Enantiomeric amplification of amino acids: part 10—extraction of homochiral crystals accompanied by catalytic racemization

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For abiogenesis to be possible, a natural process must separate proteinogenic L-amino acid enantiomers from racemic mixtures. It was shown that laboratory methods that separate L- and D-amino acid enantiomers based on differential solubility are impossible under unguided natural conditions. Preferential enrichment was used to separate these enantiomers in the solution phase. Crystallization-Induced Asymmetric Transformation was used to separate enantiomers in the solid phase. The separated L-amino acid must be replenished to continue the asymmetric transformation method. The remaining D-amino acids must therefore be racemized. This was accomplished in the laboratory using a racemizing agent in organic solvents at high temperatures. These methods cannot explain the origin of pure L-amino acids for several reasons. Organic solvents would not have existed in sufficient concentrations at high temperatures. High-quality catalysts would not have been available in high concentrations. Initial enantiomeric excesses in hot organic solvents to seed the formation of the first homochiral crystals would not be present. An unrealistically high amino acid concentration having an enantiomeric excess was necessary. Rapid stirring and glass beads in a contained volume to generate new, small seeds is not a realistic prebiotic environment. Finally, hot solvents and catalysts would have racemized any enantiomeric excess present.

We continue here our series of papers which critique possible natural explanations offered by origin of life (OoL) researchers for the origin of pure L-amino acids (AAs), necessary to produce proteins. In Part 9 of this series, we mentioned that most AAs preferentially form crystals that are 1:1 DL enantiomers, termed racemates or racemic compounds. Asparagine (Asn) and threonine (Thr) are exceptions, since homochiral DD and LL crystals (known as conglomerates) are formed preferentially.

In all cases the energy difference in crystal stability is very small, and a mixture is produced. Nevertheless, under careful laboratory conditions an enantiomeric excess (e.e.) can sometimes be obtained with careful timing by separating the solidified crystals from the solution phase after partial crystallization.

In Part 9 we discussed the example of an initial e.e. of an *(S)*-enantiomer imine being used to produce *(S)*-only crystals.[1] This required the resulting excess in *(R)*-form in solution to be reduced through rapid racemization with a special organic base, thereby replenishing the extracted S enantiomer.[2]

Chemical firms have been optimizing manufacturing processes for many years to selectively extract an enantiomer present in small excess. The key is to partially racemize the remaining mixture to replenish the enantiomer just extracted or the amount of pure enantiomer which could be obtained would be limited. For some substances (rarely proteinogenic AAs) this can ultimately yield 100% pure enantiomer.[3] However, this is not a process that occurs naturally.

Preferential enrichment

In preferential enrichment, the *solution phase* is used to obtain an e.e., taking advantage of the greater solubility of LL or DL crystals compared to DL racemates. Suppose that there is initially a small $e.e._L$ in a solution. DL will slowly precipitate out, and small LL crystals will form in the solution. It would then be increasingly difficult for a D enantiomer to encounter another D enantiomer and therefore it would attach to a DL crystal. The consequence is an increase in $e.e._L$ in the solution accompanied by an $e.e._D$ in the deposited crystals.[2,4] The liquid phase is then removed, and solvent added with heating to redissolve the crystals. Now the solution begins with an excess of D, so after cooling and deposition of DL crystals an $e.e._D$ remains dissolved accompanied by an $e.e._L$ in the deposited crystals.

Tamura and his colleagues studied the compounds shown in figure 1, by dissolving them in hot ethanol and cooling to 25°C with stirring to form crystals. Repeating the process of dissolving in solvent, crystallization, and removal of solution

phase led to new crystals having the opposite chirality after each cycle as expected, with increasing e.e. yields in the separated solution phase, but rapidly decreasing yields of product.[5–8]

Crystallization-Induced Asymmetric Transformation (CIAT)

