Racemization of amino acids under natural conditions: part 2—kinetic and thermodynamic data

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Racemization of amino acids is frequently claimed to have half-lives on the order of tens of thousands to millions of years. This is misleading. I show that L→D residue interconversion in small, soluble peptides occurs at 25°C on the timescale of only about a century for the first 10% in oceans, rendering them already unsuitable for life-related chemistry. Metal cations, especially Cu^{2+}, aldehydes and various minerals in oceans act as racemizing catalysts. L-α,α-dialkyl amino acids from meteorites also catalyze proteinogenic L-amino acid racemization. α-helices and β-sheets can slow down racemization, but these secondary structures would rarely be found under natural random conditions for putative ancestral peptides ca. 35 or fewer residues long. Hydrothermal vents, volcanos, meteorites, and rapid evaporation cycles would have prevented any build-up of L-enantiomer excess under realistic abiogenesis scenarios. The large amount of racemized amino acids built up during putative millions of years would have quickly contaminated any locally L-enriched amino acids arising through some speculative process.

Peptides require long stretches of L-amino acids to form α-helices and β-sheets. These secondary structures are indispensable to produce folded proteins able to perform necessary cellular processes. Pro-evolutionists are adamant that life must have arisen with no intelligent input and seek a naturalistic origin for homochirality of biochemicals in addition to how the correct amino acid (AA) and nucleotide sequences could have arisen. Tens or hundreds of millions of years of random chemical reactions are claimed to have produced some form of life. Here, in part 2, I will focus on the homochirality barrier and quantify the time available for any collection of optically pure AAs before too much L→D interconversion would have occurred. In part 4 I will provide a remarkable thesis: under realistic naturalistic conditions in water, a homochiral peptide will racemize faster than it would grow in length. I believe this is true for peptides of all lengths at all temperatures. If this is true, enantiopure peptides cannot have arisen naturalistically, even if originally only L-amino acids were present.

Amino acid racemization under acidic and basic conditions

Neuberger proposed, in 1948, that loss of a proton at the C2 carbon of AAs leads to a planar carbanion intermediate. The proton can be regained using either surface of the flat intermediate structure, thereby regenerating the original enantiomer or its mirror image.[1] Therefore, facilitating formation of the carbanion will accelerate racemization. Acid catalysis can help stabilize the carbanion through a C=C double bond resonance structure. Alternatively, base catalysis can function through direct extraction of the C2 proton (figure 1).

Protonation of amino groups and deprotonation of carboxyl groups on AAs lead to various ionic states, also on sidechains of many AAs such as the carboxyl group of aspartic acid (figure 2). The overall rate constant for interconversion, k_{int}, for the equilibrating reaction L⇌D results from intermediate planar carbanions from each contributing ionic state after removing a proton at the chiral carbon. The individual contributions depend on the concentration of ionic state and its ability to accommodate the carbanion charge.

Racemization rates of free amino acids in water

The rate of disappearance of the L-enantiomer as it equilibrates with the D form is given by [1]

$$\ln\left\{\frac{L_0}{2L_t - L_0}\right\} = 2k \cdot t, \qquad [1]$$

where L_0 is the initial concentration of the L-amino acid, L_t the concentation at time = t, and k, which I called k_{int} above, is the rate constant of interconversion L→D and the reverse reaction also, being identical.[3,4] I provide a derivation for equation [1] in the Appendix. Proteinogenic AAs isoleucine and threonine possess two 2 chiral carbon centres which can also racemize, leading to a more complex kinetic equation.

Equation [1] can be rewritten in terms of the optical rotation of an amino acid solution as

$$\ln(\alpha_0/\alpha_t) = 2k \cdot t, \qquad [2]$$

where α_0 and α_t are the light polarization initially and after a time interval, t.[3]

Literature discussions on abiotic racemization rates often reference the pioneering work of Professor Bada,[5] who began examining the rate of interconversion L⇌D of aspartic acid during his Ph.D. research, completed in 1968. The rates of racemization of AAs in water were determined over a range of pH values and temperatures over time. Bada showed that, for aspartic acid, k_{int} at a wide range of temperatures was about ten times faster near a pH of 3 or 11.[4] Between pH 5–8, pH changes had little effect on the k_{int} for the amino acids studied, Asp and Val.

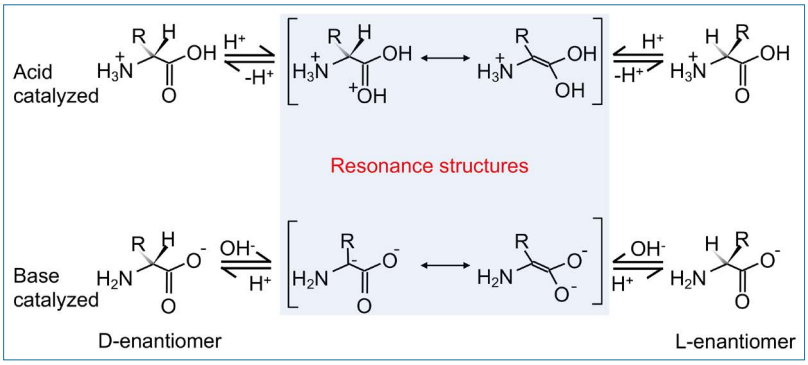


Figure 1. Acid and base-catalyzed L⇌D racemization mechanism of amino acids. Resonance structures provide free-energy stabilizing effects on the planar carbanion intermediate structure. Based on a figure in ref. 2.

