The limited value of wet–dry cycles to produce large peptides prebiotically

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The equilibrating reactions to form peptides through condensation are thermodynamically unfavourable in water. However, wet–dry cycle experiments by origin of life researchers have increased the yield and length of peptides, using carefully designed, but prebiotically implausible, conditions. High pH values had to be used; unrealistically high amino acid (AA) concentrations, and environmental catalysts which degrade AAs were avoided. The deliquescent salt mixtures used in some experiments could not have resulted from evaporation of seawater, where NaCl would have been the dominant component. Enhanced production of peptides required carefully timed periods of cooler temperatures with enough water to dissolve the reactant AAs and peptides, followed by hot periods to drive off water and prevent hydrolysis. Critically, after a few cycles, peptide yields began to decrease, since AAs regenerated from hydrolysis could also have been degraded thermally, demonstrating that the experiments were merely short-term artefacts. Avoiding hydrolysis long-term (for example, through contact with ocean water) could only have occurred if peptides had become entombed as solids in mineral matrixes, rendering them irrelevant for origin of life models. Realistically, at best, trace amounts of >8-AA peptides would have formed prebiotically.

Most proteins consist of >100 amino acids (AAs) and are indispensable as enzymes; to provide structural support; regulate genes; store and transport various molecules; transfer signalling information; and move cells.[1]

Origin of life (OoL) researchers speculate that peptides, which contain ≤50 AAs, might have served prebiotic functions, which then somehow developed into life-like organisms. The vague notion is part of the narrative offered by the amyloid world hypothesis,[2] early prions,[3] and protein world hypothesis.[4,5]

Thermodynamics of peptide condensation/hydrolysis

Hydrolysis of peptides is exergonic ($\Delta G < 0$), as shown in figure 1 going from point C to A, and occurs spontaneously in the presence of heat or a catalyst. Calculations have shown that no 9-AAs or larger peptide would have been present in prebiotic oceans at ≥ ambient temperatures at equilibrium, even assuming a high [glycine] $\approx 10^{-4}$ M.[6] Hydrothermal vent conditions are even less conducive to forming peptides.[7]

Chemists use several methods to obtain products, despite being kinetically and/or thermodynamically unfavourable, and these have been applied in OoL experiments to form short RNA, DNA, peptides, and other biopolymers. Reactions can be accelerated by lowering the transition state energy (point B in figure 1, also known as the activation energy) using special solvents and catalysts.[8] This accelerates both the forward and reverse reactions, but not the relative proportion of product/yield per se, which is determined by the difference in free energy, ΔG.

A more favourable ΔG for condensation can be obtained by increasing the free energy of the reactants (point A in figure 1) and/or lowering the free energy of the product (point C). Some solvents can have this effect, the right pH, and, in most cases, higher temperature. One technique is to chemically modify the reactants, placing them in a higher energy state.[9] Another strategy is to design an entirely different chemical process that leads to the desired product. All these principles have been applied by OoL researchers to synthesize peptides; especially modification of the -NH_2 and/or -COOH end groups of AAs. This disrupts stabilizing interactions with water and places the bond to be broken in a higher energy state. However, virtually all of the OoL experiments require conditions that are not abiotically plausible. For example, exotic chemical modifications would not have occurred naturally, and being in a high-energy state would have been unstable.

Studies based on prebiotically absurd conditions will not be reviewed here, but one technique is worth examining: the use of wet–dry cycles, which is well-known to chemists and chemical engineers.

The principle of wet–dry cycles

To form peptides, two AAs must first condense to form a dipeptide, which then condenses with another AA to form a tripeptide, and so on, as shown in figure 2.

