Enantiomeric amplification of L amino acids: part 3—using chiral impurities

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Several studies attempting to provide a natural method for L-amino acid (AA) amplification have been critically examined. (1) Stereoselective adsorption of impurities on growing crystals sometimes enhanced growth of crystals of a single AA enantiomorph. This led to conglomerates and not separated enantiomers. The supersaturated solutions would not have existed naturally and, worse, an excess of one L-AA would have enhanced forming D-AAs of a different racemic AA. (2) *α*-Methyl AAs with a slight excess of L-enantiomer are seemingly provided by meteorites. Transamination between ketoacid and L-*α*-methylvaline led to the biologically *wrong* D-AAs. (3) The *correct* enantiomers were later obtained using an unrealistically high proportion of a copper catalyst. Extrapolating to plausible concentrations showed that the enantiomeric excess (e.e.) obtainable would have been insignificant. Furthermore, under the optimized conditions, any e.e. produced would have racemized within hours. (4) Simulating wet-dry cycles in a geothermally heated rock pool containing L-AAs in montmorillonite together with L-isovaline led to rapid racemization of the AAs. Therefore, chirality could not be transferred from L-isovaline to produce L-AAs.

Enhanced chirality using single enantiomer amino acid impurities

Most biological amino acids (AAs) form crystals composed of a racemic mixture of the D- and L-enantiomers, but some AAs form conglomerates of individual (*S*) and (*R*) crystals from highly concentrated aqueous solutions. Impurities which adhere to one or more faces of the crystal can hinder further crystal growth in that direction, leading to crystals having visibly different morphologies which can be manually separated. Addadi *et al.* reported some experiments using hot supersaturated solutions of (*R*,*S*)-asparagine (i.e. racemic Asn) in the presence of optically pure glutamic acid, aspartic acid, and glutamine.[1] After 1–2 days crystals formed spontaneously upon cooling to room temperature, with crystals displaying two different morphologies. The minor variant always contained more Asn with the same chirality as that of the additive.

The same behaviour was also documented using (*R*,*S*)-threonine in the presence of small amounts of (*S*)-Glu or (*S*)-Asn. The composition of the two crystal types was confirmed by crystallizing pure (*S*)-Thr concurrently with (*R*)-Glu or (*R*)-Asn, and then in other experiments using pure (*S*)-Thr doped with (*S*)-Glu or (*S*)-Asn.[1]

The effect was due to stereoselective adsorption on the surface of growing crystals of the enantiomer having the same configuration, which slows down its growth, favouring more of the other enantiomorph being produced.[2] The effect required a strong resemblance in stereochemistry and structure between the additive and the enantiomer to be crystallized.[2] To illustrate, (R)-glutamic acid is known to crystallize preferentially from a racemic mixture in the presence of (S)-aspartic acid. These AAs differ by only a CH_2 in the side chain (see figure 1 A). As another example, (R,S)-threonine (Thr) was resolved when using Glu, Asn, Asp or Cys of a single enantiomer (see figure 1 B).[3]

Since additives and monomers incorporated into a crystal tend to bind more tightly if they have the same stereochemistry, the resulting layer won't dissolve away as easily as when mixed enantiomers are used. The pure crystals of that enantiomeric type are thus hindered from forming.[3]

The results of resolution experiments of (*R*,*S*)-asparagine (Asn) crystals in the presence of optically pure amino acids at various concentrations are shown in table 1.[2]

Crystallization was performed by cooling solutions of the racemic AA at twice the concentration of saturation with additives below their solubility limit. Typically, the AA and additives were suspended in an aqueous solution at 40°C and stirred until completely dissolved, then slowly cooled to room temperature.[2] In experiments when seeds were added to facilitate crystallization, 5% of the desired enantiomer or 10% of the R,S seeds were added to the solutions at 40°C.

Other experiments beginning with (R,S)-threonine also produced crystals of the same enantiomeric type in the presence of pure enantiomers of Asn, Glu, Cys-HCl, Asp, His-HCl, Lys-HCl, and Phe. In addition, (R,S)-glutamic acid hydrochloride was resolved by several pure AA enantiomers, including Lys, His, Ser, Cys, Leu, and Tyr. The authors point out:

> "In all cases, however, when crystallization is left to proceed for a long time or its rate is enhanced by vigorous stirring, racemic mixtures of crystals are obtained, thus confirming the exclusively kinetic nature of the resolution process."[2]

