Relative proportion of prebiotic amino acids: part 3—experiments using reduced gas mixtures

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Evolutionists believe the prebiotic atmosphere would have consisted primarily of CO_2/N_2 or CO/N_2, along with H_2O. Key Miller-type experiments reported in the literature on mixtures of highly oxidized gases using spark discharge to mimic the energetic effects of lightning produced only trace amounts of amino acids (AAs), primarily glycine. Even when high concentrations of the reducing gases CH_4 or H_2 were added, usually ~99.5% of the proteinogenic AAs produced were glycine and alanine.

Availability and proportion of prebiotic amino acids

In this 5-part series, the distribution of amino acids (AAs) produced abiotically according to origin of life (OoL) experiments in the literature are being reviewed. Part 1[1] and part 2[2] covered key Miller-type experiments using reduced gas mixtures such as CH_4, H_2, and NH_3 in water. In part 3 and part 4[3] of this series, the key Miller-type experiments using mixtures now considered to be representative of a prebiotic atmosphere (primarily CO_2 and N_2, along with water) will be reviewed. In part 5, other potential, but minor, abiotic sources of AAs will be evaluated. We will continue to draw attention to an important fact ignored in the evolutionary literature: by far most of the proteinogenic AAs formed and delivered extraterrestrially would have been only glycine plus some alanine, with only trace amounts of other AAs. Our focus here is not on the low *quantity* of AAs formed but on the *lack of variety*.

With improvement in analytical techniques, it has become possible to identify many more products from spark-discharge Miller-type experiments. A good example was provided by Wollrab *et al.* in 2016.[4] They conducted a series of experiments using the same and similar gas ratios Miller had used in the 1950s: CH_4, NH_3, H_2 = 40%, 40%, 20%, with an initial pressure of about 0.9 atm, exposed to ≈10 kV sawtooth, 20 Hz, ≈20 W, direct current sparks. The resulting chemicals were analyzed without separation using state-of-the-art mass spectrometry and NMR spectroscopy. Thousands of different compounds were formed, most in exceedingly low concentrations.[4]

OoL researchers delight in each new organic chemical identified using these experiments, overlooking that the additional substances that were generated would have *hindered* the necessary peptides from forming!

Many variables determine what products will form in Miller-type experiments, including the gas mixture, energy sources, and duration. Wollrab *et al.* selected a gas mixture which was ideal for forming a rich variety of chemicals, even though they knew that geochemists believed the expected prebiotic atmosphere mixture would have been very different. Chemists can design experiments to maximize the outcomes they wish, but, to have any relevance to OoL discussions, these would need to be calibrated or extrapolated to realistic conditions.

For example, prebiotic lightning flashes wouldn't have occurred 20 times per second in a 1-litre trapped volume, generating concentrated fragments able to combine into new stable compounds. Even reaction volume plays a role. Since reactants in Miller-type experiments are hermetically enclosed in a small volume, initially saturated products would form until hydrogen begins to be consumed, after which more unsaturated materials (which have double or triple bonds between carbon atoms) would form. Therefore, the product distribution can be affected by chemists choosing reaction durations. Even the choice of glass container has a major influence due to catalytic effects, as discussed in part 1.[1]

Reduced set of amino acids for early life-forms

Proteins are composed of 20 standard AAs, and proteins consist of about n = 300 AA on average. Therefore, the variety of possible sequences is vast, $\approx 20^{300}$, which invites the question of how the miniscule subsets of useful sequences could have arisen[5] in multiple identical copies. This led physicist and information theorist Hubert Yockey to write,[6]

> "It is concluded that *at present* there are no scientifically valid origin of life scenarios [emphasis in original]."

Truman has also used Shannon Information theory and other methods in this journal to calculate protein sequence probabilities.[7]

Table 1. Distribution of AAs for a variety of CH_4, H_2, CO, and CO_2 gas mixtures, using spark discharge as an energy source. Gas pressures in parenthesis in torr. All experiments were run in 100 mL H_2O at 25°C. Yields shown in parenthesis were based on the carbon atoms consumed. The calculated values were based on data taken from Schlesinger and Miller (1983).[14] Calculations found in Supplementary Material, sheet 'Schlesinger'.