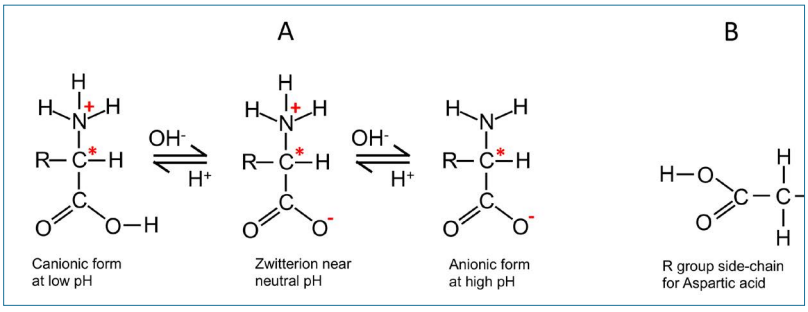


Figure 2. Amino acids can exist in various ionic states depending on the pH. **A**. Amount of protonation of the amino group and deprotonation of the carboxyl group attached at the C2 position depends on the pH. Some amino acid sidechains can also assume different ionic states. Abstraction of a proton at the chiral carbon marked with * leads to a planar carbanion intermediate which permits racemization. **B**. The side group of aspartic acid is shown.

Half-lives of amino acids in pure water

Bada and Schroeder studied the racemization half-lives of several AAs in aqueous solutions at pH 7.6 in the early 1970s by monitoring the change in optical rotation [2] over time. Extrapolating the high-temperature kinetic results to 25°C and 0°C led to the $t_{½}$ values shown in table 1.

At the half-life point $t_{½}$, equation [1] becomes

$$\ln(2) = 2 \times k_{int} \times t_{½}, \qquad [3]$$

leading to the necessary relationship:

$$\frac{L_0}{2L_t - L_0} = 2. \qquad [4]$$

A little algebraic manipulation reveals that at $t_{½}$, $L_t = 3/4L_o$ and thereby $D_t = 1/4L_o$. Therefore, AAs have D/L = 1/3 at their half-life point. This fact can be used experimentally to monitor racemization over time to find the half-life times.

Since $t_{½}$ values have been reported, we can calculate the rate constants k_{int} for several amino acids at different temperatures using [3], see table 1. k_{int} values are useful since they permit us to predict all D/L using an equation derived from [1]:[5]

$$ln\{1 + D/L) / 1 - D/L\} = 2 \times k \times t. \qquad [5]$$

Of particular interest will be the time, *t*, to attain various D/L values, which is now easy, knowing the rate constant, *k*.

The equations above assume that D/L = 0 when an experiment is initiated. If this is not the case, then a correction must be made.[7] I encounter this in part 5, where, apparently, a non-trivial amount of D aspartic acid seems to be present at the time an organism dies or is generated during the lab procedures.

The racemization half-lives, $t_{½}$, for the four AAs reported in table 1 ranged, for the two references, between about two and forty thousand years at 25°C, but long before the $t_{½}$ is reached too much contaminating D-enantiomer would have formed for origin of life purposes, as mentioned in part 1 of this series.[8]

Bada also reported that the aqueous $t_{½}$ of L-amino acids in proteins is considerably shorter than that of free AAs. To illustrate, at 100°C, pH 7–8, protein Asp $t_{½}$ was 1–3 days vs 30 days as free AA.[9] For free isoleucine $t_{½}$ ~ 300 days was calculated under these conditions.[10] This is an important general principle. Forming peptides (the precursors for big complex proteins) under abiogenesis conditions requires AAs to condense into large peptides, but peptides dissolved in water racemize far more quickly than unbound amino acids. A critic of abiogenesis does not care if an enantiomer excess of an amino acid could be produced somehow which is never able to become part of a peptide.

At higher temperatures all AAs rapidly decompose chemically, limiting the possibility that they could have arisen from sub-surface deposits on the earth.[10] Unsurprisingly, no AAs were found in 319°C hydrothermal oceanic vent waters, consistent with measured $t_{½}$ values on the order of ~ 1 min at 240°C; for example, for alanine.[10]

Table 1. Half-lives in years for racemization of amino acids in water at pH 7.6 and ionic strength 0.5 beginning with pure L-enantiomer. Since pure water was used, the rate constants for L⇌D interconversion are considerably lower than found under natural environments where chelating metals are present. Eqn. [1] was used to find the k values.

