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Guanine, Adenine, and Hypoxanthine Production in UV-Irradiated Formamide Solutions: Relaxation of the Requirements for Prebiotic Purine Nucleobase Formation

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The RNA-world hypothesis[1] has generated much interest in finding a plausible prebiotic route to RNA. The abiotic synthesis of the purine nucleobases is of particular interest, given proposals that early proto-RNA polymers might have contained only purine nucleobases.[2] Formamide is attractive as a potential prebiotic starting material for the nucleobases; it contains the four required elements (C, H, O, N) and stands out because of its likely prebiotic availability, relative stability, versatile reactivity, and low volatility compared to water.[3] Saladino, Di Mauro, and co-workers have described formamide thermochemistry in detail and have catalogued many of the potentially prebiotic compounds generated when neat formamide is heated to 160 °C in the presence of various clays and mineral catalysts.[3a,4] However, the potential for UV photons to promote nucleobase production from formamide has received comparatively little attention;[4,5] this is surprising given that the absence of an ozone layer in the prebiotic atmosphere would have allowed the passage of photons with wavelengths less than 300 nm.[6] Here, we show for the first time that guanine, adenine, and hypoxanthine can be produced from formamide in a single model prebiotic reaction at lower temperatures than previously reported, if formamide is subjected to UV irradiation during heating; this observation relaxes the requirements for prebiotic purine nucleobase formation. The yield and diversity of purines produced in heated/UV-irradiated formamide are further enhanced by the presence of inorganic catalysts, as solids or as dissolved ions. We also analyzed the products of formamide solutions to which specific hydrogen cyanide (HCN) condensation products[7] were added prior to heating. These experiments demonstrate that purine nucleobase formation through formamide chemistry is compatible with previously proposed HCN chemical pathways to the same nucleobases.

Thermal reactions of formamide, either neat or in the presence of calcium carbonate or sodium pyrophosphate, were carried out at 130 °C. Consistent with similar reactions performed at a higher temperature (160 °C),[3a] purine ($C_5N_4H_4$) was the only nitrogenous base detected in reactions containing no added inorganic catalyst (Figure 1 A). The presence of calcium

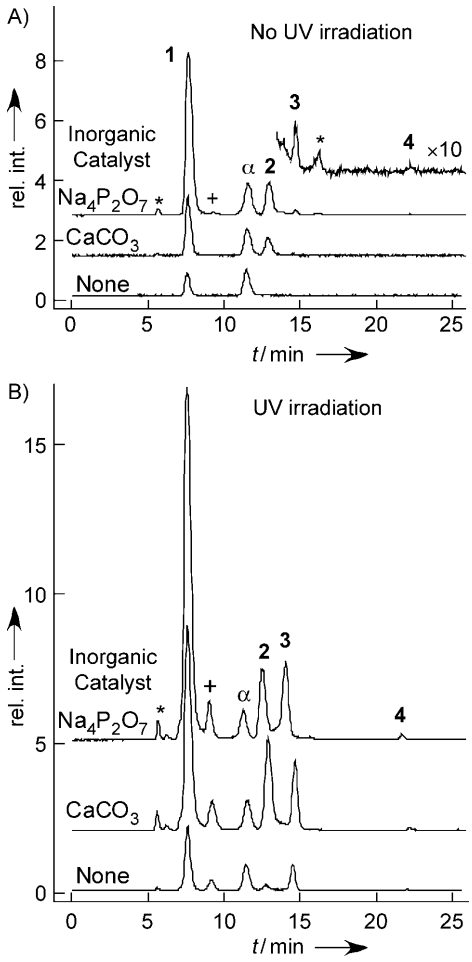

Figure 1. Combined selected ion chromatograms of formamide reactions with A) no UV irradiation and B) UV irradiation, heated to 130 °C for 48 h, in the presence and absence of inorganic catalysts. Offsets added for clarity. Products observed include purine (**1**), adenine (**2**), hypoxanthine (**3**), and guanine (**4**). Peak labeled (α) is the internal standard 2-aminopurine. Peak labeled (*) has the same product and precursor ion mass as adenine but is not adenine. Peak labeled (+) is tentatively assigned as allopurinol based upon comparison with existing standards. rel. int. = relative intensity.

