PHOTOCHEMICAL SYNTHESIS OF BIOMOLECULES UNDER ANOXIC CONDITIONS

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Abstract. We report the long-wavelength UV anoxic photosynthesis of uracil, various sugars (including deoxyribose and glycoaldehyde), amino acids, and other organic photoproducts. These reactions occur in mixtures of water, calcium carbonate, formaldehyde and hydrazine. Our data demonstrate that under several sets of conditions biomolecules can be formed in variety and abundance from reduced compounds (formaldehyde and hydrazine) derived from anoxic dinitrogen/carbon dioxide environments. The formaldehyde concentrations were varied from 10 m*M* to 0.005 m*M*, and the hydrazine concentrations were varied from 1 m*M* to 0.01 m*M*. The highest of these reactant concentrations were 500 and 6 times greater than those reported for earlier experiments upon the synthesis of these precursors from CO_2 or N_2, while the lowest of reactant concentrations employed here were 0.5 (formaldehyde) and 0.006 (hydrazine). Product yields were greatest when the hydrazine/formaldehyde ratio was 1, and when the reactant concentrations were low. These data suggest that organic products can be formed in variety from those amounts of formaldehyde and hydrazine precursors which are themselves formed under anoxic UV photochemical conditions. Hence these various reactions would seem to have prebiotic relevance. The UV 254 nm photon flux employed was 100 times higher than unattenuated solar flux. Durations of UV exposure were 24 hrs and 72 hrs. No experiments have been addressed to the possibility of UV flux dependency.

1. Introduction

Some models for the primitive Earth environment postulate a hydrogen rich atmosphere where the dominant forms of nitrogen and carbon are ammonia and hydrocarbons (Urey, 1952). Most studies on the chemical beginnings of the origin of life assume that reduced forms of carbon and nitrogen were available as simple precursors for the geochemical synthesis of biomolecules (Folsome, 1979; West and Ponnamperuma, 1970). Other geochemical models depict an anoxic environment with dinitrogen as the major atmospheric constituent, with carbon dioxide at near present day concentrations (Walker, 1977). For either of these environments, oxygen was essentially absent; formed in trace amounts by upper-atmosphere photochemical reactions (Walker, 1977; Rubey, 1955), and being rapidly consumed by geochemical reactions (Holland, 1978).

Can water, oxidized forms of carbon, and oxidized forms of nitrogen serve as suitable precursors for the geochemical synthesis of biomolecules in anoxic hydrogen-free environments? Our recent studies showed that long-wavelength UV photosynthesis of formaldehyde from carbonate and water could be effected with apparent quantum yields of 10^{-5} by model phase bounded organic structures (Folsome and Brittain, 1981). Quenched spark electrical discharges upon water, and carbonate through a nitrogen gas phase generate hydrazine, hydrazides, and oxidized forms of nitrogen

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(Folsome *et al.*, 1981). A recent review of prebiotic photosynthetic reactions of carbon dioxide and dinitrogen suggests that these might indeed serve as a source of abiological organic coumpounds (Chittenden and Schwartz, 1981). The available data suggest that organic compounds could have been formed by geochemical events upon a primitive non-reducing (anoxic) Earth, but do not demonstrate directly the synthesis of simple biological compounds.

2. Reaction Conditions

Quartz 10 × 100 mm tubes were filled with 5 ml degassed 2× dist. water, 10 mg calcium carbonate, 0.01 to 10 m*M* freshly diluted hydrazine (as hydrazine hydrate from Aldrich Chemical Co.), and 0.01 to 10 m*M* redistilled (unlabeled) formaldehyde bearing 34.7 nm 14-C labeled formaldehyde (sp. act. 48.2 mCi/m*M*). Tubes were flushed with research grade nitrogen and septum stoppered. Reaction tubes were subjected to long-wavelength UV radiation for 72 or for 24 hours using a Rayonet UV photochemical reactor (model PRP–100) equipped with Vycor low pressure Hg lamps. The UV 254 nm dose rate at the reaction vessel surface was 213.3 erg s^{-1} mm^2 as determined by thymine dimer actionometry (McLaren and Shugar, 1964) (the unattenuated solar flux at zero atmospheres at 254 nm on the Earth is 2.2 erg s^{-1} mm^2) (Levine *et al.*, 1980). Control experiments consisted of similar reaction vessels and mixtures enclosed in 2 layers of aluminium foil. After UV irradiation samples were concentrated by lyophilization and subjected to various qualitative spot tests or were separated by anion and cation exchange chromatography into sets for further organic analyses. The path of the 14-C formaldehyde label was followed by liquid scintillation counting of most fractions. 14-C formaldehyde was subjected to ion exchange chromatography on Ag–1–X8 (acetate) prior to use to remove background counts. All experiments employed three replicates each for controls and for the UV exposed tubes.

