Reports

Long-Wavelength Ultraviolet Photoproduction of Amino Acids on the Primitive Earth

Abstract. *Amino acids are produced under possible primitive Earth conditions by irradiation of gas mixtures with long-wavelength ultraviolet light, representing the most abundant useful energy source for prebiological organic synthesis. Hydrogen sulfide is the initial photon acceptor in this work; superthermal atomic hydrogen photodissociation products appear to initiate reactions leading to amino acid synthesis with an overall quantum yield on the order of* 5×10^{-5}.

A wide range of experiments (*1*) has shown that amino acids, and other molecules of importance for the origin of life, can be produced readily from a variety of gases, and with a very wide range of energy sources, provided only that the net conditions are reducing. Miller and Urey (*2*) presented a table which showed that ultraviolet light at wavelengths $\lambda < 2500$ Å provided to the primitive Earth more than 100 times more energy than all nonelectromagnetic sources combined; and from models of the evolution of the Sun, Sagan (*3*) calculated that some 4×10^9 years ago the ultraviolet photon flux at $\lambda < 2600$ Å was approximately 100 times greater than that in the vacuum ultraviolet. The shape of the Planck distribution is very steep on the Wien edge. The longest wavelengths that could accomplish organic photochemistry on the primitive Earth have not been studied, because the proposed primary constituents of the primitive reducing atmosphere—hydrogen, methane, ammonia, water, ethane, and perhaps CO and N_2—are all entirely transparent at $\lambda > 2400$ Å, and, except for a very weak absorption by ammonia, all are essentially transparent at $\lambda > 2000$ Å. What is needed is a plausible near ultraviolet photon acceptor for the primitive Earth.

In this report we consider hydrogen sulfide as such a candidate photon acceptor. In tables of cosmic abundances S ranks with such atoms as Mg and Si, coming just after H, He, O, N, and C; it is a pervasive constituent of volcanic effluvia even today, and under primitive reducing conditions sulfur should have been almost entirely in the form H_2S. Holland (*4*) calculates on equilibrium grounds that H_2S was a prominent constituent of the primitive atmosphere, with an abundance comparable to that of ammonia and water vapor. Hydrogen sulfide has a broad absorption continuum beginning at about 2700 Å and continuing down to the vacuum ultraviolet (*5*). Absorption coefficients are large enough that 6 cm-atm of H_2S absorbs $(1 - 1/e)$ of the incident 2537 Å radiation; thus reaction vessels having a capacity of a few liters are adequate for such simulations. The dissociation energy of the HS–H bond is 85 to 95 kcal/mole (*6*). The energy of a 2537 Å photon producing a photodissociation event leaves an excess energy of 18 to 28 kcal/mole; 97 percent of this excess energy is converted into translational kinetic energy of the H atom. Thus 2537 Å photodissociation of H_2S produces hot H atoms which are superthermal by 17.5 to 27.2 kcal/mole. This is only about 20 percent of the bond dissociation energy of CH_3–H, NH_2–H, and OH–H, but collisional perturbation of the state function of the larger molecule by hot H atoms of this energy is known, for example for hydrocarbons, to provide the activation energy for further reactions (*6*). Accordingly, investigations were initiated into the long-wavelength ultraviolet photochemistry of simulated primitive atmospheres containing H_2S. Other plausible long-wavelength ultraviolet photon acceptors can be imagined; and we will report separately successful experiments on the production of amino acids in which formaldehyde is the photon acceptor.

We report here the results of a series of experiments performed over several years; it was initiated at the Smithsonian Astrophysical Observatory and Harvard University and completed at Cornell University. The basic form of the apparatus is shown in Fig. 1. A 4- to 13-liter quartz or Pyrex reaction vessel (see Table 1) is provided with a Spectrosil or quartz well, fused onto it so that a Hg line emission source inserted into the well can irradiate gases in the reaction vessel in cylindrical geometry. In some experiments the source is water-cooled and thermally insulated from the reaction vessel by a vacuum dead space. With water cooling, the attenuation of the 2537 Å line is negligible, but the attenuation of the 1849 Å line is essentially complete. In other experiments dry nitrogen was used to cool, and in such cases both 1849 Å and 2537 Å irradiation occurred. Copper-constantan thermocouple gauges were inserted in various places in the reaction vessel to monitor the temperature history of the experiment. Irradiated gases were circulated through a liquid water trough by a slightly modified version of the greaseless solenoid pump of the Watson design (*7*).

