



Carbonyl Sulfide-Mediated Prebiotic Formation of Peptides

Luke Leman, *et al.*

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dynamic change of histone H4 Arg³ methylation and citrullination in HL-60 granulocytes.

Histone Arg methylation at H3 Arg¹⁷ and H4 Arg³ is known to regulate estrogen-responsive genes, such as the pS2 gene in MCF-7 cells (11, 30). The observed demethylation activity of PAD4 suggests it might regulate histone Arg methylation on specific promoters, leading to a change of gene expression. To test this idea, we first analyzed the effect of PAD4 and an enzymatically inactive form of PAD4 (PAD4^{C645S}) (fig. S3) on the activity of an EREII-luciferase reporter gene, which can be strongly induced by β -estradiol in MCF-7 cells (Fig. 5A). We found that the wild-type PAD4 effectively repressed the activity of the luciferase reporter in a dose-dependent manner (Fig. 5A), whereas the PAD4^{C645S} mutant displayed weaker inhibitory effects. Intriguingly, the PAD4^{C645S} mutant displays partial repressive activity when present at higher doses. Whether the mutant retains partial enzymatic activity, recruits additional cofactors, or heterodimerizes with endogenous PAD4 in MCF7 cells [as does wild-type PAD4 (19)] remains unclear.

The repressive activity of PAD4 on the EREII-luciferase reporter gene prompted us to test whether PAD4 plays a role in regulating the endogenous pS2 gene in MCF-7 cells after estradiol stimulation. We found both PAD4 expression and low amounts of H4 Cit³ in MCF-7 cells (28). With chromatin immunoprecipitation (ChIP) analyses, we showed that PAD4 is associated with the pS2 gene promoter before the addition of estradiol and that PAD4 amounts increased ~twofold at 40 and 60 min after estradiol induction (Fig. 5B). We observed a strong increase of H4 Arg³ methylation at 20 min and a decrease at subsequent time points, whereas H4 Cit³ increased at 40 and 60 min. Therefore, the decrease of H4 Arg³ methylation correlates with the increase of PAD4 protein and H4 Cit³ levels on the pS2 gene promoter. In addition, PAD4 was not associated with the control CIITA gene and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene promoters before or after estradiol treatment (Fig. 5C). These data suggest that PAD4 acts specifically at the pS2 promoter and that its recruitment does not simply result from increased PAD4 expression upon hormone induction. Thus, our data support the conclusion that the demethylation activity of PAD4 is likely involved in the subtle balance of the estrogen-inducible pS2 gene expression in MCF-7 cells.

Our finding that PAD4 can both deiminate and demethylinate histones suggests that PAD4 may affect chromatin structure and function via two related but different mechanisms (fig. S5). Regarding demethylation, histone Arg methylation mediated by secondary co-activators, such as CARM1

and PRMT1, has been correlated with gene activity (11–15) (fig. S5). Given the paradigm already established by reversible acetylation (31–33), it seems reasonable that Arg-directed methylation events, particularly those that lead to gene activation, would be reversible. In the case of estrogen-induced genes in MCF-7 cells, we favor the view that PAD4 also functions to remove histone Arg methylation marks, thereby reversing the transcriptional activation brought about by nuclear hormone receptor coactivators and histone arginine methyltransferases, likely in concert with other chromatin modifying activities (e.g., histone deacetylases) (fig. S5). It remains a formal possibility, however, that the repressive effect of PAD4 may be due to its deimination activity, which, in turn, prevents histone methylation by CARM1 and PRMT1. Because of the dual enzymatic activities of PAD4, deimination versus demethylation, separating any observed transcriptional or other biological effects brought about by PAD4 at target Arg residues will represent a challenge for future studies.