	0°C		25°C			0°C		25°C	
Amino acid	**$t_{1/2}$, years[a]**	**k_{int}, /year[c]**	**$t_{1/2}$, years[a]**	**k_{int}, /year[c]**		**$t_{1/2}$, years[b]**	**k_{int}, /year[c]**	**$t_{1/2}$, years[b]**	**k_{int}, /year[c]**
Phenylalanine	160,000	2.2×10^{-6}	2,030	1.7×10^{-4}		160,000	2.2×10^{-6}	2,000	1.7×10^{-4}
Aspartic acid	420,000	8.3×10^{-7}	3,460	1.0×10^{-4}		430,000	8.1×10^{-7}	3,500	9.9×10^{-5}
Alanine	1,100,000	3.2×10^{-7}	11,000	3.2×10^{-5}		1,400,000	2.5×10^{-7}	12,000	2.9×10^{-5}
Isoleucine	4,400,000	7.9×10^{-8}	34,700	1.0×10^{-5}		6,000,000	5.8×10^{-8}	48,000	7.2×10^{-6}

[a] Bada, ref. 6.
[b] Bada, ref. 3.
[c] Our calculation based on ln(2) = 2 x *k* x *t*. Using the half-life values, I calculated $k_{int} = \ln(2) / (2t_{½})$.

Table 2. Observed and extrapolated racemization rate constants (/year), using underwater sedimentary material. The rate constants at 3°C were extrapolated for the four AAs from the other respective temperatures.[14]

Amino Acid[b]	**105°C**	**77°C**	**58.5°C**	**49.5°C**	**25.0°C[a]**	**3°C**
Asx	17.3 ± 1.8	2.10 ± 0.14	0.081 ± 0.025	0.0421 ± 0.0092	0.99×10^{-3}	2.3×10^{-5}
Glx	2.98 ± 0.26	0.182 ± 0.033	0.036 ± 0.011	0.0068 ± 0.0095	0.52×10^{-3}	1.7×10^{-5}
Ser	6.68 ± 0.38	0.718 ± 0.057	0.069 ± 0.011	0.0275 ± 0.0039	1.3×10^{-3}	4.3×10^{-5}
Ala	1.33 ± 0.18	0.0639 ± 0.047	0.016 ± 0.007	0.0142 ± 0.0070	0.55×10^{-3}	4.1×10^{-5}

[a] I calculated these values using the ln(A) and E_a values reported in ref. 14 in the Arrhenius equation ln(k) = ln(A) -E_a/RT to facilitate comparison with rate constants at 25°C from other studies.
[b] Asx = Aspartate or Asparagine; Glx = Glutamate or Glutamine; Ser = Serine; Ala = Alanine.

In the next sections I will review factors which can accelerate amino acid racemization, all relevant for abiogenesis discussion purposes.

Catalytic racemization through metal cations

Stabilizing the intermediate resonance structures shown in figure 2 would render the hydrogen attached to C2 more acidic and accelerate formation of the carbanion. Although hydroxide ion is probably the base which extracts the AA α-proton in aqueous solution at neutral pH, in natural environments any base could catalyze formation of the carbanion, including phosphates and carbonates.[7]

Metal ions like Cu^{2+}, Co^{2+}, and Al^{3+} chelate amino acids, facilitating loss of the α-proton and thereby increasing the rate of racemization by several orders of magnitude.[11] The earliest experiments demonstrating this measured the rate of α-proton exchange and mutarotation using proton magnetic resonance and a polarimeter. In these experiments, L-valine and L-alanine carbanions were bound to cobalt complexes, which enhanced the lability of the α-hydrogen.[11,12]

Bada estimated the concentration of Cu to be ~ 2×10^{-7} M in an ocean. At this concentration, most amino acids would be partially chelated, with Cu^{2+} leading to oxidative deamination with a $t_{½} \approx 350$ years. Therefore, any natural source of fresh L-AA would both rapidly racemize and be destroyed through chemical transformation.[3] Based on results reported by Buckingham *et al.*[11] and estimating that about 17% of the alanine dissolved in natural waters would be chelated by Cu^{2+}, Bada calculated that at pH 7.6 and 0°C the rate constant for racemization of alanine in natural waters would be $\approx 1 \times 10^{-4}$ /year,[3] about a hundred times faster than his results in pure water (table 1). The corresponding $t_{½} = \ln(2)/2k$ would be only ≈ 3,500 years instead of 1.1 million years. Of course, most ocean water has had a temperature > 0°C. Average surface temperatures are currently ~ 17°C, where AAs and peptides are more soluble.

A putative ancient earth would have been flooded with metals such as Cu^{2+}, especially if massive meteorites had pulverized the entire terrestrial surface for tens of millions of years. Therefore, relatively slow racemization rates in pure water such as shown in table 1 are not relevant for abiogenesis discussions. It is important when reading rate constants to take the temperature into account. As we will see below, an increase of just a few degrees can accelerate L→D interconversion by an order of magnitude. The average temperature is not an accurate measure of racemization

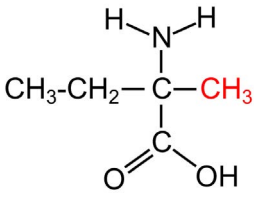
Table 3. Decrease in L-enantiomeric excess (ee_L) of alanine (Ala) and 2-aminobutyric acid (Abu) in the presence of α,α-dialkyl amino acid isovaline (Iva) in calcium montmorillonite at 150°C.[17]

A

0.5% Ala loading			
L-Iva:L-Ala			
	ee_L, 0 hrs[a]	**ee_L, 200 hrs[b]**	**ee_L, 1440 hrs**
0	86.7%	80%	61.0%
1:1	93%[b]	78%	47.6%
3:1	94%[b]	72%	41.8%
5:1	95%[b]	56%	29.9%
D-Iva:L-Ala			
0	86.7%	79%	61.0%
1:1	94%[b]	79%	51.0%
3:1	94%[b]	75%	45.8%
5:1	94%[b]	61%	34.0%

