Anoxic photolysis as a Route to Primary Metabolites on Early Earth

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Abstract

The idea of the origin of life as a complex reaction network spontaneously arising from reactions of small molecules might be wrong. The results of prebiotic chemistry experiments, where biologically relevant compounds are formed from simple monomers, such as cyanate, CO_2 or formaldehyde, are usually complex mixtures with only trace amounts of molecules of interest. Here I take an opposite approach and show that a number of primary metabolites can be derived from tar-like mixtures by Advanced Reduction Process (ARP). Tars are usually formed from polycyclic aromatic hydrocarbons (PAH), are widespread in the Universe and were likely a source of organic carbon on the early Earth. The conditions thought to exist on the surface prebiotic Earth, such as sunlight, anoxic sulfur-rich atmosphere and iron-rich dust, lead to the transformation of naphthalene and other PAH to succinic, lactic, malonic and glycolic acids, known to be primary metabolites in all living systems. The sum of reaction yields of these biologically important metabolites goes up to 40%, with individual product yields 1-15%. The simplicity of this reaction and the ubiquity of PAH on the prebiotic Earth make this process

particularly appealing, as it may have occurred on a very large scale on surfaces of the planet exposed to sunlight. These findings may point to an overlooked initial step for the origin of life on Earth, and potentially elsewhere in the Universe.

Keywords: PAH, astrobiology, carbonaceous chondrites, photolysis, abiogenesis, TCA cycle, early metabolism, origin of life

It is generally assumed that life started from chemical mixtures of simple molecules, and through a series of geochemical and chemical processes, biology emerged.[1] There is an ongoing debate concerning the initial composition of such mixtures and their similarity to modern metabolome.[2–4] Miller-Urey experiments opened the field of prebiotic chemistry[5] and, for over 70 years, combinatorial prebiotic reactions have been reported that produce biologically important molecules from simple precursors, including cyanide,[6–9] formamide,[10] and others small molecules.[11,12] Potentially the process is widespread in the Universe since irradiation of astrophysical ice analogues, rich in H_2O, CO, CO_2, NH_3 and CH_3OH, grains form multiple organic molecules, including biologically relevant aldehydes and quinones.[13–15] [2–4]

While this scenario fits well into a human narrative of "things progress from simple to complex", it might be far from reality, since relatively little progress in the area has been made since Butlerov described formose reaction over 160 years ago. Recombination of simple organic molecules can lead to the formation of enormous chemical diversity.[16,17] A natural selection process might have existed to reduce number of possible molecules to the relatively simple and conserved set of biologically relevant molecules that we see today.[18,19] Suggested selection filters include water solubility,[2] autocatalysis,[20] and partition coefficients between early protocells and the prebiotic "soup" envinroment,[21] however, experimental demonstration of any of these

scenarios is still lacking. This work suggests a new pathway for the synthesis of a constrained set of biologically important molecules.

Polycyclic aromatic hydrocarbons (PAH) are a major source of non-biological organic molecules in the Universe. These compounds constitute the bulk of carbonaceous chondrite meteorites and dust particles in solar system[22,23] unlike water soluble organic molecules that are found in *ppm* to *ppb* amounts.[24,25] PAH formation predates the formation of Earth[26] and PAH were likely the major source of carbon during the Hadean eon, and their transformation in the early Earth environment could have been the route to the organic molecules that started life. PAH are common contaminants, that are removed from atmosphere and waste water via Advanced Oxidation Process in the presence of oxygen, persulfate and ozone. Reported products of incomplete oxidation of PAH contaminants include fumaric, malonic, pyruvic, glycolic and formic acids[34] — molecules that are starting material of metabolic networks across all domains of life . Carboxylic acids, including pyruvic, are major decomposition products of such processes. While these are biologically relevant target compounds, low oxygen concentration in the early Earth atmosphere makes this route implausible.