In the Crystallization-Induced Asymmetric Transformation (CIAT) process solid crystals are used to obtain an e.e.. Now one takes advantage of the lower solubility of LL or DL crystals of some compounds compared to DL racemates. Assume once again that the L-enantiomer is desired and is provided in slight excess initially in a solution. The special feature of this method is that as the amount of L in solution is decreased as LL crystals form, racemization of D and L enantiomers in solution is deliberately accelerated and carried out continuously. Therefore, as the proportion of D increases, the conversion of D → L will exceed the reverse L → D, thereby preferentially replenishing the L lost.[9,10]

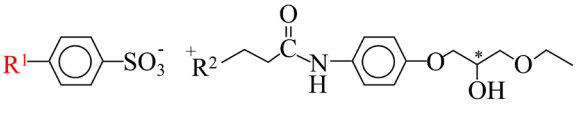

Figure 1. Compounds used to demonstrate preferential enrichment. An enantiomeric excess is amplified in the solution phase and that of the mirror enantiomer in the solid phase. Preparing a fresh solution by dissolving the crystallized material, and repeating the process, now reverses which enantiomer is amplified in the solution and solid phases.[8–11]
ST: ref 8, 9; SC: ref 10; SN: ref 11. (Figure redrawn from ref. 8.)

Almost all other processes proposed by OoL researchers would merely increase the e.e. in one location by decreasing it elsewhere. However, remixing would reverse the local excesses. With methods like CIAT, the e.e. could be deposited as crystals, in which AA racemization would be slower than in solution.

Various aliphatic and aromatic aldehydes catalyze AA racemization; see examples in table 1 using acetic acid as the solvent at 100°C for one hour.[5]

Allegedly the necessary aldehydes could have been provided through an influx from extraterrestrial sources like meteorites.[11]

The mechanism believed to racemize ***α***-AAs under acidic conditions is shown in figure 2.[5] This involves an initial protonation of the imine (Schiff base), followed by proton abstraction from the α-carbon atom by an acetate anion.

There do not seem to be restrictions on the type of aldehyde that could be used to induce racemization.

An absurdly high catalyst concentration under abiotic conditions, 0.01 mol of salicylaldehyde/mol of the pure AA, was necessary to completely racemize L-alanine in acetic acid within 1 hour at 100°C. Racemization was shown to increase rapidly with temperature. Rapid racemization also occurred when formic or propionic acid was used instead of acetic acid, as shown in table 2, but was most effective with acetic acid.