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Carbonyl Sulfide–Mediated Prebiotic Formation of Peptides

Luke Leman,¹ Leslie Orgel,² M. Reza Ghadiri^{1*}

Almost all discussions of prebiotic chemistry assume that amino acids, nucleotides, and possibly other monomers were first formed on the Earth or brought to it in comets and meteorites, and then condensed nonenzymatically to form oligomeric products. However, attempts to demonstrate plausibly prebiotic polymerization reactions have met with limited success. We show that carbonyl sulfide (COS), a simple volcanic gas, brings about the formation of peptides from amino acids under mild conditions in aqueous solution. Depending on the reaction conditions and additives used, exposure of α -amino acids to COS generates peptides in yields of up to 80% in minutes to hours at room temperature.

The first suggestion that COS might be a prebiotic condensing agent appears in a footnote of a paper by Hirschmann and co-workers on

peptide synthesis from 2,5-thiazolidinediones (1). The authors reported that traces of dipeptide are formed from phenylalanine

thiocarbamate in aqueous solution, but gave no experimental details. More recently, COS has been proposed as a possible intermediate in the hydrothermal formation of dipeptides from amino acids, but was found to be effective only in the presence of nickel and iron sulfides (2, 3). We speculated that COS might have played a more general role as a condensing agent in prebiotic chemistry.

We initially investigated the utility of COS in promoting condensation of L-phenylalanine (4). An approximately eightfold excess of COS gas (20 ml, 400 mM) was admitted into an air-free solution of phenylalanine (50 mM) in borate buffer (500 mM, pH 9.6) (5). Analysis of the reaction mixture that had stood for 2 days under argon at 25°C, by liquid chromatography–mass spectrometry (LC-MS) and ¹H–nuclear magnetic resonance (NMR) spectroscopy using authentic samples as comparators, revealed production of dipeptide in ~7% yield, along with trace amounts of urea derivative 5 (Table 1, entry 1). Analogous reactions in *N*-cyclohexylethane sulfonic acid [CHES (300 mM, pH 9.1)] or trimethylamine [Me₃N (300 mM, pH 9.4)] gave similar product yields, suggesting that buffer catalysis is not important in the rate-determining step of the condensation process.

¹Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA. ²The Salk Institute for Biological Studies, Post Office Box 85800, San Diego, CA 92186, USA.

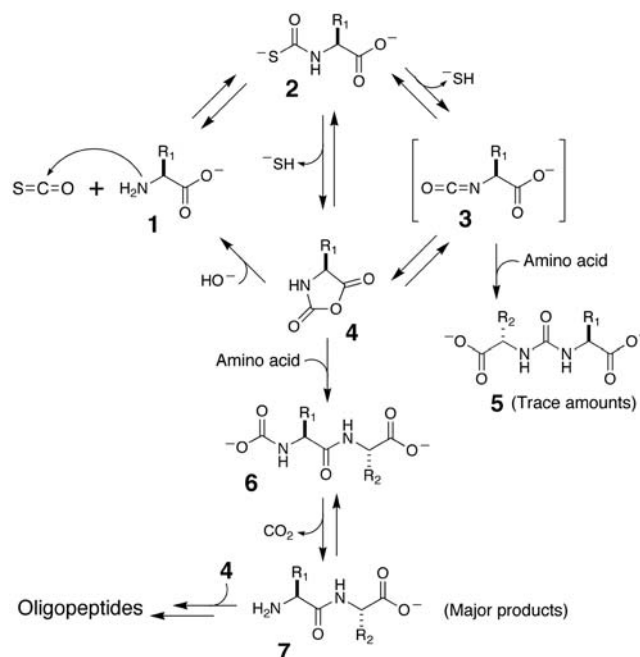
*To whom correspondence should be addressed. E-mail: ghadiri@scripps.edu

Encouraged by these initial results, we set out to explore the COS-mediated condensation reaction in greater detail.

From a consideration of the reactivity of amine nucleophiles toward COS (6), we hypothesized the reaction sequence illustrated in Scheme 1 (7). Amino acid 1 first reacts with COS to give the corresponding α-amino acid thiocarbamate 2. Intramolecular cyclization of 2 yields the α-amino acid *N*-carboxyanhydride (NCA) 4, also known as a

Leuchs anhydride. Thereafter, the well-known and efficient condensation of NCAs with amino acids would proceed through intermediates 6, to furnish the dipeptide 7 (7). The proposed reaction mechanism is supported by the following series of experiments.