	Experiment 1 N_2 (100), NH_3 (0.1), CH_4 (100)		Experiment 2 N_2 (100), H_2 (300), CH_4 (100)		Experiment 3 N_2 (100), H_2 (50), CO (100)		Experiment 4 N_2 (100), H_2 (300), CO (100)		Experiment 5 N_2 (100), H_2 (50), CO_2 (100)		Experiment 6 N_2 (100), H_2 (300), CO_2 (100)	
AA	**Relative to Gly** [a]	**Proportion overall**	**Relative to Gly** [a]	**Proportion overall**	**Relative to Gly** [a]	**Proportion overall**	**Relative to Gly** [a]	**Proportion overall**	**Relative to Gly** [a]	**Proportion overall**	**Relative to Gly** [a]	**Proportion overall**
Proteinogenic Glycine	100 (1.77%)	47.237%	100 (0.40%)	41.719%	100 (0.30%)	97.151%	100 (1.42%)	97.366%	100 (0.01%)	92.865%	100 (1.54%)	98.685%
Alanine	85	40.151%	101	42.136%	2.8	2.720%	2.4	2.337%	7	6.501%	0.87	0.859%
Valine	2.1	0.992%	1.2	0.501%	<0.001	<0.001%	0.005	0.005%	<0.001	<0.001%	<0.001	<0.001%
Aspartic acid	0.4	0.189%	1.6	0.668%	0.04	0.039%	0.09	0.088%	0.22	0.204%	0.14	0.138%
Glutamic acid	1.5	0.709%	1.5	0.626%	<0.001	<0.001%	0.01	0.010%	0.06	0.056%	<0.001	<0.001%
Serine	1.4	0.661%	3.1	1.293%	0.08	0.078%	0.15	0.146%	0.4	0.371%	0.23	0.227%
Sum:	190.4	*89.939%*	208.4	*86.942%*	102.922	*99.989%*	102.655	*99.951%*	107.681	*99.998%*	101.242	*99.910%*
Non-proteinogenic α-Aminobutyric acid	18	8.503%	30	12.516%	0.01	0.010%	0.04	0.039%	<0.001	0.001%	0.09	0.089%
Norvaline	3.3	1.559%	1.3	0.542%	<0.001	0.001%	0.01	0.010%	<0.001	0.001%	<0.001	0.001%
Sum:	21.3	10.061%	31.3	13.058%	0.011	0.011%	0.05	0.049%	0.002	0.002%	0.091	0.090%
Total AA:	211.7	*100.00%*	239.7	*100.00%*	102.933	*100.00%*	102.705	*100.00%*	107.683	*100.00%*	101.333	*100.00%*

[a] Yields relative to moles glycine

Despite the variety of gases and conditions tested, about half the proteinogenic AAs don't form at all in Miller-type experiments, as discussed in parts 1 and 2. In addition, amino acids would have also reacted with hydroxy acids, thio acids, amino sulphonic acids, and amino phosphinic acids, in addition to β-, γ- and δ-amino acids, plus α-dialkylamino acids and N-alkyl-α-amino acids.[8]

Many evolutionists assume that early life-forms, whether based on biological cells or not, could have used only a *reduced set* of AAs.[9,10] The set typically includes A (alanine), D (aspartic acid), E (glutamic acid), G (glycine), I (isoleucine), L (leucine), P (proline), S (serine), T (threonine), and V (valine).[11,12] Some claim C (cysteine) might have also been produced, but it is unstable and destroyed easily through oxidization.[13] Having fewer AAs would decrease the peptide sequence space from 20^n to 10^n but assumes that all the necessary life functions could have been satisfied with only these 10 AAs. The problem still remains that a broad range of chemicals, such as amines, alcohols, aldehydes, and carboxylic acids would also have been formed and reacted with AAs.

