Relative proportion of prebiotic amino acids: part 1—experiments using reduced gas mixtures

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The primary source of prebiotic amino acids (AAs) is often claimed to have been high energy splitting of atmospheric gases. A review of key Miller-type experiments using reducing mixtures like $CH_4/NH_3/H_2/H_2O$ showed that other compounds able to react with AAs were usually not reported. Only a very few proteinogenic AAs were obtained in most of the experiments reviewed. Typically, ~99.5% of the proteinogenic AA yields consisted of only glycine and alanine. Attempts to optimize the yield of AAs by controlling the pH, adding H_2S, or including high concentrations of anti-oxidants, like ascorbic acid, inevitably increased the variety and proportion of *non-proteinogenic* AAs. A 2021 study revealed that the borosilicate in Pyrex glassware used in Miller-type experiments catalyzes formation of organic chemicals. With Teflon reactors, only four proteinogenic AAs were produced, and ~93% of the AA yield consisted of *non-proteinogenic* AAs. Reported Miller-type experiments have failed to yield AA proportions suitable to form relevant proteins.

Proteins are indispensable for all organisms and play a prominent role in many origin of life (OoL) theories. Proteins contain 50 or more amino acids and have a distinct, complex three-dimensional structure. When the term 'peptide' is used here, simple chains of four or more AAs are meant, but all the statements referring to peptides apply to proteins also.

OoL researchers have struggled to find natural processes capable of producing sizeable peptides without a cellular system, since small peptides and huge proteins must fulfil at least ten fundamental properties *concurrently*, as summarized in table 1.

Prebiotic chemical experiments are almost always designed to optimize one of these requirements, but inevitably the conditions chosen prevent satisfying the other ones. For example, to force amino acids (AAs) to link (requirement 1), *high temperature* and pressure conditions are often used.[1] But the rate of D- ⇆ L-enantiomer racemization correlates with temperature, worsening requirement 2.[2] Also, cations like Cu^{2+} are sometimes used to facilitate requirement 1, but these also accelerate racemization.[2] To circumvent issues like racemization, glycine is often used in OoL experiments, being the only proteinogenic AAs lacking D- and L-isomers. There are 20 proteinogenic AA, manufactured in cells and used to form proteins. Of course, no useful functions (requirement 4) are produced by glycine polymers.

Furthermore, although higher temperatures produce very small peptides initially, there is a direct correlation between rate of peptide destruction and temperature. To illustrate, in experiments reported by Lemke *et al.*, gly_2 degraded slowly at 160°C, rapidly at 220°C, and at 260°C almost all would have degraded in less than 100 hr. No gly_3 was obtained at 160°C and 220°C, and a very low concentration at 260°C (a temperature simulating relatively cool hydrothermal vents). However, at 260°C gly_3 was shown to entirely degrade thermally within a few hours.[1] Therefore, OoL high-temperature experiments involving peptides are inevitably terminated after a short time.

The experimental sections in OoL publications often mention that the AA side-chain groups were *blocked*, i.e., chemically modified to prevent them from being able to react. This was necessary to obtain only linear condensation reactions (requirement 3).

The need to continuously produce specific and co-located peptides (requirements 7 and 8) is rarely mentioned. McCombs and Truman,[3] and Tan and Truman,[4] documented that the prebiotic concentration of even very small peptides would have been irrelevantly low. For example, even a high equilibrium concentration of 10^{-4} M AA would have led to an equilibrium concentration of $<4 \times 10^{-36}$ M hexapeptides at around ambient temperatures (with the concentration decreasing by about a factor of 1 million per additional AA).[4] 4×10^{-36} M corresponds to less than one molecule per 4×10^{12} litres of water.

OoL chemical experiments often require peptides with specific sequences. This prompts an important question neglected in prebiotic discussions, namely *what would the proportion of AAs have been* in the environment? This proportion would affect several of the 10 requirements:

4. Precise sequences composed of various amino acids must form to perform useful functions.

Table 1. Properties that prebiotically relevant peptides and proteins must fulfil.

No.	Requirement large peptides / proteins must fulfil for life to be possible
1	Many amino acids must be linked together, about 300 on average for proteins.
2	Only the L-amino-acid enantiomers must be included.
3	Only the linear polymers must form; i.e., the side chains of amino acids must not react.
4	Precise sequences composed of various amino acids must form to perform useful functions.
5	Other molecules, including non-biological amino acids, must be avoided in the peptides.
6	The long chains must adopt a suitable 3-dimensional structure.
7	A vast number of peptide copies must be produced continually for millions of years.
8	The correct proportion of peptides having a specific sequence must be co-located.
9	The entire system or organism must self-replicate, including all necessary peptide copies.
10	The polymers and 3-dimensional structure must be formed under prebiotically realistic conditions.

Table 2. Template and substrates mixture to form amyloids in experiments by Rout *et al.*[5]
F = phenylalanine, E = glutamate, R = arginine.

Experiment	Peptide	Abbreviation	Structure
	Template	$(FE)_4$	$H_3C-C(=O)-NH-FEFEFEFE-C(=O)-NH_2$
Experiment 1	Substrate	$R(FR)_3$	$H_2N-C(=O)-RFRFRFR$
Experiment 2	Substrate	$(FR)_3$	$H_2N-C(=O)-RFRFRF$
Experiment 3	Substrate	$R(FR)_2$	$H_2N-C(=O)-RFRFR$

5. Other molecules, including non-biological amino acids, must be avoided in the peptides.
7. A vast number of peptide copies must be produced continually for millions of years.
8. The correct proportion of peptides having a specific sequence must be co-located.

This is related to the *information challenge*: where did the guidance come from to produce thousands of correct proteins, considering that any of 20 of the proteinogenic AAs could be located at every position? Without informational guidance, polymer sequences are determined by chemical principles and not a future biological benefit.