To investigate the production and reactivity of the putative intermediate 2, we probed by ¹H-NMR the reaction of L-phenylalanine (25 mM) in D₂O (500 mM borate, pD 8.9) with COS (bubbled slowly



Scheme 1.

Table 1. The yields of major products observed in the COS-mediated condensation of L-phenylalanine under various conditions. All reactions were performed in a 25-ml Schlenk tube with a 2-ml total reaction volume. Reaction yields were determined by reversed-phase HPLC analysis against added acetamidobenzoic acid (ABA) as the internal standard.

Entry	L-Phe (mM)	L-Phe thiocarbamate (mM)	COS (mM)	Additive* (mM)	Final pH†	Time (hours)	Dipeptide (% yield)	Tripeptide (% yield)
1	50	–	400‡	–	8.7§	56	6.8	–
2	–	50	–	–	9.2	65	7.2	–
3	25	25	–	–	9.4‡¶	60	5.6	–
4	25	25	–	5 (EDTA)	9.4	41	7.2	–
5	50	–	400#	50 (PbCl ₂)	9.1	11	48.1	1.6
6	50	–	400‡	50 (PbCl ₂)	8.2	3.5	34.2	1.2
7	25	–	400#	25 (PbCl ₂)**	8.4	3	20.3	1.6
8	50	–	400‡	50 (CdCl ₂)	8.4	16	40.0	0.6
9	50	–	400‡	100 [K ₃ Fe(CN) ₆]	8.8	20 min	36.3	Nd††
10	25	25	–	50 [K ₃ Fe(CN) ₆]	9.2	1.5	55.2	4.4
11	–	70	–	100 [K ₃ Fe(CN) ₆]	8.9§	5 min	63.1	12.8‡‡
12	25	25	–	25 (PbCl ₂)	9.5	27	21.6	0.8
13	25	25	–	25 (CdCl ₂)	9.2	16	48.0	4.0
14	25	25	–	25 (FeCl ₂)	9.2	64	23.2	0.8
15	25	25	–	25 (FeCl ₃)	8.8	53	13.6	Nd††
16	25	25	–	25 (ZnCl ₂)	8.8	17	4.0	–
17	25	25	–	25 (bromoacetate)	9.0	31	33.6	0.8
18	–	25	–	25 (bromoacetate)	9.1	31	23.2	2.8
19	25	25	–	50 (benzylbromide)§§	9.8	32	32.8	–
20	–	50	–	50 (benzylbromide)§§	9.8	32	27.3	–

*Metal sulfide precipitates were formed in the reaction mixtures containing divalent metal ions. †Reactions were performed in 300 mM sodium CHES buffer unless otherwise noted. ‡Approximate amounts of COS gas bubbled into the reaction mixture. §500 mM sodium borate, pH 9.6. ¶300 mM Me₃N, pH 9.4. ||400 mM sodium CHES buffer. #Approximate amounts of COS gas admitted into the Ar-purged reaction vessel. **Reaction was performed in a water sample obtained from the Pacific Ocean, La Jolla, CA. ††Undetermined yield. Product(s) observed by LC-MS. ‡‡Additional products include 2.8% tetrapeptide and traces of penta- and hexapeptide. §§Inhomogeneous reaction mixture due to low aqueous solubility of benzylbromide.