Besides H_2S and the equilibrium vapor pressure of H_2O above the liquid, these experiments employed ammonia, methane, and, in all but one case, ethane. Ethane is known both experimentally (*8*) and by equilibrium thermodynamic calculations (*9*) to be produced in reducing atmospheres of cosmic composition, and photochemical calculations for the Jovian planets indicate that large quantities of ethane may result from the short-wavelength ultraviolet photodissociation of methane (*10*). In the only other experiments on long-wavelength ultraviolet irradiation of primitive atmospheres that we know of, a Hg sensitization experiment in which Hg vapor was the photon acceptor (*11*), glycine, alanine, and perhaps α-aminobutyric acid were produced, but only in the presence of ethane; in making amino acids it is evidentally desirable to begin with preformed C–C bonds. In all of our experiments, gases were transferred to the reaction vessel through a spiral tube kept at −23° to −64°C to prevent small quantities of Hg vapor or any trace of stopcock grease from passing into the experimental portion of the vacuum system.

With the precursor gases, one or two Hg resonance lines, the gas temperature (*12*), and the time history of ir-

Table 1. Summary of experiments.

Composition and volume (in cm³) of reaction vessel	Precursors (quantities in cm³)	Emission line (Å)	Gas temperature (°C)	Trap temperature (°C)	Irradiation time	Amino acids detected in liquid solution	Other products	Appearance of liquid solution
				Experiment 1				
Quartz, 3780	CH_4, 1128; NH_3, 1150; H_2S, 1150; liquid H_2O, 10	2537	20	−23	14 hours	None	$S_1, \ldots, S_8, \ldots$	Pale yellow
				Experiment 2				
Quartz, 4195	CH_4, 591; C_2H_6, 2010; NH_3, 1000; H_2S, 549; liquid H_2O, 15†	2537, 1849	400	−23	(2537 + 1849), 2 days; 2537, ½day; (2537 + 1849), 9 days	Ala, Gly, Ser, Glu, Asp, Cys_2*	$S_1, \ldots, S_8, \ldots$ and unidentified high mass number products	Deep viscid orange-brown
				Experiment 3				
Quartz, 4189	CH_4, 612; C_2H_6, 2024; NH_3, 1004; H_2S, 542; liquid H_2O, 16	2537	215–360	−64	30 hours	Ala, Gly, Ser, Glu, Asp, Cys	Unexamined	Deep viscid orange-brown
				Experiment 4				
Pyrex, 5673	CH_4, 801; C_2H_6, 2738; NH_3, 1366; H_2S, 726, liquid H_2O, 21	None	300–400	−64	11 days	None	Unexamined	Colorless
				Experiment 5				
Pyrex, 12686	CH_4, 1638; C_2H_6, 6055; NH_3, 3035; H_2S, 1678‡; liquid H_2O, 50	2537	50	−64	25 days	Ala, . . . §	Unexamined	Greenish yellow: brown droplets formed in glassware

* Only amino acids confirmed both by ^{14}C-autoradiography and an amino acid analyzer are listed. † Liquid water was isolated from gas phase photolytic products for first 15 hours of this experiment. ‡ After 2 days' irradiation an additional 989 cm³ of H_2S was introduced into the system. § Yield after acid hydrolysis is ten times greater and includes Gly, Ser, Glu, Asp, and Cys.

radiation as variables, five experiments were performed (see Table 1). Experiments 1 and 2 were extremes: experiment 1 was ethane-free, 2537 Å only, cool and brief; experiment 2 added ethane and the 1849 Å line, was performed at higher temperatures and for an extended period. In experiment 3, 1849 Å radiation was removed, the gas temperature was dropped, and the irradiation time was shortened. Experiment 4 was a high-temperature radiation-free control; experiment 5, a lengthy room-temperature, 2537 Å radiation run. These experiments are now described in more detail.

Experiment 1. The purest commercially available gases were procured and purified by repeated fractional distillations through constant-temperature baths until they were found to be tensiometrically homogeneous. Purity was also checked with a PE model 620 high-dispersion, double-beam spectrophotometer. Analysis of the gas sample after irradiation for 14 hours with a CEC model 10C 180-deg sector mass spectrometer revealed the presence of H_2. The quantity of noncondensable gases at −196°C (H_2, N_2, or possibly some CO) collected at the end of the experiment indicates that several hundred cubic centimeters of this fraction comes from CH_4, NH_3, or H_2O. Fractional distillation of the gas products at the end of the experiment and subsequent infrared spectroscopy revealed no new molecules. There seems little doubt that photolytic hot H atoms from H_2S did initiate chain reactions. Mass spectra of an evaporated aliquot of the aqueous solution revealed the presence of polymeric sulfur, $S_1, \ldots, S_8, \ldots$. The reaction vessel was removed from the vacuum system and rinsed with water. The presence of elemental S was confirmed by melting point analysis. After repeated washings and dryings the quantity of S collected indicated that at least 22 percent of the initial H_2S was photodissociated in the experiment. After 14 hours the color of the aqueous solution was a pale yellow. Paper chromatography of this solution performed immediately after the experiment revealed no ninhydrin-positive features; ion-exchange chromatography on a Beckman model 120C amino acid analyzer several months after the experiment also gave negative results.