Advanced reduction process (ARP) is used for water purification[31] that does not require molecular oxygen for PAH decomposition. Photolysis of bisulfite (HSO_3^-) and ferric ions under mid-range UV irradiation produce highly reactive •OH and •H radicals[16,28–30] that lead to advanced reduction process of PAH.[27,32] Environmental conditions on early Earth—high intensity ultraviolet (UV) light, liquid water, high volcanic activity and iron-rich dust— would be similar to the conditions used in ARP and could potentially present a natural filter that reduced number of organic molecules of the prebiotic "soup" to the few relevant one to the origin of life. Milder oxidation conditions on Earth, due to the absence of oxygen and other strong oxidants from the atmosphere, could lead to partial oxidation of PAH with carboxylic

acids as end products. To test the hypothesis that iron-sulfur chemistry could lead to the photolytic formation of biologically relevant molecules, we studied ARP of PAH in anoxic aqueous solutions of ferric ions and sulfite.

I started with the study of naphthalene, which is a most abundant molecule in PAH mixtures.[35] PAH are poorly soluble in water and likely would have formed a floating layer on the surface of lakes and oceans. I constructed a microfluidic flow photoreactor to simulate photolysis of PAH under these conditions. A mercury vapor lamp provided a wide spectrum of UV light with a maximum at 365 nm, simulating sunlight on the surface of the planet in the absence of an ozone layer. Flow rate controlled exposure times of the solution to the UV light which varied from 3 to 30 minutes. Solvent fractions were analyzed by HPLC, GCMS and NMR.

Disappearance of naphthalene was monitored by HPLC. Naphthalene concentrations dropped to 50% after being exposed to UV light for 3 minutes and disappeared completely after 10 minutes exposure. Only trace amounts of other aromatic molecules were detected by HPLC at various times during the reaction, disappearing completely after 10 minutes of irradiation.