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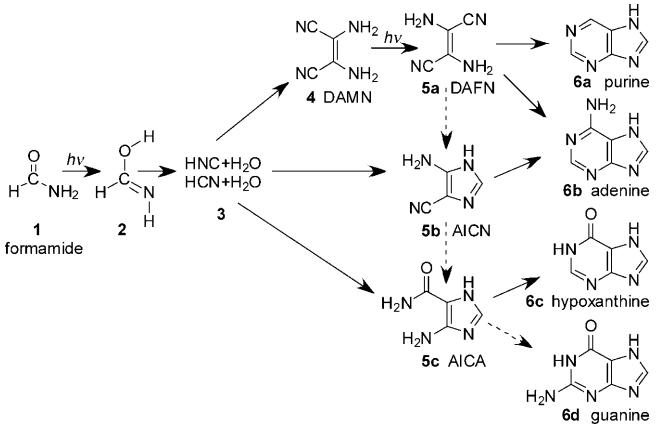
carbonate or sodium pyrophosphate enhanced purine yield and enabled the production of adenine and small amounts of hypoxanthine; additionally, a trace amount of guanine was formed in the presence of sodium pyrophosphate (Figure 1 A, inset).

Irradiation of formamide samples with 254 nm light increased the yield and diversity of purine nucleobases produced during heating at 130 °C for all samples tested. The nucleobases identified included purine, adenine, hypoxanthine, and guanine (Figure 1 B). While the yields of all purines identified increase with UV-irradiation, the relative increases in the yields of hypoxanthine and guanine were most pronounced.

Several pathways have been proposed for the generation of nucleobases from HCN. As these reactions are also likely involved in the thermal/photoinduced processes in formamide solutions, we briefly summarize the reaction pathway described in previous studies (Scheme 1).[3a,7–9] The primary pathways are thought to involve diaminomaleonitrile (DAMN, **4**), diaminofumaronitrile (DAFN, **5a**), aminoimidazolecarbonitrile (AICN, **5b**), and aminoimidazolecarboxamide (AICA, **5c**) as intermediates. In these pathways, DAMN and DAFN are proposed precursors to purine (**6a**) and adenine (**6b**), AICN to adenine, and AICA to hypoxanthine (**6c**) and guanine (**6d**).



Scheme 1. Putative pathways for production of nucleobases from formamide (**1**). Energy input in the form of heat and/or UV irradiation isomerizes formamide to formamidic acid (**2**), which is then converted into hydrogen cyanide and/or hydrogen isocyanide and water (**3**). Further thermal or photochemical transformations are believed to generate the reactive intermediates diaminomaleonitrile (DAMN, **4**), diaminofumaronitrile (DAFN, **5a**), aminoimidazolecarbonitrile (AICN, **5b**), and aminoimidazolecarboxamide (AICA, **5c**). These intermediates react further to form purine nucleobases including purine (**6a**), adenine (**6b**), hypoxanthine (**6c**), and guanine (**6d**).

As a means to explore the possible intermediates of the UV-enabled purine nucleobase formation process, DAMN, AICN, and AICA were each added to neat formamide (10 mM) before heating and UV irradiation. The progression of selected product yields for these reactions and for neat formamide was monitored over a 96 hour period. Heating 10 mM DAMN and 10 mM AICA in neat formamide for 48 hours without UV irradiation at 130 °C yields only modest quantities of adenine; heating 10 mM AICN in neat formamide yields greater amounts (Figure 2 A). This reaction reaches a steady state after 48 hours and stays constant within experimental error. When these same reactions are carried out in the presence of UV light, similar adenine yields are observed for samples spiked with AICN. In the UV-irradiated AICN-containing solution, there appears to be a photoinduced decrease in adenine yield at longer reaction times; after 48 hours, AICN or adenine might undergo photodecomposition, or the formation of higher molecular weight chemical species might be stimulated. In cases in which 10 mM DAMN is initially present, irradiation results in an approximately 15-fold increase in adenine yield—from 20 μg adenine per gram of formamide (0.2 mM) to about 300 μg per gram of formamide (2.5 mM; Figure 2 B). This observation is consistent with a photochemical step in the production of adenine from DAMN-containing formamide solutions; this is likely the formation of DAFN, which is the *trans* isomer of DAMN. UV irradiation of samples containing 10 mM AICA did not result in an appreciable increase in adenine production.