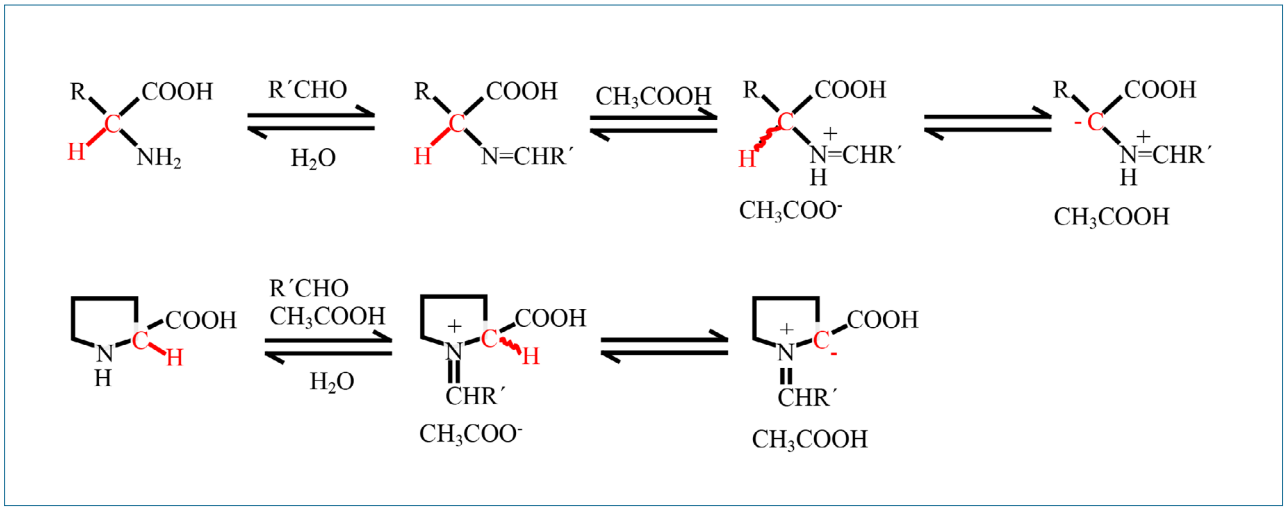

Figure 2. Racemization mechanism of amino acids under acidic conditions.[4]
The chiral C which racemizes is shown in red. (After scheme 1 on p. 91 of ref. 4.)

Table 1. Ability of various aldehydes to racemize some amino acids. A mixture of L-amino acid (1.5 mmol), aldehyde (0.3 mmol), and acetic acid (6 ml) was heated in a sealed tube in an oil bath at 100°C for 1 hour. Data is from table 2 of ref. 5.

		Degree Racemization, %			
Aldehyde	**Reaction Temp, °C**	**L-Ala**	**L-Met**	**L-Phe**	**L-Pro**
None	80	7	0	35	0
None	100	13	24	35	3
Formaldehyde	100	83	95	100	63
Acetaldehyde	100	97	100	100	98
Propionaldehyde	100	78	100	100[a]	87
n-butyraldehyde	80	97	95[a]	100[a]	99
n-heptylaldehyde	80	100	100[b]	100[b]	100
Benzaldehyde	100	72	100	100	72
Salicylaldehyde	80	100	100	100	91

[a] A ninhydrin test revealed degradation products containing an amino group.

[b] Considerable decomposition was found.

Table 2. Comparison of kinds of aliphatic acid solvent on the racemization of amino acids. A mixture of L-amino acid (1.5 mmol), aldehyde (0.3 mmol), and acetic acid (6 ml) was heated in a sealed tube in an oil bath at 100°C for 1 hour. Data is from table 4 of ref. 5.

Aliphatic acid	**L-Ala**	**L-Lys**	**L-Met**	**L-Phe**
Formic acid	81	43[a]	49	100
Without salicylaldehyde	53	19[a]	18	95
Propionic acid	9[b]	99[a]	96[a]	100[a]
Without salicylaldehyde	2[b]	15[a]	19[a]	100[a]
Acetic acid	100	100	100	100
Without salicylaldehyde	13	9	24	35

[a] A ninhydrin test revealed degradation products containing an amino group.

[b] Low solubility of the amino acid in the aliphatic acid.

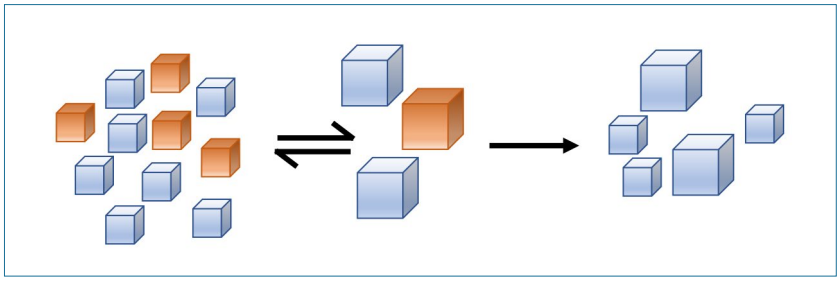

Figure 3. Without attrition by rapid stirring with glass balls, larger crystals are favoured due to the decrease in surface area per volume. An enantiomeric excess can be used to form one crystal form preferentially in solution. Racemization using a catalyst can be used to replenish the now-depleted enantiomer still dissolved.[13] (After scheme 2 from ref. 13.)