To illustrate how precise sequences are used in allegedly plausible OoL experiments, Rout *et al.* showed, in an often-cited paper, that preferential addition of a particular AA could be achieved by *designing pairs of carefully tailored peptides*.[5] The peptide substrates and templates were constructed with alternating hydrophilic and hydrophobic L-only AAs residues to produce amphipathic β-strands which could produce amyloids. The peptides used are shown in table 2.

After extensive analysis of how to form the necessary β-strands, three specific AAs were selected (phenylalanine (F), arginine (R), and glutamic acid (E)) to form *peptides with specific sequences*. The likelihood of obtaining many identical copies of these sequences, necessary to form amyloids, would depend on the proportion of the three AAs in the environment. Of the three, *only glutamic acid would have been present* about 4 Ga ago, but in an insignificant proportion compared to, for example, glycine and alanine.[6] Truman documented many other reasons why these peptides would not have been available prebiotically, including that the AA had to be first chemically activated using CDI (1,1'-carbonyldiimidazole, $(C_3H_3N_2)_2CO$) and that all the AA would have been present as racemic mixtures of D- and L-enantiomers.[6]

Nevertheless, other evolutionists found no reason for concern. Maury commented on the paper by Rout *et al.* claiming that[7]

> "… it *demonstrates* that an amyloid formed from short peptides can direct the synthesis of its own constituent peptides *under plausible prebiotic Earth conditions* [emphasis added]."

Quite the contrary. Requiring *thousands of identical peptide pairs* to have formed at a precise location where each peptide has a vanishingly low sequence probability of being formed at all is indistinguishable from requiring a miracle.

Amino acids from Miller-type reactions

In Miller-type experiments, simple gaseous molecules are fragmented, using energy sources and combinations of simple molecules, like H_2O, CH_4, H_2, NH_3, O_2, CO, CO_2, and H_2S.

Gishlick, from the National Center for Science Education, wrote a paper critiquing Jonathan Wells' observation that the original Miller-type experiments were based on an incorrect highly reducing combination of gases, having used high concentrations of CH_4, and H_2.[8] In figure 1 of his article, Gishlick mentioned 13 series of experiments others had conducted after the classical 1953 publication. Although these used various reactants and energy sources, almost no AAs were found in these later experiments. In the last column of the figure, Gishlick documented the likelihood that the experiments reflected plausible prebiotic conditions at all, using terms like 'unlikely' and 'under special conditions'. The highest rating offered was a very dubious 'possible'. None of his examples reflected a probable atmospheric composition.

We will not devote effort here to review gas mixtures or conditions nobody felt, or feels, worthy of championing;[8] for example, using temperatures like 10,000 K or irradiation with very high-energy 40 MeV protons which were not relevant for OoL purposes.[9] In any event, these experiments led to very few AAs, almost entirely glycine, like all the other experiments, and also in very low yields.

Here, in part 1 of a 5-part series, we have reviewed the key experiments found in the OoL literature based on highly reduced gas mixtures.

CH_4, NH_3, and H_2 in water

Miller published, in 1953, a seminal paper based on the gas mixture CH_4, NH_3, and H_2 in a volume ratio of 2:2:1, with 200 mL water in a 5-litre flask.[10] A continuous electrical discharge was used between a pair of electrodes for 1 week to form free radicals. Detection and identification of AAs were conducted using paper chromatography, a very crude analytical method by today's standards. Five AAs were reported: glycine, α-alanine and β-alanine were identified, but aspartic acid and α-aminobutyric acid (AABA) were less certain, since the spots were quite weak.

The experiments were repeated in 1959, and the products shown in table 3 were identified using newer analytical techniques.

All the compounds in table 3 can react with AAs, leading to either peptides or interfering with their formation. From the data in table 3, the following conclusions can be made:

- ~79% of the moles AAs were proteinogenic (i.e., 19.935% / (19.935% + 5.309%)).
- ~21% were proteinogenic AAs vs non-AA interfering compounds (i.e., 19.935% / (19.935% + 74.756%)).
- ~19.9%, of the overall yield was proteinogenic, as shown in table 3-A.
- ~99% of the proteinogenic yield was Gly + Ala (i.e., (100 × (63 + 34) / 98)).

Table 3. Yields from sparking a mixture of CH_4, NH_3, H_2 (2:2:1), and H_2O. Based on data from table 2 of Miller and Urey (1959).[11] Calculations found in Supplementary material 1, sheet 'Miller'.

A

Proteinogenic AA	10^{-5} moles	Proportion overall
Glycine	63	12.815%
Alanine	34	6.916%
Glutamic acid	0.6	0.122%
Aspartic acid	0.4	0.081%
Sum:	98	*19.935%*

B

Non-proteinogenic AA	10^{-5} moles	Proportion overall
β-Alanine	15	3.051%
Sarcosine	5	1.017%
α-Amino-n-butyric acid	5	1.017%
N-Methylalanine	1	0.203%
α-Aminoisobutyric acid	0.1	0.020%
Sum:	26.1	*5.309%*

C

Non-AA	10^{-5} moles	Proportion overall
Formic acid	233	47.396%
Glycolic acid	56	11.391%
Lactic acid	31	6.306%
Acetic acid	15	3.051%
Propionic acid	13	2.644%
Iminodiacetic acid	5.5	1.119%
α-Hydroxybutyric acid	5	1.017%
Succinic acid	4	0.814%
Urea	2	0.407%
Iminoacetic-propionic acid	1.5	0.305%
N-Methyl urea	1.5	0.305%
Sum:	367.5	*74.756%*

Table 4. Yields from sparking a mixture of CH_4, N_2 (1:1), H_2O, and trace amount of NH_3. Data from table 1 of Ring *et al.* (1972).[13] Only the amino acids were reported. Calculations found in Supplementary material 1, sheet 'Ring'.