into the NMR tube over 10 min). Surprisingly, the reaction was complete, affording a quantitative yield of phenylalanine thiocarbamate **2** (fig. S1) that was reasonably stable toward hydrolysis under the reaction conditions studied (8). To establish that thiocarbamate **2** is a competent reaction intermediate in COS-mediated peptide bond formation, we prepared an authentic sample of analytically pure phenylalanine thiocarbamate **2** (*I*). Anaerobic solutions of L-phenylalanine (25 mM) and phenylalanine thiocarbamate **2** (25 mM) (Table 1, entries 3 and 4) or phenylalanine thiocarbamate **2** (50 mM) alone (Table 1, entry 2), upon standing for 40 to 60 hours, gave dipeptide in 6 to 7% yield. Presumably the free phenylalanine required for dipeptide formation in the latter reaction is produced in situ from the decomposition of phenylalanine thiocarbamate **2** during the course of the reaction. As expected, the yield of peptide from phenylalanine thiocarbamate **2** is pH dependent in the range studied (pH 7.6 to 10.4), with maximum amounts of peptide formed around pH 9.0 (fig. S2). In the lower pH range, an increased rate of thiocarbamate decomposition to the free amino acid and amine protonation can decrease the flux through the productive reaction pathway. At higher than optimal pH values, yields can be reduced by hydrolysis of the thiocarbamate **2** and the NCA intermediate **4**.

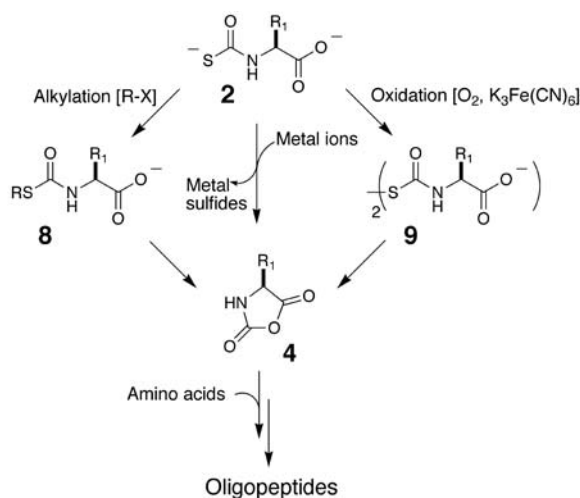
Because the thiolate anion is a relatively poor leaving group, the anaerobic intra-

molecular cyclization of thiocarbamate **2** to give NCA **4** is likely to be the rate-limiting step in the overall condensation process (9). To uncover more efficient and thermodynamically more favorable pathways for the conversion of **2** to **4**, we tested a number of additives that could potentially enhance the leaving-group ability of the thiolate anion and provide additional thermodynamic driving force for the reaction (Scheme 2). Dramatic rate accelerations and enhanced product yields were obtained in the presence of metal ions, oxidizing agents, or alkylating agents (Table 1, entries 5 to 20).

The COS-mediated condensation of phenylalanine or the conversion of phenylalanine thiocarbamate **2** to peptide products is substantially enhanced in the presence of stoichiometric amounts of Pb²⁺, Fe²⁺, or Cd²⁺ ions (Table 1, entries 5 to 8, 12 to 16; fig. S3) (10). Analogous experiments performed with Pb²⁺ or Cd²⁺ under anaerobic conditions gave similar results (Fig. 1A). A reaction was performed in filtered Pacific Ocean water with added Pb²⁺ buffered to pH 9.0 to determine the effect of high salt concentrations and trace inorganic and organic impurities (Table 1, entry 7). Good yields of di- and tripeptide produced after 3.5 hours confirmed the robustness of the reaction. In a similar vein, S-alkylation of the thiocarbamate **2** should enhance the leaving-group ability of the thiol and accelerate its cyclization to the NCA **4** (Scheme 2). The

presence of either bromoacetate or benzyl bromide increased the yield of dipeptide formed by a factor of ~4 relative to the basal reaction (Table 1, entries 17 to 20).

Recent reports of oxidative acylation of amines by thioacids (11) suggested that oxidative reaction conditions might also accelerate conversion of **2** to **4** via the bis-aminoacyl thiocarbamate disulfide **9** (Scheme 2). We confirm that oxygen introduced into the reaction did indeed improve dipeptide yields (fig. S4). However, it is now generally believed that the Earth's primitive atmosphere was free of oxygen (12). Alternative plausible prebiotic oxidizing agents are nitrate, nitrite, and ferricyanide ions (13). Solutions containing stoichiometric amounts of phenylalanine thiocarbamate **2**, phenylalanine, and potassium ferricyanide afforded greater than 50% yields of dipeptide along with appreciable quantities



Scheme 2.