B

2% Ala or Abu loading			
α-H-amino acid + Iva	**ee_L, 0 hrs[a]**	**ee_L, 200 hrs[b]**	**ee_L, 1440 hrs**
L-Ala	96.7%	73%	46.2%
L-Ala + D-Iva (1:1 molar)	100%	71%	35.0%
L-Ala + L-Iva (1:1 molar)	100%	65%	29.9%
L-Abu	100%	81%	52.9%
L-Abu + D-Iva (1:1 molar)	100%	81%	43.3%
L-Abu + L-Iva (1:1 molar)	100%	74%	36.8%



Isovaline (Iva)[c]

[a] Measured at 35°C during sample preparation, before heating to 150°C.
[b] ee_L values were estimated from figures provided in ref. 17.
[c] Isovaline is a non-biological α,α-dialkyl amino acid which lacks a labile C2-hydrogen and thus does not racemize readily unlike proteinogenic amino acids.

rate, due to the over-proportional effect of small temperature increases.

In 2010 Johnson and Pratt studied the racemization of metal-catalyzed amino acids in iron sulfate brine conditions intended to mimic Martian surface conditions. They reported that racemization was dramatically accelerated, several orders of magnitude faster than previously reported, due only to the effects of minerals AAs would encounter.[13]

Racemization rate of bound and unbound amino acids under natural aqueous conditions

Bada's pioneering work examined AA racemization in pure water, which could not have included various catalytic effects I will further discuss below. Amino acids created by some process would not all remain dissolved in pure water, and charged sediment surfaces could potentially stabilize racemization transitional states. Therefore, Steen and colleagues collected sedimentary material from under ocean water 16 m deep, which contained AAs in a natural setting, both in free form and as part of larger biomolecules. Samples were collected from 0–10 cm, ~ 30 cm, and ~ 340 cm below the seafloor. Racemization rates were determined for all three depths. I reasoned that, for abiogenesis purposes, deeply buried layers removed from water would be less relevant for abiogenesis chemistry, and focused on the results from the uppermost layer. ^{14}C measurements indicated the uppermost layers had been deposited about 25 years before. Being of biological origin, this provided AAs in free form and in peptides in high L-form proportions.

The samples were homogenized using a mortar and pestle, and then wet portions (2 g) were placed into 5-ml sterilized, airtight glass vials.[14] D- versus L-AA ratios were measured over time to obtain the rate of interconversion, k_{int}, at 105°C (over 168 hours), 77°C (602 hours), 58.5°C (165 days) and 49.5°C (165 days).[14] Before measuring D and L concentrations using HPLC, bound amino acid was freed by hydrolyzing in 6 N HCl.

The experimental results were extrapolated to obtain the k_{int} for aspartic acid, glutamic acid, serine, and alanine at 3°C, table 2.[14] These four AAs are the most frequently found in sediments.

Comparing the rate constants for Asx and Ala at 25°C and in the 0–3°C range from table 1 and table 2, I conclude that Bada's use of pure water and free AAs underestimates racemization rates in relevant oceanic environments by about 1 to 2 orders of magnitude.

As a rough rule of thumb, if a sample were to be 100% pure L-amino acid initially, about 0.1% of the residues would become D-enantiomers per year at 25°C until about the $t^{1/2}$ point, at which time enough D would exist for the D→L reverse reaction to become relevant. In other words, in only about a century 10% of the residues would have converted to D. This assumes no contamination by AAs from the rest of the world, although these would have consisted of almost only racemic AAs. In part 1, I conclude that once only 5–10% D is present the L-peptides become worthless for biology-type purposes.[8] The 1% per thousand years per residue assumes that the internal peptide positions racemize as fast as the N-end only, which may be too fast an estimate.[15]

It has been theorized that peptide-bound amino acids won't racemize as fast as the residues in a terminal position. However, hydrolysis of an internal position would generate two new fragments with terminal residues. Also, fast in-chain racemization of Asn, Asp, and Ser is known to occur.[15,16]

Catalytic racemization through non-proteinogenic amino acids

Experiments were reported in 2020 by Fox *et al.* at the University of Hohenheim in Germany, designed to model a geothermally heated rock pool that contained amino acids in the clay mineral calcium montmorillonite.[17]

Hot volcanic islands are assumed by evolutionists to have existed before continents arose, which would have provided an environment to concentrate organic materials such as L-alanine (L-Ala) and L-2-aminobutyric acid (L-Abu). Fox *et al.* mixed homochiral samples of these AAs with D- and L-Iva (α,α-dialkyl amino acid isovaline (Iva)), which lack a hydrogen at the C2 position and thus racemize only under very harsh conditions, such as γ-irradiation. L-Iva enantiomeric excesses of up to 18.5% have been reported in some carbonaceous meteorites,[18] and many researchers claim that L-Iva could transfer enantiomeric excess to proteinogenic L-AAs.[19] This is based on wishful speculation, not chemical experiments, as I will now show.