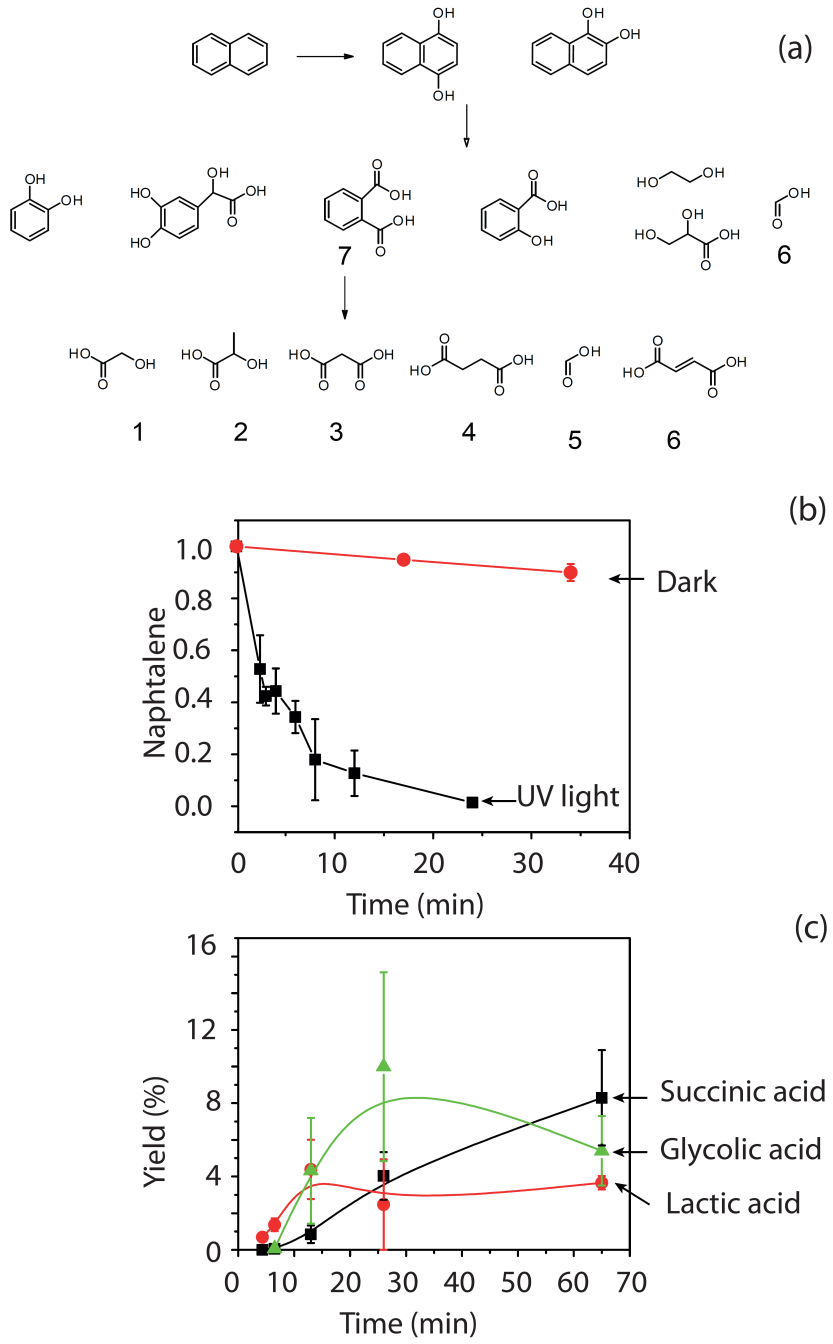

Figure 1. (a) Suggested steps of the photolysis of naphthalene, inferred from the products detected by GC-MS; (b) Naphthalene oxidation by Fe^{3+} sulfite system followed by HPLC; (c) Yields of lactic, glycolic and succinic acids as a function of reaction times, as determined by GCMS.

Table 1. Yields of carboxylic acids obtained by hydrolysis of naphthalene at 50 µL/min flow rate (23 minutes exposure time). Yields calculated from ^{1}H NMR peaks from 3 different runs against TMS internal standard.

Acid	**Yield Range, %**
Formic	5.2-16.5
Glycolic	1.4-3.8
Malonic	0.5-3.6
Succinic	1.3-9.3
Lactic	0.9-6.3

Exposure of naphthalene to UV light for 1-5 min induced oxidative opening of the aromatic rings and formation of salicylic, phthalic and mandelic acids. Exposure times longer than 10 minutes led to the accumulation of several carboxylic acids and trace amounts of other organic compounds (Figure 1). We observed gas formation in the reactor, which suggests that part of the naphthalene underwent complete oxidation to CO_2. While individual product yields were relatively modest (1-10%) overall yield of biologically important carboxylic acids (succinic acid, formic acid, glycolic acid and lactic acid) measured up to 40% of initial naphthalene concentration (Table 1 and ESI).

In the absence of UV light naphthalene was slowly oxidized (50 % of the compound was consumed over 72 h). The main products of the "dark" reaction, as detected by ^{1}H NMR, were 2,6-dihydroxybenzoic, formic and glyceric acids. We, however, did not observe succinic acid, or other biologically relevant carboxylic acids.

For the photolysis of other PAH we started with a turbid suspension of insoluble molecules in water. After 10 minutes of radiation the solution was transparent with all large molecules fully oxidized to water soluble compounds. The photolysis of the mixture of PAH compounds, including pyrene, antracene and other 2 and 3 ring molecules led to the formation of a greater diversity of products. Concentration of lactic, glycolic and succinic acids were higher than expected for the yields detected from naphthalene alone, suggesting that acids could be formed from multiple sources. Interestingly, fumaric and tartaric acids were also detected in these reactions, which, in addition to succinic acid, may suggest a route for the formation of other citric acid cycle metabolites (i.e., oxaloacetate and pyruvate).