Miller-type experiments using CO_2/N_2 and CO/N_2 mixtures

As discussed in part 4 of this series, CO_2 and N_2, along with H_2O, are believed by evolutionists to have dominated the prebiotic atmosphere, with small amounts of other gases. Schlesinger and Miller performed a series of experiments using combinations of N_2, NH_3, CH_4, H_2, CO, and CO_2 in 100 mL H_2O at 25°C.[14] The spark discharge was run continuously for 48 h at 25°C. Then the aqueous phase was allowed to equilibrate for 48 h to allow amino nitriles to form. The AAs were obtained by subsequent hydrolysis of the nitrile groups in 3 M HCl, leading to the results shown in table 1.

The team determined the amount of AAs produced at different partial pressures and found that the yield of AAs was approaching a maximum at around 100 torr (see figure 4 in Schlesinger and Miller (1983)).[14] Other experiments also showed that sparking beyond four days would not have increased the yields of AAs very much (see figure 3 in Schlesinger and Miller (1983)).[14]

Experiments 1 and 2 in table 1 confirmed what all other researchers have found; namely, that using CH_4 instead of CO or CO2 produced a much greater variety of AAs. This was also shown in parts 1 and 2 of this series. However, very little CH_4 would have been present in a prebiotic atmosphere.

Also consistent with other reports, a high concentration of H_2 or CH_4 was necessary to obtain AAs (i.e., H_2O as vapour was not sufficient). As reported in experiment 5 of table 1, even a very high H_2:CO_2 ratio of 0.5 in an equimolar mixture of CO_2 and N_2 yielded only 0.01% AAs based on carbon atoms consumed (see value in parenthesis in the 'Relative to Gly' column of experiment 5).

In the absence of H_2, a mixture of CO_2, N_2, and NH_3 (100 torr, 100 torr, and 0.1 torr, respectively) led to an AA yield of only 5×10^{-3}%, and without NH_3 even less, 6×10^{-4}%, according to figure 2 in Schlesinger and Miller (1983).[14] These low AA yields were comparable with the amounts that may have arisen from contamination from the reagents used to detect them.[14]

Yields of AA were based on the carbon atoms consumed, and the pNH_3 used was produced by using a 0.05 M NH_4Cl solution with NaOH to control the pH to around 8.7. The authors admitted that the partial pressure of NH_3 of ~0.1 torr produced was higher than would have been found under prebiotic conditions, but they needed this to synthesize some AAs.

In experiment 3, CO was used instead of CO_2, in a ratio of H_2:CO = 0.5. The AA yields (0.3%) were also very low, but CO is a relatively reactive gas, and the authors noted that higher partial pressures would not have accumulated prebiotically.

The data in table 1 leads to the following statistical conclusions:

- Proteinogenic AAs: 90% (experiment 1); 87% (experiment 2); 99.9% (experiment 3); 99.9% (experiment 4); 99.9% (experiment 5); 99.9% (experiment 6).
- Gly + Ala vs all the proteinogenic AAs: 97.2% (experiment 1); 96.4% (experiment 2); 99.9% (experiment 3); 99.8% (experiment 4); 99.4% (experiment 5); 99.6% (experiment 6). However, experiments 1 and 2, which contained no CO or CO_2, are not credible. If experiments 1 and 2 are ignored, then on average ~99.7% of the proteinogenic AAs would have been Gly or Ala, and ~96.9% only Gly.

With CO and CO_2 atmospheres, glycine was virtually the only AA produced. By contrast, when CH_4 was used instead of CO or CO_2 (experiments 1 and 2), then about as much alanine was produced as glycine. However, now also substantial amounts of α-aminobutyric acid formed, a non-proteinogenic AA.

