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Amino Acid Dependent Formation of Phosphate Anhydrides in Water Mediated by Carbonyl Sulfide

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Carbonyl sulfide (COS) is a component of volcanic gas emissions on present-day Earth,[1] a major constituent of the atmosphere of Venus,[2] and one of the molecules observed in interstellar gas clouds.[3] It brings about the efficient formation of oligopeptides from amino acids under a variety of potentially prebiotic conditions.[4] Here we investigate the reactions of COS in the context of phosphate activation in neutral aqueous solutions. We show that COS is an effective reagent for the synthesis of aminoacyl phosphates from amino acids and inorganic phosphate or aminoacyl adenylates from amino acids and adenylic acid. We further show that the mixed anhydrides of amino acids and inorganic phosphate, in the presence of certain divalent ions, are phosphorylating agents, producing pyrophosphate from phosphate. The amino acid dependent activations of phosphate reported here, which occur under mild aqueous conditions in parallel with the production of peptides, suggest that peptide synthesis and phosphoryl transfer reactions might have shared a common activated precursor on the prebiotic Earth.

Scheme 1. Phosphate Activations Mediated by Carbonyl Sulfide

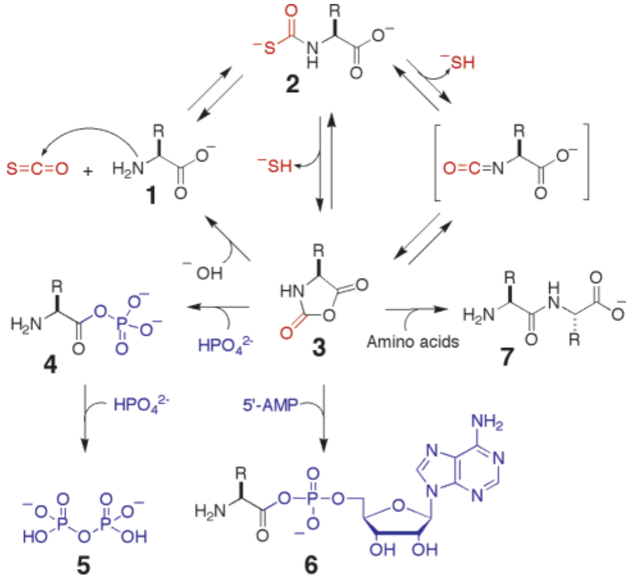
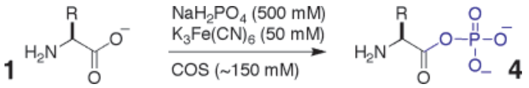


Table 1. COS-Mediated Formation of Aminoacyl−Phosphate Anhydrides (**4**) for Various Amino Acids (**1**)[a]



amino acid (**1**) (20 mM)	final pH	time (min)	AA–anhydride (**4**) (% yield)
L-arginine	7.1	25	26
L-phenylalanine	7.2	35	24
L-glutamic acid	7.2	30	23
L-aspartic acid	7.2	20	22
glycine	7.2	50	19
L-alanine	7.2	85	14
L-cysteine	7.2	30	6.0
L-serine	7.1	45	not observed
L-histidine	7.2	30	not observed

[a] Reactions were carried out in bis-Tris propane buffer (pH 7.5, 400 mM) at 25 °C. Yields were determined by integration of ^{31}P NMR peaks.