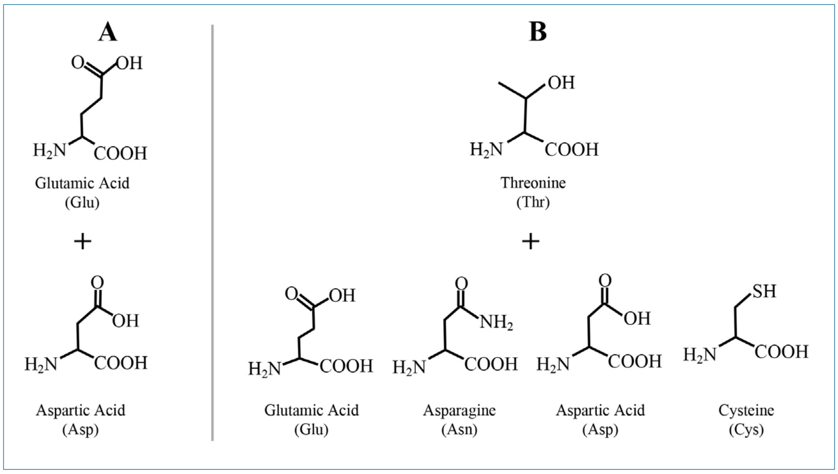

Figure 1. Some racemic amino acids can be resolved by doping with a single enantiomer of a structurally similar amino acid. A: Glutamic acid can be resolved by aspartic acid. B: Threonine can be resolved by glutamic acid, asparagine, aspartic acid or cysteine.

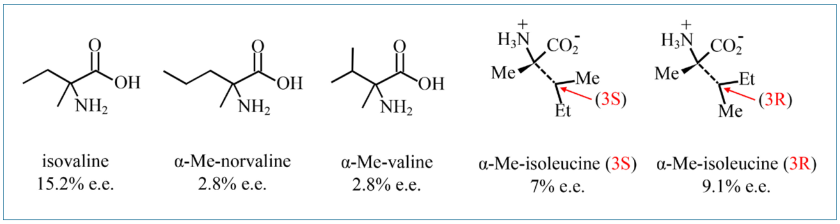

Figure 2. *α*-Methyl amino acids found on the Murchison chondritic meteorite which have the S configuration (generally referred to as the L-enantiomer) and their % enantiomeric excess (e.e.). Based on figures found in refs. 10 and 13.

The graphical data in the paper confirmed that racemization was almost complete within just a few hours, even without stirring or shaking.[2]

Critical evaluation

- The enantiomorphs which sometimes formed remained intimately mixed as conglomerates and in the same enantiomeric proportions overall. No isolation of D-AAs was achieved.
- These kinds of processes would be devastating for Origin of Life (OoL) purposes, since if the desired excess of L-AAs could have somehow arisen, they would have inhibited growth of crystals of similar AAs containing L-enantiomers. (The change in D vs L content of the large *solution phase* would be negligible, so this would not be a method to enrich L-AAs). It has been known for many decades that small amounts of impurities at the surface of growing crystals can slow down crystallization rates by several orders of magnitude.[4]
- Generating super-saturated solutions of (R,S) AA solutions by chance is implausible, and the different proportions of D vs L in two enantiomorphs would be only short-lived.

One should keep this work in mind when reading about amplification processes which require pure AAs. Proteins require 19 kinds of L-only AA enantiomers, but they would have interfered with each other's enrichment.

Enhanced chirality via amino acid and sugar synergisms

In part 2 of this series, we discussed the effect of biological D-sugars on enantioselectivity of AAs and why this does not provide an answer to the origin of pure L-AAs.[5] Sugars have also been used in other OoL experiments, as we will see next.

Some meteorites of the carbonaceous chondritic type deliver unusual AAs with *α*-methyl groups, which have an excess of the L-isomers.[6–9] It has been proposed that this e.e. could be transferred to biological AAs. Breslow has also shown that decarboxylative transamination reactions under special conditions alleged to be relevant for a prebiotic world could also produce these kinds of AAs, having a preference for the L-isomer.[10,11]

Chirality transfer using L-*α*-methylvaline

In 2010 Breslow *et al.* claimed:

> "We show how the amino acids needed on prebiotic earth in their homochiral L form can be produced by a reaction of L-alpha-methyl amino acids—that have been identified in the Murchison meteorite—with alpha-keto acids under credible prebiotic conditions."[12]

In the same paper they also stated:

> "… with our results, we think we can make a good case that there is a credible story for this origin of homochirality in the prebiotic world. It has the advantage—over various hypothetical ideas—of being a proposal for which there is significant evidence."[12]

As we will see, this is overstating matters, since they actually obtained the wrong enantiomers in excess (D-AAs) and had to experiment extensively to find some special conditions to obtain the opposite of what would be produced naturally. They do admit candidly:

> "Apparently no alpha-keto acids have been found in the meteoritic deposits, so our process is relevant only if we can explain how they could arise."[12]

Table 1. Enantiomeric excess from (*R,S*)-asparagine (Asn) in the presence of optically pure amino acids at various concentrations. 300 mg / 3 ml Asn was used at room temperature.[2]