Two competing processes determine the concentration of peptide of length *n*:

$$\text{Rate of condensation: } k_{cond}[\text{peptide}_{n-1}][\text{AA}] \rightarrow \text{peptide}_n + H_2O \qquad (1)$$

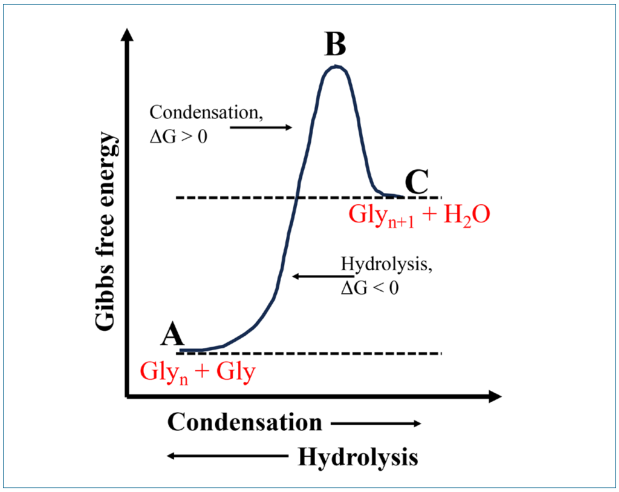

Figure 1. Condensation of glycine (Gly) with another glycine or a peptide is endergonic (unfavourable), whereas the reverse hydrolysis is exergonic (favourable).

$$\text{Rate of hydrolysis: } k_{hyd}[\text{peptide}_n][H_2O] \rightarrow \text{peptide}_{n-1} + \text{AAs} \qquad (2)$$

where k_{cond} and k_{hyd} are the rate constants for condensation and hydrolysis, respectively, under specific conditions of temperature, solvent, pH, and anything else affecting the rate. Since $[H_2O]$ is usually in large excess and thus essentially unchanged, $k_{hyd}[H_2O]$ is usually combined into the reported value of k_{hyd}.

At equilibrium, the rate of condensation is equal to the rate of hydrolysis so that eqn 1 = eqn 2, leading to the equilibrium constant:

$$K_{eq} = k_{cond} / k_{hyd} = [\text{peptide}_n] / [\text{peptide}_{n-1}][\text{AA}]. \qquad (3)$$

K_{eq} can be increased by hindering hydrolysis. Initially, enough water must be present to dissolve peptide_{n-1} and AA to permit condensation. But by later removing H_2O, hydrolysis of peptide_n can be prevented. To then form peptide_{n+1}, water must be added, which now allows hydrolysis. However, *by carefully controlling* the temperature and duration during several cycles of adding and evaporating water, condensation can be favoured over hydrolysis.

Forming dipeptides is thermodynamically the most unfavourable step, since both AAs form stable charged zwitterions, $NH_3^+–CHR–CO_2^-$.[10] The interaction with water stabilizes Gly by about 9.9 kcal/mole at from 25°C to 37°C.[11] Therefore, two principles are involved in hindering hydrolysis: the absence of water as a reactant and too little water available to effectively stabilize all the resulting AAs. Heat drives off water and also weakens the stabilizing interactions of AAs with water. The same principles apply to each AA involved in further chain elongation.

Highly optimized conditions to form peptides

Rodriguez-Garcia *et al.* developed an automated system to simulate wet–dry cycles under a wide range of conditions,[12] obtaining the highest yields of peptides previously reported from similar reactions by optimizing the key parameters.[8] An approximately 0.0875 M aqueous solution of glycine was rapidly injected into hot vials, and the water allowed to evaporate at various constant temperatures (90°C, 110°C, or 130°C). For subsequent dehydration–hydration cycles, the water was replaced and allowed to evaporate again. Longer duration of the evaporation step (to remove more water) initially increased the total yield of peptides and the greatest length, but soon a maximum was reached (e.g., 15 h at 130°C and about 70 h at 100°C) when *thermal degradation then began to decrease the yield* (figure 3). The experiment at 90°C was discontinued after 100 h.

Increasing the number of cycles also steadily decreased the yield (figure 4, table 1).

