Perspective

Investigating the Evolution of Biomolecular Homochirality

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The molecular property of chirality has intrigued both scientists and laymen for more than a century and a half. van't Hoff[1] was the first to postulate the existence of chiral molecules—nonsuperimposable forms that are mirror images of one another, as are left and right hands—as early as 1874, a quarter century after Pasteur[2] had shown that salts of tartaric acid exist as mirror image crystals.* Synthesized in the laboratory in the absence of a directing template, the left- and right-handed molecules of a compound, or enantiomers, will form in equal amounts (a "racemic" mixture). However, chiral molecules present in nature as part of living organisms are most often produced exclusively in one enantiomeric form. This property of single chirality is critical for molecular recognition and replication processes and would thus seem to be a prerequisite for the origin of life.[3] Enantiopure molecules such as enzymes help to direct the synthesis of further enantiopure molecules in living organisms, with prominent examples being the D-sugars and L-amino acids that make up DNA and proteins, respectively. Similar strategies are used in laboratory asymmetric synthesis and catalysis, where we draw upon the natural pool of chiral molecules to provide building blocks for constructing new enantiopure molecules or catalysts. This leads logically to the question: what served as the original templates for biasing production of one enantiomer over the other in the chemically austere, and presumably racemic, environment of the prebiotic soup?

This Perspective highlights two models for the evolutionary routes that may have been taken by simple molecules in a racemic prebiotic world to arrive at the high levels of enantiopurity inherent in modern biological molecules.[3] (See ref. 3 for a discussion of other models.) Short of constructing a time machine, we have no way of elucidating precisely the chain of events that led to modern biology. However, this article illustrates the application of chemical reaction engineering and equilibrium thermodynamics, two basic intellectual building blocks of chemical engineering, to the development of plausible mechanisms for this fundamental process. The first model invokes "far from equilibrium" autocatalytic processes following the early theoretical work of Frank[4] and of Calvin[5]. Soai's landmark discovery[6] of an autocatalytic reaction following these theoretical descriptions, and the subsequent mechanistic studies of the Soai reaction by the groups of Blackmond[7,8] and Brown,[7,9] provide proof of concept for such models. The second model[10,11,12] is an alternative mechanism based on the equilibrium phase behavior of ternary systems of amino acid enantiomers and solvent. Both models focus on identifying means for amplifying a small imbalance in enantiomeric concentrations ("asymmetric amplification"), while the possible origin of this initial imbalance is the subject of other discussions.[3] These models offer dramatically different approaches for addressing the fundamental question of how molecular homochirality evolved in the complex molecules required for recognition, replication and ultimately for the chemical basis of life.

Autocatalytic Model for the Evolution of Homochirality

More than sixty years ago, Frank[4] developed a mathematical model for "spontaneous asymmetric synthesis", or the autocatalytic production of enantiomerically enriched molecules from a near racemic mixture in the absence of an added chiral template.[13] He showed that a substance that acts as a catalyst in its own self-production, and at the same time acts to suppress production of its enantiomer, provides a simple and sufficient model for the evolution of enantiopure molecules from a near-racemic mixture. The challenge to discover a reaction with these features was posed in the last sentence

*For a delightful discussion of all aspects of symmetry, see: "Reflections on Symmetry in Chemistry...and Elsewhere," by E. Heilbronner and J.D. Dunitz, Verlag Helvetica Chimica Acta, Basel: 1993.

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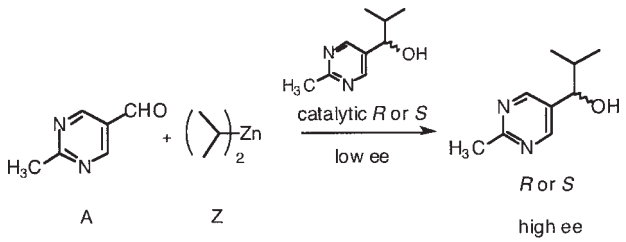


Scheme 1. The Soai reaction.

of this purely theoretical article: "A laboratory demonstration may not be impossible."*

More than forty years later, the first experimental proof of this concept was found when Soai and coworkers reported the autocatalytic alkylation of pyrimidyl aldehydes with dialkylzincs[6] (Scheme 1), in which the reaction rate is accelerated by addition of catalytic amounts of its alcohol product. In addition, and most strikingly, this reaction was shown to yield the autocatalytic product in very *high* enantiomeric excess (ee = $(R-S)/(R+S)$; R = right-hand enantiomer; S = left-hand enantiomer) starting from a very *low* enantiomeric excess in the original catalyst. Since this initial discovery, Soai's group has presented remarkable observations of this asymmetric amplification in the reaction of Scheme 1. Enantiomeric excesses as high as 85% were reported for a reaction initiated with a chiral amine at 0.1% ee.[15] These chiral initiators could be produced at low ee by exposing racemic mixtures to circularly polarized light. Asymmetric amplification has also been observed for the reaction initiated by inorganic chiral materials such as quartz.[16] However, the mechanism of the reaction, and the question of whether it exemplifies the Frank model, remained unexplored until the work of Blackmond and Brown in 2001.[7] This work was based on experimental kinetic studies and kinetic modeling, extending to the special case of this remarkable autocatalytic reaction theoretical models that had been developed previously for rationalizing observations of asymmetric amplification in asymmetric catalysis.[17]