Exp.	Amino acid additive	Additive, mg/mL	Crystal yield, %	$[\alpha]_D$, deg	(*R*) ee configuration	Crystal morphology	Time before filtration, days	Seeds to start crystallization
1	(*S*)-Asp	16.6	14	−29.9	98.0	prisms	2	*R,S*
2	(*S*)-Asp	6.6	17	−23.7	77.7	prisms and plates	4	
3	(*S*)-Asp	3.3	38	−9.6	31.4	prisms and plates	4	
4	(*R*)-Asp	6.6	6	+30.5	100	prisms	6	
5	(*S*)-Glu	16.6	22	−26.0	85.2	prisms and polyhedra	4	
6	(*R*)-Glu	17.0	14	+24.2	79.3	prisms and polyhedra	6	
7	(*S*)-Ser	25.0	21	−15.8	51.8	prisms and octahedra	4	*R,S*
8	(*S*)-Ser	16.6	33	−17.1	56.1	prisms and octahedra	1	*R,S*
9	(*S*)-Ser	8.3	43	−8.8	28.8	prisms and octahedra	1	*R,S*
10	(*S*)-Lys-HCl	33.3	25	−14.7	48.2	prisms and octahedra	7	
11	(*S*)-Lys-HCl	8.3	53	−7.7	25.2	prisms	1	*R,S*
12	(*S*)-His-HCl	16.6	48	−21.3	69.8	prisms and octahedra	1	*R,S*
13	none		43	0	0	prisms	2	
14	(*S*)-Glu	17.0	34	0	0	powder	2	

In other words, they did not begin with the data and conditions expected in meteorites or a prebiotic Earth to evaluate what would result naturally, but invested considerable expertise to design reaction conditions in a laboratory, which could be set up just right to produce the desired outcomes.

Let us review these interesting experiments. Breslow and his team proposed that *α*-methyl AAs found on meteorites, such as shown in figure 2, could transfer their chirality in the synthesis of biological AAs.[10]

Any meteorite plunging through the earth's atmosphere would arrive with the ~10 K temperature or so of space in its interior but would be heated on its outside by friction. "However, the thermal conductivity of chondrites is so low that the inner core would retain a temperature well below 0°C", and thus organic molecules near the core would not be expected to be destroyed through heating.[12]

The non-methylated AAs, as used by proteins, found in carbonaceous chondritic meteorites were found to be racemic.[10]

Some *α*-methylated AAs delivered to Earth by meteorites seem to possess up to 15% e.e.$_L$ of non-terrestrial origin. Breslow and Levine reasoned that perhaps this excess could be transferred to form L-AAs. They experimented with D-*α*-methylvaline reacted with pyruvate and with phenylpyruvate salts, to form alanine and phenylalanine respectively (see figure 3). They wished to see if an e.e. would result and, if so, of which handedness.[14] The hope was to transfer excess L-*α*-methylated AAs formed to produce biological L-*α*-H amino acids. D-*α*-methylvaline was used in the experiments, but the L alternative was expected to produce the mirror effect, a reasonable assumption, which nevertheless should be confirmed.

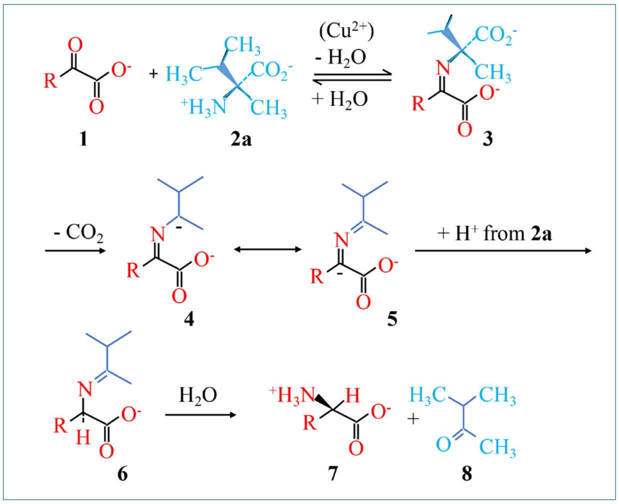

Figure 3. Transamination between ketoacid (1) and *α*-methylvaline (2a) to form amino acids. For 1: sodium pyruvate (R = methyl) or sodium phenylpyruvate (R = Ph-CH$_2$) were used.[10,14]

Table 2. Enantiomeric excess of L-phenylalanine generated by the reaction of phenylpyruvate and D-*α*-methylvaline as a powder mixture at high temperature[a,14]

Temperature	1 min	5 min	10 min	30 min
100°C	[b]	9.5 ± 1.5%	9.4 ± 1.6%	6.87 ± 0.01%
120°C	8.3 ± 1.2%	6.0 ± 0.6%	5.8 ± 1.7%	3.5 ± 0.7%
160°C	4.0 ± 1.1%	Racemic		

[a] All values are enantiomeric excess of L-amino acid and the average of 2 runs.

