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A likely possible origin of homochirality in amino acids and sugars on prebiotic earth

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This Letter is dedicated to Harry Wasserman on the occasion of his 90th birthday. Few have accomplished so much to advance organic chemistry

ABSTRACT

For life to start on earth and elsewhere, it is critical that the building blocks—amino acids and sugars—be in predominant homochiral form. Over the past century, the origin of terrestrial prebiotic homochirality has been the subject of many speculations. In this Letter I summarize the experimental evidence for ways in which some meteoritic components could have led to the dominance of L amino acids and D sugars on earth, and the most likely way in which the original chiral excesses in the meteorites were formed.

The homochirality of amino acids is critical to their function in proteins. If proteins with L amino acids had occasional random placements of the D enantiomers they would have varying and random conformations. While this is no problem in current biology, where the L amino acids are produced by the action of specific enzymes, the mode of formation of dominant L amino acids on prebiotic earth before the existence of such enzymes is less obvious. This problem has excited interest and speculation for at least 100 years, but the field has lacked convincing experimental support for the various theories until recently. The same problem exists for sugars, which have the D configuration in modern biochemistry.

The amino acids generally have a single center of chirality whose three-dimensional geometry at a particular carbon defines the L configuration—the carbon bearing an amino group, a carboxyl group, a hydrogen atom, and a side-chain group (which only in threonine and leucine has an additional chiral center). However, the sugars such as ribose and glucose have several chiral centers; they are classified as D sugars, as in D-ribose and D-fructose, based on the configuration of the chiral center furthest from the carbonyl group of the sugar. Thus, for the sugars, the question is: how did this particular carbon become preferentially formed with the D configuration on prebiotic earth?

A striking relevant recent result was the discovery that some meteorites have landed on Earth containing organic compounds, including some amino acids that are in proteins today and also some special amino acids with a methyl group in place of the hydrogen on the chiral center of normal L amino acids.[1–10] The protein (2H) amino acids are racemic; they could have started with an excess of the L enantiomer, by the processes to be described below, but over time they could have been converted to the racemate by reversible loss of a proton on the chiral center. However, no racemization is possible if that hydrogen is replaced by a methyl group. In the meteorite that landed near Murchison Australia in 1969, five α-methyl amino acids were found (Fig. 1), all of which had a small but real excess of what were described as the L enantiomers; in modern chemical notation these are the S enantiomers—structures with the methyl group attached where the hydrogen atom would be in L amino acids.

This raises two questions: why do they have this excess of the *S* enantiomers, and how could they have played a role in generating the normal L amino acids and D sugars on earth? For the first question, the best evidence is the finding by astronomers that there is an excess of right circularly polarized light in this sector of the universe.[11,12] Bonner showed that irradiating a racemic mixture of a normal amino acid with right circularly polarized light in the ultraviolet region led to selective destruction of the D enantiomer, resulting in a mixture with a few percent excess of the L enantio-

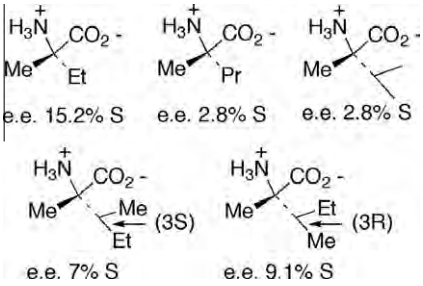

Figure 1. α-Methyl amino acids discovered in the Murchison meteorite, all of which have the *S* configuration that was described as L.

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mer.[13] There has long been speculation that circularly polarized light could have caused such excesses to be formed on earth, but the short wavelengths needed for absorption by ordinary amino acids could not penetrate the prebiotic atmosphere of the earth (carbon dioxide) or the current one. The new idea is that racemic mixtures of the α-methyl amino acids were formed in the asteroid or Kuiper belt, from which the meteorites originate, by Strecker reactions using compounds—HCN, ammonia, and carbonyl compounds—that have been identified by microwave spectroscopy to be present in interstellar space.[14] Then they were selectively decomposed by unshielded right circularly polarized light, and the α-methyl amino acids with an excess of the *S* form were delivered to the earth by chondritic meteorites.

Some have worried that frictional heating as the meteorites entered our atmosphere should have decomposed any organic compounds, but of course it did not. Since the meteorites are chondritic—pieces of rock with little thermal conductivity—and started at 10 K, they arrive still very cold and freeze water vapor when they are cracked open.