Table 2. COS-mediated formation of mixed peptides. Abbreviations for the amino acid residues: A, Ala; F, Phe; L, Leu; S, Ser; Y, Tyr.

Entry*	L-Phe (mM)	Reactant 2 (mM)	PbCl ₂ (mM)	Final pH	Time (hours)	Observed dipeptides†	Observed tripeptides†
1	10	L-Tyrosine (10)	20	7.2	3	FF, YY, (YF), (FY)	YYY, (YYF), (YFF), FFF
2	25	L-Leucine (25)	50	7.1	3	FF, LL, (FL)	(LLF), (LFF), FFF
3	25	L-Alanine (25)	50	5.9	3	FF, (AF)	(AAF), (AFF), FFF
4	25	L-Serine (25)	50	6.3	3	SS, FF, SF, FS	SSS, (SFF), FFF

*Each experiment was initiated by admitting ~20 ml of COS gas to an argon-purged reaction vessel containing 2 ml of the reaction mixture indicated dissolved in 500 mM Me₃N buffer, at an initial pH of 9.1. Peptide products were identified by LCMS after quenching the reaction at 3 hours. †Peptides for which product masses were observed but primary amino acid sequences which were not determined are indicated in parentheses.

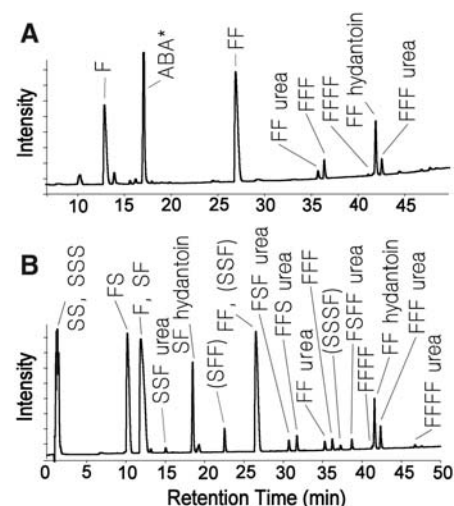


Fig. 1. HPLC-MS chromatograms of COS-mediated condensation of (A) L-Phe (Table 1, entry 6) and (B) a mixture of L-Phe and L-Ser (Table 2, entry 4) in the presence of PbCl₂ analyzed at reaction times of 3.5 hours (wavelength λ = 250 nm) and 3 hours (λ = 220 nm), respectively. Peptides for which product masses were observed but primary amino acid sequences were not determined are indicated in parentheses. ABA was added to the reaction mixture before HPLC analysis as the internal concentration standard. See supporting material for urea and hydantoin chemical structures (fig. S5A). Abbreviations for the amino acid residues: F, Phe; S, Ser.

of tri- and tetrapeptides (Table 1, entry 10). When an excess of the oxidizing agent was used with phenylalanine thiocarbamate **2**, LC-MS established that a 63% yield of dipeptide was obtained in just 5 min, along with 13% tripeptide, 3% tetrapeptide, and traces of penta- and hexapeptide (Table 1, entry 11).

In experiments in which a mixture of L-serine (Ser, 50 mM) and the phenylalanine thiocarbamate **2** (25 mM) in CHES (400 mM, pH 9.0) were allowed to react, either in the presence of CdCl₂ (25 mM) or K₃Fe(CN)₆ (25 mM), a mixture of peptides was produced corresponding to Phe-Ser, Phe-Phe, Phe-Phe-Ser, and Phe-Phe-Phe. No homopolymers of serine were observed. In another experiment, a mixture of L-serine and L-phenylalanine was exposed to COS (Table 2, entry 4). In contrast to the previous reaction, Ser-Ser and Ser-Ser-Ser were produced, along with polymers of phenylalanine and mixed peptides (Fig. 1B). These observations strongly suggest that the activated α-aminoacyl compound derives from the thiocarbamate structure and that, once activation has occurred, peptide formation proceeds via nucleophilic attack by a second α-amino acid molecule on the in situ–formed NCA. The generality of the COS-mediated α-amino acid condensation reactions in the presence of Pb²⁺ was established with reaction mixtures containing equimolar mixtures of L-phenylalanine and either L-tyrosine, L-leucine, L-alanine, or L-serine (Table 2, fig. S5). In all reactions, efficient production of mixed dipeptides and tripeptides was observed.