[b] The chemical reaction did not occur.

In the mechanism shown in figure 3, the *α*-methylvaline performed the transamination that converted a ketoacid to an AA and provided the proton to the *α*-carbon of the product AA in a stereoselective manner.

The researchers used first D-*α*-methylvaline and sodium pyruvate in solution but failed to obtain any transamination product. In the next attempts they dissolved the reactants in a methanol/ethanol mixture or in water to produce a well homogenized mixture; then the solvent was removed. The resulting solid was heated to temperatures ranging from 100 to 160°C for 1 to 30 mins, which the authors claimed, "mimics the common prebiotic concept of chemistry in a dried lake bed".[14] Finally, the imine produced was hydrolyzed.

Using sodium pyruvate produced at most 3% e.e.$_L$ alanine under all the times and temperatures tested (R = methyl in figure 3), but slightly higher selectivity was obtained using sodium phenylpyruvate (R = Ph–CH$_2$ in figure 3), see the results in table 2.

Since using D-*α*-methylvaline produced a slight excess of an L-AA, using instead L-*α*-methylvaline, as allegedly found in meteorites, must produce the biologically *wrong* D AAs. Noteworthy also is that the e.e.$_L$ of phenylalanine decreased with increasing temperature and over time on the timescale of only minutes.

Reacting in a dry state minimized providing protons which would facilitate racemization. In fact, when the reactions were carried out on montmorillonite clay, no e.e. resulted, presumably because protons could now be provided from different directions.

An alternative scenario proposed would use *α*-methyl AAs of extraterrestrial origin to catalyze formation of some D-sugars, as Pizzarello and Weber have done and then use those sugars to catalyze the formation of the normal L-AAs.[15] However, credible prebiotic examples of the latter process have not yet been produced.[13]

Critical evaluation

- L-*α*-methylvaline did not lead to the hoped-for L-alanine and L-phenylalanine. These experiments were claimed to have been executed under prebiotically realistic conditions but provide examples of how an e.e.$_D$ of AAs could have been produced; i.e. of the wrong handedness for biological purposes. This would make the generation of an excess of L-AAs even more difficult.
- These experiments provided examples of how researchers can manipulate reaction conditions to obtain the results desired. The reactions were terminated quickly since it was apparent that racemization was occurring rapidly. An e.e. which only lasts a few hours would not lead to plausible OoL models.

Chirality transfer using L-*α*-methylvaline with a copper catalyst

In a new series of experiments, instead of using a 2.8% e.e. L-*α*-methylvaline as found in a meteorite, Breslow *et al.* began with an e.e.$_L$ of 96% which, when reacted with phenylpyruvate, led to L-phenylalanine with up to 37% e.e., and when reacted with pyruvate, produced L-alanine with up to 20% e.e. In these experiments the scheme in figure 3 was modified to include a copper sulphate catalysis in extremely high concentration. Both copper and zinc are present in some meteorites and in low average concentrations on Earth, but with zinc (II) the researchers obtained the same result as without a metal ion, namely a small amount of formation of the *wrong* D-AA![12]

This novel copper-catalyzed decarboxylative transamination was also used to synthesize L-valine from L-*α*-methylisoleucine.[10]

Were these designed experiments representative of what would occur under unguided conditions? The transamination reaction in the presence of copper (II) sulphate was carried out using 1 equivalent of cupric sulphate, 1 equivalent of sodium pyruvate or sodium phenylpyruvate, and 4 equivalents of *α*-methyl AA dissolved in water. The reaction mixture was *stirred* at room temperature to ensure complete dissolution, and the parent reaction mixture was then subdivided into vials (approximately 10 mg/vial). Water was removed from the vials in vacuo, and the resulting solid reaction mixtures were heated for various times and temperatures.[10] One of the products was an *amino acid*, see figure 3.

Anhydrous copper (II) sulphate is found naturally as the very rare mineral chalcocyanite. No information was provided for the effectiveness under these experimental conditions when various alternative copper (II) sulphates are used, which have the chemical formula $CuSO_4(H_2O)_x$. These crystal minerals are also rarely found in nature, though.

Table 3. Enantiomeric excesses of L-amino acids produced from the pathway shown in figure 3 in the presence of extremely high cupric sulphate. Data from the Supplemental Materials of ref. 10.