Experiment 2. Here ethane was added, 1849 Å radiation was introduced, and the temperature of the reaction vessel was raised to 400°C. In addition, the gas phase photolysis products were isolated from the liquid water bath for the first 15 hours of the experiment. Finally, 2 mc of $[^{14}C]CH_4$ and 1 mc of $[1\text{-}^{14}C]C_2H_6$ were added to the gas mixture, bringing the amino acid detectivity into the picomole range by autoradiographic paper chromatography. The temperature of the aqueous solution was varied with heating tapes between 70° and 90°C for the first 9 days of the experiment, providing significant quantities of water vapor in the reaction vessel, and then dropped to 25°C for the last 3 days.

A deep orangish-brown viscid (polymeric) material appeared within minutes of turning on the Hg discharge and continued to develop throughout the experiment. Mass spectra of this dark liquid performed on an AEI model MS-902 mass spectrometer revealed peaks well above the background at mass-to-charge ratios (m/e) of 188, 220, 260, and 280, among others. No attempt is made here to interpret these data. The liquid was examined for amino acids by two-dimensional chromatography on Whatman No. 1 chromatotographic paper. The solvents were mixtures of methanol, *n*-butanol, water, and ammonia (10 : 10 : 5 : 2) and acetone, *n*-butanol, water, and ammonia (10 : 10 : 5 : 2). The paper chromatograms were examined by ninhydrin spray and by autoradiography with Kodak medical x-ray film type NS-54T after exposure for 1 month. In addition, a Hitachi model KLA-3B amino acid analyzer was employed. Identifications were confirmed by adding standard chromatographically pure solutions of candidate amino acids to the experimental liquid. Amino acids found by both autoradiography and ion-exchange chromatography are listed in Table 2. This represents the first successful synthesis of the sulfur-containing amino acid cystine; we discuss the synthesis of sulfur-containing amino acids under prebiological conditions in more detail elsewhere (*13*).

After two decades of experience with prebiological organic chemistry, experimenters rarely check to see whether the molecules they discover could be produced by microorganisms metabolizing other experimental products. To check this remote possibility, we mixed 0.25 ml of the solution from experiment 1 with 0.2 ml of the solution from experiment 2. This mixture was divided into two parts, the first immediately analyzed with an amino acid analyzer; the other after standing 1 month.

The concentration of all amino acids remained fixed within experimental error, except for serine which declined in abundance significantly after 1 month; but serine is (with threonine) the most thermolabile amino acid known (*14*).

Experiment 3. This experiment differed from experiment 2 in that 1849 Å radiation was not employed, and the gas phase photolytic products had access to the liquid solution at all times in the experiment. The liquid solution again turned a deep viscid orange-brown. Alanine, glycine, serine, and cysteine were observed.

Experiment 4. This control experiment on the absence of ultraviolet was performed by constructing the entire reaction vessel of Pyrex borosilicate glass which is ultraviolet-opaque. In contrast with previous experiments, the liquid medium here remained colorless throughout the experiment. Also in contrast with the previous three experiments, the residual vapor pressure over a liquid nitrogen trap at the end of the experiment was only 10 mm-Hg, indicating no evolution of H_2. Both paper chromatography and the amino acid analyzer gave negative results for amino acids.

Experiment 5. This 2537 Å irradiation was performed at room temperature. After 7 hours of photolysis, 2 mc of $[^{14}C]CH_4$ was introduced; after 2 days of photolysis, additional H_2S was introduced into the reaction vessel. Drops of brownish liquid were observed forming during the course of the experiment, but the coloration of the liquid medium at the end of 25 days of photolysis was a slightly greenish yellow. The quantity of residual gas (mainly H_2) not condensable at -196°C after 2 days was calculated to be several liters.