3. Qualitative Results

No detectable organic products were recovered upon UV irradiation of water, nitrogen and $CaCO_3$ alone. This confirms our previous work which reported an apparent quantum yield of 9×10^{-8} of organic carbon products from these precursors (Folsome and Brittain, 1981). Neither HCN nor alkylnitriles were present in any experiment, although the spot test method used, pyrohydrolysis and benzidine acetate-copper(II) staining (Folsome *et al.*, 1981), can detect nanomolar amounts of these organic functional groups. The absence of HCN and nitrile functions is a consistent feature of these photochemical reactions. The presence of reducing sugars was suggested by positive aniline phthalate tests (Peschke, 1965), and by the anisaldehyde-sulfuric acid test (Lisoba, 1964). The Dische colorimetric test (Dische and Schwarz, 1937) for deoxy sugars was positive: absorbance of the colored derivative showed a maximum at 600–700 nm characteristic of deoxyribose, although this is not to be taken by itself as a definite identification.

4. Scheme for Group Separation

4.1. ANION EXCHANGE CHROMATOGRAPHY

Aqueous phase from the quartz reaction vessels was decanted, brought to 0.1 N NH_4OH and applied to an AG 1 × 8 (Bio-Rad) acetate charged anion exchange column (1 × 15 cm) (Simmonds, 1969). Elution of products was effected by successively applying 10 bed volumes (10 ml) of 0.1 N NH_4OH (fraction A), and 20 bed volumes of 0.1 N HCl (fraction B). As our reconstruction experiments have shown, fraction A consists of peptides, amino acids and sugars, while fraction B contains purines and pyrimidines.

4.2 CATION EXCHANGE CHROMATOGRAPHY

Fraction A (the first anion column rinse) was concentrated, dissolved in 1 N HCl and applied to a 1 × 15 cm AG 50 × 8 H^+ (Bio-Rad) column. This column was rinsed with 30 ml 1N HCl (fraction C), and then with 15 ml 2N NH_4OH (fraction D). Fraction C would contain neutral organic compounds as sugars, etc., while fraction D would contain amino acids and peptides.

For experiment No. 1 (see Table I) 14-C formaldehyde label was distributed among these fractions as:

Fraction B: (pyrimidines/purines), 6.0×10^4 dpm (1.6 %)
Fraction C: (neutrals/sugars), 2.7×10^5 dpm (7.3 %)
Fraction D: (amino acids/peptides), 1.1×10^5 dpm (2.9 %)
(Input 14-C formaldehyde counts were 3.7×10^6 dpm.)

(Counts above background recovered from identically processed unirradiated controls were subtracted from appropriate irradiated reaction mixtures.)

TABLE I
Yields as a function of reactant concentrations[a]

Exp. No.	Reactant concentration formaldehyde	hydrazine	UV 254 nm exposure	[b] Products recovered from AG1X8 (acetate)
1	10 m*M*	10 m*M*	72 hrs	1.6 %
2	1 m*M*	1 m*M*	72 hrs	8.7 %
3	0.01 m*M*	0.01 m*M*	72 hrs	14.2 %
4	0.5 m*M*	0.01 μ*M*	72 hrs	15.4 %
5	0.01 m*M*	1 m*M*	24 hrs	1.0 %
6	0.01 m*M*	0.01 m*M*	24 hrs	7.8 %

[a] Other reaction conditions were held the same: 5 ml d.HOH, 10 mg $CaCO_3$, under N_2 gas. Controls were wrapped in foil. Yields corrected for control background. All experiments and controls were performed in triplicate.