Schlesinger and Miller proposed that a wildly unrealistic H_2:CO_2 ratio of 2:1 or more would have been necessary to produce an adequate quantity of AAs, but, according to experiment 6, 98.7% of the AAs (that is, proteinogenic plus non-proteinogenic) would have still been glycine, and almost all the rest alanine. The OoL community claims that around 4 Ga ago the atmospheric concentration of CO_2 was probably at least 10 times greater than today's 3×10^{-4} atm. This implies that the mixing ratios of H_2 and CO_2 would have to be about 6×10^{-3} and 3×10^{-3} atm, respectively. That represents 300 times more H_2 than generated by current volcanos.[14]

Schlesinger and Miller pointed out correctly that other terrestrial sources of H_2 would not have been able to generate the necessary high production of H_2, such as from submarine hot springs; transformation of organic matter by abiotic processes; or photolysis of water in the atmosphere.[14]

Neutral CO_2/N_2 mixtures with antioxidants

Cleaves *et al.* suspected that the main reason for the low yields of AAs reported was that low concentrations of hydrogen cyanide (a key intermediate) formed.[15] In CO_2/N_2 mixtures, most of the N atoms produced by splitting N_2 combine with O atoms to form NO_x species instead of HCN.[16] Cleaves *et al.* were also aware that electric discharges acting on CO_2/N_2 mixtures over water and on N_2 over water produced large amounts of HNO_2 and HNO_3. Nitrate and nitrite oxidized AAs to nitrosamines under acidic conditions, so they decided to include various oxidation inhibitors in some experiments.

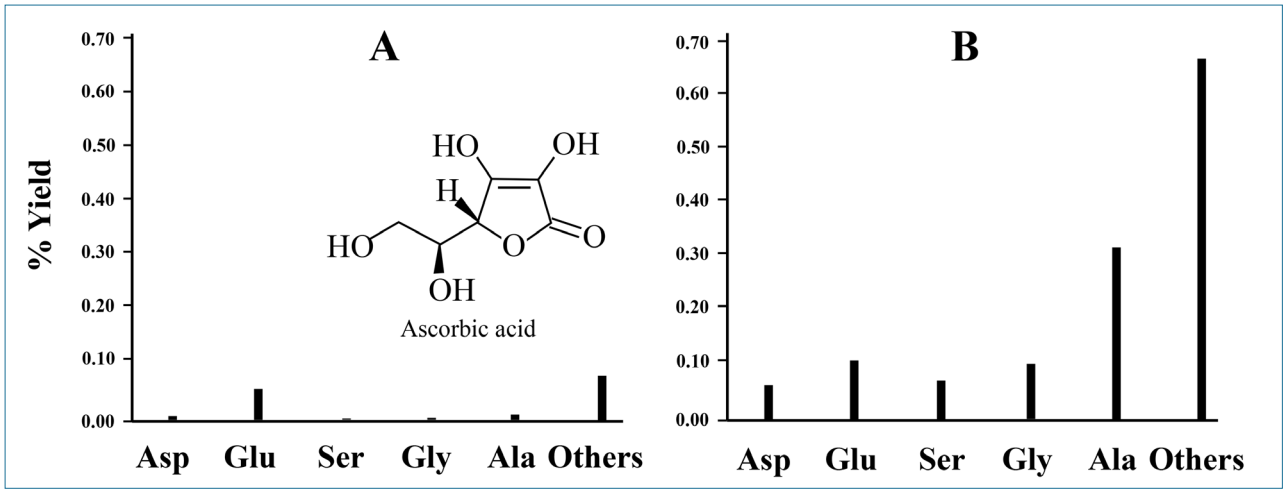

Figure 1. Yields of AAs using a mixture of CO_2 and N_2 (each 0.13 bar) from spark discharge experiments (48 hours at room temperature) in the presence of the biological antioxidant ascorbic acid.[15] A: without $CaCO_3$ B: with 2 mmol $CaCO_3$. Redrawn by R. Truman from Cleaves *et al.* (2008).[15]

Table 2. Distribution of products estimated from the data shown in figure 1 and table 2 from Cleaves *et al.* (2008)[15]