Other racemization procedures use an aldehyde and a metal ion which forms a chelate compound with the initially formed Schiff base, and the reactions are carried out under neutral or weakly alkaline conditions instead of pure organic acid.[5] This highlights the fact that metal cations would have been ubiquitous in primordial oceans. Why is this important? Loss of a proton at the α-carbon of an AA produces a planar carbanion intermediate, and re-protonation could occur at either plane, leading to either D- or L-enantiomers. Bada calculated that chelation by dissolved Cu^{2+} in oceans would have facilitated the removal of these protons, increasing the rate constant for racemization of for example alanine by about a hundred-fold (at pH 7.6 and 0°C).[12] However, *indiscriminate and unavoidable racemization would have resulted for all amino acids in the solution; not the outcome wished for by OoL researchers*.

Aspartic acid in acetic acid with catalytic salicylaldehyde

Heating promotes the preferential dissolution of smaller crystals, as illustrated in figure 3.

This is related to the Gibbs–Thomson effect, whereby small crystals are observed to be in equilibrium with their liquid melt at a lower temperature than large crystals.[14] The observation that small crystals dissolve and redeposit onto larger crystals leading to preferential growth of larger crystals is known as 'Ostwald ripening'.[13,15] This effect was described by Wilhelm Ostwald in 1896.[16,17]

OoL researcher Viedma *et al.* studied aspartic acid with an initial e.e. of each of the enantiomers under racemizing conditions in acetic acid at 90–160°C, using salicylaldehyde as the catalyst even though it does not form abiotically.[13] Asp was selected

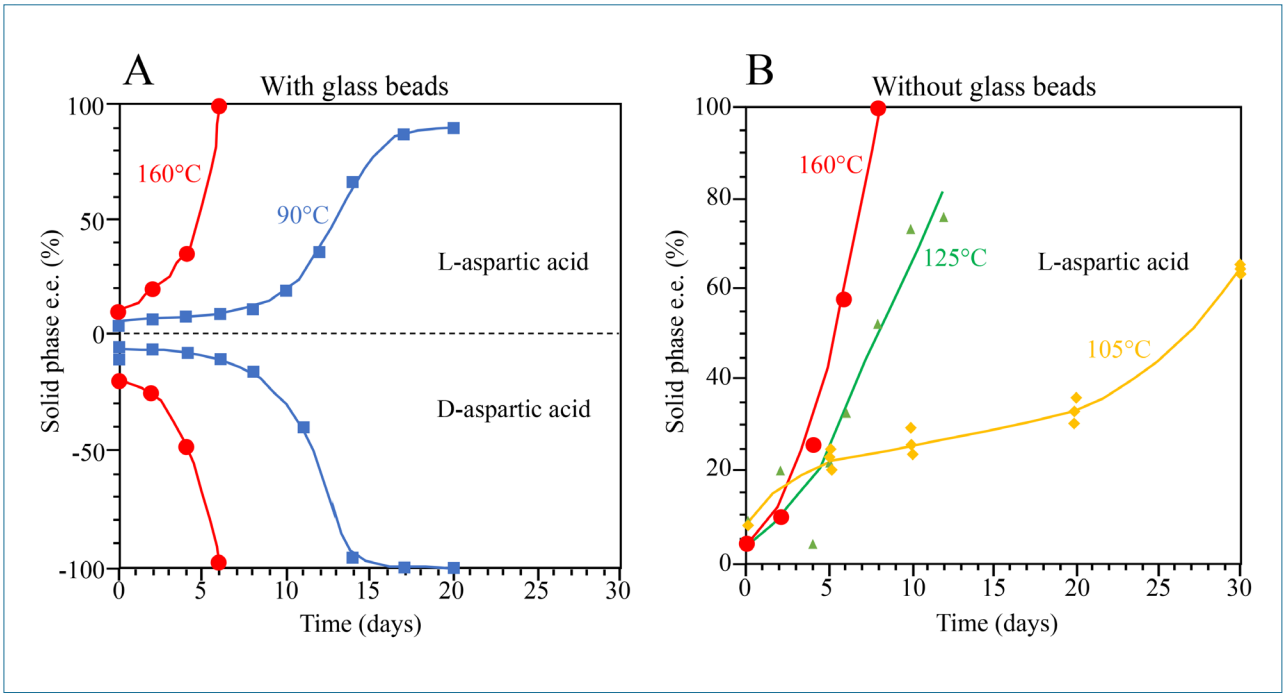

Figure 4. Progress of solid phase e.e. for D- and L-aspartic acid while racemizing.[13] A: Using attrition-enhancement. Red: gradient heating to 160°C. Blue: isothermal at 90°C. Positive e.e. values refer to the biological L-asp. B: Stirring only, without attrition enhancement. Red: gradient heating to 160°C. Green: under reflux at 125°C. Yellow: isothermal heating to 105°C. (After figure in ref. 13.)