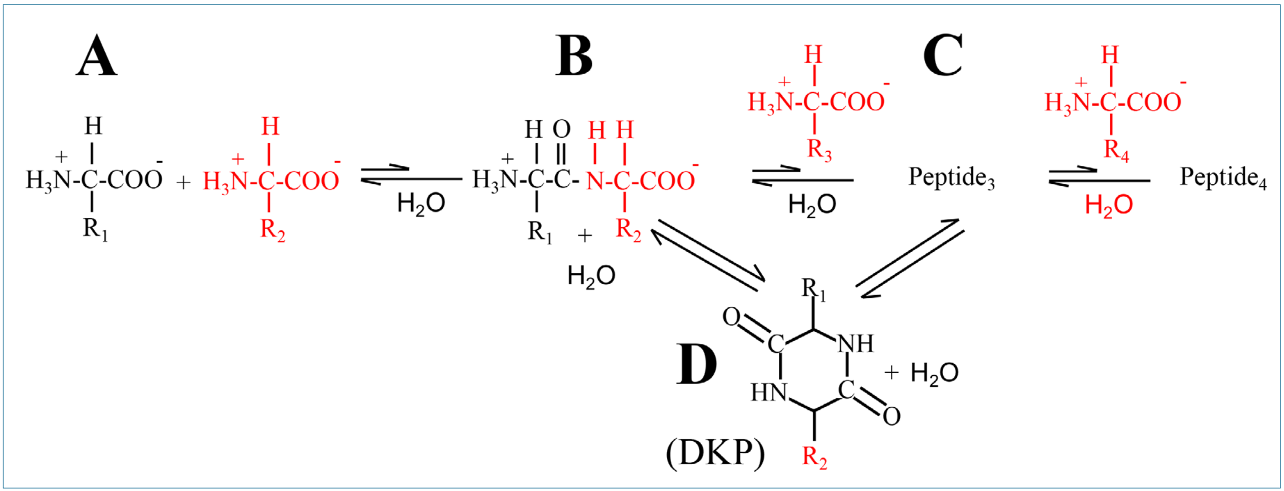

Figure 2. Condensation/hydrolysis reactions involving amino acids and peptides. DKP = Diketopiperazine.

The percentages in table 1 might be misunderstood, since they are not concentrations, but rather represent the amount of starting material accounted for by each Gly_n. To convert into relative concentrations with respect to the initial concentration of Gly, each entry must be divided by the number of glycines, *n*, in that peptide. For example, after 4 cycles at pH 9.8, $[\text{Glys}_{13}] = 0.25\%/13 = 0.019\%$ of the initial Gly.

Formation of peptides is favoured at high pH.[13] However, prebiotic ocean water, hydrothermal water, and water volcanos would have been slightly acidic. Importantly, the optimal but prebiotically irrelevant pH 9.8 selected produced about 20 times more peptide yield than obtained at pH 4–7, at 130°C for 24 h (Supplementary table 2, ref. 12); in fact, *no peptide*$_n$ *with* $n > 8$ *formed at all in that pH range*. (Supplementary figure 13, ref. 2).

Campbell *et al.* experimented with 24 h wet–dry cycles using deliquescent[14] molar mixtures of 32.3% glycine, 48.2% NaCl, 16.3% KCl, 2.4% NaOH, and 0.8% KOH.[15] 50 mg samples were placed in vials and subjected to 1, 2, 3, 5, or 10 cycles.