A

Proteinogenic AA	10^{-5} moles	Proportion overall
Alanine	79.0	43.90%
Glycine	44.0	24.45%
Aspartic acid	3.4	1.89%
Valine	1.95	1.08%
Leucine	1.13	0.63%
Glutamic acid	0.77	0.43%
Isoleucine	0.48	0.27%
Serine	0.50	0.28%
Proline	0.15	0.08%
Threonine	~0.08	~0.04%
Sum:	131.46	*73.05%*

B

Non-proteinogenic AA	10^{-5} moles	Proportion overall
α-Amino-n-butyric acid	27.0	15.00%
α-Hydroxy-γ-amino-butyric acid	7.4	4.11%
Norvaline	6.1	3.39%
α,γ-Diamino-butyric acid	3.3	1.83%
α-Aminoiso-butyric acid	~3.0	~1.67%
Norleucine	0.6	0.33%
Alloisoleucine	0.51	0.28%
Isovaline	~0.5	~0.28%
Allothreonine	~0.08	~0.04%
tert-Leucine	<0.002	<0.00%
Sum:	48.49	*26.95%*

These kinds of statistical results will be examined throughout this 5-part series to demonstrate that they are inconsistent with OoL requirements. Seemingly only *proteinogenic AAs* are *assumed* to have been present prebiotically, since only they are considered in the OoL experiments. Even if all the AAs had been proteinogenic, then, according to this experiment, on average *99% of every position for all peptides and proteins would have consisted of only glycine or alanine* (with ⅔ being glycine). It would have been impossible to obtain the wide variety of sequences necessary for life, each in multiple identical copies.

Furthermore, each mole of glutamic acid + aspartic acid present (see table 3-A) would have competed with 26.1 moles of non-proteinogenic AAs (see table 3-B). To make matters even worse, about 75% of chemicals formed (see table 3-C) were not even AAs but would have reacted with them, preventing peptides from forming at all. A more detailed analysis of these issues is available in a separate paper.[12]

CH_4, N_2, and NH_3 (trace concentration) in water

Ring *et al.* experimented with mixtures of CH_4, and N_2 (1:1) plus NH_3 in trace amounts in 100 mL of 0.05 M NH_4Cl with NH_3 added to bring the pH to 8.7 in a 3-litre flask.[13] The spark discharge was run for 48 hours, and the products identified are shown in table 4. Similar results were reported by others using a mixture of CH_4, H_2O, H_2, plus N_2 instead of NH_3 although the yields were lower.[14]

Table 4 includes only amino acids, since a dedicated amino-acid analyzer was used. These experiments showed the following distribution of products:

- ~73%, of the AAs were proteinogenic, according to table 4-A.
- ~93.6% of the proteinogenic yield was Gly + Ala (i.e., (100 × (790 + 440) / 1314.6)).

Glycine and alanine were found to be the most prevalent AA obtained, as in the earlier experiments addressed above, but this time more alanine than glycine was obtained, the opposite of what has almost always been reported. This is difficult to explain chemically. Perhaps highly water-soluble glycine was lost during experimental processing.

In this paper, we find the only example we are aware of in the OoL literature of the significant problem that only one or two proteinogenic AAs are produced predominantly in these kinds of experiments poses.[13]

> "It seems unlikely that the amino acids that were important in prebiotic polypeptides were present in the primitive ocean in about equal concentrations."

This was quite an understatement, since the amount of alanine + glycine shown in table 4 represents about 94%

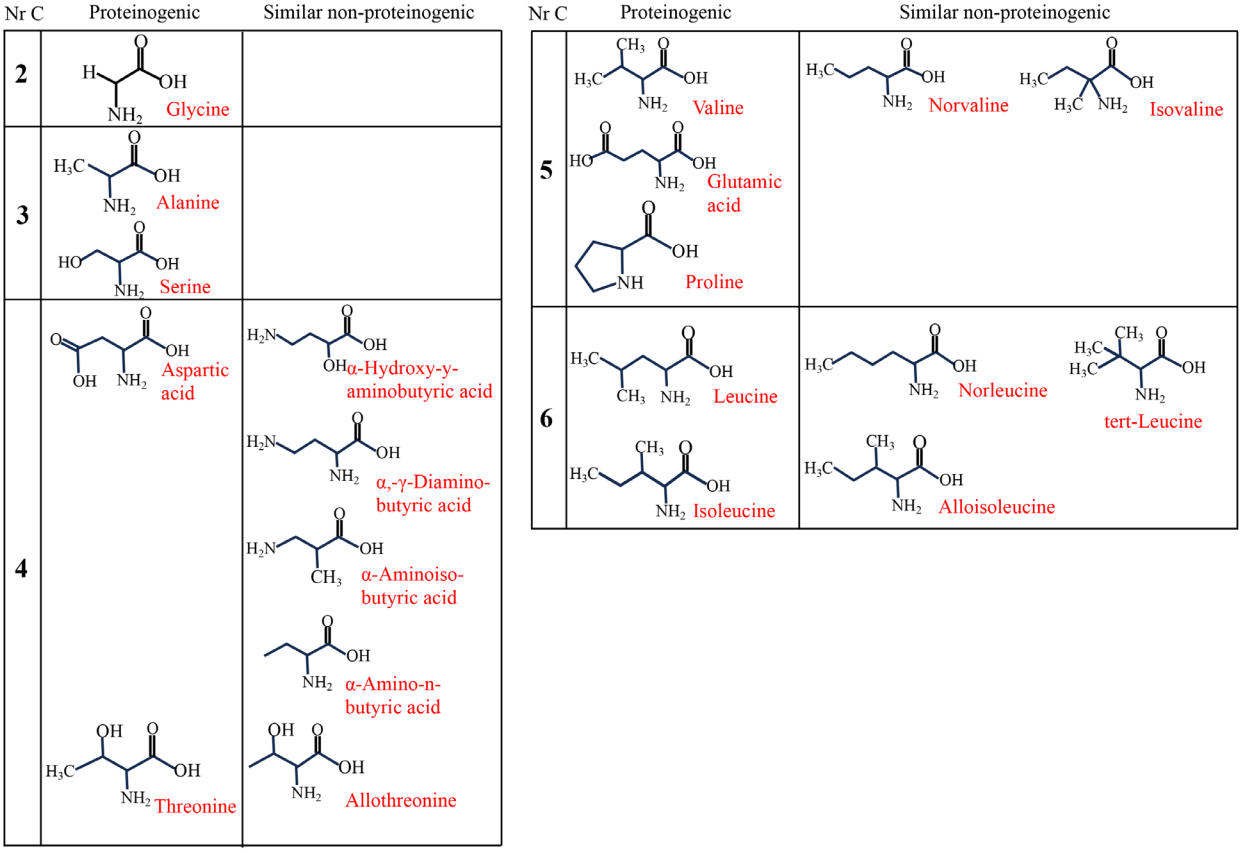

Figure 1. The structures of proteinogenic and non-proteinogenic amino acids formed in Miller-type experiments are similar or comparable (drawn by R. Truman).