One mechanism corresponding to the mathematics of the Frank model is the case developed by Kagan[17] as the ML_2 model. The ML_2 model is based on the formation of dimers from the enantiomeric catalyst molecules R and S: two homochiral (RR and SS, Eqs. 1 and 2), and one heterochiral (SR, Eq. 3) dimer. The relative concentrations of the dimers at any given relative concentration of total R and S depends on the dimerization-equilibrium constant K_D (Eq. 4). It may be seen that if the initial monomer concentrations of R and S are unequal, the relative concentrations of R and S ultimately present as monomers in equilibrium with the dimers will be different from their initial relative concentration.

$$R + R \overset{K_{\text{homo}}}{\rightleftharpoons} RR \tag{1}$$

$$S + S \overset{K_{\text{homo}}}{\rightleftharpoons} SS \tag{2}$$

$$R + S \overset{K_{\text{hetero}}}{\rightleftharpoons} SR \tag{3}$$

$$K_D = \frac{2 \cdot K_{\text{hetero}}}{K_{\text{homo}}} = \frac{[SR]^2}{[RR] \cdot [SS]} \tag{4}$$

Two possibilities exist for catalytic systems based on such a model: (a) the enantiomers R and S act as catalysts and the dimers serve as inactive reservoirs;[17,18] or (b) the dimers themselves are active as catalysts. These two possibilities are shown in Scheme 2.

In the first case, where monomers are active catalysts, the key to amplification of the concentration of one enantiomer over the other lies in the value of the dimerization equilibrium constant K_D. A high K_D value means that the heterochiral dimer is more stable than the homochiral dimer, producing a distortion in the monomer concentration toward the enantiomer present in excess. In an autocatalytic reaction, this trend is propagated through repeated catalytic cycles as the newly formed monomer products participate in the dimer equilibria. On the other hand, if the two types of dimers exhibit equal stability (stochastic dimer formation, $K_D = 4$), then the relative concentrations of R and S monomers will remain the same as the overall relative concentration considering all the R and S included in both monomer and dimer species. No nonlinearity between catalyst and product ee will be observed in the case of stochastic dimer formation.

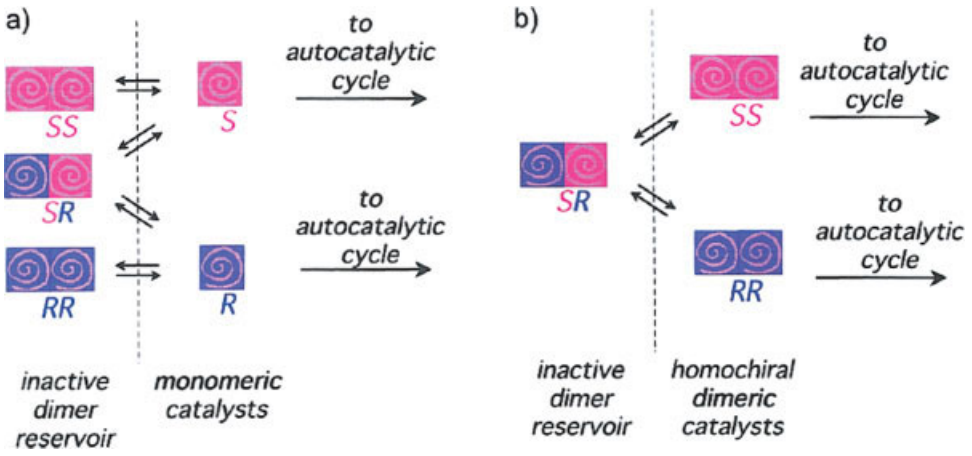
In the second case, where dimers themselves are active as catalysts, asymmetric amplification depends both on K_D and on the relative reactivity of homochiral and heterochiral dimers. One notable difference between this and the former case of monomer-active catalysts is that here amplification of ee may occur even for the case of stochastic dimer formation, as long as the homochiral dimers are more active as catalysts than are the heterochiral dimers.