Table 3. Data from the open circles in figure 4 at 90°C[13]

Day	e.e. (d), %	e.e. (l), %
0	5	5
11	40	28
15	100	75
20	100	90

knowing that of the 20 biogenetic AAs aspartic acid is one of only two that form separate crystallize D and L crystals under ambient conditions. Salicylaldehyde was known from industrial experience to be an unusually effective racemizing agent and, as mentioned above, acetic acid was the best choice to accelerate this racemization. Experiments were carried out both in the presence and absence of 2.5 mm glass beads (which, when stirred rapidly, lead to continuous crystal attrition).[13] In part 9 of this series, we documented how attrition caused by stirring glass beads can sometimes cause homochiral crystals to form before the more stable racemic ones.[1]

For the attrition-enhanced experiments (i.e., glass beads were added) summarized in figure 4 A, temperatures of 90°C and 160°C were used with 0.5 g total Asp, 5 ml acetic acid and 0.3 ml salicylaldehyde placed in a screw-capped bottle. The initial e.e. and rpm of rotation were not identical (for reasons not explained).[13]

For the three experiments summarized in figure 4 B, which did not use attrition enhancement, surprisingly the proportion of Asp to acetic acid and Asp to salicylaldehyde used by the researchers all differed considerably. The reason for this decision was not explained in the paper and was only apparent upon examining the separate Supporting Information document. The solid phase enrichment was found to occur about three times faster at 125°C than at 105°C, and even faster at 160°C. In the absence of glass beads, the e.e. only reached 58% after 30 days. For comparison purposes, one must also consider that the proportion of Asp to acetic acid used at 105 °C was much higher than that used at 125°C and 160°C. It is noteworthy that the experiments were not continued for extended periods, since this would have resulted in the thermal destruction of aspartic acid under these conditions.

The experiments at 105°C were repeated three times with results that agreed with each other quite well. Unfortunately, the counterpart experiments using D-Asp were not performed at this temperature, as is discussed below.

Reporting bias of results

Notice in figure 4 that e.e.$_D$ increased considerably faster over time than e.e.$_L$. In addition, the L-form attained a maximum of ~90% but the D-form reached an e.e. of ~100%. The results for both enantiomers are summarized in table 3.

Why was this difference not mentioned by the authors? This seems to be an example of reporting bias, which occurs all too frequently in science. The results which support the desired outcome are reported and discussed extensively, whereas those which don't are often downplayed, ignored, or attributed to experimental error. This does not necessarily reflect dishonesty but rather a prior conviction as to what the correct results should be and a desire not to confuse with flawed data.[18]

According to figure 4 B, the e.e.$_D$ also increased more rapidly than e.e.$_L$ at 160°C. But this is inconclusive since, according to the Experimental section, an initial e.e.$_L$ of only 9% was used vs. an initial e.e.$_D$ of 20% e.e., hindering a comparison. Additional experiments should have been performed and the e.e.s documented at identical time intervals. What we see instead is an example of another kind of bias that can occur in research. Experiments that offer the potential to support a favoured theory are often performed, rather than those offering the potential to discredit the theory.[18] For events from the distant past that are impossible to prove in a laboratory, statements like "the bulk of the evidence indicates" may well be an artifact of bias in deciding what kinds of research to perform.

The OoL community rejoices over all examples of AAs producing an e.e.$_L$, no matter how small. However, some small differences in D/L could simply reflect measurement errors. The same effort is not devoted to finding, communicating, or justifying examples of e.e.$_D$. Taking all the data into account would emphasize how, under natural conditions, maximum entropy is the expected outcome over time, in which the concentration of D- and L-AAs is equal.