A. L-phenylalanine by reacting L-*α*-Me-valine and sodium phenylpyruvate with cupric sulphate.

Time (min)	120°C L-e.e., %	Error margin	140°C L-e.e., %	Error margin	160°C L-e.e., %	Error margin
1	4.6	±2.7 (2 trials)	0.0	(3 trials)	0.0	(3 trials)
5	0.0	(3 trials)	6.3	±4.2 (3 trials)	15.1	±1.1 (3 trials)
10	0.0	(3 trials)	8.4	±4.0 (3 trials)	10.5	±1.1 (3 trials)
30	6.1	±2.3 (3 trials)	14.1	±8.5 (3 trials)	32.0	±20.3 (3 trials)
45	2.2	±0.4 (3 trials)	2.6	±1.8 (3 trials)	33.3	±18.5 (3 trials)
60	4.7	±2.9 (3 trials)	13.6	±13.9 (6 trials)	37.0	±14.8 (3 trials)
120	5.6	±6.1 (2 trials)	23.3	±12.9 (4 trials)	26.5	±17.1 (3 trials)
Average:	2.2		6.2		11.6	

B. L-alanine by reacting L-*α*-Me-valine and sodium pyruvate with cupric sulphate.

Time (min)			140°C L-e.e., %	Error margin	160°C L-e.e., %	Error margin
1			4.9	±2.9 (2 trials)	3.7	±2.7 (2 trials)
5			14.1	±21.7 (3 trials)	5.0	±4.0 (3 trials)
10			9.4	±6.2 (3 trials)	11.8	±4.9 (3 trials)
30			6.9	±6.1 (3 trials)	23.0	±13.8 (2 trials)
45			8.3	±1.6 (3 trials)	8.9	±5.5 (3 trials)
60			16.9	±9.5 (3 trials)	19.6	±5.1 (3 trials)
120			19.6	±5.0 (2 trials)	12.9	±7.6 (3 trials)
		Average:	11.4		12.1	

C. L-valine by reacting L-*α*-Me-isoleucine and sodium 3-methyl-2-oxobutanoate with cupric sulphate.

Time (min)			140°C L-e.e., %	Error margin	160°C L-e.e., %	Error margin
1			7.7	±6.7 (3 trials)	0.6	±1.6 (3 trials)
5			--		0.6	±0.4 (2 trials)
10			--		1.2	±0.6 (3 trials)
30			1.1	±0.3 (3 trials)	3.6	±2.4 (3 trials)
45			1.1	±2.4 (3 trials)	6.9	±2.5 (3 trials)
60			3.2	±1.3 (3 trials)	7.0	±1.4 (3 trials)
120			7.5	±5.0 (3 trials)	11.3	±10.7 (3 trials)
		Average:	3.2		7.2	

Yields were not reported. A variety of reaction times (1–120 mins) and temperatures (120–160°C) were screened, and the best results were obtained at 160°C for 60 mins, wherein L-phenylalanine was obtained in 37% e.e. Shorter reaction times or lower reaction temperatures led to much lower e.e.s.[10]

The authors admitted a key point:

> "*Heating 96% enantiopure L-R-methylvaline with sodium pyruvate and cupric sulfate at 160°C for 60 min under solvent-free conditions yields L-alanine in 20% ee. In the absence of copper, only low ee's of the undesired D-alanine were obtained.*"[10]

L-α-methylisoleucine (found on meteorites in 7.0% e.e.$_L$) reacted with 3-methyl-2-oxobutanoate also in the presence of high proportions of cupric sulphate to produce L-valine with up to 23% e.e. "In the absence of copper, no reaction occurred."[10] Also, zinc salts did not lead to the reversal of chiral introduction they obtained using the copper catalyst.

The authors had succeeded in finding conditions which reversed the enantioselectivity found in the earlier work,[14] whereby L-α-methylvaline with a copper catalyst now led to L-phenylalanine preferentially. The authors claimed that the reaction conditions (solvent-free, 120–160°C) mimicked plausible conditions on prebiotic Earth.[10] However, upon eventually encountering water at such high temperatures, the L-AAs produced would have racemized rapidly, on a timescale of days.[16–19]

Recall that no α-keto acids have been found in the meteoritic deposits, and these would have had to be available in a free chemically unbounded form in order to react. So, at best these would have been present in ppb concentration in a minority of meteorites. Suitable α-methylamino acids would also be available in only very low concentrations. After the meteorite had broken into pieces while hurtling through the atmosphere and then crashing unto the earth, both classes of material would have been further diluted by orders of magnitude in terrestrial water or crust. Both chemicals would almost always be present concurrently in the *absence* of Cu in a suitable salt form, leading to the *wrong* D-AAs. Cupric ion does accelerate the decarboxylation process, but this cannot compensate for the vastly greater degree of reaction which would have occurred in the absence of a suitable copper catalyst overall.[12] This is a highly contrived and implausible scenario to generate a reliable source of L-AAs to create proteins for a primitive life-form.

The relative e.e. would also have been drastically diluted through mixing with existing racemic amino acids, so that millions of years would have been needed to have accumulated the minimum concentrations needed, during which time all AA e.e. would have been lost through racemization.