Kinetic modeling applied to experimental kinetic profiles from *in situ* monitoring of the Soai autocatalytic reaction of Scheme 1, employing the reaction product at different initial ee values as catalyst showed that homochiral and heterochiral dimers are formed stochastically.[7,19] This has been verified experimentally by ^{1}H NMR spectroscopic characterization of solutions of enantiopure and racemic alkanols.[7,9,20,21]** Because amplification of ee is observed in this reaction, stochastic dimer formation rules out the monomer active model. The experimental results are consistent with the dimer model of Scheme 2.

This dimer model provides an elegant and simple solution to one mystery of the evolution of homochirality. This concerns the level of complexity associated with modern asymmetric catalysts. Amplification of ee in asymmetric catalysis is typically achieved by creating a specific stereochemical bias toward a highly stable inactive heterochiral dimer. By modern methods, synthesis using a complex asymmetric template provides this bias, and we can draw upon the extensive natural chiral pool of molecules for our building blocks to construct such templates. This resource was not available to prebiotic molecules. The significance of our finding that asymmetric amplification occurs in a statistical distribution of dimer-active cata-

*This statement has been described as "a challenge to all red-blooded chemists" See Ref. 14.

The veracity of a model developed from kinetic investigations alone requires confirmation from other methods, since a unique fit to a single mechanism is unlikely. Spectroscopic and structural identification of species predicted by a kinetic model can add significant weight to a mechanistic proposal and may help to eliminate possibilities. This important point has been neglected in several recent studies of the Soai reaction that proposed alternative mechanisms based on kinetic modeling alone.See the discussion in Refs. 8–10 in Blackmond, DG, *Testrahedron Asymm.* **2006, 17,584. Blackmond DG., *Tetrahedron Asymm.*, 2006;17:584.



Scheme 2. Comparison of (a) monomer model, and (b) dimer model for autocatalytic reactions. See text and equations 1–4.

lysts is that it tells us that *no* stereochemical bias was required to produce a distortion in the ee of the active catalyst. Amplification of ee under these conditions requires *not* sophisticated stereoselection but *only* different activities for the homochiral and heterochiral dimers, repeated over many autocatalytic cycles. Difference in activities for diastereomeric species (i.e., stereoisomers that are not enantiomers) such as these dimers is more likely than not to be the case. If, as in the Soai reaction, the relative reactivities happen to give the activity edge to the homochiral species, amplification is ensured even for nonselective dimer formation. In an autocatalytic reaction, the extent of this amplification is limited only by the number of cycles the reaction undergoes. Thus statistics (stochastic dimer formation), and one stroke of luck (lower activity of the heterochiral dimer), are sufficient prerequisites to account for the evolution of our homochiral world today.

Equilibrium Phase Behavior Model for the Evolution of Homochirality

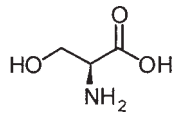
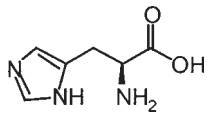
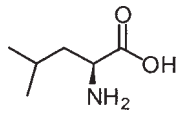
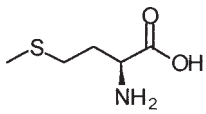
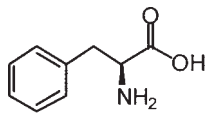
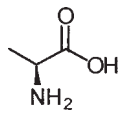
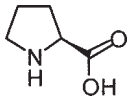
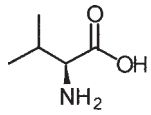
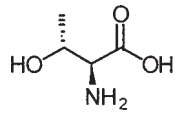
While the Soai reaction serves as a mechanistic model for the evolution of homochirality, this dialkylzinc chemistry is unlikely to have been of importance in an aqueous prebiotic environment. Therefore, speculation has continued concerning other processes that might have been directly responsible for the development of high enantiomeric excess in biological systems. Amino acid chemistry seems a plausible area for investigation, as amino acids have been implicated in prebiotic catalysis and have been found in nonracemic form in meteorites.[22] The discovery that proline catalyzes aldol and related reactions with high enantioselecitivity marked the birth of a new and highly active field of research now known as organocatalysis.[23] Our initial studies of two proline-mediated reactions, the α-aminoxylation[24] and the α-amination[25] of aldehydes, revealed intriguing features including an accelerating reaction rate and asymmetric amplification of product enantiomeric excess. While at first glance these features appear similar to those characterizing the Soai autocatalytic reaction, in fact these amino acid mediated reactions are autoinductive rather than autocatalytic, characterized by involvement of the reaction product in the catalytic cycle.[26] This "improved" catalysis does not alter the enantiomeric excess of the reaction product, but such product acceleration remains interesting in the context of development of complexity: a reaction product that enables higher-efficiency in its own catalytic production suggests a tool for evolutionary development of primitive amino acid catalysts.