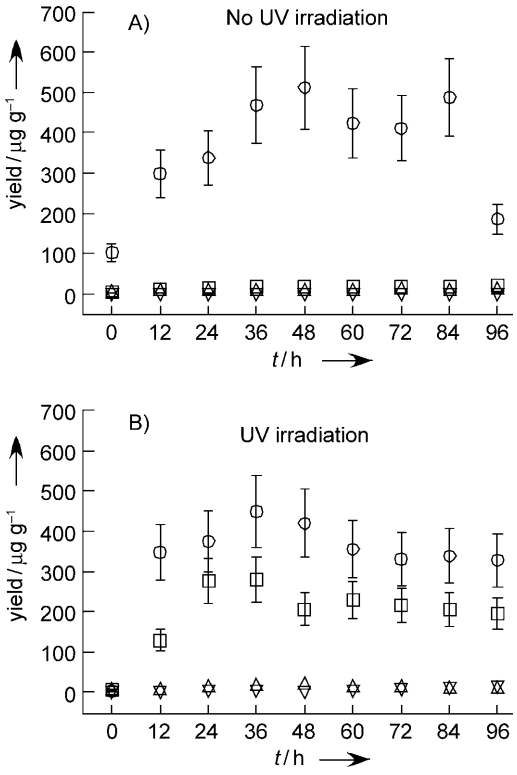


Figure 2. Yield of adenine by headed formamide solutions A) with no UV irradiation, and B) UV irradiation, as determined by LC-MS (see the Supporting Information). Solutions of neat formamide (▽), and formamide with 10 mM DAMN (□), 10 mM AICN (○), or 10 mM AICA (△) were maintained at 130 °C; samples in (B) were irradiated with 254 nm light.

Heating neat formamide or a formamide solution containing 10 mM AICN to 130 °C without irradiation yields very little hypoxanthine by a purely thermal process; in contrast, a solution containing 10 mM AICA yields appreciable hypoxanthine (Figure 3 A). The yield of hypoxanthine rises to approximately 240 μg hypoxanthine per gram of formamide (2 mM) in

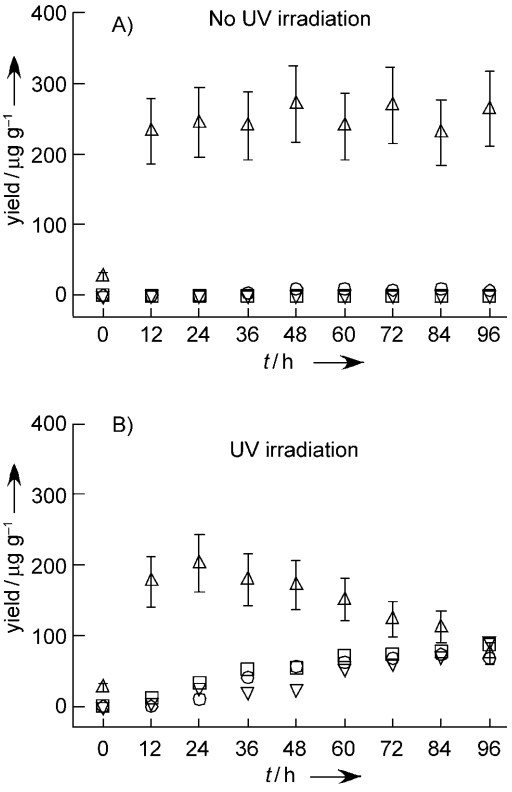


Figure 3. Yield of hypoxanthine by formamide solutions A) with no UV irradiation and B) UV irradiation, as determined by LC-MS (SI). Solutions of neat formamide (▽), and formamide with 10 mM DAMN (□), 10 mM AICN (○), 10 mM AICA (△) were maintained at 130 °C in A) and B) and irradiated with 254 nm light in B) only.

12 hours; it then remains approximately constant with sustained heating.

Hypoxanthine yields for all UV-irradiated and heated formamide solutions studied reach a plateau of approximately 100 μg hypoxanthine per gram of formamide (1 mM; Figure 3B), whether spiked with a putative intermediate or not. Formamide solutions initially with 10 mM AICA exhibited greater hypoxanthine yield (similar to the thermal-only experiments), but yields declined over time in the UV-irradiated samples; this suggests that approximately 100 μg hypoxanthine per gram of formamide (about 1 mM) is a steady-state condition. This observation is consistent with an increased importance of pathways mediated by DAMN and AICN and decreased importance of the AICA-mediated pathways with regard to hypoxanthine production in irradiated reactions.

Overall, our observations for the effects of DAMN, AICN, and AICA on purine nucleobase formation in formamide are consistent with these molecules being possible intermediates under our reaction conditions. We note that LC-MS/MS and HPLC analysis did not show the presence of DAMN, AICN, or AICA as final products in any reactions investigated; this suggests that if these molecules are truly intermediates in purine production, their conversion to purines is fast relative to their production from formamide.

While the purine nucleobases were the focus of this study, these compounds represent a subset of all products generated in the formamide reactions. Representative chromatograms from LC-MS and LC-UV, which provide some illustration of the breadth of other compounds generated, are provided in the Supporting Information (Figures S1 and S2).

We have worked with solutions of neat formamide because, for practical experimental considerations, it is the obvious starting point to study the effects of UV-irradiation on nucleobase production from formamide. However, it is difficult to envision a situation in which neat formamide was collected on the prebiotic Earth, as water, which is miscible with formamide, was most likely present[10] and more abundant than formamide. Nevertheless, given the lower volatility of formamide and the fact that azeotropic mixtures are not formed by water and formamide,[11] one could imagine scenarios in which primarily aqueous pools that initially contained small amounts of formamide would lose water through evaporation, during hot and dry periods, to give rise to concentration solutions of formamide.