Fox *et al.* reported that several percent racemization already occurred with L-Ala samples merely during the process of preparing the amino-acid–mineral suspension, repeatedly dried at 35°C for a short time.[17] This racemization occurred for pure L-Ala, and when mixed with either L-Iva or D-Iva. In fact, fast loss of homochirality for AAs at a temperature as low as 35°C is known to occur during wet–dry cycles,[20,21] a fact not mentioned in speculative models for concentrating AA through repeated flooding and evaporation cycles on the banks of ancient lakes.

The Fox *et al.* experiments demonstrate that L-AAs and the non-proteinogenic amino acids L-2-aminobutyric acid and a proteinogenic amino acid were converted to D-AAs with increased concentration of both D- and L-Iva, although the effect is greater when L-Iva is used, as shown in table 3. In other words, instead of chirality transfer, L-Iva accelerated racemization of proteinogenic L-AAs! The authors suggested that hydrogen-bonded L-Ala (L-Abu)–Iva dimers would form more readily than the L–D diastereomer for steric reasons, and thus racemize faster. Therefore, the search by evolutionists for the highest concentrations possible for L-Iva in meteorites as a key contributor for the origin of L-AA enantiomeric excess seems misguided, since the 'wrong' proteinogenic enantiomers are preferentially formed.

Unfortunately, the researchers did not perform longer experiments with repeated mixing and drying cycles at 35°C of Iva and various proteinogenic L-AAs to draw attention to how loss of homochirality under plausible conditions is often on the timescale of days, a disastrous empirical observation abiogenesis advocates should be aware of.

Table 4. Effect of various aldehydes on racemization of four amino acid [a,22]

		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[b]	87
n-butyraldehyde	80	97	95[b]	100[b]	99
n-heptylaldehyde	80	100	100[c]	100[b]	100
Benzaldehyde	100	72	100	100	72
Salicylaldehyde	80	100	100	100	91

[a] 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 80 or 100°C for 1 h.
[b] A small amount of degradation was observed via thin-layer chromatography (TLC).
[c] Considerable decomposition was detected by TLC.

Table 5. Comparison of effect of aliphatic acid solvent on L-amino acid racemization, beginning with pure L-enantiomer [a,22]

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

[a] A mixture of L-amino acid (1.5 mmol), aldehyde (0.3 mmol), and aliphatic acid (6 ml) was heated in a sealed tubåe in an oil bath at 100°C for 1 h.
[b] A small amount of degradation was observed via TLC.
[c] Because of low solubility, the reaction was carried out under heterogeneous conditions.

Catalytic racemization through aldehyde catalysis

Loss of enantiomeric excess of D- or L-AAs can be facilitated by various chemicals, including aliphatic and aromatic aldehydes (table 4).[22] The assumed mechanism was shown in part 1.[8]

Table 6. Racemization rate constants of Asp residues in an α-helix and β-sheet[6]

A) Values reported in ref. (6)

	k x 10^2 per day (°C)					
	90	80	70	60	50	37
$(Asp-Leu)_{15}$ [a]	3.37	1.37	0.455	0.145	0.03	0.0059[c,d]
$(Leu-Asp-Asp-Leu)_8$-Asp [b]	4.46	1.985	0.9	0.485	0.175	0.055[c,e]

B) Our calculations for other temperatures using the Arrhenius relationship $\ln(k) = \ln(A) - E_a/RT$. Parameters from ref. (6): $(Asp-Leu)_{15}$ ln(A) = 34.593, E_a = 27.31; $(Leu-Asp-Asp-Leu)_8$-Asp ln(A) = 22.333, E_a = 18.38

	k per year (°C)					
	105	77	58.5	49.5	25	3
$(Asp-Leu)_{15}$ [a]	65.87	3.61	0.405	0.127	3.9×10^{-3}	0.980×10^{-4}
$(Leu-Asp-Asp-Leu)_8$-Asp [b]	44.67	6.32	1.45	0.666	63.3×10^{-3}	53.5×10^{-4}

[a] Forms β-sheets

[B] Forms α-helices

[c] Extrapolated from the rate constants at the other five temperatures using an Arrhenius plot

[d] *k* = 0.0214 / year

[e] *k* = 0.2020 / year

Table 7. Time for Asp residues located in α-helices and β-sheets to reach various D/L proportions at 37°C in years, starting with pure L-enantiomers[6]

Sequence	E_a,kcal /mol	k_{37}/year	D/L	Years[a]
β-sheet: $(Asp-Leu)_{15}$	27.3	0.0214	0.10	4.7
	—	—	0.334	16.2
	—	—	0.99	123.7
α-coil: $(Leu-Asp-Asp-Leu)_8$-Asp	18.4	0.2002	0.10	0.5
	—	—	0.334	1.7
	—	—	0.99	13.2

[a] Years = ln[(1 + D/L) / (1 − D/L] / 2*k*. I assume D/L = 0 at time = 0.

Some of the aldehydes would have been present in comparable concentration as some proteinogenic amino acids in abiogenesis models, such as through influx from extraterrestrial sources. The often-dramatic loss of homochirality documented in table 4 occurred at fairly elevated temperatures and required high concentrations of AA and aldehyde. On the other hand, the experiments were carried out for only one hour; longer evolutionary time would lead to the same trend, accelerated racemization over time.