Critique of these studies

Two centuries of experimentation and careful thought by physical chemists have produced a deep understanding of phase changes between solids, liquids, and gases and the circumstances which permit an e.e. to be distributed across them. Researchers like Viedma, who have devoted their careers to OoL topics, design their experiments based on deep chemical knowledge, benefiting from a plethora of techniques and special equipment to drive chemical changes in the manner they wish. Chemists are trained to guide chemical processes to attain a predetermined goal, and this has produced many valuable products. The non-chemist rarely understands why specific details were necessary and can be misled to think that with enough time the same outcomes might have occurred naturally. Someone with a comparably low level of understanding of how art is produced, using the same logic, might also be led to believe that natural processes could have produced all the works of art in the Louvre Museum.

In the experiments described above, several important design principles were indispensable. The researchers needed to cause rapid racemization of the mixture to replenish the desired enantiomer being extracted, but avoid racemization of the initial mixture which contained an e.e. of the desired enantiomer (necessary to produce the first homochiral crystal seeds). Being competent chemists who have mastered many 'tricks of the trade', they skilfully did this. They knew that crystallization required the interaction of two or more molecules of the enantiomer in initial excess. Therefore, the rate is a higher order in concentration; see eqn (1). Loss of e.e., however, is first order with respect to the concentration of the e.e., see eqn (2).

$$\text{Rate of formation of initial homochiral seeds} \sim k_1 \times [\text{e.e.}]^n, n \geq 2 \quad (1)$$

$$\text{Rate of loss of enantiomer excess} \sim k_2 \times [\text{e.e.}] \quad (2)$$

The initial homochiral seeds can be caused to form as fast as possible by beginning with a high concentration of Asp already having an e.e. Additional enhancement by stirring, and especially through adding glass beads to break the crystals formed causes the rate of formation to be greater than the rate of loss.

The researchers also knew that Asp would be one of the two AAs likely to produce homochiral crystals.[19,20] To optimize equilibration and high concentrations, the reactions were carried out in equipment that constrained the volume. Acetic acid boils at 118°C, but most of the experiments were carried out in screw-capped bottles so that temperatures above the boiling point could be used, giving the best results.[21] Racemization had to be perfectly timed and balanced to replenish the depleted enantiomer. This required a special solvent instead of water. Acetic acid was typically used since it was known to provide the best results among all the carboxylic acids tested.

One might think that the ideal conditions used were only for researcher convenience and that more time might have adequately compensated for less ideal conditions. Let us extrapolate to provide some insight into this possibility. Instead of an initial absurd concentration ≥0.5 g Asp in 5 ml acetic acid (i.e., 0.1 kg/l), what concentration might have been more realistic? After all, this is about three times

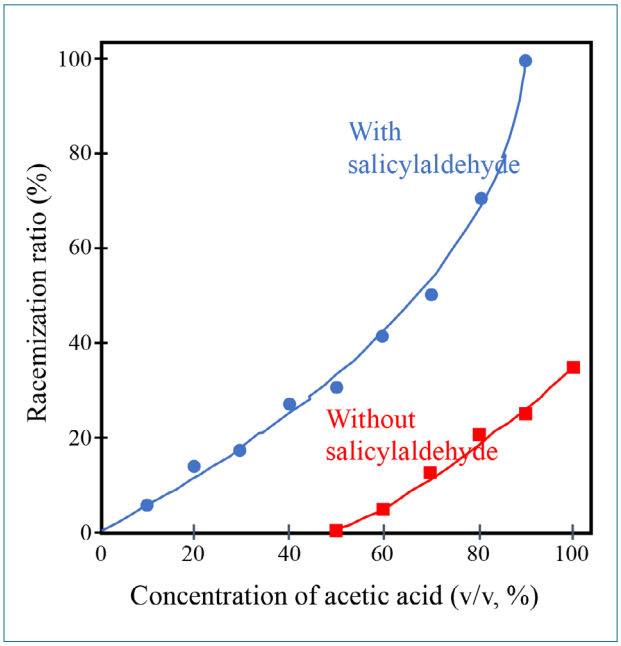

Figure 5. Racemization of L-phenylalanine (0.1 g) at 100°C in a sealed tube of aqueous acetic acid (3 ml) for 1 hour. Blue: With 0.2 molar equivalent salicylaldehyde racemizing agent. Red: Without salicylaldehyde. (Redrawn from figure 3 of ref. 5.)