We have performed experiments to test whether this "drying pool" model can give rise to solutions of formamide that also produce nucleobases. In one such experiment, 10 mol% formamide in water was heated to 100 °C for an extended period of time. Our results indicate the loss of water, the hydrolysis of formamide to ammonium formate at a rate of approximately 1% per 24 h (Figure S3), and the formation of purine, adenine, and hypoxanthine in detectable amounts over the course of 96 h (Figure 4).

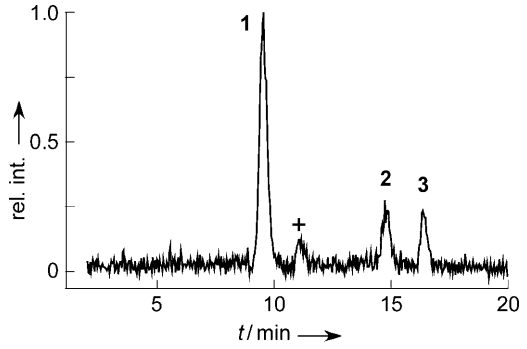


Figure 4. Selected ion chromatogram demonstrating nucleobase production after heating a 10 mol% formamide/90% water mixture at 100 °C for 96 h. Products observed include purine (**1**), adenine (**2**), and hypoxanthine (**3**). The peak labeled (+) has the same mass as hypoxanthine and is tentatively identified as allopurinol based on comparison with an authentic standard.

While the yields of these nucleobases are lower than those observed for the neat formamide reactions, the confirmation of nucleobase production from an initially mixed water–formamide solution supports the prebiotic relevance of the experiments presented above, and further relaxes the requirements for prebiotic purine nucleobase production. We also note that the mixed-solvent, inorganic catalyst-free, nonirradiated reaction produced hypoxanthine and adenine; under the same reaction conditions these products were not observed in neat formamide reactions. Given the previously reported observation that ammonium formate facilitates adenine produc-

tion[8a,d,12] and our observation that ammonium formate was produced over the course of heating and water evaporation, it is possible that the in situ formation of ammonium formate is an important factor in nucleobase production in this mixed-solvent system.

The observation that 254 nm UV light induces the formation of adenine, hypoxanthine, and guanine from neat formamide, suggests that formamide is a photoactive entity. The electronic absorption spectrum of formamide nominally begins at 206 nm,[13] which corresponds to a higher energy than that of the photons used in the present study. However, the tail of the UV absorption spectrum of a formamide solution extends to long enough wavelengths that the extinction coefficient of formamide at 254 nm is nonzero (approximately 0.6 $cm^{-1}\,M^{-1}$). Recently, Duvernay and co-workers outlined a photochemical pathway from formamide (**1**) to formimidic acid (**2**), which then decomposes to HCN/HNC and water (**3**).[9] HCN and HNC are believed to then undergo polymerization reactions to produce DAMN, AICN, and AICA under thermal and photochemical/thermal conditions (Scheme 1). Assuming that optical excitation of formamide is one of the important photochemical transitions, we can see from Duvernay's results that incident photons could make water available even in neat formamide to help initiate and facilitate the chemical steps leading to nucleobase formation.[9]

Here, we have demonstrated the production of adenine, hypoxanthine, and guanine in UV-irradiated formamide solutions. These "one-pot" reactions occur due to the synergy of thermal and photochemical processes. The reactions' yields and product distributions are enhanced in cases in which inorganic catalysts (as solids or dissolved ions) are present. The lower temperature at which our study was conducted combined with the equalizing effect that UV light has on purine nucleobase production (regardless of the presence of inorganic catalyst), further relax the chemical and environmental requirements for the formation of biopolymer building blocks on the prebiotic Earth.

Experimental Section

Reaction conditions: Formamide solutions that contained calcium carbonate or sodium pyrophosphate had these inorganic catalysts present at 4.4% *w/w*. Formamide solutions that contained putative purine intermediates (DAMN, AICA, or AICN), had these compounds present at 10 mM. Reactions described as UV-irradiated were exposed to a low pressure mercury lamp (Pen-Ray, primary emission at 254 nm; no emission below 200 nm), during heating. All reactions were heated at 130 °C for 48 h, unless otherwise noted.

Sample analysis: LC-MS was carried out by using a Merck SeQuant ZIC-HILIC HPLC column on an Agilent 1100 binary HPLC system coupled to a Micromass Quattro LC triple quadrupole mass spectrometer. Information on temperature and gradient is given in the SI. Reverse-phase HPLC was performed on an Agilent 1200 Series RRLC system with a diode array detector. A Phenomenex Synergi Polar RP column was used for separations.

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