[b] Predominantly uracil and related photodecomposition products. Data expressed as percentage of input 14-C label corrected for control background.

Note on concentrations: 1 m*M* formaldehyde represents 0.15 mg formaldehyde per reaction vessel.

Fraction B, presumed purines and pyrimidines, was evaporated to dryness, the trimethylsilyl derivative was prepared (Folsome *et al.*, 1973) and subjected to gas chromatographic (GC) separation using a 4 m × 2 mm nickel 3 % OV–17 column (temperature programmed 4 °C/m from 90 °C to 200 °C). One major GC peak at 135 °C was present. Combined GC/mass spectrometry revealed that the major unknown component had an identical mass spectrum and GC retention temperature to that of a bis-TMS uracil standard. The UV absorption maxima for uracil and for this photoproduct also demonstrated equivalent pH displacements of maxima; (to 260 nm at pH 7, and to 272 nm at pH 13). The yields of uracil for various reaction mixtures (see Table I) accounted for about 1.6 % to 15.4 % of the input formaldehyde label. Aliquots of fraction B were applied to silica gel TLC plates and separated using a variety of solvent systems (I; water: II; methanol / water / 11 N HCl, 70 / 30 / 1: III; n-butanol / methanol / water / 10 N NH_4OH, 60 / 20 / 20 / 1). Spots were located under 254 nm UV illumination and were coincident with standard uracil. TLC plates were sprayed with chlorine-o-tolidine reagent (Isaq and Ban, 1977) and only unknown spots with Rf's identical to standard uracil were present. The method of Wright and Satchell (1971) for the specific detection of adenine in amounts as low as 10^9 *M* on TLC plates was also employed, with negative results. Hence we positively identify uracil as a major UV photoproduct of these reactions.

Fraction C, presumed sugars, which had been washed through both anion and then cation exchange columns, contained 7.3 % (2.7×10^5 dpm) of the 14-C formaldehyde label. After concentration this material was subjected to two-dimensional silica gel TLC separation (first dimension; dichloromethane / methanol / 11 N NH_4OH; 95 / 5 / 1: second dimension; ethyl acetate / water saturated n-butanol; 50 / 50). The plate was divided into 2 × 2 cm squares which were removed for determination of radiolabel by liquid scintillation counting. Developed TLC plates were also stained with aniline phthalate (Peschke, 1965) or iodine vapor prior to determination of radiolabel distribution within 2 × 2 cm squares. Most of these squares contained significant radiolabel which indicated that a wide range of sugars and related compounds were present. The only areas which were consistently above this product background were spots which co-chromatographed with glycoaldehyde and with deoxyribose. Hence we identify tentatively glucoaldehyde and deoxyribose as major products contained in this sugar fraction.

Fraction D, amino acids, released from the cation exchange column, contained 2.9 % (1.1×10^5 dpm) of the 14-C formaldehyde label (for exp. No. 1). This material was concentrated and separated by two-dimensional TLC (first dimension; isopropanol / formic acid / water, 80 / 4 / 20: second dimension; n-butanol / acetone / triethylamine / water, 30 / 30 / 6 / 15). Squares 2 × 2 cm were scraped from the developed plate and 14-C activity determined by liquid scintillation counting. A mixture of known and unlabeled amino acids was co-chromatographed and the plates were ninhydrin stained prior to removing 2 × 2 cm squares for some plates. 14.6 % of the total fraction D counts (1.6×10^4 dpm) co-chromatographed with amino acid standards and are identified as alanine (2.7 %), glycine (3.9 %), glutamate (1.4 %) leucine (3.0 %), valine

(3.6 %) and serine (< 1 %). For one experiment, an aliquot of this fraction was hydrolyzed in 6 N HCl for 30 min. and subjected to a Beckman quantitative amino acid analyzer which revealed glycine (371 nm/ml), alanine (3.5 nm/ml), glutamate (15 nm/ml), leucine (2.6 nm/ml), valine (trace), and serine (16 nm/ml). Relative abundances of hydrolyzed and unhydrolyzed fractions were different, suggesting release of some amino acids from an acid labile precursor. Several large ninhydrin positive peaks (in addition to NH_3) were noted at the buffer change zone. In all, these data show that 14-C labeled input formaldehyde carbon can be recovered in a variety of amino acids. Other discrete 14-C labeled zones were noted on the two-dimensional TLC plate which might be correlated with non-protein amino acids, although no standards were available for these determinations.