Evaporates from the liquid solution were analyzed on a Beckman model 120B amino acid analyzer. In addition, 0.5 ml of this solution was dried under N_2 and hydrolyzed for 22 hours in 5.7*N* HCl at 105°C. It was then dried under vacuum and also introduced into the amino acid analyzer. From the first sample 0.006 μmole of alanine per milliliter was detected. The acid hydrolysis fraction, however, yielded 0.077 μmole of alanine per milliliter some 13 times more than in the first sample. Glycine, serine, cystine, and aspartic and glutamic acids were also detected in the acid hydrolysis fraction. This result strongly suggests that amino acids in such experiments are not primarily made as free amino acids, but rather in some other form—possibly nitriles or polypeptides—from which they are released on hydrolysis. By ^{14}C counting of the products of this experiment, we found that more than 2 percent of the initial methane had gone into liquid or solid products, establishing that chain reactions initiated by 2537 Å photolysis of H_2S eventually lead to CH_4 dissociation and participation in subsequent reactions. Hydrocarbon sulfides such as $(C_2H_5S)_2$ and $(C_2H_5)_2S$ are known gas phase intermediaries in such experiments (*15*).

Table 2. Amino acid yields (in micromoles per mole of H_2S) from experiment 2.

Alanine	Glycine	Cystine	Serine	Glutamic acid	Aspartic acid
597	56	25.7	6.1	6.1	3.06

We can perform a crude calculation of the energetics and quantum yields in such experiments from the results of experiment 2, where a total of the order of 1 μmole/cm^3 or approximately 10^{19} molecules of amino acids were formed. The Hanovia SC 2537 and S654 A lamps employed emitted $\sim 1.6 \times 10^{13}$ ergs at 2537 Å during the experiment. We neglect the much smaller amounts of 1849 Å and other line and

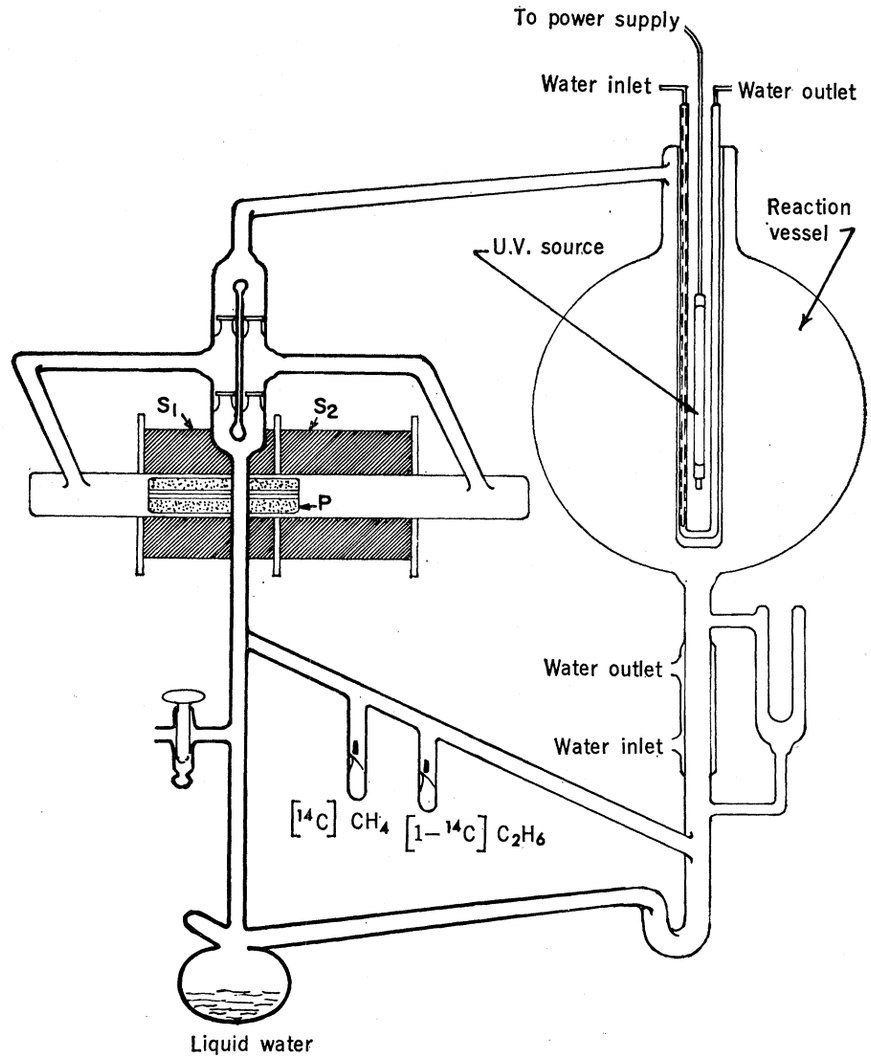

Fig. 1. Schematic diagram of a typical experimental setup. Gases are irradiated by cooled ultraviolet (*U.V.*) source at upper right and circulated by greaseless solenoid pump at upper left through water bath at lower left. The solenoid windings are indicated by S_1 and S_2, and the glass-enclosed iron plunger by *P*. The four glass gravity valves of the pump are shown in the closed position.

