetrical urea **4a** (Scheme 2), the breakdown of which also turned out to be accompanied by additional processes

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Table 1. Nature and Yields of Obtained Peptides Determined by CE

entry	reactants	time[a]/days	residual **1a**	peptide (yield)[b]
1	Ala[c]	53[d]		Ala_2 (<0.2%)[e]
2	**1a**[f]	28[g]	21%	Ala_2 (1.7%)[h]
3	**1a**[f] + Ala[c]	53[g]	42%	Ala_2 (12.7%)[h] Ala_3 (1.9%)
4	**1a**[f] + Gly[c]	53[d]	7%	Ala−Gly (2.1%) Gly_2 (6.8%)
5	**4a**[f]+ Ala[c]	35[g]		Ala_2 (20.0%)[h] Ala_3 (1.2%)
6	**4a**[f] + Gly[c]	35[d]		Ala−Gly (18.5%)
7	urea[f] + Ala[c]	56[g]		Ala_2 (7.2%)[h]

[a] Incubation at 80 °C. [b] Yields relative to activated agent (by EC) unless otherwise mentioned. [c] At 100 mM. [d] At 50 mM NaH_2PO_4/50 mM Na_2HPO_4 buffer, pH 6.8 at 25 °C. [e] Yield relative to Ala (by NMR). [f] At 20 mM. [g] At 50 mM $NaHCO_3/CO_2$ buffer, pH 7.1 at 25 °C. [h] Mixture of isomers.

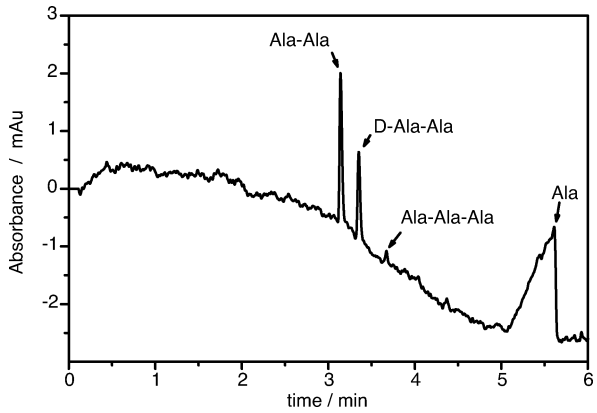

Figure 2. Capillary electrophoresis (CE) of crude reaction medium obtained after incubating 20 mM CAA **1a** with 100 mM Ala for 53 days; buffer 50 mM $NaHCO_3$ under CO_2 atmosphere. Electrophoretic conditions: fused silica capillary, 50 μm i.d. × 27 cm (19.6 cm up to the detector). Electrolyte: 100 mM phosphate buffer, pH 2.56. Applied voltage: + 15 kV. Sample injection: 1s, 0.5 psi.

(Supporting Information). Then, peptide production via NCA is a minor process hardly detected from the reaction of CAA **1a** alone (Table 1). However, the reversibility of the elimination process in the presence of excess Ala gave us the opportunity to artificially select the products of ammonia elimination and to suppress the pathway (b) leading to cyanate hydrolysis. Despite slower rates, substantial degrees of conversion were reached (1 or 2 months). At that time, capillary electrophoresis (CE) indicated the presence of Ala peptides as diastereomers due to the occurrence of an epimerization process (Figure 2 and Table 1). The absence of peptide production in a control experiment carried out without any activating agent (entry 1) and the production of Gly_2 and Ala−Gly from **1a** in the presence of excess glycine are consistent with the processes displayed in Scheme 2 and the transient formation of cyanate that can be trapped by any added AA. On the contrary, the symmetrical urea **4a**, only yielding isocyanate **2a** by decomposition, also produced Ala−Gly, but not Gly_2, in the presence of excess Gly, though a side reaction giving the N-terminal hydantoin **5a** was observed. Introduced in the medium as a cyanate precursor, urea also promoted peptide formation in the presence of Ala (entry 7). Urea thus behaves as an unanticipated peptide-coupling agent.

Since CAA decomposition takes place via an elimination pathway, the intermediacy of NCA **3** is highly probable. Indeed, the cyclization of anion **2a**−H^+ is likely to be easier than that of neutral acid **2a** from which NCA has been isolated under acidic conditions.[4] This new pathway illustrates the theoretical possibility of obtaining NCAs from moderately activated AA derivatives.[6] It may be helpful in understanding the processes that contributed to the origin of life. NCAs are likely intermediates in the primordial formation of peptides,[6] so investigations aimed at finding prebiotically plausible synthetic pathways have been carried out recently.[7] The ability of NCAs to promote phosphate and nucleotide activation[8] may have contributed to the emergence of the translation apparatus. This contribution and a putative role of energy carriers at early stages of the evolution of living organisms[6] would require the availability of straightforward abiotic pathways of synthesis of NCAs sufficient to feed the first living organisms with energy and allow them to survive in varied environments. Cyanate is produced by electric discharges in reducing mixtures of N_2, CO_2, and H_2,[9] which is consistent with a recent reassessment of the composition of the primitive Earth atmosphere.[10] Furthermore, the Bücherer−Bergs synthesis[11] may have directly yielded CAAs as well.[2a] Then the pathway reported here starting from CAAs extends the availability of NCA to reducing conditions, whereas it has been mainly demonstrated in mild oxidizing environments[4] or in the presence of oxidizing agents.[7] Then, the emergence of life and early stages of biochemical evolution may have taken advantage of the unique features of theses simple activated forms of AAs over the varied environments of the primitive Earth.

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Supporting Information Available: Experimental procedures, NMR spectra, plots of reaction time course, and reaction conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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