Rapid racemization also occurred when formic or propionic acid were used instead of acetic acid, as shown in table 5.[22]

Unfortunately, experiments were not reported at various lower temperatures or lower concentrations of aliphatic acids which would permit extrapolation to more realistic conditions, to determine what the $t_{½}$ of loss of homochirality would be.

Racemization can also be catalyzed under non-acidic conditions. Traditional laboratory racemization procedures using an aldehyde catalyst employ a metal ion which forms a chelate compound with the initially formed Schiff base under neutral or weakly alkaline conditions.[22]

Rapid racemization of amino acids located in stable secondary structures

Generally, strict alternation of hydrophilic (hi) and hydrophobic (ho) amino acids induces a β-sheet structure, whereas a tetrapeptide periodicity (-hi-hi-ho-ho-) induces an α-helix conformation when Zn^{2+} cations are present.[23,24] To obtain a β-sheet, the hydrophobic amino acids must display their hydrophobicity to a marked degree.

Stable secondary polypeptide structures can slow down racemization. Brack *et al.* measured L→D rate constants for Asp at different temperatures for an $(Asp-Leu)_{15}$ peptide which forms a β-sheet and for an $(Leu-Asp-Asp-Leu)_8$-Asp which forms an α-helix, as shown in table 6.[6]

I calculated rate constants in table 6B for the six temperatures used in table 2 using the available E_a and ln(A) parameters in the Arrhenius eqn. to facilitate comparisons. L→D is shown to be much faster for Asp, even when present in designed α-helices and β-sheets, than all the AAs reported in table 2.

The average racemization half-life for each Asp at 37°C was around 2 and 16 years when present in $(Leu-Asp-Asp-Leu)_8$-Asp and $(Asp-Leu)_{15}$, respectively, indicating that Asp residues racemize much faster in α-helices (table 7), ref. 6. The authors pointed out that without ~ 0.5 equivalent per Asp residue of zinc chloride the secondary structures don't form, and these 30- and 33-residue peptides remain as random coils in water. This implies that for Asp located in randomly produced peptides the E_a must be much lower, and

the rate constant for L→D must be faster than when found in α-helices: k_{int} >>10^{-3} at 3°C, and k_{int} >> 10^{-2} at 25°C in water.

Aspartic acid is probably the most easily racemized AA.[6]

Analysis of the rate constants

Asx had a k_{int} in the middle range of the four AAs studied by Steen,[14] so I used it to illustrate how L-enantiopurity is lost over time, as shown in figure 3 and figure 4.

Loss of L-enantiomer will be faster the higher the proportion of L, since little D is available for the back-reaction D→L (figure 4). The trend is linear until enough D builds up (figure 4B), so that k × years in this region predicts [D]/[L] closely. Thus, for Asx a ratio of [D]/[L] = 5% is reached in ≈ 2,174 years at 3°C (0.05 / 2.3 x10^{-5}) for each Asx present in a peptide. A mere 5% contamination of D-residues for an average-size protein of 300 residues represents 15 randomly distributed D-enantiomers, which would generally not be expected to remain functional.[25]

A high near racemic D ≈ L ratio is not necessary for larger polypeptides to be 'ruined'. Even at low temperatures, enough L→D interconversions would occur in at most the timeframe of hundreds of years for small peptides, and less for larger peptides, even assuming such large peptides would form in water (a topic discussed on part 3). Since various AAs are involved, those which naturally racemize faster due to their chemical properties will specify the time needed to reach the 5–10% maximum permitted D/L ratio.

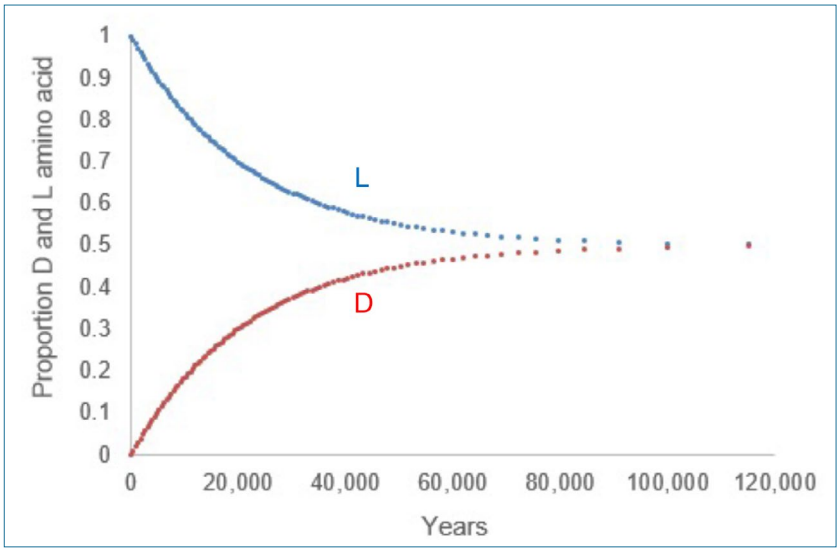


Figure 3. Initially enantiopure amino acids would lose optical purity rapidly and converge to a racemic mixture under natural conditions. Data derived from ln{(1 + D/L) / (1 − D/L)} = 2 x k_{int} x t, using k_{int} = 2.3 x 10^{-5} / year for Asx at 3°C in aqueous slurry from ref. 14.