Astronomers do not agree on the origin of the excess right circularly polarized light in our sector of the universe. A popular idea is that a neutron star acts as a cyclotron, emitting circularly polarized light of opposite chirality above and below the circulation plane, and that the nearest one dominates and was oriented to send us the right polarized beam.[14] Some astronomers prefer the idea that the circularly polarized light seen in the universe could arise from processes involving magnetic white-dwarf stars, but the other implications are the same. By either idea, other sectors of the universe could have an excess of left circularly polarized light, producing D amino acids in a world the mirror image of our own. Also, if there were sectors with no excess of right or left circularly polarized light one would not expect to see an excess of L or D amino acids, so spontaneous synthesis of polypeptides or proteins with defined geometries would be difficult and therefore life would be less likely.

We addressed the question of how the small excesses of *S* α-methyl amino acids found in the Murchison meteorite could translate into an excess of L enantiomers in the normal amino acids of biology. We devised a process of decarboxylative transamination in which α-methyl amino acids could react with α-keto acids to form the normal amino acids, and with chiral transfer so that a small excess of *S* in the α-methyl amino acids would lead to a small excess of L in the normal amino acids.[15] The process was credible under prebiotic conditions, involving only heating the components in the presence of some catalytic Cu(II) ion, which is a component of meteorites. As we described, DFT calculations indicated that a Cu(II) complex of two imines formed from the α-methyl amino acid and the keto acid would decarboxylate one of them that would then protonate to form the L amino acid steered by the chirality of the second ligand, as we observed. However, with the few percent of S excess in the meteoritic amino acids we expected only 1% or so of enantioexcess in the product amino acids. Amplification of the resulting chiral excess would be critical to achieve a dominance of the L amino acids large enough that new organisms would select them for evolutionary success.

Morowitz had proposed a process by which a small enantioexcess of an amino acid could be amplified under simple prebiotic conditions.[16] His treatment was published in 1969. We conceived the same idea in 2006,[17] not aware of his work, and Blackmond also published a version of the concept in 2006.[18–21] The Morowitz treatment is based on the general fact that most amino acids form racemic compound crystals that are less soluble, and with higher melting points, than the crystals of the L or the D enantiomers. In such racemic crystals neighboring L and D molecules interact to lower the free energy compared with the crystals where an L molecule has only other L neighbors. The idea is that a mixture of D and L amino acids with some excess of the L component (or on another planet perhaps the D component) would dissolve in water, and as the water evaporates the less soluble racemate would precipitate leaving a solution with an increased richness in the L component. Because the precipitation of the racemate depends on the solubility product [L][D], while that of the homochiral L compound depends only on the solubility [L], there is a feedback to increase the precipitation of the racemate as the concentration of L increases. Thus starting with a small excess of the L amino acid, the final L/D ratio becomes very large with even a modest difference in the solubilities. The treatment is shown below.

$$S(\mathrm{L}) = [\mathrm{L}]$$

$$SP(\mathrm{DL}) = [\mathrm{D}][\mathrm{L}]$$

$$[\mathrm{D}] = SP(\mathrm{DL})/[\mathrm{L}]$$

$$[\mathrm{L}]/[\mathrm{D}] = S(\mathrm{L})^2/SP(\mathrm{DL})$$

The SP(DL) is equal to the square of half the solubility of the racemate. By this equation, if the homochiral crystals were only twice as soluble as were the racemates the final ratio [L]/[D] would be 16:1, a solution with 94% of the L and 6% of the D. As we will describe later, this treatment is not quite correct and can overestimate the selectivity for reasons that we have demonstrated.

Kinetics can sometimes outdo thermodynamics. With equilibrium solubilities we saw L tryptophan amplified to 94.5% L and 5.5% D, starting with any arbitrarily small excess (the treatment is true for any initial ratio, limited only by the practical need to achieve saturation in both the racemate and homochiral compound, so that smaller amounts of water would be needed with smaller initial L excess).[15] However, we found that this ratio was raised to 99:1 when we poured a small amount of water through the final mixture, imitating the effect of rainwater. The homochiral and racemic crystals differ in their activation energies for subtracting (or for adding) units, a form of dissociation (or binding), and the homochiral crystals dissolve faster beyond the requirements of the equilibrium constants.