AA	Without ascorbate	With ascorbate	Without ascorbate	With ascorbate
	Without $CaCO_3$		With $CaCO_3$	
Aspartic acid	~0.001%	0.005%	0.001%	0.055%
Glutamic acid	~0.001%	0.05%	0.001%	0.095%
Serine	~0.001%	0.001%	0.001%	0.06%
Glycine	~0.001%	0.003%	0.001%	0.09%
Alanine	~0.001%	0.005%	0.001%	0.3%
Sum proteinogenic:	–	*0.064%*	*0.005%*	*0.6%*
Non-proteinogenic:	–	*0.07%*	*0.001%*	*0.67%*

The team first experimented with a CO_2/N_2 mixture (both 100 torr pressure) in 100 mL water, exposing them to an electric discharge for 48 h at 23°C.[15] The pH dropped to 3.2 and AA yields were only ~10^{-2}% based initial amount of N_2, similar to those reported by other teams.[15] What these trace amounts of AAs were was not reported.

Cleaves *et al.* proposed several mechanisms that could explain the formation of AAs: (1) the Strecker synthesis; (2) the Bucherer–Bergs reaction; and (3) hydrolysis of HCN oligomers.[15] Formation of non–α-amino acids implied the presence of additional mechanisms. Low pH would inhibit the Strecker synthesis shown in figure 3-A of part 1 of this series[1], which depends on the nucleophilicity of both ammonia and cyanide anion.[15]

The experiment was repeated using a saturated concentration of $CaCO_3$ as a pH buffer. The AA yield increased to ≈0.19% but, according to their figure 1-II, only glycine was found.[15] The low levels of free cyanide, aldehydes, and ammonia produced were interpreted to mean that the intermediates were likely bound as nitriles or other precursor compounds.[15]

They experimented next with 0.15 M oxidation inhibitors, ~100-fold excess over total nitrite + nitrate formed.[15] The only proteinogenic AAs identified were serine, glutamic acid, glycine, and alanine, aspartic acid, and a similar total amount of non-proteinogenic AAs, including α-aminoisobutyric acid, γ-aminobutyric acid, and β-alanine. The best results were obtained using ascorbate, shown in figure 1.[15,17]

Other potential oxidation inhibitors such as $FeCl_2$, Na_2S, Na_2SO3, and sodium formate were also tested, but Cleaves *et al.* reported that only pyrites and $FeSO_4$ "were found to be able to protect against degradation to a significant degree using this molar ratio of reactants."[15] And indeed, even when these inhibitors were present in 100-fold molar excess.

The amount of AAs produced was close to zero, except when high concentrations of both ascorbate and $CaCO_3$ were added. The data from table 2 leads to the following conclusions:

- On average ≈48% of the moles AAs formed were proteinogenic in the presence of a high concentration of ascorbate.
- ≈65% of the proteinogenic yield was Gly + Ala after ascorbate and $CaCO_3$ were added.
- Since only AAs were identified, the proportion of proteinogenic AAs vs non-AA interfering compounds can't be estimated.

According to figure 1-B, about the same amount of proteinogenic and non-proteinogenic AAs (e.g., β-alanine, γ-aminobutyric acid, and α-aminoisobutyric acid) were identified in the presence of saturated $CaCO_3$ and ascorbic acid. Of the 20 proteinogenic AAs, only Asp, Glu, Ser, Gly, and Ala, were produced, where Gly + Ala represented about ⅔ of the total proteinogenic yield. Remarkably, after admitting that ascorbate gave the best results but is not a plausible prebiotic oxidation inhibitor, the authors did not report the results for the oxidation inhibitors that would have been present.