Present-day levels of COS in volcanic gases have been reported up to 0.09 mol % (14). Because the gas hydrolyzes rapidly on a geological time scale, it is unlikely to have accumulated to a high concentration in the atmosphere. Thus, if COS was important in prebiotic chemistry, it is likely to have functioned in localized regions close to its volcanic sources. Although it may be unlikely that a substantial proportion of any amino acids present would have been converted to thiocarbamates, this would have been no obstacle to a “polymerization on the rocks” scenario (15, 16) in which peptides long enough to be irreversibly adsorbed near the source of the COS were subject to slow chain elongation. The direct elongation of peptide chains using COS as a condensing agent and the condensations catalyzed by Fe²⁺ or Pb²⁺ ions seem plausible as prebiotic reactions (17). The very efficient polymerizations brought about by oxidizing agents are more problematic as prebiotic reactions, but [Fe(CN)₆]³⁺ has been discussed as a potential prebiotic oxidizing agent (13).

It remains to be determined whether COS could have participated in prebiotic chemistry in other ways—for example, as an interme-

diate in the reduction of CO₂ (18, 19) and as a condensing agent in phosphate chemistry (20, 21).

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- The observed half-life of phenylalanine thiocarbamate **2** (25 mM in D₂O, pD 8.6) formed in situ from the amino acid and COS was 10 hours. In a separate NMR study using an authentic sample of **2** (50 mM in D₂O, pD 9.0), a hydrolysis half-life of ~20 hours was observed.
- Condensations of NCAs with free amino acids (100 mM each in borate buffer pH ≈ 10) at 4°C are typically complete in less than 2 min (1, 24).
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Materials and Methods

Figs. S1 to S5

Reference

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Genome Sequence of a Polydnavirus: Insights into Symbiotic Virus Evolution

Eric Espagne,^{1*†} Catherine Dupuy,^{1†‡} Elisabeth Huguet,¹ Laurence Cattolico,² Bertille Provost,¹ Nathalie Martins,² Marylène Poirié,¹ Georges Periquet,¹ Jean Michel Drezen¹

Little is known of the fate of viruses involved in long-term obligatory associations with eukaryotes. For example, many species of parasitoid wasps have symbiotic viruses to manipulate host defenses and to allow development of parasitoid larvae. The complete nucleotide sequence of the DNA enclosed in the virus particles injected by a parasitoid wasp revealed a complex organization, resembling a eukaryote genomic region more than a viral genome. Although endocellular symbiont genomes have undergone a dramatic loss of genes, the evolution of symbiotic viruses appears to be characterized by extensive duplication of virulence genes coding for truncated versions of cellular proteins.

Once regarded as a rare biological event, symbiosis is now known to be central to the

origin of eukaryotic cellular organelles. The genomes of mitochondria and plastids are known to be dramatically reduced compared with those of their ancestors—free-living bacteria (1). There are also examples of viral symbionts, but almost nothing is known about the genome rearrangements these have undergone during their evolution.

Polydnaviruses (PDVs) are used by parasitoid wasps to facilitate development of their progeny within the body of immunocompetent insect hosts, which are typically lepidopteran larvae (2). Viral particles are produced in the

¹Institut de Recherche sur la Biologie de l’Insecte, CNRS UMR 6035, UFR Sciences et Techniques, Parc de Grandmont, 37200 Tours, France. ²Genoscope, Centre National de Séquençage, 2 rue Gaston Crémieux, CP 5706, 91057 Evry, France.

*Present address: Institut de Génétique et Microbiologie, Université Paris Sud, Bat. 400, 91405 Orsay cedex, France.

†These authors contributed equally to this work.

‡To whom correspondence should be addressed. E-mail: catherine.dupuy@univ-tours.fr