Destruction of any L-e.e. produced

Elevated temperatures such as required by these experiments would not have permitted crystallization in a drying lakebed near a site of meteorite landing to produce amplification.[10] What are we to make of the statement that "the best results were obtained at 160°C for 60 min"? They did not draw attention to the fact that a plateau was reached at this very short time. 'Best results' suggest that conditions removed from these times and temperatures produced less of the amino acid and/or lower e.e., so I examined the Supplemental Materials provided. The results are summarized in table 3, referring to the reaction pathway shown in figure 3 to produce some L-amino acids.[10]

Let us review the results summarized in table 3.

Production of L-phenylalanine

From table 3 A we observe that enantiomerically *pure* L-α-Me-valine added to sodium phenylpyruvate was able to transfer a maximum of 37% chirality to L-phenylalanine when reacted at precisely 160°C for 60 minutes. If not then quickly removed from the heat, more than 10% of the e.e. was lost within just one hour. Of course, the e.e. of L-α-methyl valine found in the Murchison meteorite was not 100% but only 2.8%. The total amount present in a meteorite would have been in the ppb range; not all of it would have leached out immediately at the crash site; and all or most would have remained in large complexes. The conclusion is unavoidable that even under the best possible conditions <<1% e.e. L-phenylalanine could have been produced this way at some terrestrial location.[12]

It is absurd to expect a meteorite to crash and produce an environment around exactly 160°C for just one hour. Suppose the temperature was lower. The results reported for 120°C seem to vary randomly. An e.e.$_L$ of 4.6% was found after 1 minute, but none for five and for 10 minutes. The values found between 30 and 120 minutes don't follow any trend. Given that one hour was ideal at 160°C, what e.e.$_L$ would have resulted at 120°C? Only about 4.7% instead of 37% chirality could have been transferred assuming an unrealistic ideal stochiometric proportion.

The results from the higher temperature of 140°C were also not promising; for example only ~3.2 % e.e. L-valine was produced on average for durations of 30–120 minutes.

The authors concluded that a temperature around 160°C produced the highest e.e. values when forming phenylalanine.[10] But this creates a dilemma. From table 3, in two out of three of the AAs, going from one hour reaction time to two hours significantly *decreased* the e.e.$_L$. (Except for table 3 C, the error margin is about the size of the e.e. itself, and the e.e. of alanine was the smallest of the AAs reported). The OoL community is searching for a natural

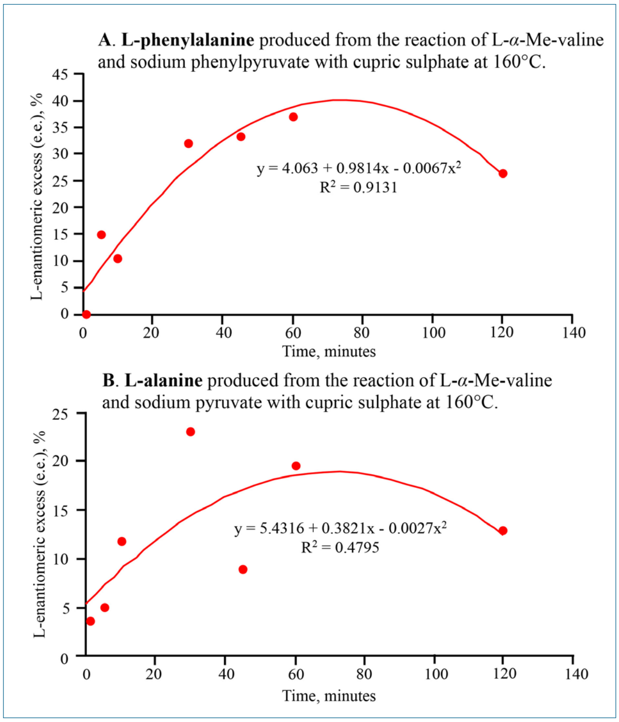

Figure 4. The data from tables 3 A and B at 160°C demonstrate that after only a few hours under the best reaction conditions all or most of the enantiomeric excess would have been lost. Data from the Supplemental Materials of ref. 10. Curve fitted using a second order polynomial function with Excel. Average of 2 or 3 trials from ref. 10 were used.

way to produce 19 AAs with high e.e.$_L$ but the e.e. produced would have been rapidly destroyed. Figure 4 shows that within only a few hours at 160°C, the e.e. would have been entirely eliminated.

Under unguided natural conditions, these materials, at temperatures of 120–160°C, would eventually come in contact with water where racemization would be very rapid.[16–19] There are many realistic scenarios where AAs would be exposed to high-temperature water, such as in or near hydrovents.