For the cool-humid phases, the vials were placed in a temperature and humidity-controlled chamber for 20 h, set to 40°C and either 30, 50, or 70% RH (relative humidity). For the hot-dry phases, the vials were transferred to a hot plate for 4 h, set to 120°C. Overall, the results for the 50 mg samples reported after 10 cycles were:

- At 30% RH: <1 mg water was absorbed, with total peptide yield 1.29%. Gly_8 yield was 0.01%, and Gly_9 was the largest peptide obtained.[16]
- At 50% RH: <1 mg water was absorbed, with total peptide yield 2.56%. Gly_6 was the largest peptide, with 0.05% yield.[17]
- At 70% RH: 50 mg water was absorbed, with total peptide yield = 17.33%. Gly_8 yield was 0.71%, and Gly_{13} was the largest peptide obtained, with 0.05% yield.[18]

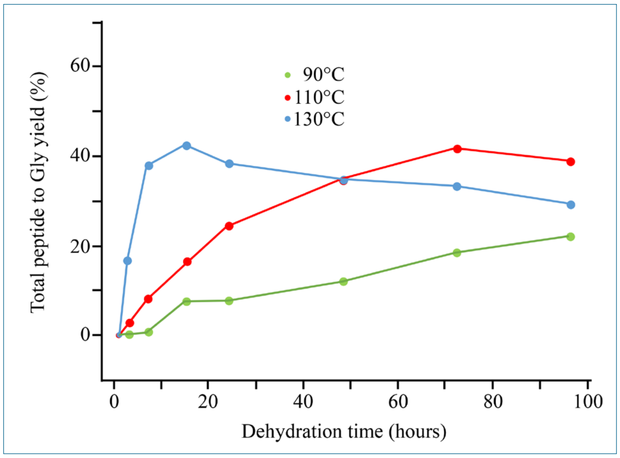

Figure 3. Dehydration initially increased yield of peptides and then decreased it. Initial [glycine] = 0.0875 M, [NaCl] = 0.25 M, pH = 9.8. Redrawn from Rodriguez-Garcia *et al.*, ref. 12.

However, the total yield of peptides increased only for a limited number of cycles and then decreased steadily, as shown by the red line in figure 5 (the yield decreased ~3% in only five 1-day cycles); this is consistent with the results from Rodriguez-Garcia *et al.*, above. Lowering the temperature to 100°C slowed down the rate of thermal destruction (blue line in figure 5).

In additional experiments, 20 ml deionized water was added after each cycle to simulate rainfall.[15] Very little peptide

