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Amino Acid *N*-Carboxyanhydrides: Activated Peptide Monomers Behaving as Phosphate-Activating Agents in Aqueous Solution

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α-Amino acid *N*-carboxyanhydrides (NCAs), **1**, are activated building blocks that can yield polypeptides with high degrees of polymerization.[1] This unique ability prompted investigations[2] of the prebiotic significance of NCA polycondensation even though no convincing prebiotic pathway was available for their synthesis. Their stereoselective polycondensation has also been considered as a possible pathway for the abiotic origin of single-handedness in nature.[3] The disclosure of a possible mechanism for their formation under the conditions of the primitive Earth through the activation of carbamoyl amino acids by NO/O_2[4,5] made their prebiotic status more likely. In the course of a kinetic study of NCA polymerization in aqueous solution,[6,7] the competitive hydrolysis was observed to be subject to buffer catalysis by various buffers including phosphate,[7] generalizing the previous observation of acetate buffer catalysis.[8] Usually, buffer catalysis corresponds to general acid−base catalysis, but the alternative possibility of nucleophilic catalysis has to be considered in every case.[9] Nucleophilic mechanisms involving carboxylic−phosphoric mixed anhydrides have thus been reported for several reactions of acyl donors including activated esters,[10] thioesters,[11] or anhydrides.[12] A nucleophilic mechanism would then be expected for phosphate buffer catalysis of NCA hydrolysis (Scheme 1) because the carbamate group must be a better leaving group than phosphate dianion from the tetrahedral intermediate **2** as expected from the values of ca. 5[13] and 7.2 for the respective pK_A's of the conjugated acids. Because of their close relationship with aminoacyl adenylates,[14] the intermediates of amino acid activation involved in protein biosynthesis, and their potential phosphorylating ability,[15] we devised that the transient formation of aminoacyl phosphates **3** would have important consequences in the field of prebiotic chemistry. Here we show that an intermediate displaying properties consistent with an aminoacyl phosphate is formed from the corresponding NCA in diluted phosphate buffer and that its breakdown proceeds through phosphoryl transfer.

The reaction of 10 mM Val-NCA in phosphate buffers was monitored by NMR spectroscopy. An intermediate was indeed observed in the course of the reaction (Figure 1), and both its formation and its decay progressed according to first-order kinetics. Since rates were affected by a change in the concentration of HPO_4^{2-} but not that of $H_2PO_4^-$,[16] then phosphate dianion must be the reactive species, which is in agreement with previous kinetic measurements.[7] However, when the concentration of the acid component of the buffer was reduced, the final mixture was complicated by the formation and precipitation of polypeptides due to the increased pH value promoting the direct formation of peptides from Val-NCA. In agreement with the structure of the mixed phosphoric−carboxylic anhydride **3a** (R = $CHMe_2$) for the intermediate, a signal at −1.5 ppm (apart from the large peak of inorganic phosphate, δ = 0.2 ppm) displaying a similar kinetic

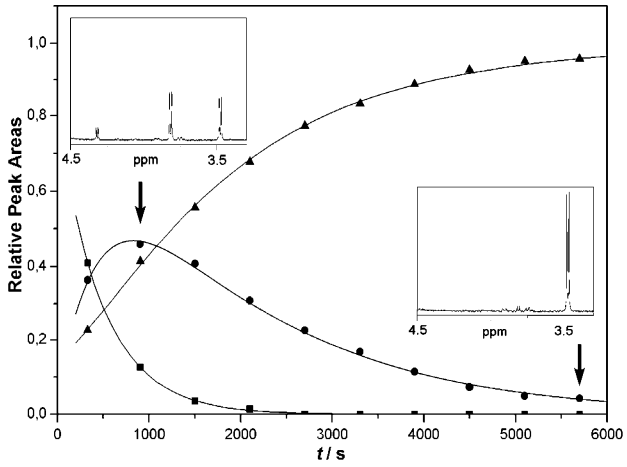
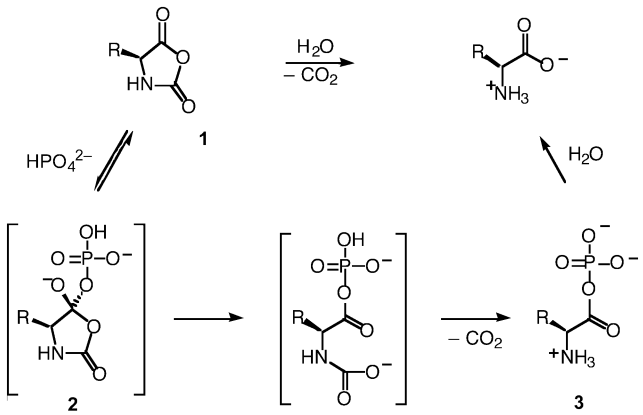


Figure 1. Progress of the reaction of 10 mM Val-NCA in a 20 mM Na_2HPO_4/200 mM NaH_2PO_4 solution in D_2O at 21 °C (pD 6.2) monitored by ^1H NMR spectrometry (300 MHz). Relative areas of CHα signals of Val-NCA (■, δ = 4.32 ppm, J = 4.3 Hz), **3a** (●, δ = 3.81 ppm, J = 4.5 Hz) and Val (▲, δ = 3.48 ppm, J = 4.4 Hz). Experimental points and curves fitted assuming exponential decays for Val-NCA (2.1×10^{-3} s^{-1}) and for **3a** (5.7×10^{-4} s^{-1}).