Production of L-alanine

From table 3 B, enantiomerically pure L-α-Me-valine added to sodium pyruvate transferred a maximum of 19.6% at 140°C when exactly 120 minutes was used. The highest e.e., 23% transfer, was achieved at 160°C when exactly 30 minutes was used, but at all longer durations the e.e.$_L$ decreased. The concerns discussed in the section above apply here also.

Production of L-valine

From table 3 C, enantiomerically pure L-α-methyl isoleucine, another meteoritic component, converted sodium 3-methyl-2-oxobutanoate to L-valine with a maximum e.e. of 11.3% (at 160°C and 120 minutes). At 140°C, an e.e. of 7.7% was obtained after 1 minute. But then at 30 and 45 minutes, the valine was about racemic within experimental error. Of all the experiments only the e.e. results for valine were not reported at 5 and 10 minutes. After 120 minutes an e.e. of 7.5% was reported, almost as high as found at 1 minute. Unfortunately, reaction times of a few days were not reported, in particular for 160°C, to see when loss of e.e. becomes apparent. Individual values were not provided nor was the meaning of ±, which might mean a range or standard deviation. The huge error margin of ±10.7 at 160°C raises the concern of a single unrepresentative outlier distorting the results.

As mentioned above, when Breslow and Levine used 100% R α-methylvaline to transaminate α-keto acids they obtained AAs with insignificant e.e. (L-alanine with ~3% e.e., and L-phenylalanine with ~9.5% e.e.).[14]

Critical evaluation

In addition to the realities presented above, which demonstrate that the authors have reported a laboratory artefact which would not have occurred naturally, we would like to emphasize three facts.

- In the absence of the extremely high proportion of copper catalyst, only small e.e. of the 'wrong' D-alanine were obtained.[12] The probability of finding exactly all the best components concurrently is much lower vs. all except for the Cu catalyst. The occurrence of all these events would have averaged out to a net excess of the wrong AA enantiomers.
- Clearly the transamination of α-keto acids to transfer a small fraction of chirality from pure *R* α-methylvaline to form AAs was only a laboratory artefact with no plausible relevance for OoL purposes.
- We have here an example of the often-observed fact that to obtain an e.e. another molecule possessing a much higher e.e. must be used.

Acceleration of amino acid racemization by isovaline (Iva)

In a popular evolutionary scenario, rock pools on active volcanic islands before permanent continents had formed provided favourable locations for prebiotic organic reactions.[20] Allegedly, large amounts of organic compounds could have accumulated in high concentrations, instead of being diluted in the ocean. It is claimed that different types of gradients (e.g. temperature and pH) could have helped drive chemical reactions. Moreover, minerals such as montmorillonite—a weathering product of basaltic material—could have affected the behaviour of some reactions. For

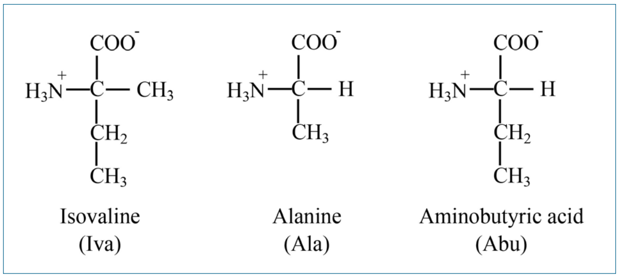

Figure 5. Structures of the L amino acids used in this study: Iva (2-amino-2-methylbutanoic acid), Ala (2-aminopropanoic acid), and Abu (2-aminobutyric acid).[22]

example, the intercalation of AAs hinders their sublimation at higher temperatures.[21] Some OoL researchers propose that the source of L-AAs derived from chirality transfer from *α*-methyl variants of proteinogenic AAs. For example, Breslow and Cheng claim:

> "… evidence has now accumulated that the L handedness of amino acids was derived from a special group of amino acids, with an extra methyl group that prevented loss of their small excess of the L form."[11]

This brings us to some experiments conducted by Fox *et al.* at the University of Hohenheim in Germany and reported in 2020. The experiments were designed to model a geothermally heated rock pool containing AAs in montmorillonite.[22] The team examined the effect of D- and L-isovaline (L-Iva) on the thermal racemization of L-alanine (L-Ala) and L-2-aminobutyric acid (L-Abu), see chemical structures in figure 5.

The AAs were integrated into the clay mineral calcium montmorillonite at 150°C. Recall from above that non-natural *α*-methylated AAs such as isovaline are believed to have possessed a small e.e.$_L$ in meteorites landing on Earth.