The reaction between inorganic phosphate and an aminoacyl *N*-carboxyanhydride[5] (NCA, **3**, Scheme 1) has recently been shown to generate a mixed aminoacyl−phosphate anhydride (**4**).[6] Despite indications from the literature that concentrated phosphate solutions can rapidly hydrolyze COS gas to CO_2 and H_2S,[7] we wondered if *N*-carboxyanhydrides might still form in the presence of phosphate and then react with phosphate to yield an aminoacyl−phosphate anhydride. Our studies indicate that mixed aminoacyl−phosphate anhydrides (**4**) are rapidly produced from amino acids (**1**) and inorganic phosphate in the presence of COS and ferricyanide—a potentially prebiotic[8] oxidizing agent that greatly enhances the efficiency of COS-mediated peptide synthesis[4] (Table 1, Figures 1 and S1). Yields of the aminoacyl−phosphate anhydrides were as high as 25% (Table 1). The participation of aminoacyl thiocarbamate **2** as an intermediate in the formation of aminoacyl−phosphate anhydride **4** was confirmed by initiating a reaction with an authentic sample of L-phenylalanine thiocarbamate **2** without COS (Scheme 1). Further, in the absence of amino acid or COS, no phosphate anhydrides were observed. Product identities were established by comparison of ^{31}P NMR chemical shifts (Figure S4) with those of authentic samples.[6,9] Product yields depended markedly on the concentration of phosphate in the reaction mixture (Figure S3); maximum yields were obtained at phosphate concentrations of ~500 mM. The optimal pH value of ~7.5 for the formation of **4** (Figure S3) is somewhat lower than the optimal pH value of ~9.0 for COS-mediated peptide synthesis.[4] The reaction of amino acids with NCA at near-neutral pH values is less favorable than that at higher pH values as a result of protonation of the α-amine. Also, the most abundant phosphate ion at pH 7.5 is the dianion (HPO$_4^{2-}$), which is reportedly the reactive species in attacking the NCA.[6] The reaction of L-phenylalanine (Table 1) still generated a ~6% yield of dipeptide (**7**), a ~1% yield of tripeptide, and a trace of tetrapeptide (observed by HPLC−MS) in just 35 min.

In principle, aminoacyl−phosphate anhydrides could act as acylating agents[10] and/or as phosphorylating agents[6,11,12] by reacting via C−O or P−O bond cleavage, respectively. We have found that aminoacyl−phosphate anhydrides, in the presence of certain alkaline earth metal ions that form phosphate precipitates, can generate pyrophosphate (**5**) in yields of up to 30% based on the yield of

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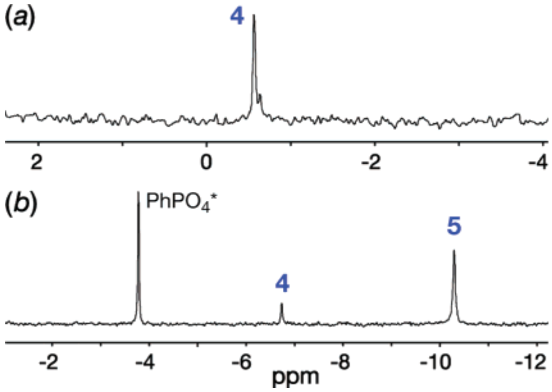
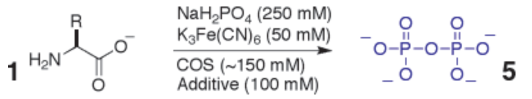


Figure 1. ^{31}P NMR spectra illustrating phosphate anhydride formations. (a) Formation of L-phenylalanine phosphate (**4**) in a reaction as described in Table 1. (b) Formation of L-alanyl phosphate (**4**) and its conversion to pyrophosphate (**5**) in a reaction as described in Table 2. $PhPO_4$* is phenyl phosphate, an internal concentration standard added after quenching the reaction. The differences in chemical shift for the two spectra result from pH differences in the two samples.[9]

Table 2. Pyrophosphate (**5**) Formation under Various Conditions[a]