more Asp than the weight of all salts currently found in a litre of ocean water![22] Leading OoL researcher Professor Bada estimated that the maximum concentration of AAs in ancient oceans would have been only about 10^{-8} g/l water, a staggering difference of a factor of 10^{10}.[23] Worse, the specific sample of Asp would have required an e.e.$_L$. of at least 5% and be present in a suitable highly concentrated organic solvent at a temperature of 100°C or higher. Under those conditions, Asp as an amino acid, and any e.e.$_L$ could only have survived a few hours. Even without a racemizing agent AAs would racemize rapidly in the hot organic acid at various levels of aqueous concentration, as shown in the example in figure 5 for after only 1 hour.

A naturally occurring location and chemical environment would also have had to be hermetically enclosed to force equilibration and production of racemic Asp. Suppose, by miraculous good fortune, a sample satisfied all these constraints, which we'll endow with an Asp concentration ~10^{-6} M. This is significantly lower than used in the above reports but still far too concentrated to be plausible for pure Asp created abiotically.

Using $n = 2$ in eqn (1) reveals that the first homochiral seeds would form at a rate ~10^{12} times lower than reported in the laboratory experiments. Some additional corrections would be needed to permit realistic extrapolations. Rapid stirring (600–1,200 rpm) and the presence of ideally sized glass beads or anything analogous in a clean, closed environment would not have existed, decreasing the rate given by eqn (1) by many more orders of magnitude. Comparing the laboratory data which is expressed in hours with the fact that a year consists of <10^4 hours demonstrates that billions of years would not have compensated for the guiding expertise provided by the chemists.

The unavoidable conclusion is that once again OoL researchers have spent decades exploring how L-only AAs might arise naturally, using deep knowledge. This has led to expertly designed experiments that could not possibly have any natural relevance—even in the most wildly conceived optimistic scenarios. Here are some specific objections to the above reports:

- Without intelligent guidance, racemizing chemicals like salicylaldehyde would have eliminated any initial e.e. needed to act as seeds.
- The presence of racemizing agents in an appropriate solvent at high temperature would have indiscriminately racemized *all* proteinogenic AAs present in nature.
- There is no natural analogy for an enclosed high-temperature volume containing a pure suitable organic acid. How would Asp have been placed there?
- All the concentrations were carefully selected and are unrealistic for OoL purposes. Pure, highly concentrated Asp with an e.e.$_L$ could not arise naturally. Even the concentration of the racemizing agent was carefully selected. If racemization occurred randomly, there would have been no initial e.e.
- The e.e. required a very hot, pure organic solvent. Lowering the temperature of acetic acid from 125°C to 105°C in the absence of glass beads decreased the maximum e.e. obtained dramatically and increased the time needed for e.e. to build up. At acetic acid temperatures under 50°C, no measurable amount of enantiopure crystals would likely have formed.
- Loss of e.e. occurs at a kinetic rate which is first order with respect to its concentration but at a higher order to form homochiral crystals. For the initial e.e. to form the first seed crystals instead of racemizing, a very high initial concentration of the A.A. had to be quickly mixed in a hot solvent. Realistically, the initial e.e. would not have been available.
- An e.e.$_L$ which remained in a solid crystalline state would have been irrelevant for OoL purposes. It would have to eventually dissolve in water, where racemization would then occur, plus contamination with already racemized dissolved AAs. Truman and Schmidtgall showed that for both kinetic and thermodynamic reasons, the rate of racemization will always be faster than the formation of peptides under any known natural aqueous conditions.[24]

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