of the proteinogenic AAs produced, implying very minor proportions of the remaining 18/20 proteinogenic AAs.

The authors suggested that evaporation of seawater in a lagoon might have led to partial precipitation of AAs, with the more soluble glycine and alanine being preferentially washed out. The researchers overlooked that such a process would have also concentrated the non-proteinogenic AAs, *which were all less soluble than alanine and glycine*!

This was a serious oversight. The most soluble AAs in table 4 are all proteinogenic (glycine, alanine, and serine). So, by their reasoning, any peptides formed would have consisted primarily of non-proteinogenic AAs. To make matters worse, many of the α-hydroxy acids having the generic structure HO-CHR-COOH, which also formed in these kinds of reactions, would have also been less soluble[14]. So would all the large amines, alcohols, and carboxylic acids not reported by the authors.

Many of the proteinogenic and non-proteinogenic amino acids formed in the experiments by Ring *et al.* are very similar, as shown in figure 1, and have similar solubilities For example, threonine/allothreonine are mirror-images (enantiomers) on their side chain and so are isoleucine/alloisoleucine. Their concentrations were reported to be the same, and their solubility in water must be identical. It would not have been possible to selectively precipitate out only the 'right' AA under randomly fluctuating temperatures.

CH_4, NH_3, H_2 in water—the Miller volcanic spark discharge experiment

In 2008, Johnson *et al.* reanalyzed some samples stored in sealed vials from Miller's original experiments.[15] Miller had used CH_4, NH_3, H_2 in the volume ratio 2:2:1 in 500 mL water. The samples were analyzed with high-performance liquid chromatography and liquid chromatography–time of flight mass spectrometry, having a sensitivity able to identify AA at the sub-picomolar ($<10^{-12}$ M) level.

The products from three experiment variants were examined.

Experiment 1 was the original setup apparatus reported in 1953.[10]

Table 5. α-Amino acids detected in three experiments conducted by Miller. Gases: CH_4 (200 torr), NH_3 (200 torr), and H_2 (100 torr) in 500 mL boiling water, sparked with a Tesla coil for 1 week. Relative moles were compared to glycine = 1. Mole proportion compared to all moles of chemicals found in each experiment. Data taken from table S1 of Johnson *et al.* (2009).[15] See main text for description of the three experiments. Calculations found in Supplementary material 1, sheet 'Johnson'.

nd = Not detected at the sensitivity level of $<10^{-12}$ M.

A

	Experiment 1		Experiment 2		Experiment 3	
Proteinogenic AA	**Relative to Gly**	**Proportion overall**	**Relative to Gly**	**Proportion overall**	**Relative to Gly**	**Proportion overall**
Glycine	1	96.081%	1	45.157%	1	28.024%
Alanine	2.7×10^{-2}	2.594%	0.9	40.641%	1.4	39.233%
Serine	1.0×10^{-4}	0.010%	1.6×10^{-3}	0.072%	2.7×10^{-3}	0.076%
Aspartic acid	6.0×10^{-4}	0.058%	2.0×10^{-4}	0.009%	2.5×10^{-3}	0.070%
Valine	3.3×10^{-5}	0.003%	1.1×10^{-4}	0.005%	nd	nd
Glutamic acid	2.0×10^{-4}	0.019%	1.0×10^{-4}	0.005%	1.3×10^{-3}	0.036%
Phenylalanine	nd	nd	2.0×10^{-6}	0.000%	nd	nd
Sum:	1.0279	*98.765%*	1.9020	*85.889%*	2.4065	*67.439%*

B

	Experiment 1		Experiment 2		Experiment 3	
Non-proteinogenic AA	**Relative to Gly**	**Proportion overall**	**Relative to Gly**	**Proportion overall**	**Relative to Gly**	**Proportion overall**
β-Alanine	3.0×10^{-3}	0.288%	0.3	13.547%	9.0×10^{-1}	25.221%
α-Amino-isobutyric acid	1.1×10^{-3}	0.106%	3.7×10^{-3}	0.167%	7.1×10^{-2}	1.990%
Isovaline	4.0×10^{-4}	0.038%	2.6×10^{-3}	0.117%	9.9×10^{-3}	0.277%
β-Amino-isobutyric	3.2×10^{-5}	0.003%	9.0×10^{-4}	0.041%	4.8×10^{-2}	1.345%
β-Amino-butyric acid	5.0×10^{-4}	0.048%	6.0×10^{-4}	0.027%	4.7×10^{-2}	1.317%
γ-Amino-butyric acid	1.0×10^{-4}	0.010%	6.0×10^{-4}	0.027%	1.4×10^{-2}	0.392%
α-Amino-butyric acid	7.0×10^{-4}	0.067%	2.0×10^{-4}	0.009%	nd	nd
Isoserine	nd	nd	1.4×10^{-4}	0.006%	nd	nd
β-hydroxy-aspartic	nd	nd	1.3×10^{-4}	0.006%	nd	nd
Norvaline	5.4×10^{-5}	0.005%	1.9×10^{-5}	0.001%	nd	nd
2-Methyl serine	nd	nd	1.6×10^{-5}	0.001%	nd	nd
α-Amino adipic acid	nd	nd	3.8×10^{-6}	0.000%	nd	nd
Homoserine	nd	nd	3.4×10^{-6}	0.000%	nd	nd
Ornithine	nd	nd	2.5×10^{-6}	0.000%	nd	Nd
2-Methyl glutamic acid	nd	nd	2.4×10^{-6}	0.000%	nd	nd
Sum:	0.0070	*0.566%*	0.0036	*13.950%*	1.0899	*30.543%*
Overall total:	1.0408	100.00%	2.2145	100.00%	3.5684	100.00%

