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A route to enantiopure RNA precursors from nearly racemic starting materials

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The single-handedness of biological molecules is critical for molecular recognition and replication processes and would seem to be a prerequisite for the origin of life. A drawback of recently reported synthetic routes to RNA is the requirement for enantioenriched reactants, which fails to address the puzzle of how the single chirality of biological molecules arose. Here, we report the synthesis of highly enantioenriched RNA precursor molecules from racemic starting materials, with the molecular asymmetry derived solely from a small initial imbalance of the amino-acid enantiomers present in the reaction mixture. Acting as spectators to the main reaction chemistry, the amino acids orchestrate a sequence of physical and chemical amplification processes. The emergence of molecules of single chirality from complex, multi-component mixtures supports the robustness of this synthesis process under potential prebiotic conditions and provides a plausible explanation for the single-handedness of biological molecules before the emergence of self-replicating informational polymers.

Recent advances in the synthesis of the canonical pyrimidine[1] and purine[2] nucleotides have brought a prebiotically accessible pathway to RNA closer to realization[3,4]. However, these encouraging findings leave unsolved a central puzzle of the origin of life—the single-handedness of biological molecules, including that of the simple sugars in these nucleotide syntheses. The observed suppression of D-nucleotide polymerization in the presence of their mirror-image L-nucleotides (known as enantiomeric cross-inhibition[5], or more informally as the problem of 'original syn'[6]) places a severe limitation on the 'RNA world'[7] hypothesis, in the absence of a means of constructing these monomers in enantiopure form. Furthermore, the incorporation of L-enantiomers into growing chains could result in the production of families of diastereomers for any nucleobase sequence, making development of the phenotypic properties required of RNA problematic. The current study reports the synthesis of activated pyrimidine nucleoside intermediates of single chirality via a concerted sequence of physical and chemical amplification processes that make use of a small initial imbalance in amino-acid enantiomers as the only molecular asymmetry in the system. Our work provides further support for recent findings suggesting that both physical and chemical processes played essential roles in early biology prior to the emergence of evolved biochemical capabilities[8,9].

Others have demonstrated the synthesis of ribonucleotide precursors to RNA from simple building blocks including simple carbohydrates, high-energy nitrogen-containing molecules and inorganic phosphate[1]. This synthesis is remarkable in that it bypasses the problematic intermediacy of free ribose and nucleobases while providing a prebiotically plausible route to RNA. However, the provenance of the homochirality of the D-glyceraldehyde used in that work remains a critical stumbling block. Without access to highly enantioenriched sugars, the nucleotides formed in these syntheses would not lead to informational polymers capable of establishing a genetic code.

Results and discussion

The homochirality of biological molecules requires a means of obtaining both sugars and amino acids in enantiopure form. Racemic synthesis of amino acids has been shown to be prebiotically accessible[10], and amino acids have been detected at significant concentrations in chondritic meteorites[11], with partial enantioenrichment of up to 18% enantiomeric excess (e.e.)[12,13] of the L-enantiomer over the D-enantiomer (e.e. = (L − D)/(L + D)). Our previous work[9,14–17] and that of others[18–23] demonstrated that further enantioenrichment is possible by manipulation of amino-acid phase behaviour. Because the functional groups present in amino acids and sugars can each mediate the chemical reactions of the other, we reasoned that enantioenriched amino acids may play a role in the chemistry leading to RNA synthesis, even though these compounds are not themselves components of RNA.