Scheme 1. Nucleophilic mechanism of catalysis of NCA hydrolysis by phosphate dianion



behavior was observed by ^{31}P NMR spectroscopy. These NMR data are also consistent with the values reported for valyl ethyl phosphate **4a**.[14a]

The structure of the mixed anhydride **3a** was confirmed by an independent preparation from the Boc/tBu-protected derivative **4c** adapted from the synthesis of the ethyl phosphate derivative **4b**.[14a] Boc and tBu protecting groups were removed from **4c** with trifluoroacetic acid (TFA), and the acidic product was dissolved in 0.02 M Na_2HPO_4 buffered D_2O displaying NMR spectra and a

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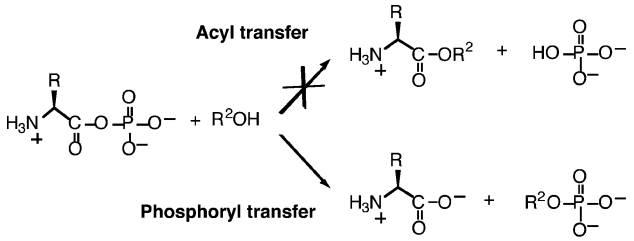
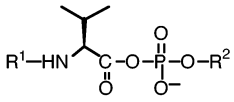
Scheme 2



kinetic behavior in agreement with that observed for the intermediate of Val-NCA hydrolysis.



4a R^1 = H, R^2 = Et
4b R^1 = Boc, R^2 = Et
4c R^1 = Boc, R^2 = tBu

Because aminoacyl phosphates are carboxylic−phosphoric mixed anhydrides with potential acyl and phosphoryl transfer abilities, two different mechanisms of hydrolysis are conceivable (Scheme 2, R^2 = H). To investigate this point, the intermediate **3a** was formed in aqueous solution, then excess methanol (R^2 = CH_3), was added and the product was analyzed by NMR spectroscopy after removal of the solvent under reduced pressure. Methyl phosphate [^{1}H NMR (D_2O) δ = 3.39, J (P,H) = 10.4 Hz; ^{31}P NMR (D_2O) δ = 4.55, in agreement with reported data[17]] was identified as a product, whereas Val-OMe was not detected provided that NCA was no longer present at the time of methanol addition. This result is consistent with a cleavage of the P−O bond of the mixed anhydride. It is also in agreement with the well-known reactivity of acetyl phosphate near neutrality,[18] which is similar to that of monosubstituted phosphates esters that predominantly undergo a dissociative mechanism of phosphoryl transfer through a metaphosphate-like transition state.[19]

NCAs are therefore capable of activating inorganic phosphate in diluted aqueous solution, which may have important consequences for the processes that led to the emergence of life. It is remarkable that the mixed anhydride pathway is prevalent even at low (10−20 mM) concentrations of phosphate dianion and that high temperatures are not required for phosphoryl transfer. Amino acid chemistry was not expected to be able to provide a mechanism for prebiotic phosphorylation and then to have possibly participated in nucleotide and RNA synthesis. The connection revealed by the mixed anhydride pathway could be an indication of an early coevolution involving both amino acid/peptide and nucleotide/RNA chemistries. Because of its highly evolved character, it seems unlikely that an early connection of that kind could have been lost at a later stage, which challenges the occurrence of an RNA-only world where living organisms used RNA for both catalysis and information storage.[20] Furthermore, the process by which the chemical energy present in NCA is transferred to a phosphorylated intermediate simulates modern biological metabolic pathways. In this view, the carbon−oxygen bond in NCAs can be considered as an energy-rich bond, according to Lipmann's terminology.[21] This process may have constituted an original entry into the core of a protometabolism in which "high-energy" species such as acyl phosphates, thioesters, and ATP have been connected to each other, which has been previously conceived as the consequence of a thioester world.[22] Further developments such as the research of conditions or catalysts allowing phosphorylation of selected prebiotic molecules of interest are currently under investigation in our group.

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Supporting Information Available: Reaction of **3a** with methyl alcohol and procedures for the synthesis of compounds **4c** and **3a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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