C	Experiment 1		Experiment 2		Experiment 3	
Non-AA	Relative to Gly	Proportion overall	Relative to Gly	Proportion overall	Relative to Gly	Proportion overall
Methylamine	5.0×10^{-3}	0.480%	2.8×10^{-3}	0.126%	nd	nd
Ethylamine	1.7×10^{-3}	0.163%	7.4×10^{-4}	0.033%	nd	nd
Ethanolamine	2.5×10^{-4}	0.024%	1.9×10^{-5}	0.001%	7.2×10^{-2}	2.018%
Isopropylamine	1.3×10^{-5}	0.001%	5.7×10^{-6}	0.000%	nd	nd
n-Propylamine	3.8×10^{-6}	0.000%	2.6×10^{-6}	0.000%	nd	nd
Sum:	7.0×10^{-3}	*0.669%*	3.6×10^{-3}	*0.161%*	0.072	*2.018%*

Experiment 2 incorporated an aspirating nozzle to increase the gas flow rate through the apparatus, forcing steam and gas directly into the spark.

Experiment 3 also incorporated the aspirator device but used a silent discharge instead of electrodes.

Table 5 summarizes the findings by Johnson *et al.*, but secondary amines (which have an H replaced by another group) were not reported:[15]

> "Analyses of secondary amines such as sarcosine, imino-diacetic acid, and imino-acetic propanoic acid, which are not reactive with OPA/NAC, are not reported."

They also stated that

> "Besides the amino acids listed, several 6-carbon amino acids were also detected but these were not quantified."

This is an example of systematic bias in interpretations of the results of OoL experiments.[16]

The data in table 5 leads to the following statistics, based on moles of the chemical produced in experiments 1, 2, and 3:

- Proteinogenic AAs: 99.4%; 86.0%; 68.8%.
- Proteinogenic AAs vs non-AA interfering compounds: 99.3%; 99.8%; 97.1%.
- Proteinogenic AAs in the overall yield: 98.8%; 85.9%; 67.4%.
- Gly + Ala vs all the proteinogenic AAs: 99.9%; 99.9%; 99.7%.

Many chemicals able to interfere with forming peptides, such as amines and larger carboxylic acids, were not reported, since the purpose was only to identify as many proteinogenic AAs as possible.

Unfortunately, the sealed vials were half a century old, and the experiments were not repeated to validate these values.

The proportion of non-proteinogenic AAs varied considerably. For experiments 1, 2, and 3, they found 0.6%; 14%; 31%, respectively. This illustrates a principle encountered in these kinds of experiments. Whenever the conditions or gas mixtures are optimized to yield more AAs and/or a wider variety, *the proportion of non-proteinogenic AAs increases over-proportionally*. Recall also that the authors reported that several 6-carbon amino acids were also detected but not quantified.[15]

It is noteworthy how much the Gly/Ala ratio varied for experiments 1, 2, and 3 (37.0; 1.1; 0.7, respectively). In experiments 2 and 3, the gases were forced directly into the spark and, on average, about the same amounts of glycine and alanine were formed, whereas in experiment 1, 37 times more glycine than alanine was produced.

On average, glycine and alanine comprised more than 99.8% of the proteinogenic AAs! Of all the remaining AAs, the proportion of *proteinogenic AA* compared to total AAs for experiments 1, 2, and 3 was only 13.7% 0.6%; 0.6% respectively. Therefore, if only AAs had been present in some prebiotic environment, then *any peptides formed would have consisted of almost entirely glycine and alanine, with the rest being almost entirely non-proteinogenic*!

Johnson *et al.* focused on experiment 2, claiming that it might mimic reactions caused by lightning in steam-rich volcano eruptions. They admitted that evolutionary geoscientists currently don't believe a primitive atmosphere would have had the highly reducing composition Miller used. However, they argued that some local environments might have, temporarily. They further suggested[15]

> "Amino acids formed in volcanic island systems could have accumulated in tidal areas."

This is highly implausible, considering the violent conditions predicted based on evolutionary assumptions, with average tides about 30 times higher than currently.[17] The aerosols released would also have seeded increased rainfall, flushing soluble chemicals into the ocean.

It is noteworthy that none of the three experiments produced any of the other 13 proteinogenic AAs at a sensitivity level of $<10^{-12}$ M.

CH_4, H_2S, NH_3, and CO_2 in water

After Stanley Miller died, some vials with residues from his early experiments were found and analyzed by Bada[18] and Parker *et al.*,[19] using the latest analytical methods. One series of experiments used a gas mixture of CH_4, H_2S, NH_3,