The yield of proteinogenic AAs was only about 0.6% based on the amount of N_2 consumed, according to figure 1-B, using their best but prebiotically irrelevant oxidation inhibitor, ascorbate. The 0.15 M concentration of $FeSO_4$ used (i.e., 1.5×10^{-4} moles/mL) corresponds to 22.8 g/L (i.e., 0.15 mol/L × 151.91 g/mol). The current concentration of all oceanic sulphates (not only $FeSO_4$) is about 2.7 g/L[18], around a tenth of the amount used in the experiments. Furthermore, since sulphates are discharging rapidly into oceans from industrial waste, and $FeSO_4$ would have been accumulating in water for billions of years, the concentration of $FeSO_4$ would have been much lower than 2.7 g/L about 4 billion years ago.

These observations indicate that oxidation inhibitors would have made a negligible contribution to raising the amount of proteinogenic AAs formed, and a predominantly $CO_2/N_2/H_2O$ atmosphere would have only led to a negligible amount of Gly and virtually no other AAs.

Neutral CO/N_2 atmosphere

Most evolutionary geochemists believe that the prebiotic atmosphere was composed of CO_2, N_2, and H_2O, combined with lesser amounts of CO, CH_4, and H_2.[19–21] However, experiments using different energy sources using this mixture have consistently yielded only minute concentrations of AAs.

Miyakawa *et al.* reasoned that a less oxidized gas mixture would be more suitable for OoL purposes.[22] The triple bond of CO is too strong to be broken in spark discharge experiments but could be ruptured with high-energy proton beams. However, in the presence of water molecules, this

Table 3. Yields of AA using a mixture of N_2 (8 torr) and CO (16 torr) in 300 mL distilled water (~37°C, pH 7.5–8.0), from spark discharge experiments (~15 kV) lasting 6 months. Most of the CO_2 produced was removed by an absorbent ($Ca(OH)_2$ = 40 g). The N_2 and CO consumed were replaced. Calculations based on data from figure 3 of Hirose *et al.* (1990).[23] Calculations found in Supplementary Material, sheet 'Hirose'.

AA	Sample 1 [a]		Sample 2 [b]	
	Relative to Gly [c]	Proportion overall	Relative to Gly [c],[d]	Proportion overall
Glycine	100 (5.6%) [e]	87.390%	100 (5.2%) [e]	96.315%
Alanine	2.44	2.132%	1.3913	1.340%
Aspartic acid	0.36	0.315%	0.78261	0.754%
Serine	0.12	0.105%	0.56522	0.544%
Gly-Gly	5.6	4.894%	0	0
Sum proteinogenic:	*114.12* [f]	99.729%	*102.739*	98.953%
Sarcosine [g]	0.16	0.140%	0.52174	0.503%
β-Alanine	0.15	0.131%	0.56522	0.544%
Sum non-proteinogenic:	*0.31*	0.271%	*1.08696*	1.047%
Overall total:	114.43	100.00%	103.826	100.00%

[a] Labelled as Sample-C in ref. 23. After 6 months sparking, the aqueous portion was heated for a month at 65–70°C; then a portion was heated in sealed tubes at 100°C for 322 hrs.
[b] Labelled as Sample-D in ref. 23. After 6 months sparking, the aqueous portion was heated for a month at 65–70°C; then a portion was hydrolyzed with 6N HCl at 100°C for 24 hrs.
[c] Yield in moles relative to glycine
[d] Values in figure 3 in ref. 23 adjusted by setting glycine = 100
[e] % yield based on the amount of carbon consumed
[f] Gly-Gly was multiplied by 2 since after hydrolysis 2 glycine would form.
[g] Sarcosine peak might have included some glutamic acid.

would have decreased the concentration of CO steadily through oxidization to much less reactive CO_2.

Nevertheless, Miyakawa *et al.* argued that a primarily CO atmosphere might have existed temporarily, perhaps due to reduction of CO_2 to CO by hot metal iron resulting from impacts of chondrite meteorites.[22] In some experiments, they added a gas mixture of CO (350 torr) and N_2 (350 torr) to H_2O (5 mL at 297 K) and irradiated this atmosphere with 2.5–3.0 MeV protons from an accelerator for 3 hrs.[24] (High energy protons are a major component of cosmic rays). Although some organic compounds were identified, no AAs were detected according to table 1 in their paper.[22]

In 1990 Hirose *et al.* also experimented with a CO/N_2 mixture (~2:1) under other conditions.[23] The initial mixture contained CO (16 torr) and N_2 (8 torr) in 300 mL distilled water at ~37°C and pH 7.5–8.0. CO and N_2 were added as they were consumed so that the partial pressures varied between CO ~10–30 torr and N_2 ~5–10 torr. The spark discharge (~15 kV) experiment lasted 6 months.