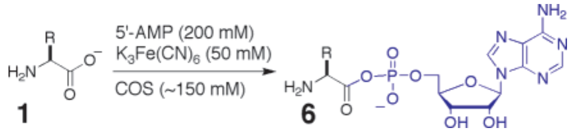
amino acid (**1**) (20 mM)	additive (100 mM)	time (h)	pyrophosphate (**5**) (% yield)[b]
L-glutamic acid	$CaCl_2$	27	30 (5.4)
L-glutamic acid	hydroxylapatite[c]	37	12 (2.2)
L-glutamic acid	$SrCl_2$	46	2 (0.4)
L-glutamic acid	$BaCl_2$	46	6 (1.1)
L-glutamic acid	$MgCl_2$	46	not observed
L-glutamic acid	$PbCl_2$	46	not observed
L-arginine	$CaCl_2$	21	23 (4.1)
L-phenylalanine	$CaCl_2$	27	13 (2.3)
L-alanine	$CaCl_2$	40	18 (3.2)
L-aspartic acid	$CaCl_2$	40	32 (5.8)
glycine	$CaCl_2$	40	17 (3.1)
L-histidine	$CaCl_2$	40	5.0 (0.9)

[a] Reactions contained PIPES buffer (pH 7.5, 200 mM) at 25 °C. [b] Yields were determined by ^{31}P NMR integration and are based on the maximum concentration of intermediate **4**. The values in parentheses represent yields based on amino acid. [c] Hydroxylapatite is a naturally occurring $CaPO_4$ mineral; 150 mM NaH_2PO_4 was used in this reaction.

aminoacyl−phosphate anhydride (Figure 1, Table 2). No pyrophosphate formed in otherwise identical reaction mixtures lacking amino acid or COS. It is notable that the phosphoryl transfer reactions occur at very low concentrations of the mixed anhydride **4** (2−4 mM). Two lines of evidence suggest that the phosphoryl transfer occurs at the surface of the insoluble metal−phosphate salt. First, the product pyrophosphate was found adsorbed to the insoluble material. Second, the Mg^{2+} ion, which does not precipitate phosphate ions, did not catalyze pyrophosphate formation. Since the abundant calcium phosphate mineral, hydroxylapatite, was a suitable substrate for the reaction, we believe the scheme to be a plausibly prebiotic mechanism of phosphorylation. In principle, pyrophosphate could act as a prebiotic phosphorylating agent in place of ATP; pyrophosphate can replace ATP in several enzymatic phosphorylation reactions.[13]

Aminoacyl adenylates (**6**) are critical intermediates in contemporary protein biosynthesis and would be produced if NCA reacted with the AMP in the same way as it does with inorganic phosphate. Indeed, adenylates (**6**) were generated with a variety of amino acids under similar conditions to those employed for the formation of **4** (Figure S5). While accurate determinations of aminoacyl adenylate yields are complicated by their hydrolytic instability, yields of ~10% have been observed for several amino acids (Table 3). In the case of phenylalanine, product identities were confirmed using reported ^{31}P NMR chemical shift values[14] and by comparison of HPLC−MS and ^{31}P NMR profiles with those of an authentic sample.[9,15]

Table 3. Aminoacyl Adenylate (**6**) Formation Induced by COS[a]



amino acid (**1**) (20 mM)	initial/final pH	time (min)	AA–adenylate (**6**) (% yield)
glycine	7.0/6.7	110	12
L-arginine	7.0/6.8	150	8
L-phenylalanine	7.0/6.7	105	7
L-aspartic acid	7.0/6.8	160	7
L-alanine	7.0/6.9	180	6
L-glutamic acid	7.0/6.8	120	5
L-serine	7.0/6.8	120	not observed

[a] Reactions contained PIPES buffer (pH 7.0, 400 mM) at 25 °C. Yields were determined by integration of ^{31}P NMR peaks using 0.2% phosphoric acid in a sealed capillary tube as an external concentration standard.

The studies reported here suggest that COS, a simple volcanic gas, could have mediated both phosphoryl transfer and peptide synthesis reactions via a single intermediate, aminoacyl *N*-carboxyanhydride, under mild aqueous conditions on the prebiotic Earth.

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Note Added in Proof. An account of reactions between NCA and nucleotides has recently been reported. See: Biron, J.-P.; Parkes, A. L.; Pascal, R.; Sutherland, J. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 6731−6734.

Supporting Information Available: Experimental procedures, ^{31}P NMR spectra, plots of reaction time course, product formation versus pH and phosphate concentration, and product characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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