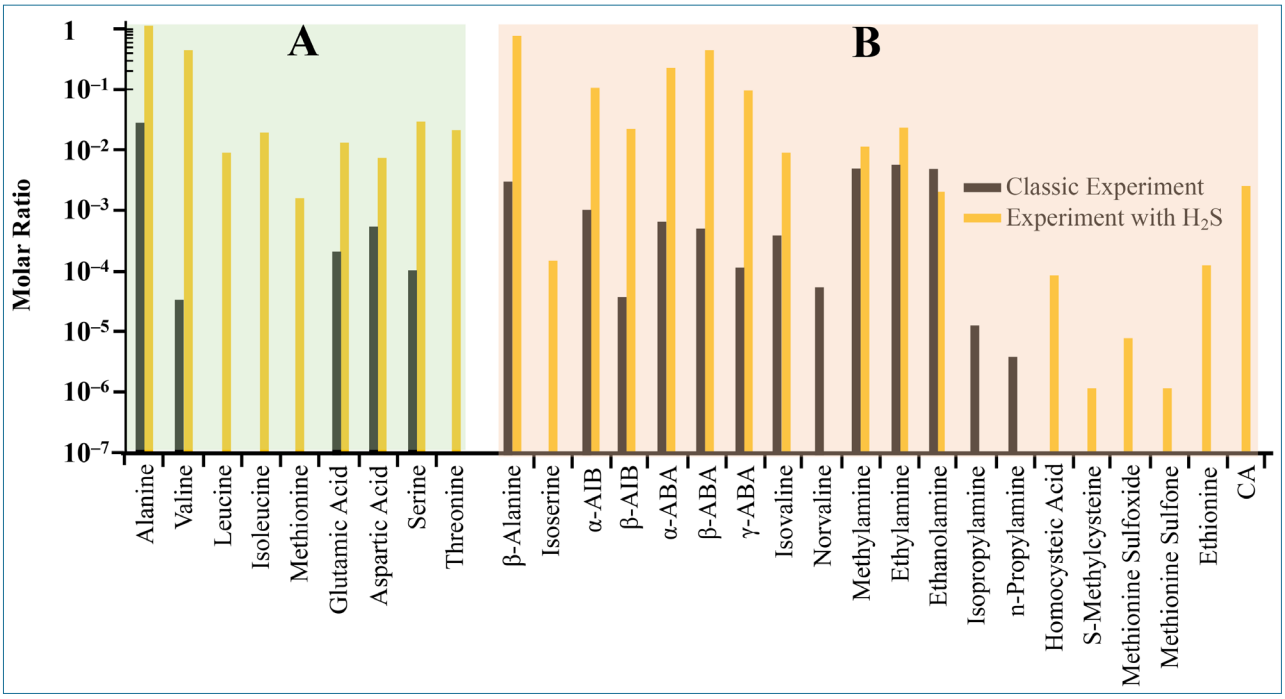

Figure 2. Molar proportion of amino acids compared to glycine (=1) from Miller's spark discharge experiments.[18,19] Black bars: using CH_4, NH_3, H_2 (2:2:1) in 200 mL boiling water. Spark discharge for three days using tungsten electrodes. Orange bars: using CH_4, H_2S, NH_3, and CO_2 at partial pressures of 258 torr, 100 torr, 250 torr, and 87 torr, respectively, with 300 mL water. A, in green background: proteinogenic amino acids; B, in orange background: non-proteinogenic amino acids. Redrawn with some modifications by R. Truman based on figure 5 in Bada (2013).[18] Abbreviations: α-AIB: α-Amino-Isobutyric Acid; β-AIB: β-Amino-Isobutyric Acid; α-ABA: α-Amino-Butyric Acid; β-ABA: β-Amino-Butyric Acid; γ-ABA: γ-Amino-Butyric Acid; CA: Carbamoyl-aspartate?

and CO_2 at partial pressures of 258 torr, 100 torr, 250 torr, and 87 torr, respectively, in 300 mL boiling water, forced through a nozzle into the electric spark. This might resemble steam-rich volcanic eruptions or geysers exposed to lightning.

For unknown reasons, Miller had not analyzed the samples, which should raise concern, since a thin-layer chromatography (TLC) analysis would only have required a small amount of effort. Parker *et al.* analyzed the samples in 2011, almost 60 years later but unfortunately did not repeat the experiments themselves.

The yield and diversity of amino acids synthesized in this 'volcanic' experiment in general exceed those obtained in the original 'classic' experiments using the gas mixture CH_4, NH_3, and H_2 in water, as shown in figure 2.

It is difficult to extrapolate these results to realistic prebiotic conditions. Most of the lightning near erupting volcanoes has been observed at two different heights, one 1–4 km and the other ~10 km above the vent.[20] As the volcanic plume rises, it spreads in every direction and dilutes with the surrounding atmosphere. More damaging to the OoL argument is that, besides water vapour, *CO_2 dominates the output of volcanic eruptions*; CH_4 and NH_3 are present in only trace proportions, the opposite of the composition Miller had used.

The data from table 6 leads to the following statistics:

- ~61% of the moles AAs were proteinogenic (i.e., 60.563% / (60.563% + 38.626%)).
- ~84% of the proteinogenic yield was Gly + Ala (i.e., (100 × (1 + 1) / 2.387)).
- The proportion of proteinogenic AAs vs non-AA interfering compounds can't be estimated, since only AA and amines were identified.

This was viewed as a major success for prebiotic chemistry compared to the former Miller experiments.[18,19] However, glycine + alanine comprised about 84% of the proteinogenic AAs and would have dominated the content of peptides formed prebiotically. Of all the AAs other than glycine and alanine reported in table 6, ~80% would have been non-proteinogenic and potentially incorporated into the peptides. (The proportion would have been much higher than this, since the researchers did not identify non-proteinogenic AAs having secondary amino groups[19]). To illustrate how non-proteinogenic AAs would have contaminated peptides, both β-alanine and β-ABA would have been present in far greater concentration than all the remaining (i.e., non-glycine or alanine) proteinogenic AAs combined.