The synthesis of RNA precursors was carried out by reacting racemic glyceraldehyde *rac*-**1** and 2-amino-oxazole[24] **2** according to published procedures[1], but modified to include amino acids **3** as additives in the reaction mixture (see Supplementary Information). In the absence of amino acids, the formation of racemic amino-oxazolines dominated by the ribo- and arabino-compounds **4** and **5** was confirmed, which may be converted to racemic activated pyrimidine nucleotides[1]. Interestingly, in a number of cases, the presence of an amino acid induces a degree of enantioselectivity in the formation of the nucleotide precursors, as shown in Table 1. Proline (Pro, **3o**) provides the most selective reaction, yielding the amino-oxazolines in ∼55–58% e.e. from racemic glyceraldehyde under these unoptimized screening conditions. Also showing discernable enantioselectivity are alanine (Ala, **3a**) and tryptophan (Trp, **3r**), with ∼8–10% e.e. (D), and histidine (His, **3i**), which gave ∼8% e.e. to the unnatural L-amino-oxazolines under these conditions. A side product is formed in these reactions that sequesters the amino acid. Figure 1 shows the main reaction and this side reaction for the case of proline.

The observation of chiral selectivity induced by proline warranted further study of this system. NMR spectroscopy and mass spectrometry enabled the assignment of the major by-product structure **6** shown in Fig. 1 (see Supplementary Information). It is postulated that this compound forms in a three-component reaction analogous to that described recently by Powner *et al.*[1], with the amino acid playing the role they reported for an achiral amino-imidazole.

As a probe of the chiral selectivity of the sugar–amino acid interaction, the reaction of enantiopure D-glyceraldehyde D-**1** and

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Table 1 | Formation of enantioenriched amino-oxazolines in the presence of L-amino acids.

Amino acid	Three-component product* 6	Ribose amino-oxazoline D-4 (% e.e.)	Arabinose amino-oxazoline D-5 (% e.e.)
Ala (**3a**)	++	8.9	8.1
Arg (**3b**)	++	4.1	7.3
Asn (**3c**)	+	1.1	0.5
Asp (**3d**)	+	2.1	1.4
Cys (**3e**)	+++	n.a.	1.4
Gln (**3f**)	+	1.2	1.1
Glu (**3g**)	+	0.8	0.1
Gly (**3h**)	++	-	-
His (**3i**)	++	7.5 (L)	8.1 (L)
Ile (**3j**)	+	2.1	0.5 (L)
Leu (**3k**)	+	1.1	2.1
Lys (**3l**)	+++	n.a.	n.a.
Met (**3m**)	+++	n.a.	n.a.
Phe (**3n**)	+++	2.5	5.4
Pro (**3o**)	++	55	58
Ser (**3p**)	+++	3.0	1.9
Thr (**3q**)	++	1.1	2.6
Trp (**3r**)	++	10.2	9.8
Tyr (**3s**)	+	0.5	2.6
Val (**3t**)	++	2.0	1.0 (L)

*Yield of side product **6**: +, low; ++, medium; +++, high. n.a., no products isolated or observed by chiral LC

2-amino-oxazole **2** was monitored in the presence of either L-proline or D-proline in separate experiments. The reaction rate was found to be accelerated by a factor of 2.5 for the D–D (or L–L) sugar–amino acid interaction when compared to the D–L (or L–D) case, accompanied by an increase in the ratio of the three-component and two-component reaction products (from 1:2 to 4:1). This D–D (or L–L) preference reveals that the three-component reaction in a substrate pool containing the proteinogenic L-proline, racemic glyceraldehyde and 2-amino-oxazole will select for the L-hand of the sugar, allowing an increasing proportion of the natural D-glyceraldehyde to accumulate in the substrate pool over time. Thus, the amino acid does not participate directly in the two-component reaction, and the degree of asymmetric amplification exhibited by the product amino-oxazolines depends on the rate and selectivity of the three-component side reaction compared to the rate of the main two-component reaction. Because the side reaction is driven by amino-acid concentration, enantioenrichment in the amino-oxazolines increases with increasing proline concentration. As shown in Fig. 1, enantioenrichment of greater than 90% e.e. in D-glyceraldehyde may be obtained. This process effectively acts as a kinetic resolution of racemic glyceraldehyde.