A key experimental feature was to remove as much of the CO_2 generated as possible using the absorbent $Ca(OH)_2$ to lower the ratio of CO_2:H_2. The resulting mixture included CO_2 at 3–4 torr pressure and H_2 at 0.2–0.6 torr; i.e., CO_2:H_2 ≈ 10:1, a ratio for which some AAs are expected to form. Removal of atmospheric CO_2 was assumed to have occurred by calcium and magnesium ion absorption in the ocean.[23]

Another important experimental detail was to provide a cold trap at –15°C to capture the simple molecules formed and prevent their decomposition by additional spark discharges. Every 3.5 hrs, the sparking was discontinued, and an infrared lamp in the trap was turned on for half an hour to melt the icy content, thereby enriching the aqueous phase.

Every 10 days, CO and N_2 gases were added to replace the amount consumed, allegedly simulating a continuous supply of volcanic gas into the primitive atmosphere.

After 2 weeks of discharges, about 60 mM of NH_3 had formed in the water phase, and, at the end of the experiment, a visual estimate indicates that the amount of NH_3 was comparable to the amount of all the AAs combined (figure 3 of Hirose *et al.* (1990)).[23]

After 6 months, the aqueous portion in the vessel was heated for a month at 65–70°C. Sample 1 shown in table 3 resulted from further heating of samples placed in sealed tubes at 100°C for 322 hrs. Sample 2 resulted from samples hydrolyzed with 6N HCl at 100°C for 24 hrs. The authors did not identify the complex mixture of molecules formed but did report finding the AAs summarized in table 3.

The data from table 3 leads to the following statistics:

- On average, over 99% of the moles of AA identified were proteinogenic. However, since no effort was made to identify all AAs formed, the proportion of non-proteinogenic AAs is understated by an unknown amount.
- On average, ~97% of the proteinogenic yield was Gly + Ala.
- Since only AAs were identified, the proportion of proteinogenic AAs to non-AA interfering compounds unfortunately can't be estimated.

Several aspects of the experiment are dubious. The CO to CO_2 ratio was maximized to obtain as much organic product as possible and not to simulate most likely abiotic atmospheric conditions. For example, volcanic eruptions increase the proportion of CO_2:CO in the atmosphere, the opposite of what they claimed to be simulating. In addition, the cold trap located only centimetres away from the spark discharge does not reflect natural conditions, nor does exposure of atmospheric CO_2 to a pure, solid antioxidant absorber.

Experiments not covered

Several experiments were not reviewed in detail here which used mixtures and/or conditions that were not plausible for OoL purposes. An example are the experiments by Miyakawa *et al.*, who heated a mixture of $CO/N_2/H_2O$ to a plasma at 10,000 K, producing nitrogen, oxygen, hydrogen atoms, and CN radicals.[24] In any event, this resulted in only three proteinogenic AAs: Gly, Ala, and Asp, in yields of 0.1%, 0.03% and 0.007%, respectively (based on carbon yields), plus 0.01% non-proteinogenic β-Ala. In other words, ~95% was Gly + Ala, and the other products, formed in trace amounts, consisted of more non-proteinogenic than proteinogenic AAs after excluding Gly and Ala. This is broadly consistent with the results from the key studies examined here in detail.