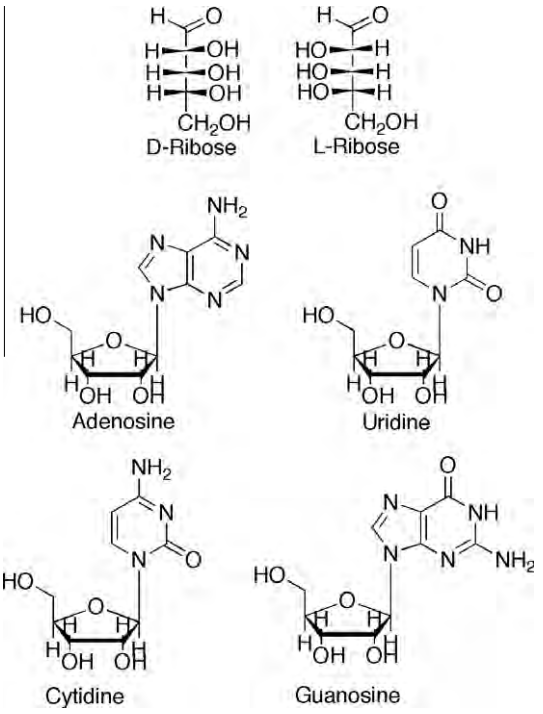

Figure 2. D and L ribose and the nucleosides that were examined.

We decided to examine the possibility of amplification of chiral excesses with ribose and with ribonucleosides. In contrast to the situation with amino acids, we saw that D-ribose and DL-ribose (Fig. 2) had the same solubility.[22] Ribose forms a racemate that is a solid solution, with essentially identical properties, solubilities, and melting points at all compositions. In the crystals a D-ribose molecule can equally well have either a D or an L as its neighbor. For this reason, D-ribose cannot be amplified by the selective solubility method. Thus we examined the ribonucleosides.[22] We synthesized L-uridine and made a 1:1 mixture with D-uridine, producing crystals of the racemate that had a melting point of 176 °C, 21 °C higher than the 155 °C for D-uridine. We found that D-uridine had a water solubility at 22 °C of 454 mg/mL, while the racemic uridine had a solubility of only 87 mg/mL, 5.2 times smaller. This should give a D/L ratio of 108:1 at saturation with both crystals by the Morowitz calculation. We then dissolved both the D-uridine and the DL-uridine crystals to saturation together in water at 22 °C, filtering away the excess, and saw 96% D and 4% L in solution, a ratio of 24:1, a large ratio that would make the D-uridine dominant, but less than predicted by the Morowitz treatment.

We then examined the situation with adenosine, synthesizing L-adenosine and mixing it with D-adenosine to produce racemic crystals. In this case the melting point of the racemate was 243 ± 1 °C, while for D-adenosine the mp was 230 ± 1 °C. The solubility of D-adenosine in water at 22 °C was 5.2 mg/mL, 6.3 times as large as the 0.8 mg/mL for the DL crystal. From the Morowitz treatment there should have been a 160:1 D/L ratio at saturation equilibrium, but we observed a 99:1 ratio of D/L adenosine. Again very large, but less than that predicted by the Morowitz treatment with cytidine we saw a decomposition on the melting of both the D and the DL crystals, but the solubilities predicted an even larger amplification than for the other two nucleosides, with D-cytidine at 192 mg/mL in 22 °C water, while DL-cytidine had a solubility of 24.3 mg/mL. The Morowitz equation predicts a D/L ratio of 250:1 at saturation, while we observed a 199:1 ratio.

Obviously all three of these nucleosides can be amplified to very high D/L ratios by selective solubilities, but the Morowitz treatment overestimated the ratios. We concluded that this reflected the fact that the solubilities being measured were in pure water, but the solubilities relevant to the amplification experiments are in water along with a second component. In the experiment, the solubility of the racemate that is relevant is that of the presence of the homochiral component also in solution. We concluded that the homochiral dissolved material was acting as a cosolvent, increasing the solubility of the racemate over that in pure water.[14] That is, the homochiral dissolved component has two roles. In one it helps drive the racemate out of solution by its role in the solubility product of the racemate, but in the other role it increases the solubility of the racemate by acting as an antihydrophobic cosolvent like ethanol, with which relatively hydrophobic substrates such as nucleosides have greater solubility than in pure water. To test this idea, we examined the solubility of racemic uridine in water with D-cytidine added to saturation.[14] This can mimic the cosolvent effect of D-uridine, but does not play a role in the solubility product of DL-uridine. We found that the D-cytidine increased the solubility of DL-uridine by 40% over that in pure water. Thus the Morowitz treatment overestimates the amplifications a bit because of the cosolvent effect, but they are still so high that they could lead to the dominance of the D-nucleosides on prebiotic earth even starting from a very small initial excess.