The L-Ala used was almost 100% enantiopure initially. Remarkably, merely upon loading into montmorillonite, the L-e.e. decreased by a few percent (but this did not occur for L-2-aminobutyric acid (L-Abu)). This effect was particularly pronounced for the experiment using only 0.5% Ala, where the L-e.e. dropped to 86.7%, even though the temperature did not exceed 35°C. Such rapid racemization (within hours) at a low temperature had already been observed during multiple wet-dry–cycle experiments when loading into montmorillonite.[20]

In a series of tests, different Iva-to-Ala molar ratios (1:1, 3:1, and 5:1) were used to monitor the racemization of L-Ala over a period of 8 weeks. About thirty L-e.e. values were measured, leading to smooth trend curves with L-e.e. decreasing over time.[22] The rate of racemization increased with increasing concentration of Iva. When 0.5%-pure L-Ala enantiomer was used, in the absence of Iva, the L-e.e. dropped to 61.0% after eight weeks. In the presence of L-Iva, final L-e.e. values of Ala decreased to 47.6% (1:1 Iva-to-Ala molar ratio), 41.8% (3:1) and 29.9% (5:1) (see table 4).[22]

The same trends were obtained when 0.5%-pure D-Iva enantiomer was mixed with L-Ala, but for all three molar ratios the rate of racemization was less pronounced (see table 4).[22]

The smaller effect of D-Iva compared to L-Iva was also observed when higher concentrations of L-Ala and of L-Abu (2%) were used in 1:1 molar ratio with each of the Iva enantiomers, as shown in table 4.[22]

HPLC analysis showed that decomposition and sublimation were not a significant issue short-term, since, in all cases, at least 67% of the initial Ala and Abu were still present after the 8-week-long experiments. Of course, decomposition over deep time would have destroyed the AAs in these kinds of environments with fluctuating temperatures.

The acceleration in racemization seems to be due to direct interaction between two AA molecules. The higher the concentrations, the faster this occurred. Hydrogen-bonded L-Ala or L-Abu–Iva dimers would exist as two L–L and

Table 4. Rate of racemization of L-alanine and L-2-aminobutyric acid by D- and L-*α*,*α*-dialkyl amino acid isovaline

0.5% Ala			
L-Iva: L-Ala	**L-e.e. final**	**D-Iva: L-Ala**	**L-e.e. final**
0	61.0%	0	61.0%
1:1	47.6%	1:1	51.0%
3:1	41.8%	3:1	45.8%
5:1	29.9%	5:1	34.0%

2% Ala or Abu	
***α*-H-amino acid + Iva**	**L-e.e. final**
L-Ala	46.2%
L-Ala + D-Iva (1:1 molar)	35.0%
L-Ala + L-Iva (1:1 molar)	29.9%
—	—
L-Abu	52.9%
L-Abu + D-Iva (1:1 molar)	43.3%
L-Abu + L-Iva (1:1 molar)	36.8%

Ala: alanine
Abu: 2-aminobutyric acid
Iva: *α*,*α*-dialkyl amino acid isovaline

L–D diastereomers, where, for steric reasons, the L–D dimer apparently forms less readily than homodimers.[22]

The authors found that D- and L-Iva molecules are more effective at accelerating racemization of L-Ala than a second L-Ala molecule. The reasoning is based on data from table 4.

i. When 0.5% L-Ala was mixed 5:1 Iva:Ala molar ratio, an e.e.$_L$ of 29.9% was obtained when mixed with L-Iva but 34.0% with D-Iva.

ii. The same L-e.e. values were obtained, within experimental error, when 2.0% L-Ala concentration was mixed 1:1 molar with L-Iva and then with D-Iva. This is remarkable, since comparing i) and ii) shows that much less L-Ala was used in i (0.34 mmol) than in ii (1.4 mmol), whereby the compensating greater amount of L- or D-Iva was small: 1.7 mmol for i) vs 1.4 mmol for ii).[22]

The mechanistic basis for Iva causing faster racemization of L-Ala than a second L-Ala was unknown. Deprotonation at the α-C atom to form a carbanion is the most common mechanism for AA racemization and is catalyzed by a Bronsted base. The negative charge withdrawing carboxylate group of a second (zwitterionic) AA may assist the deprotonation step. A hydrogen-bonded dimer involving the carboxylate group of the AA Iva might be more basic and/or have better proton accessibility than L-Ala in the L-Ala–L-Ala interaction.[22]

We agree with the authors' conclusion:

> "Accelerated racemization of amino acids could have been an obstacle to the development of homochirality."[22]

Under natural conditions, the loss of chirality would have been slower since the conditions were optimized for laboratory convenience. But complete racemization would have occurred eventually for AA trapped in such hot, rocky crevices, which survived thermal decomposition. There would not have been a transfer of chirality from L-Iva to racemic AAs to generate more of the L-enantiomer.

These results contradict the results reported above[10] and confirm that the unnatural L-methylated AAs delivered by meteorites would have catalyzed formation of the biologically *wrong* D-AAs. The experiments producing the *wrong* AAs were far more likely to resemble a putative prebiotic Earth.

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