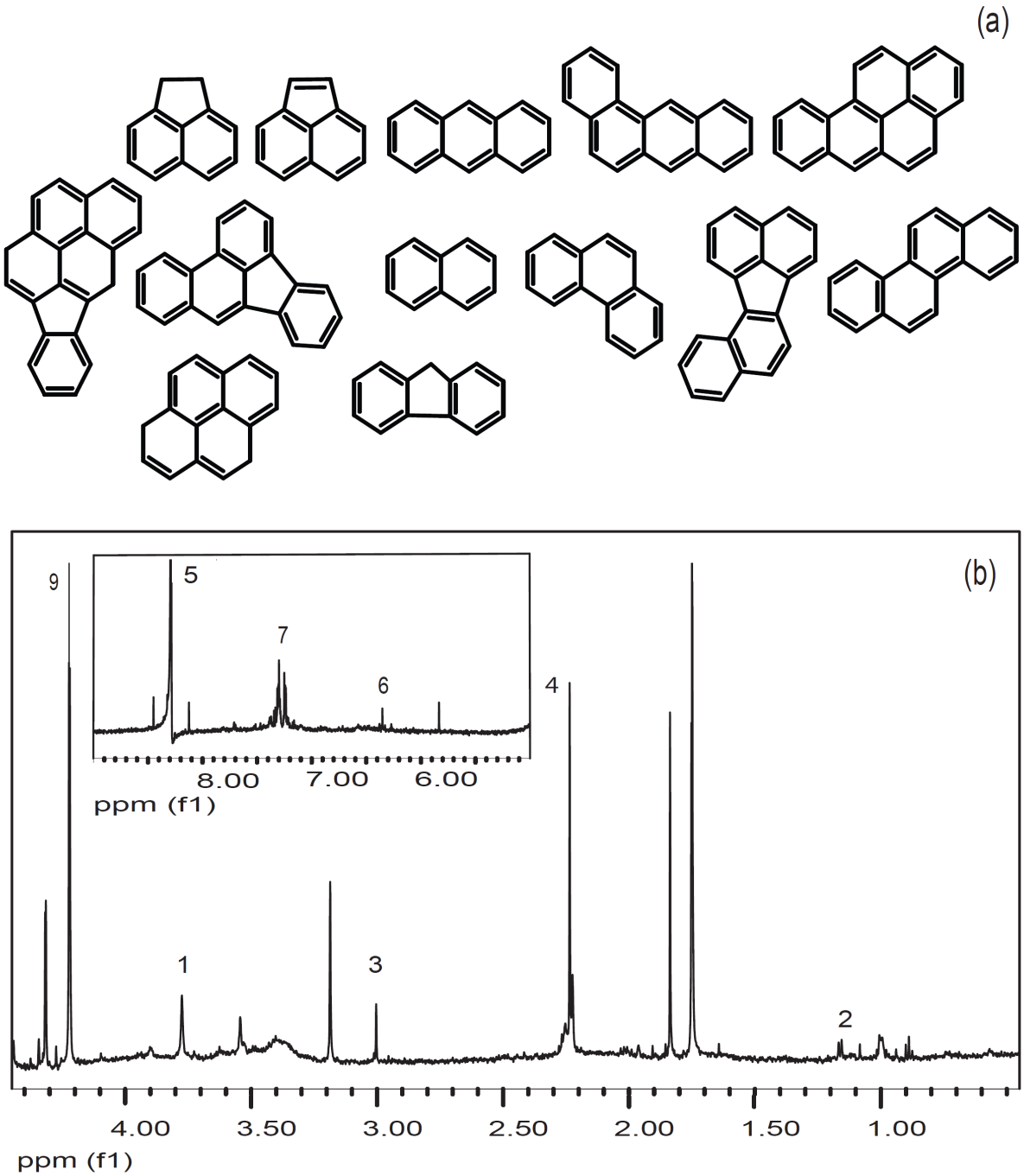

Figure 2. (a) ^{1}H NMR spectrum of products of naphthalene photolysis at 50 µL/ min reactor flow (26 minutes exposure time) and (b) ^{1}H NMR spectrum of products of PAH mixture photolysis at 50 µL/ min reactor flow (26 minutes exposure time). Acids identified by comparison with internal standards (see SI).