The situation with guanosine was different.[22] Again D-guanosine and the DL-guanosine crystals melted with decomposition, but we could examine their solubilities in 22 °C water. DL-Guanosine had a solubility of 0.84 mg/mL while D-guanosine had a solubility of 0.46 mg/mL, essentially half that of the racemate. This indicated that the racemate belongs to the third class of racemic crystals. It is not a solid solution like ribose, nor a racemic compound crystal like those of uridine, adenosine, and cytidine, it is a racemic conglomerate of D and L crystals, each with its own solubility. Such a conglomerate is like that with sodium ammonium tartrate that allowed Pasteur to separate the D and L crystals by hand. This inversion in solubilities, with the racemate more soluble than the homochiral compound, makes it impossible to amplify D-guanosine by selective solubilities, but of course the D-ribose from hydrolysis of the other three nucleosides could have been used to synthesize D-guanosine on prebiotic earth.

The previous work all assumes that ribose was available on prebiotic earth with some excess of the D enantiomer and that ribose could be incorporated into nucleosides under prebiotic conditions. We are still working on the second problem, but have made some progress with the first one. We have investigated the formation of the simplest sugar belonging to the D series, D-glyceraldehyde.

It is generally believed that sugars were prebiotically synthesized by a process called the formose reaction.[23] A sugar such as ribose ($C_5H_{10}O_5$) is a formal pentamer of formaldehyde (CH_2O), whose simple dimer is glycolaldehyde and whose trimer is glyceraldehyde. When formaldehyde is treated with a mild base such as calcium hydroxide there is a period when nothing happens until there is the sudden conversion of the formaldehyde to glycolaldehyde and glyceraldehyde and even larger sugars, the formose reaction. Many years ago we proposed and demonstrated the mechanism of this process in this journal, by an autocatalytic cycle (Fig. 3).[24]

A first molecule of glycolaldehyde is formed by an unknown process, possibly involving cosmic radiation, and this then reacts with formaldehyde to form glyceraldehyde. This is in equilibrium with dihydroxyacetone by enolization and ketonization, and this then reacts with another formaldehyde to form a four-carbon ketosugar. By enolization and aldehyde formation a four-carbon aldehyde is formed that can undergo a reverse aldol reaction to form two molecules of glycolaldehyde. Then another turn of the cycle makes four glycolaldehydes, etc. In our recent work we focused on the formation of glyceraldehyde, a three-carbon sugar that is the simplest one with a chiral center.[25] In the absence of a chiral catalyst this reaction would form both D-glyceraldehyde and its enantiomer L-glyceraldehyde in equal amounts (Fig. 4), but we wondered what would happen if the reaction were catalyzed by a chiral amino acid. As we have described above, we can trace the formation of L amino acids back to the small excesses of S α-methyl amino acids in the Murchison meteorite, so the question was simple. Would L amino acids catalyze the formation of D-glyceraldehyde preferentially, and if so could the preference be amplified to a sufficient excess that the dominant D-glyceraldehyde would be selected by incipient life? If so, all

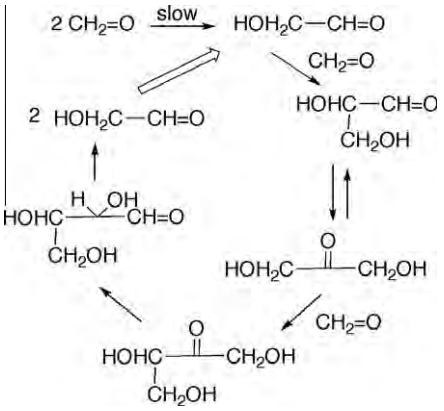

Figure 3. The formose cycle by which formaldehyde can be converted into glycolaldehyde and glyceraldehyde. The first molecule of glycolaldehyde is formed slowly, perhaps by cosmic radiation processes, but then it acts to catalyze its own formation by the cycle with aldol addition reactions, isomerizations, and eventually a reverse aldol reaction forming two glycolaldehydes from the initial one. For simplicity no stereochemistries are implied or shown.

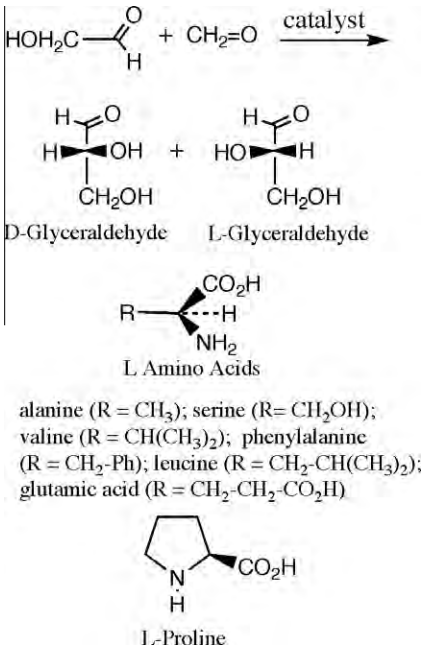

Figure 4. The aldol addition of formaldehyde to glycolaldehyde will form both D and L glyceraldehyde, and catalysis by L amino acids induces a preference for forming the D enantiomer. The exception is catalysis by L-proline, forming the L enantiomer preferentially. The results are in Table 1. D-Glyceraldehyde is the basis on which all D sugars can be formed.

the D sugars, built on the D-glyceraldehyde by adding pieces to its aldehyde group, would be part of the general origin of homochirality tracing back to the meteorites. If not, a new source of homochirality would need to be found for the sugars.