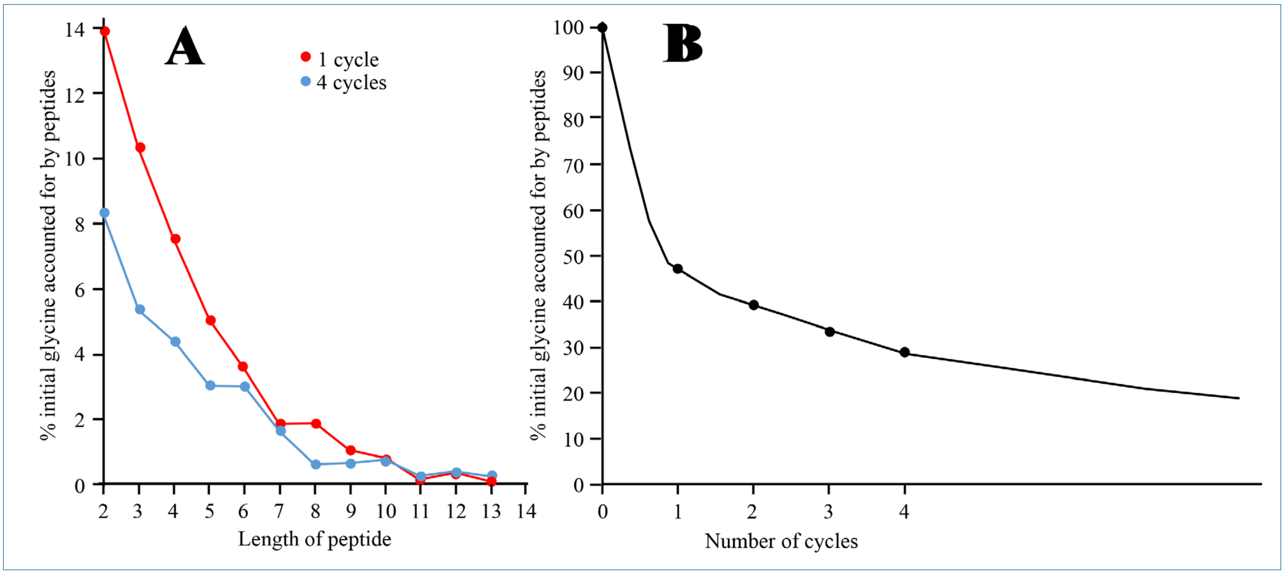

Figure 4. Increasing the number of wet–dry cycles decreases the total yield of peptides, shown for 130°C, 24 h cycles, at pH 9.8. A: redrawn from Supplementary figure 14[12]; B: based on data from Supplementary Materials, table 3 in ref. 12. The amounts of glycine present as peptide at the end of each cycle were calculated in table 1 below.

Table 1. Peptide yields shown as a percentage of the glycine starting material accounted for after 1–4 hydration–dehydration 24 h cycles, at 130°C, pH 9.8. Initial glycine = [0.0875] M.[12]

Oligomer	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Gly2	13.96	10.26	9.42	8.36
Gly3	10.4	7.7	6.46	5.41
Gly4	7.61	5.95	5.11	4.41
Gly5	5.11	4.23	3.53	3.07
Gly6	3.64	3.71	3.37	3.03
Gly7	1.91	2.07	1.94	1.67
Gly8	1.91	2.05	1.07	0.64
Gly9	1.09	1.3	0.77	0.66
Gly10	0.81	0.93	0.81	0.74
Gly11	0.2	0.32	0.28	0.26
Gly12	0.4	0.56	0.4	0.38
Gly13	0.11	0.34	0.3	0.25
Total Gly in peptides	*47.15*	*39.42*	*33.45*	*28.9*

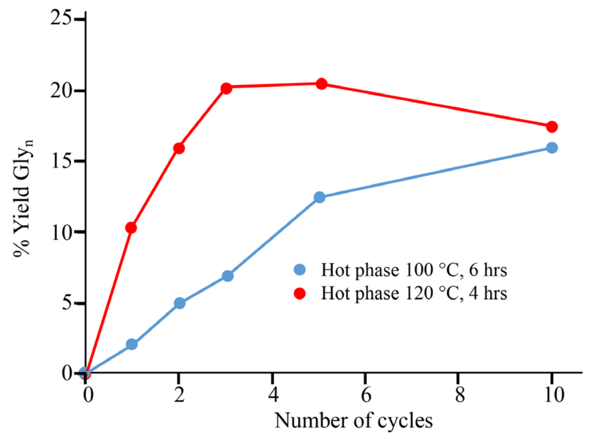

Figure 5. Total yield of oligomers at 70% relative humidity after 1, 2, 3, 5, and 10 cycles. Red: 20 h at 40°C, 4h at 120°C. Blue: 18 h at 40°C and 6 h at 100°C. From Campbell *et al.*, ref. 15.

was obtained after 10 cycles. A single heavy rainfall or influx of water would have hydrolyzed a considerable proportion of the peptides, especially under high temperature conditions. The Campbell *et al.* study, which applied to only highly optimized deliquescent mixtures of pure chemicals, clarified how a small amount of water was necessary to increase diffusion of reactants to favour condensation over hydrolysis, and that subsequent rapid evaporation to dryness was critical to obtain longer oligomers.