Kinetic resolution has been implicated as a mechanism for prebiotic chiral amplification by preferential destruction of one enantiomer of a racemic mixture in a photoresolution process driven by circularly polarized light[25]. However, most chiral compounds exhibit only a very weak preference in this context, and enantiomeric yields of ~20% e.e. may be realized only at the expense of ~99% photodestruction of both enantiomers[26,27]. The enantioenrichment observed in the chemical reaction reported here exhibits a greater than 50-fold increase in the yield of the desired enantiomer compared to the photoresolution process (see Supplementary Information). This work also indicates that the system may proceed effectively either as a resolution of proline by glyceraldehyde, or of glyceraldehyde by proline, as long as one or the other is available in non-racemic form. In principle, such a resolution could be coupled with other approaches for chiral amplification of either amino acids or sugars.

Sutherland and colleagues demonstrated that the ribo-amino-oxazoline selectively crystallizes as an enantiopure compound from solutions containing a mixture of different amino-oxazolines that are partially enantioenriched above 60% e.e.[28]. Optimization of this process in the presence of amino acids led to the finding that enantiopure ribo-amino-oxazoline crystals may be obtained from solution e.e. values as low as 20% e.e., directly from crude reaction mixtures containing the reaction products, amino acid and the three-component side product. The enantiopure ribo-amino-oxazoline crystals can then be converted to enantiopure arabino-amino-oxazolines by phosphate-catalysed isomerization, as demonstrated by Sutherland and co-workers[29].

A likely prebiotic scenario suggests that the reaction medium might contain a complex pool of molecules, including several different amino acids. To test the robustness of these chirality amplification processes in multicomponent mixtures, the reaction of racemic glyceraldehyde with 2-amino-oxazole was carried out in mixtures of a variety of different amino acids. Enantiopure crystals of ribo-amino-oxazoline were successfully obtained directly from crude reaction mixtures containing as many as 14 (Pro, His, Ala, Trp, Leu, Ile, Asp, Asn, Glu, Gln, Val, Tyr, Phe, Thr) of the 19 chiral, proteinogenic amino acids shown in Table 1 (see Supplementary Information).

The prebiotic plausibility of this route to RNA from racemic sugars requires a means of obtaining enantioenrichment in the amino acid. Previous work[15–18,20] has demonstrated that highly enantioenriched solutions may be obtained from a small initial enantiomeric imbalance for many amino acids, including proline, via physical amplification processes that predominantly sequester the minor enantiomer as a racemic solid. The synthesis of enantiopure RNA precursor molecules from racemic glyceraldehyde should therefore be possible starting from amino acids with low enantioenrichment. When a sample of L-proline amplified from an initial 1% e.e.