continuum emission. From the initial proportion of H_2S employed, the 5-cm mean light path through the reaction vessel, and the 2537 Å absorption coefficient of H_2S (*5*), we calculate that approximately 10 percent of the radiation emitted at the beginning of the experiment was absorbed by H_2S; and rather less toward the end of the experiment. Thus $\sim 6 \times 10^6$ amino acid molecules were produced per erg absorbed. We compare this efficiency number with the 5×10^{10} per erg found in comparable shock experiments (*16*). Thus it appears that shocks are approximately 10^4 times more efficient than ultraviolet for prebiological organic synthesis. But $\lambda < 2600$ Å ultraviolet was at least 10^3 times more abundant on the primitive Earth. If we allow for the facts that (i) only free amino acids and not the products of acid hydrolysis were analyzed in experiment 2; that (ii) at $\lambda < 2537$ Å the H_2S photolytic products are more energetic than at 2537 Å; and that (iii) other photon acceptors—for example, HCHO—may be effective at $\lambda < 2537$ Å, we conclude that overall ultraviolet and shock energies were of comparable importance in the prebiological synthesis of amino acids. A previous conclusion (*16*) that shocks were more important relied on earlier ultraviolet quantum yields from Hg sensitization experiments (*11*). The quantum yield per 2537 Å photon found in our experiment is $\phi \sim 5 \times 10^{-5}$, about an order of magnitude more than found in (*11*). Assuming all $\lambda < 2600$ Å photons were absorbed by H_2S on the primitive Earth, and that no subsequent destruction of amino acids occurred, this ϕ and the photon flux for the early Sun (*3*) imply approximately 200 kg of amino acids produced per square centimeter in 10^9 years. Destruction, of course, must have occurred, but a very high concentration of organic compounds and conditions quite congenial for the origin of life seem to be suggested.

The principal conclusions of this work follow: (i) H_2S is an acceptable long-wavelength photon acceptor for prebiological organic chemistry. The 1849 Å line is not necessary for the production of amino acids, but initial ethane is. Ethane is a likely product of electrical discharge and short-wavelength ultraviolet irradiation of methane. Temperatures higher than plausible mean surface temperatures are not required. (ii) A quantum yield $\phi > 5 \times 10^{-5}$, and an efficiency number $\gtrsim 6 \times 10^6$ amino acid molecules per erg are implied. Over 10^9 years of ultraviolet irradiation of the early Earth, this is the equivalent of 200 kg of amino acids produced per square centimeter, a huge number suggesting congenial conditions for the origin of life. (iii) Cystine and therefore perhaps other sulfur-containing amino acids can be produced in simulated prebiological conditions.

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References and Notes

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12. The most reasonable estimate for the temperature of the primitive Earth is something around room temperature, but there may have been localized times and places when surface temperatures were higher; upper atmospheric temperatures were of course much higher. Methane is not successfully attacked by radicals and hot hydrogen until it is brought to a temperature of some 300°C (*17*). Accordingly in some experiments temperature of 300° to 400°C were employed. Amino acids have been produced by heat alone from a reducing atmosphere, but temperatures of 900° to 1100°C are required (*18*); we show below that temperatures of 300° to 400°C alone will not produce detectable amino acids.
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Countercurrent Chromatography with Flow-Through Coil Planet Centrifuge

Abstract. *We have developed a new method of countercurrent chromatography which employs a vertical helical tube in the centrifugal field. The helical tube is arranged so that it does not rotate as it revolves, thus eliminating the need for rotating seals. When the gyrating tube is filled with either phase and the other phase is introduced into the tube in the proper direction, an equilibrium state results in which the two phases are split into multiple alternating segments within the coil. Each phase oscillates to and fro with the rotation as the moving phase is steadily eluted out through the other end of the tube. Consequently, solutes introduced into the tube are subjected to a rapid partition process, resulting in an efficient chromatographic separation without the complications arising from solid supports. The method is illustrated by the microanalytical separation of dinitrophenyl amino acids and can be used on a preparative scale.*

Helix countercurrent chromatography (*1, 2*) provides various advantages over the liquid partition techniques with the coil planet centrifuge (*3*), because of its continuous flow-through system. The versatility of the method is, however, limited by the fact that the solvent is fed from a ro-