Deliquescence mixtures prebiotically implausible

The proportions of glycine, NaCl, KCl, NaOH, and KOH obtained from evaporated seawater could not have produced deliquescent mixtures. The proportion NaCl : KCl ≈ 3 was selected because the most water would be absorbed through deliquescence although about 50 times more Na^+ than K^+ is found in seawater.[19] The 2.4% NaOH proportion was also unrealistic, and selected to ensure an ideal highly alkaline pH >13.[20]

A more serious problem is that evolutionists estimate [glycine] was ≈ 10^{-10}–10^{-4} M in prebiotic oceans,[6] whereas [NaCl] ≈ 0.6 M.[21] A molar proportion of NaCl / glycine = 1.5 was used in the experiments, whereas a local dried oceanic salt mixture would have contained ≈ $0.6/10^{-10}$ to $0.6/10^{-4}$ more NaCl than glycine, ignoring all the other chemicals present in seawater. Much water would have been necessary to dissolve the miniscule proportion of glycine embedded in NaCl. The 0.05 g samples of optimized deliquescent salt combination absorbed only 0.05 g water, leading to exceedingly concentrated [Gly]. However, the very dilute prebiotic Gly would have condensed very slowly, favouring hydrolysis, which would have resulted in virtually no longer peptides being produced.

Favourable results depended on expert design

OoL wet–dry experiments either produced only very short peptides in trace amounts or, like the studies above relied on, expertly designed conditions not relevant prebiotically. Peptide elongation requires condensation to proceed faster than hydrolysis and successful experiments were designed with this in mind.

Eqn (1) shows that forming [peptide_n] depends on both [AA] and [$\text{peptide}_{n\text{-}1}$] whereas, as peptides grow larger, more positions become available for hydrolytic attack. Therefore, *high [AA] must be used*. Table 2 documents the dramatic effect [AA] has, especially on the key first step, AA + AA → AA_2, which is thermodynamically the most unfavourable.[11] Using $[\text{Gly}]_{\text{initial}} = 10^{-1}$ M led to $[\text{Gly}_3] = 1.75 \times 10^{-3}$ M, whereas $[\text{Gly}]_{\text{initial}} = 10^{-4}$ M to $[\text{Gly}_3] = 3 \times 10^{-6}$ M, even though water had been rapidly removed to prevent hydrolysis. The rate of production of [Gly_4] would have been very slow with low concentrations of both Gly_3 and Gly_2 whereas hydrolysis would not have slowed down. This effect is incremental for each larger peptide. Furthermore, Gly is consumed to produce all peptides of length 2 to *n*, leaving less to produce Gly_{n+1}.

H_2O is added during the wet phase, and, once heated, AAs are regenerated. Consequently, during each cycle, additional AAs can degrade to simpler chemicals, in numerous ways.[23,24] Even trace amounts of many substances present in nature catalyze destruction of AAs, especially in mineral clays,[25] hindering formation of peptides and decreasing total peptide yields with additional cycles.[26,24] Fluctuating temperatures would have worsened matters. During periods of temperature far above the boiling point of water, peptide and glycine

Table 2. [Peptide] formed depends strongly on the initial concentration of amino acid, even when water is removed to prevent hydrolysis.[22]

[Starting] (M)	[Gly_2] (mM)	[Gly_3] (mM)
10^{-4}	0.007	0.003
10^{-3}	0.159	0.015
10^{-2}	0.376	0.155
10^{-1}	1.292	1.750

would have quickly degraded thermally, but, at temperatures considerably under the boiling point, almost only hydrolysis would have occurred instead of condensation.

Exposure to ocean water would have diluted peptides, facilitated hydrolysis, and added more contaminants like NaCl.

Peptides not dissolved in water would have become irrelevant for OoL purposes, and slowly degraded via radiation, radioactivity, and other processes.

In conclusion, wet–dry and similar experiments[26] are short-time artefacts, quickly discontinued before the peptides formed and would be destroyed thermally. Under plausible prebiotic concentrations of AAs, pH, unguided temperature changes, and the expected contaminants, it is unlikely that peptides >8 AAs would have formed above trace concentrations. Another important reason that smaller peptides are favoured is that the rate of condensation is bimolecular (eqn 1), whereas the rate of hydrolysis is unimolecular (eqn 2) as long as water is present in abundance.

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