As Figure 3 shows, glyceraldehyde is proposed to be formed in the formose cycle by the reaction of formaldehyde with glycolaldehyde. This is an aldol reaction, and many such reactions are known to be catalyzed by amines, which react with the glycolaldehyde to form an enamine that adds to the other carbonyl component. Thus we carried out the reaction of glycolaldehyde with formaldehyde in the presence of a variety of L amino acids. We found (Table 1) that all the L amino acids caused the formation of glycolaldehyde with a small excess of the D enantiomer, with one exception. L-Proline catalyzed the formation of an excess of L-glyceraldehyde.

The preferences were not large, with a 60:40 ratio of D/L glyceraldehyde catalyzed by L-glutamic acid, for instance, and smaller ratios with the other L-amino acids. Proline was probably not present in large amounts among the early amino acids (but is present in the Murchison meteorite), since it is formed in two secondary reactions from glutamic acid. We argued that the results with the other amino acids did support the idea that the preference for D-glyceraldehyde—and then the other D sugars derived from it—was simply the result of the formation of the other L amino acids on prebiotic

Table 1
Ratio of D to L glyceraldehydes synthesized from glycolaldehyde and formaldehyde in the presence of amino acids

Amino acid	Ratio (D/L)
L-Serine	50.3/49.7
L-Alanine	50.8/49.2
L-Phenylalanine	52.2/47.8
L-Valine	52.2/47.8
L-Leucine	54.4/45.6
L-Glutamic Acid	60.7/39.3
L-Proline	28.9/71.1

earth. However, the next question was whether there was a likely process for amplifying the small excess of D-glyceraldehyde formed in this way, preferably by using the selective solubilities that had worked with the amino acids and most of the nucleosides.

Here, Nature was on our side. D-Glyceraldehyde is a syrup with essentially complete water solubility, but racemic DL-glyceraldehde is a solid with a melting point of 145 °C and a limited water solubility. An X-ray structure determination showed that DL-glyceraldehyde exists as a six-membered ring dioxane dimer of one D and one L molecule, with all the substituents equatorial, so this flat dimer molecule packs easily into a crystal.[26] By contrast, with the same structure based on a dimer with D-glyceraldehyde alone one large group in the six-membered DD ring would be axial, making the dimer less stable and also making crystal packing less favorable. The result is that we could take the 60:40 ratio of D/L glyceraldehyde formed by catalysis with glutamic acid and turn it into a 92:8 D/L ratio in water solution by slow evaporation of water that caused the racemic crystals to precipitate.

This work fills some of the cracks in the general idea that the unusual amino acids delivered to earth by the Murchison meteorite and related ones could have led to the dominance of L amino acids and D sugars that would permit life to start. Of course showing that it could have happened this way is not the same as showing that it did. Proper theories need the possibility of falsification, and this account would be in trouble if significant examples were found in which meteorites contained R α-methyl amino acids instead of the S examples in the Murchison meteorite. Also, an alternative scenario to ours would use the Murchison α-methyl amino acids to catalyze the formation of some D sugars, as Pizzarello and Weber have shown,[27] and then use those sugars to catalyze the formation of the normal L amino acids. Good credibly prebiotic examples of the latter process have not yet been produced.

Further work is needed to show credibly prebiotic versions of the conversion of D-glyceraldehyde to D-ribose, D-glucose, D-fructose, and D-2-deoxyribose, efforts underway in our lab. We also need a credible way in which nucleosides and nucleotides could be formed from these sugars and other components. Finally, of course, we all need ways in which these and other sensible building blocks could assemble into structures with the exciting properties of life. An implication from this work is that elsewhere in the universe there could be life forms based on D amino acids and L sugars, depending on the chirality of circular polarized light in that sector of the universe. Since such life forms could well be advanced versions of dinosaurs, assuming that mammals did not have the good fortune to have the dinosaurs wiped out by an asteroidal collision as on earth, we may be better off not finding out.

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