Racemization at relevant temperatures

One must always be cognizant that AAs in solution exposed to elevated temperatures for even very short time periods racemize exceedingly quickly, and prebiotic scenarios are rich in hydrothermal vents, meteorites, and rapid evaporation cycles. Circulating water cannot be assumed to have always remained at a very low temperature for millions of years.

Frigid temperatures of 0–3°C would indeed slow down racemization, but at such temperatures, and realistic amino acid concentrations, large polypeptides will not form, a topic I cover in part 3. The evolutionist now faces a dilemma, since higher temperatures are needed to condense AAs, but racemization

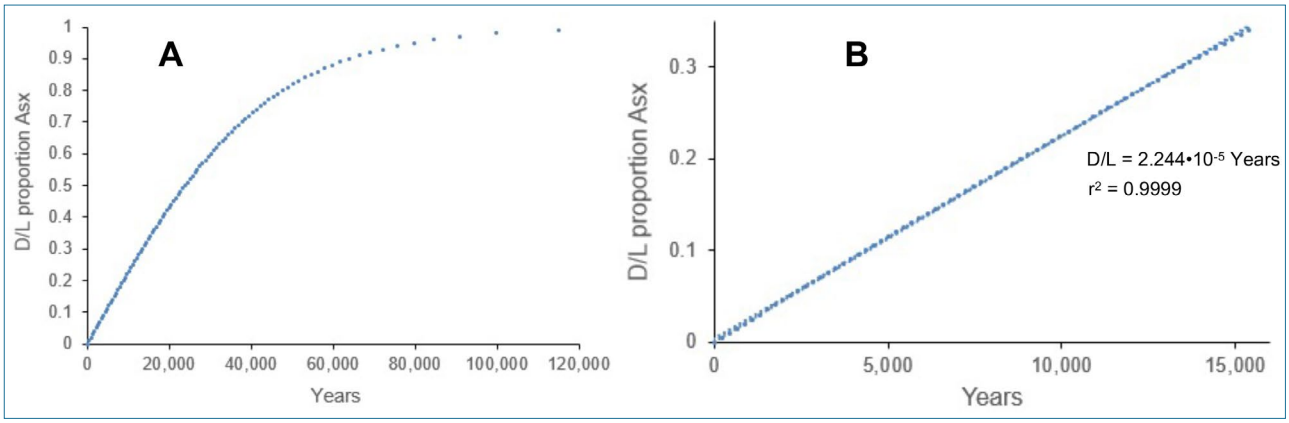


Figure 4. Proportion D/L beginning with no D-enantiomer present in a sample. Data derived from ln{(1 + D/L) / (1 − D/L)} = 2 x k_{int} x t, using k_{int} = 2.3 x 10^{-5} / year for Asx at 3°C in aqueous slurry from ref. 14. **A**: Relative rate of production of D-enantiomer slows as the proportion of D increases. **B**: At low concentrations of D-enantiomer, its production increases almost perfectly linearly with time up to about the racemization half-life.

accelerates rapidly with increases in temperature. From table 2, on average, k_{int} is about 3×10^4 times greater at 77°C than at 3°C for the four AAs studied.

Using an average temperature over time can significantly underestimate the amount of D enantiomer produced. Suppose a sample of Asx spent half its time at 49.2°C and half at 3°C, for an average of 26.1°C. From table 2, after t = 1 year at 25°C (close enough to 26°C to illustrate), a fraction of ~ 0.001 D would be produced. But using the individual rate constants shows that the amount of D-enantiomer would be more than 20 times greater: ½(0.0421) + ½(2.3 x 10^{-5}) = 0.021, and thus about 20 times less time to obtain a fraction of 0.001 D. (For relatively high D/L values equation [5] must be used to demonstrate this effect.)

To help visualize the dominant effect of temperature on loss of enantiopurity, I graphed our calculated D/L values over time using the data provided for 77°C by Steen[14] (figure 5).

In part 1, I showed that just ~ 5% L→D interconversion prevents forming small, stable 3-dimensional peptide structures under realistic naturalistic settings even at low temperatures.[8] How long would it take to reach a D/L of 5% at 77°C as described above? Using $\ln\{L_0/(2L_t-L_0)\} = 2kt$ or approximating by dividing 0.05 by the rate constants shows that only 9 (Asx) to 286 (Ala) days are needed, with an average of ~ 105 days for all the AAs.

During the millions of years assumed by origin of life researchers, AAs would have been exposed to periods of intense heat, due to volcanism, hydrothermal vents, meteorite crashes, concentration in shallow evaporating pools of water, and so on. Surface water temperatures near the equator today are around 30°C, and racemized AAs would distribute rapidly during the vast time assumed. Meteorites are a favourite theoretical source of excess L-AAs (but nobody claims D/L values anywhere near 0.1, of course). A meteorite crash would have produced enormous temperatures surrounding any L-AAs delivered from the meteorite.