In these experiments, HCN and aldehydes/ketones were produced, presumably according to scheme (1)[8]

Table 6. Distribution of products estimated from the orange bars in figure 2, i.e., using a gas mixture of CH_4, H_2S, NH_3, and CO_2. Data extracted from figure 5 in Bada (2013).[18]

A

Proteinogenic AA	Compared to Gly	Proportion overall
Glycine	1	25.372%
Alanine	1	25.372%
Valine	0.3	7.612%
Isoleucine	0.02	0.507%
Serine	0.02	0.507%
Threonine	0.02	0.507%
Glutamic acid	0.01	0.254%
Leucine	0.009	0.228%
Aspartic acid	0.007	0.178%
Methionine	0.001	0.025%
Sum:	2.387	*60.563%*

B

Amines	Compared to Gly	Proportion overall
Ethylamine	0.02	0.507%
Methylamine	0.01	0.254%
Ethanolamine	0.002	0.051%
Sum:	0.032	*0.812%*

C

Non-proteinogenic AA	Compared to Gly	Proportion overall
β-Alanine	0.8	20.297%
β-ABA	0.4	10.149%
α-ABA	0.1	2.537%
α-AIB	0.1	2.537%
γ-ABA	0.1	2.537%
Isovaline	0.01	0.254%
β-AIB	0.01	0.254%
Carbamoyl-aspartate	0.002	0.051%
Ethionine	0.0001	0.003%
Homocysteic acids	0.0001	0.003%
Isoserine	0.0001	0.003%
Methionine sulfoxide	0.00008	0.002%
Methionine sulfone	0.000001	0.00003%
S-Methylcysteine	0.000001	0.00003%
Sum:	1.522	*38.626%*
Overall total:	3.941	100.00%

$$CH_4 + NH_3 + H_2O \rightarrow RCOR' + HCN \quad (1)$$

These dissolved in the water, whereupon AAs could be synthesized by the Strecker reaction pathway figure 3-A, but so could hydroxy acids via the cyanohydrin pathway (figure 3-B). The products of reaction 3-A contain an $–NH_2$ end group and can polymerize to form amide bonds. The products of reaction 3-B are identical except for the –OH end group but would polymerize to form ester bonds. Unless NH_3 is present in huge excess, the polymers would be a mixture of both reactions, in a proportion dependent on temperature and pH. This fact emphasizes why most of the results from Miller-type reactions are misleading: OoL researchers neglect to report or emphasize *the enormous number of chemicals produced which would have interfered with forming proper peptides*.

In these experiments, the spark discharges apparently split water into H· and OH· radicals (one of the most reactive chemical species known), which then led to a wide variety of molecules. However, Miller only separated out the AAs from the reaction mixture (shown in figure 2), although *hydroxylated compounds are preferentially synthesized* in the 'volcanic' experiment.[8]

Catalytic effect of borosilicate silicates in glassware

In 2021, Criado-Reyes *et al.* performed a variant of the Miller electric spark experiment which has the potential of invalidating virtually all published Miller-type experiments.[21] They suspected that the borosilicate glass surface of the Pyrex containers used was catalyzing reactions in a manner not relevant abiotically, so they compared outcomes using this kind of glass reactor with a Teflon reactor. A combination of gases was chosen which was known to be one of the *most effective Miller atmospheres*: NH_3 (200 mbar), CH_4 (200 mbar), and N_2 (100 mbar). 1.33 mbar = 1 torr.

As they had anticipated, far higher yields and a much broader range of products were obtained using the conventional borosilicate glass (Pyrex) container, as shown in table 7.[21]

It is noteworthy that, of the 48 major compounds identified, all but four can react readily with amino acids, since they contain one or more carboxyl or amino functional groups.

A few hours after sparking, the surface of only the borosilicate flask was covered by a thin brown film composed mainly of HCN oligomers, which explained the origin of large *insoluble* polycyclic aromatic hydrocarbons (PAHs). This film did not form in the water layer or anywhere else in the Teflon reactor. The film was likely due to interaction with silanol groups on the surface of the glass reactors.

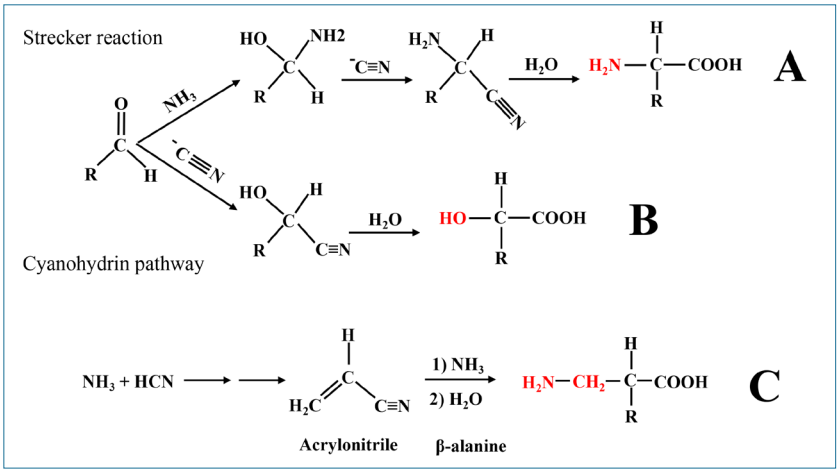

Figure 3. A: Strecker reaction pathway to form amino acids. B: Cyanohydrin reaction pathway to form α-hydroxy carboxylic acids. C: Possible synthetic route to form β-amino acids (which are not proteinogenic). Drawing by R. Truman using information from Bada (2013).[18]

The data in table 7 leads to the following conclusions, based on moles of the chemical produced, using a Pyrex (Pyr) and Teflon (Tef) reactor:

- Proteinogenic AAs vs total AAs: 57.5% (Pyr); 7.2% (Tef).
- Proteinogenic AAs vs sum of proteinogenic AAs and non-AA interfering compounds: 27.1% (Pyr); 5.4% (Tef).
- Proteinogenic AAs in the overall yield: 22.6% (Pyr); 3.2% (Tef).
- Gly + Ala vs all the proteinogenic AAs: 54.6% (Pyr); 0% (Tef).

These results force a reconsideration of virtually all Miller-type experiments, since reactant gases were always being forced into contact with borosilicate catalysts. Under prebiotic conditions, virtually all the energy from lightning strikes would have been restricted to the atmosphere, far away from catalyzing minerals. Therefore, the results when using Teflon containers should be the most relevant approximation for prebiotic purposes.