5. Discussion

Our results demonstrate that formaldehyde in the presence of hydrazine can serve as a precursor to the photosynthesis of uracil. In addition, our data indicate that a variety of sugars and amino acids are formed in these reactions. Although our identifications for specific members of these series are tentative, the point to be stressed here is that a variety of sugars are indeed recovered. The data show that over 7 % of the input formaldehyde can be recovered in the total sugar and neutral fraction (fraction C). While we have tentatively identified only the most major products as glycoaldehyde and deoxyribose, it is clear that other related compounds are present. Ponnamperuma and Mariner (1963) had previously reported the UV photosynthesis of 5- and 6-carbon sugars from formaldehyde. We were unable to detect any 6-carbon sugars, and could only identify (tentatively) deoxyribose in these experiments. However, our reaction conditions were different, being pH buffered by the solubility product of excess $CaCO_3$, and employing lower concentration of reactants.

The high specificity of these reactions for uracil is of interest: from 1.6 % to 15.4 % of the starting formaldehyde is recovered as uracil (as computed from the UV extinction coefficients of material recovered by column chromatography or by radiolabel recovery from 14-C formaldehyde into that fraction), while no other pyrimidines nor purines were found. UV irradiation also degrades uracil to pyrimidine dimers, hydropyrimidines and other photodecomposition products. We performed reconstruction experiments in which 0.2 μM uracil was UV irradiated (in 2 × dist. water, saturated with $CaCO_3$ under nitrogen) for comparable UV doses and found that 40 % of the input uracil could be recovered by our experimental procedures.

The absence of any other detectable pyrimidines and of any purines in these reactions of interest. Stoks and Schwartz (1979) reported the presence of uracil and purines in carbonaceous chondrites. Folsome *et al.* (1973) recovered only pyrimidines which were not amino substituted from carbonaceous chondrites.

Our previous work showed that phase bounded microstructures were required for the UV photosynthesis of formaldehyde from carbonate and water (Folsome and Brittain, 1981). The formaldehyde concentrations which we had reported for micros-

tructure mediated UV photosynthesis of formaldehyde were 2.25 μM, while the concentrations of formaldehyde used for these experiments were varied from 0.5 μM to 10 mM. We had also reported that hydrazine is generated by electrical discharges through N_2 over $CaCO_3$-saturated water (Folsome *et al.*, 1981). The hydrazine yields for these earlier experiments were of the order of 0.16 mM, while the concentration employed for these experiments ranged from 10 mM to 0.01 mM. Clearly the concentrations of both formaldehyde and hydrazine used in these experiments encompasses the ranges that our previous work suggested and intimates this to be an adequate simulation for primordial organic systheses. However, the experimental photon fluxes we used were 100 fold higher than the nominal unattenuated solar flux at 254 nm. Since we do not yet know whether the synthesis is photon flux dependent we cannot at this time propose that these systheses are meaningful in an origin-of-life context from this aspect. This work indicates that the UV photochemistry of aqueous anoxic carbonate buffered formaldehyde and hydrazine mixtures can lead effectively to the synthesis of a variety of amino acids, to uracil, and to sugar-like products (including deoxyribose). To complete the scenario for anoxic primordial biogenesis, precursors to organic phase bounded structures are required which do not require reduced carbon. Our recent observations suggest that a surface organic coating becomes accreted upon $CaCO_3$ crystals during prolonged UV irradiation of nitrogen, $CaCO_3$, water mixtures. We suggest that such a membranous coating might serve the functional role of such model organic microstructures. Surface coatings on coccoliths (Burki *et al.*, 1982) have been reported and methods to characterize such coating have been developed. Our current work is directed towards isolation and characterization of these surface films.

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