Another example of experiments rarely discussed in the OoL literature involved irradiation of a $CO/N_2/H_2O$ gas mixture using high-energy 40 MeV protons, mentioned in a paper by Miyakawa *et al.* (see their table 1).[22] It is worth stating that the same general trends were also found: only 4 proteinogenic AAs were obtained (Gly, Ala, Asp, and Ser) in yields of 0.22%, 0.034%, 0.013%, and 0.00139% respectively (based on carbon yields). Glycine + alanine thus represented ~ 94% of the proteinogenic AAs obtained, and the non-proteinogenic β-Ala (0.02%) represented more than the remaining proteinogenic AAs (Asp + Ser) combined.

Discussion

A variety of molecules can be generated when gases are mixed in a closed container and fragmented into radicals and charged ions using high-energy particles,[25] UV irradiation,[26] or thermal energy.[27] For OoL purposes, all the experimental details, including interfering factors, must reflect true undesigned abiotic conditions.

The Rout *et al.* study discussed in part 1 illustrated an important principle to understand OoL research methodology: assumptions are cherry-picked to optimize a specific outcome instead of considering multiple independent constraints.[28] When convenient, evolutionists argue that fewer AAs would have been necessary for life to have arisen, but then researchers like Rout *et al.* and others designed experiments allegedly prebiotically plausible which depend on AAs that would, at best, have been present in trace proportions. Instead of offering a proof-of-concept, experiments like these provide examples of non-feasible possibilities. *With enough negative examples like these, eventually all naturalistic possibilities would be exhausted*.

The plausibility of peptide sequences used in OoL experiments must be evaluated by considering the AA proportions which would have been present prebiotically along with all chemicals able to react with AAs. A large number of *identical* peptides could not have been produced which required specific very low probability AAs in the same position. The most probable atmospheric composition would have been dominated by CO_2, N_2, and H_2O in which glycine and alanine would have been virtually the only proteinogenic AAs formed. Therefore, peptides formed abiotically would have consisted of primarily these two AAs.

Several researchers have examined atmosphere compositions which included high concentrations of gases like CH_4 and H_2 to increase the variety of AAs formed. The experiments discussed above showed, however, that this inevitably increased the concentration and variety of compounds which would have *interfered* with forming peptides.

Since evolutionists cannot offer realistic scenarios to satisfy the ten constraints mentioned in part 1, which OoL-relevant peptides must satisfy, a plethora of conceptual speculations have arisen. For example, contrary opinions are expressed on whether to retain the notion of a primordial soup. Lane *et al.* reject the notion entirely.

> "Despite thermodynamic, bioenergetic and phylogenetic failings, the 81-year-old concept of primordial soup remains central to mainstream thinking on the origin of life. But soup is homogeneous in pH and redox potential, and so has no capacity for energy coupling by chemiosmosis."[29]

Bada, however, disagrees, writing in 2013,[30]

> "The results discussed here challenge claims that the 'primordial soup' concept of the origin of life is 'well past its sell-by date' and as such is null and void.[29] In fact, the 'primordial soup' theory as a model for the processes needed to produce the raw material used in subsequent reactions involved in the origin of life is very much alive and doing very well indeed!"

Specific proposed prebiotic environments are argued for or against, depending on which property scientists wish to emphasize, since no single environment is suitable for all of them. Instead of taking into account that the requisite polymeric peptides, RNA and DNA, would have hydrolyzed in water, in the above reference Bada focused only on whether raw materials (i.e., some component AAs) might have been present at all.[30] Therefore, after admitting that almost all evolutionary geophysicists believe the primitive atmosphere consisted of CO_2 and N_2, he nevertheless argued that reducing atmospheric gases (like H_2, CH_4, and NH_3) might have concentrated somewhere. His creative considerations included the possibility of a Titan-like atmospheric organic haze of gases protecting from UV photochemical degradation.[31–33]

In part 4 of this series, we will summarize the experiments discussed here in a manner analogous to the way part 2 summarized the results from reduced gas mixtures. Then the evidence for alternative atmosphere compositions proposed will be evaluated. In part 5 the proportion of proteinogenic

AAs claimed to have formed abiotically in other less important ways will be summarized.[34]

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