The use of UV as a source of energy in origin of life experiments is not new, but here it used it to destroy unnecessary chemical bonds and "clean up" or constrain product diversity. Previous work by Sutherland, where UV light eventually destroys undesired side products in nucleotide synthesis,[36] suggests, that similar selection rules can be applied for nucleotides.

Our results suggest that under geologically plausible conditions, carboxylic acids might have been predominant components of the prebiotic "soup." Previous work by other groups provides insight into how these compounds might have led to more complex molecules. For instance, formic acid in the presence of ammonia can be converted to formamide, a starting compound for the synthesis of nucleotides.[37] Lactic acid can be oxidized to pyruvic acid in the presence of pyrite[38] and pyrrhotite[39] minerals. Pyruvic acid chemistry and its relevance to the origins of life has been extensively explored in the literature.[40,41] Succinic acid can be transformed to succinic anhydride and participate in formation of amide bonds[42] or thioester formation.[43,44]Thioester formation can potentially be the first step in experimental demonstration of "Thioester World" scenario, proposed by Christian de Duve.[21,45,46] Succinic acid is not only a component of the citric acid cycle but it is an important "hub" chemical that links together several metabolic pathways in modern cells (Figure 3), including the formation of thioester succinyl CoA,[43,44] phosphorylation of GDP, the electron transport chain,[47] and in at least three known carbon fixation pathways (rTCA, 4HB and 3HP pathways).[48] Succinic acid seems to be a compound that was both common on early Earth and central to modern biochemistry and more studies exploring its roles in first prebiotic networks are needed.

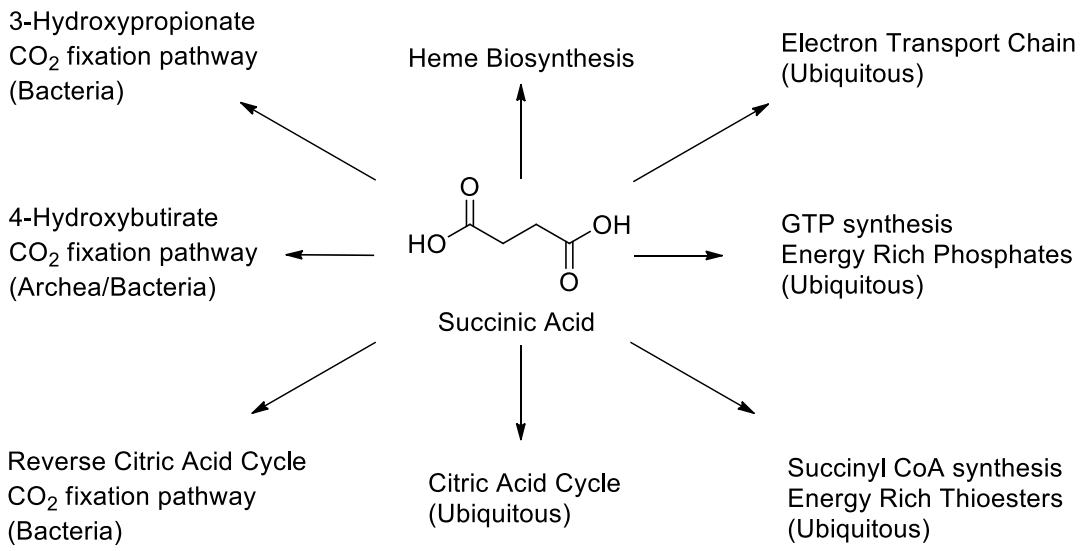

Figure 3. Role of succinic acid in modern biochemistry.