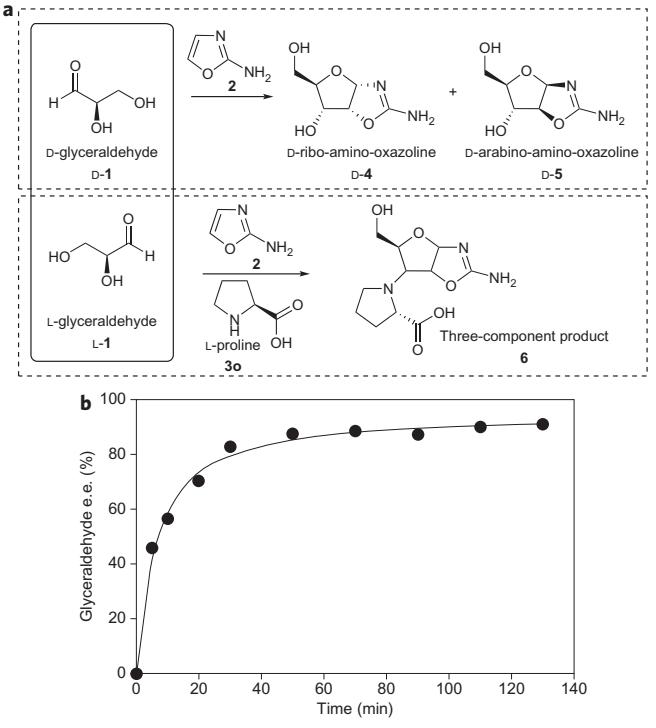

Figure 1 | Racemic glyceraldehyde is converted into enantioenriched RNA precursors in the presence of a chiral amino acid. a, In the presence of an enantioenriched L-proline (**3o**), the diastereoselective formation of a three-component side product (**6**) effectively sequesters the unnatural L-glyceraldehyde (L-**1**). **b**, The side reaction acts as a kinetic resolution of glyceraldehyde, giving enantiorichment of greater than 90% e.e. D-**1**, which reacts with **2** to form the enantioenriched amino-oxazoline RNA precursors D-**4** and D-**5**. e.e. values are ±2%.

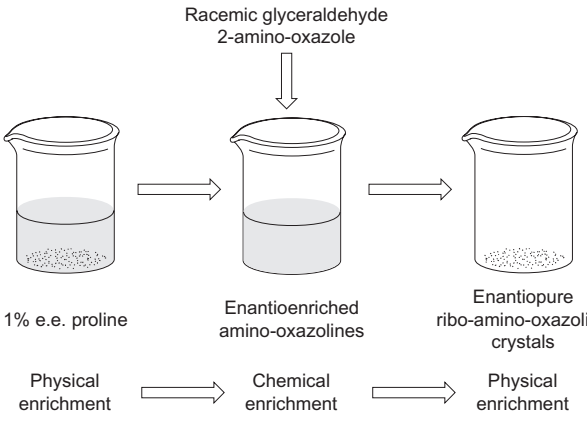

Figure 2 | Route to enantiopure RNA precursors from racemic glyceraldehyde and slightly enantioenriched amino acid. 1% e.e. L-proline (**3o**) is suspended in solvent (either $CHCl_3$ or EtOH). After equilibration, the remaining solid is removed and the solvent is evaporated from the supernatant. Racemic glyceraldehyde DL-**1** and amino-oxazole **2b** are then added and the mixture is dissolved in water. The ensuing reaction produces amino-oxazolines **4** and **5** in 20–80% e.e. Cooling the mixture to 4 °C induces crystallization of enantiopure ribo-amino-oxazoline crystals.

was added to the reaction of racemic glyceraldehyde with 2-amino-oxazole, enantiopure crystals of the ribo-amino-oxazoline were obtained. Enantiopure crystals of the unnatural L-ribo-amino-oxazoline were produced in a similar manner starting from 1% e.e. of the unnatural D-proline and racemic glyceraldehyde, eliminating the possibility that the observed amplification might be caused by cryptochiral impurities present in the modern environment.

Figure 2 outlines this production of enantiopure RNA precursors from racemic starting materials and nearly racemic amino acids. The chemical amplification achieved in the kinetic resolution of Fig. 1 is augmented by two physical amplification processes: enantioenrichment of the amino acid before the resolution, and selective crystallization of enantiopure ribo-amino-oxazoline following the resolution.

A set of simple physical and chemical amplification steps are presented that combine to give enantiopure pyrimidine ribonucleoside precursors to RNA from racemic starting materials. The only asymmetry present in the system at the outset is a small imbalance in amino-acid enantiomers, the role of which is a selective diversion of the unnatural hand of the sugar component away from the main synthetic reaction. The emergence of molecules of single chirality from complex, multi-component mixtures supports the robustness of the processes under potential prebiotic conditions. This work provides a prebiotically plausible scenario for the synthesis of enantiopure RNA starting from simple racemic sugars and nearly racemic amino acids.

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Author contributions

D.G.B. conceived this work and wrote the paper. J.E.H. designed and carried out the experiments. E.T. carried out preliminary work.

Additional information

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