Eleven proteinogenic AAs were identified using the conventional Pyrex container, as shown in table 7-A. This agrees with the work of others who also used unrealistic highly reducing atmospheres composed primarily of CH_4 and NH_3. The data in table 7-C shows, however, that in the absence of the borosilicate catalysts only four proteinogenic AAs were obtained, even in the highly reducing gas mixture chosen, and these represented *a mere 3% of the compounds which would have reacted readily with AAs*. Remarkably, no glycine or alanine were found.

These are very sobering results, considering that Criado-Reyes *et al.* had selected an exceptionally effective, but prebiotically unrealistic, gas mixture. Importantly, of the miniscule proportion of four proteinogenic AAs formed, just two, histidine and lysine, jointly represented about 92%, and neither are members of the reduced set of AAs assumed to have been available prebiotically.[22,23]

Discussion

A variety of molecules can be generated when chemicals are mixed in a closed container and fragmented into radicals and charged ions using high-energy particles,[24] UV irradiation,[25] or thermal energy.[26] For OoL purposes, however, all the experimental details must reflect true undesigned abiotic conditions.

The Rout *et al.* study discussed above illustrates an important principle to understand OoL research methodology: assumptions are cherry-picked to optimize a specific outcome instead of considering multiple independent constraints.[5] When convenient, proponents of evolutionary theory argue that fewer AAs would have been necessary for life to have arisen but then publish experiments based on entirely different AAs, since otherwise the peptides wouldn't possess the necessary properties. This goal-oriented research permitted Rout *et al.* to satisfy protein requirement no. 4 in table 1, but in a manner contradicting other evolutionary assumptions. Instead of offering a proof-of-concept, experiments like these provide examples of non-feasible possibilities. *With enough negative examples like these, eventually all naturalist possibilities would be exhausted*. In other words, OoL research is not making progress but steadily eliminating possible answers.

The plausibility of peptide sequences used in OoL experiments must be evaluated by considering the AAs proportions that would have been present prebiotically. A large number of *identical* peptides would not have existed having mostly low probability AAs.

Since evolutionists cannot offer realistic scenarios to satisfy the ten constraints in table 2, 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:[27]

> "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."

Bada disagrees, however, writing in 2013:[18]

> "The results discussed here challenge claims that the 'primordial soup' concept of the origin of life is 'well

Table 7. Major products and relative molar proportions of organic chemicals using the usual Pyrex (borosilicate glass) reactors vs a Teflon reactor. Most of the values are the average of 3 replicates. Gas mixture: NH_3 (200 mbar), CH_4 (200 mbar), and N_2 (100 mbar) in water at room temperature, buffered with NH_4Cl at pH 8.7. The data from table S2 in Criado-Reyes *et al.* 2021[21] was used to calculate relative moles of each chemical reported. Calculations found in Supplementary material 2, sheet 'Reyes'.

A

No.	Proteinogenic AA	Proportion overall	
		Pyrex [a)]	Teflon [b)]
5	Glycine	4.30%	0%
6	Alanine	8.02%	0%
7	Valine	0.78%	0%
8	Leucine	0.90%	0%
9	Proline	2.96%	0.24%
10	Serine	0.48%	0%
11	Asparagine	0.45%	0.02%
12	Aspartic acid	0.68%	0%
13	Glutamic acid	0.22%	0%
14	Lysine	1.43%	1.33%
15	Histidine	0.35%	1.61%
22	Glycylglycine (2×)	2.00%	0%
	Sum:	*22.57%*	*3.19%*

B

No.	Non-proteinogenic AA	Proportion overall	
		Pyrex [a)]	Teflon [b)]
16	β-Alanine	0.66%	0%
17	Isovaline	~0%	~0%
18	α-NH2-isobutyric acid	~0%	~0%
19	γ-NH2-butyric acid	1.54%	10.47%
20	N-formylglycine	4.72%	1.61%
21	N-formylleucine	1.00%	0%
23	1-Butanamine	8.65%	29.15%
24	Isobutylamine	0.12%	0%
	Sum:	*16.71%*	*41.24%*

C

No.	Compounds which interfere with forming peptides	Proportion overall	
		Pyrex [a)]	Teflon [b)]
1	Formamide	18.69%	19.67%
2	Formic acid	27.67%	19.25%
3	Urea	3.79%	3.23%
25	Glycolic acid	1.85%	0%
26	Oxalic acid	2.48%	0.62%
27	Pyruvic acid	1.19%	5.35%
28	Lactic acd	0.10%	0.92%
29	Maleic acid	0.24%	0.48%
30	Malic acid	0.14%	0%
31	Oxaloacetic acid	0.24%	0%
32	2-Ketoglutaric acid	0.06%	0%
33	Hexanoic acid	0.04%	0.02%
34	Nonanoic acid	0.39%	0.35%
35	Gentisic acid	0.38%	0%
36	Adenine	0.71%	1.43%
37	Guanine	0.03%	0%
38	Uracil	0.85%	0%
39	Cytosine	0.04%	1.50%
40	Thymine	0.04%	0.66%
41	Parabanic acid	0.72%	0.02%
42	3.5-diNH2-1.2.4-triazole	0.69%	0.56%
43	1H-Indole-3-methanamine	0.01%	1.52%
44	9-Acridinamine	0.21%	0%
45	Hydroxy-naphthalene	0.16%	0%
46	1.8-Dihydroxy- naphthalene	0.03%	0%
	Sum:	*60.73%*	*55.57%*

a) Pyrex reactor (borosilicate), type usually used in Miller-type experiments
b) Teflon reactor

past its sell-by date' and as such is null and void.[27] 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 was focusing only on whether AAs could have been present.[18] 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 existed somewhere. His creative speculations included the possibility of a Titan-like atmospheric organic haze of gases protecting from UV photochemical degradation.[28–30]

OoL researchers believe almost unanimously that the atmosphere when life was supposed to have arisen would have consisted almost entirely of oxidized gases, and mainly CO_2. However, special conditions have been envisioned to temporarily produce a prebiotic atmosphere consisting primarily of reduced gases. This will be discussed in part 2 of this series.[31]

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