A decomposition of large storage molecules into simpler building blocks and their recombination into new complex molecules can be seen as proto-catabolism occurring on a planetary scale and early Earth might have acted as a giant prebiotic chemical reactor.[6,49] The prebiotic chemistry similar to ours might have been common to small rocky planets with sulfate cycles everywhere in the Universe.

Experimental

$FeCl_3$(>99.9%), Naphthalene (99+%), H_2SO_4 10N were purchased from Aldrich; Na_2SO_3 (BioUltra, anhydrous, >98.0%) from Sigma. Carboxylic acids used as standards (glycolic, malonic, lactic, succinic, phtalic, etc.) were purchased from Aldrich. PAH calibration mixture of 10 µg/mL in acetonytrile of Acenaphthene, Acenaphthylene, Anthracene, Benz[*a*]anthracene, Benzo[*a*]pyrene, Benzo[*b*]fluoranthene, Benzo[*ghi*]perylene, Benzo[*k*]fluoranthene, Chrysene, Dibenz[*a*,*h*]anthracene, Fluoranthene, Fluorene, Indeno[1,2,3-*cd*]pyrene, Naphthalene, Phenanthrene, and Pyrene was used for PAH photolysis experiments.

We developed a continuous flow microfluidic photoreactor to study photolysis of Naphthalene . The reactor had an arc mercury lamp in the center and was surrounded by a quartz jacket. A PFA tubing with ID of 250 µm was wrapped around the quartz jacket. The total volume of the reactor was 1.3 mL. All the reactants were pumped through the PFA tubing via a Harvard Instrument syringe pump. The residence time was adjusted via changing the pumping speed. We used Hamilton Airtight 10 cc glass syringes in the experiments.

Standard reaction conditions were Naphthalene 0.05 mM pH 4.0, $FeCl_3$ 0.5 mM, $NaHSO_3$ 10 mM, N_2 atmosphere (COY Humidity Control Glovebox). All solutions were prepared from N_2 inside the glove box with residual O_2 concentration of 0.5-1% and was flushed with N_2 for 10 minutes. No attempts to further reduce O_2 concentration were made. 10 cc Hamilton Airtight

syringes were filled with reaction mixture. To achieve steady state conditions 2 mL of solution were pumped through the system at desired speed rate before the collection of the samples were started.

For HPLC analysis 200 µL aliquots were collected in amber glass vials under N_2 atmosphere, reaction was quenched with 10 µL of MeOH and immediately analyzed by HPLC. Column: Agilent Poroshell 120 SB-C18 2.7 µm 4.5mm×75 mm; Solvent: A: 0.1% TFA in H_2O; B: 0.1% TFA in ACN; gradient : 5 to 100% B in 8 minutes; detection: UV light 252 nm.

For NMR analysis 6 mL aliquots were collected in amber glass vial under N_2 atmosphere, quenched with MeOH, neutralized to pH 11 with NaOH and lyophilized for 24h. White precipitate was dissolved in 1mL of D2O, TMS was added as an internal standard to calculate yields (1mM total proton concentration), solution was centrifuged to remove iron oxide precipitate and measured by Bruker 600Hz NMR. Peak shifts were compared against the peaks of internal standards (with pH adjustment).

For GCMS analysis to 200 µL of sample 20 uL of 6M HCl and 200 mg of NaCl ware added, and extracted with 1 mL of Ethyl Acetate. Extracts were neutralized with Et3N and evaporated under N_2 flow at 50°C. The residue was derivatized with MSTFA reagent (Thermo) for 30 minutes at 50°C (100 µL ACN, 50 µL MSTFA, 10 µL 2,5-dichlorphenol 300 µg/mL as an internal standard). 1 µL of the prepared solution was injected into Agilent 7890A GC System with 5975C inert XL MSD with Triple-Axis Detector. Main products were identified by NIST 2014 database search and confirmed against elution times of standard solutions of each molecule. Yields were calculated from calibration curved from initial amount of naphthalene (in moles).

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