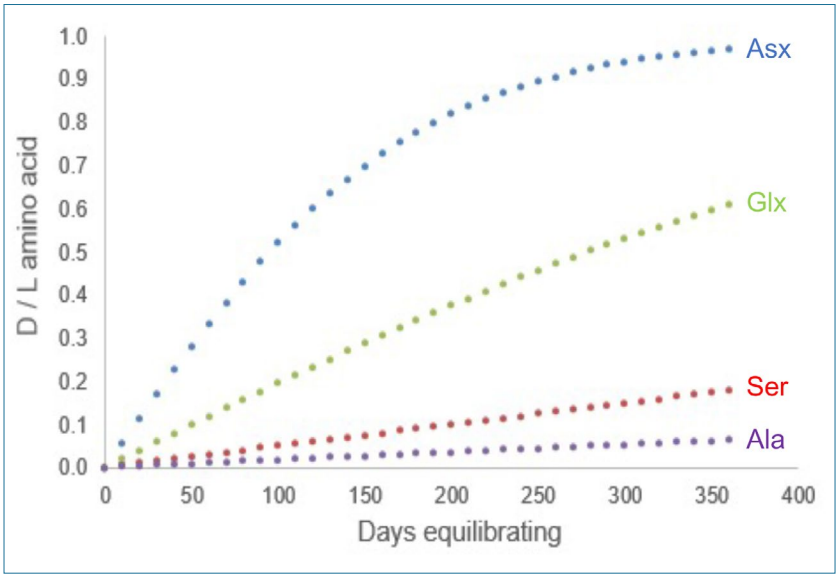


Figure 5. D/L values calculated using $L_t = ½(1+e^{-2kt})$ derived from $\ln\{L_0/(2L_t-L_0)\} = 2kt$, where $L_0 = 1$ and $D_t = 1 - L_t$. D/L (i.e. D_t/D_t) values graphed for every tenth day. Rate constants at 77°C from ref. 14. Per day rate constants used: Asx 5.75 x 10^{-3}; Glx 4.99 x 10^{-4}; Ser 1.97 x 10^{-3}; Ala 1.75 x 10^{-4}. Asx = Aspartate or Asparagine; Glx = Glutamate or Glutamine; Ser = Serine; Ala = Alanine.

Conclusions

Vast amounts of time are claimed to offer more opportunities for life to arise, but increasing time decreases any enantiomeric excess which may have formed. Furthermore, during tens of millions of years on lifeless Earth, the total quantity of racemic AA (in free and bound form) would have increased continually with time. This would have made contamination of any L excess which arose much later by some unknown cause ever more inevitable.

I commonly encounter claims that amino acids have half-lives on the order of tens of thousands to millions of years, ignorant of or downplaying the drastic limitation the lack of pure L-amino acids really places on abiogenesis speculations. I believe Bada's old publications, using pure water around the freezing point, lacking the chelating Cu^{2+} found in ocean water, and without considering racemization of residues temporarily bound in peptides (table 1), is at the root of this misperception. My data shows that starting with pure L-AAs, soluble peptides exposed to temperatures of ~ 100°C would convert enough L→D to render them functionally useless on a timescale of merely days. Events such as bombardment by meteorites, violent volcanism, and plate subductions produce intense heat. Evolutionists recognize that extreme heat would have destroyed a large proportion of existing AAs. However, one finds very few references where they candidly point out that such events would also eliminate any L excess, should it arise somehow. All young-earth Genesis Flood models predict much warmer oceans than we have today up to the end of the Ice Age, so tens of thousands of years were not necessary to produce organic remains with high D/L values.

Many different L-AAs would be needed to form relevant polypeptides, and these will not fold reliably to form proteins if enough D-enantiomers are also present. For abiogenesis purposes, then, the commonly used AAs which racemize the fastest will narrow yet more the 'window of abiogenesis opportunity'. The naturalist models are

forced to posit a steady production of polypeptides based on only L-AAs in high concentrations having relevant sequences, but this is wishful speculation and will not occur under naturalistic conditions.

Appendix

I could not find a derivation for equation [1] in the literature and offer my version here.

At all times during amino acid interconversion,

$$L \rightleftharpoons D$$

Since $D = (1 - L)$ and the rate constant k for the forward and backward reactions are identical, the rate of loss of L can be expressed as

$$-\frac{dL}{dt} = kL - k(1 - L) = k(2L - 1) \qquad [6]$$

We integrate separately by time and concentration of L

$$-\int_{L_0}^{L_t} \frac{1}{2L - 1} dL = \int_{t_0}^{t_t} k\, dt \qquad [7]$$

Using the well-known solution for the left-hand side,

$$\int \frac{c}{ax+b} dx = \frac{c}{a} ln|ax + b|$$

$$-\frac{ln(2L_t - 1) - ln(2L_0 - 1)}{2} = k(t_t - t_0) \qquad [8]$$

Since ln(a) – ln(b) = ln(a/b), and at time = 0, t_0=0 we can simplify

$$ln\frac{(2L_0 - 1)}{(2L_t - 1)} = 2\,k\,t \qquad [9]$$

Since at time 0, $L_0 = 1$ (i.e. only L-enantiomer is present),

$$ln\frac{(2L_0 - L_0)}{(2L_t - L_0)} = ln\frac{(L_0)}{(2L_t - L_0)} = 2\,k\,t \qquad [10]$$

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