This leaves the question of asymmetric amplification in amino-acid catalyzed reactions. Kagan ML_2 models, such as were applied to the Soai reaction appear to be unlikely, because solution formation of dimers has not been observed in these systems under reaction conditions. In studies of proline-mediated aldol reactions, we observed further unusual nonlinear behavior between catalyst ee and reaction product ee for reactions carried out in DMSO solvent that could not be explained by such models, giving a *constant* product ee over a wide range of proline ee values. Recognizing that the low solubility of proline in most organic solvents means that these reactions often take place under heterogeneous conditions, we hypothesized that this nonlinear effect could have a physical, rather than a chemical origin, and we turned to an examination of the phase behavior of proline, and other amino acids in organic solvents and water.[10]

Although the solid-solution phase behavior of racemic and enantiopure compounds was studied more than a century ago,[27] and is well understood,[28] much less attention has been paid to scalemic (nonracemic, nonenantiopure) mixtures. When equilibrium is established between solid and solution in a scalemic mixture of most proteinogenic amino acids[†] and solvent under isothermal conditions, this ternary system will consist of dissolved amino acid in the solution phase and two separate solid phases: a racemate as 1:1 cocrystals of D and L enantiomers, and a second solid phase of the pure enantiomer that is in excess. At a given temperature and pressure, the composition of the solution phase at equilibrium, known as its eutectic, is fixed by the phase rule and can have an ee value anywhere between 0–100% ee. We found, for example, that the eutectic

[†]Seventeen of the proteinogenic amino acids crystallize as 1:1 DL co-crystals, known as a "racemic compound". More rarely, some chiral compounds crystallize as "conglomerates", forming separate solid phases for d and i species. A third, very rare type of solid is a "solid solution" in which the two enantiomers form a continuous solution at all relative concentrations. The phase rule dictating the eutectic composition applies to racemic compounds and to conglomerates, but not to compounds forming solid solutions, which do not exhibit a eutectic. The discussion here focuses on racemic compounds.

Table 1. Experimental and Predicted Eutectic ee Values for Selected Amino Acids

Ser $ee^{eut} = 99\%$ (99%) 	His $ee^{eut} = 94\%$ (93%) 	Leu $ee^{eut} = 88\%$ (91%) 
Met $ee^{eut} = 85\%$ (87%) 	Phe $ee^{eut} = 83\%$ (87%) 	Ala $ee^{eut} = 60\%$ (59%) 
Pro $ee^{eut} = 50\%$ (50%) 	Val $ee^{eut} = 47\%$ (45%) 	Thr $ee^{eut} = 0\%$ 

Values in parentheses are calculated from Eq. 5. Experimental values measured in water at 25 °C, except for proline, measured in DMSO.

for proline in DMSO at 25°C is ca. 50% ee. Under solid-solution equilibrium, this eutectic value thus dictates the solution ee for all values of scalemic proline ee employed, which in turn dictates the product ee that may be achieved in solution phase reactions catalyzed by proline. This fact rationalized the unusual observation of a constant product ee in asymmetric aldol reactions over a wide range of total proline ee values, which is manifested as a positive nonlinear effect at overall proline ee values below the eutectic ee, and as a negative nonlinear effect at proline ee values above the eutectic ee. The nonlinearity with respect to the overall proline ee is due to a distortion between the ee values in the solid phase and the solution phase, where catalysis occurs.

This work demonstrates an effect opposite to practical crystallization processes, where the goal is production of a solid of high enantiopurity. However, these findings suggested that aiming for the opposite effect—high-solution enantiomeric excess and lower solid ee—provides a means for realizing asymmetric amplification in solution, where catalytic reactions occur. This prompted examination of the phase behavior of a number of amino acids to explore their potential for higher asymmetric amplification than is possible with proline and its eutectic positioned at ca. 50% ee. Table 1 reveals that in fact several of the proteinogenic amino acids exhibit high eutectic ee values. For example serine, with its eutectic at $> 99\%$ ee, provides a virtually enantiopure solution from a nearly racemic sample under solid-liquid equilibrium conditions.

Each chiral compound exhibits its own characteristic eutectic composition,[28] but no means has yet been discovered of predicting what this value will be *a priori*. Studying amino acid phase diagrams, as shown in Figure 1 for histidine and isoleucine, reveals that the eutectic *ee* is related to the relative solubility of the racemate compared to the enantiopure compound. High eutectic *ee* is achieved in cases where the racemate (rac) is much less soluble than the enantiopure compound (ep). This solubility ratio has been defined as a parameter termed α.[28] Based on the concept of solubility product applied to enantiomer mixtures, a simple model was developed that successfully predicts the eutectic ee values as a function of α for a range of chiral compounds.[11] This is shown in Eq. 5, and values calculated according to this equation are given in parentheses for the amino acids in Table 1. The model has been successfully applied to common chiral ligands, as well as to these amino acids, demonstrating that the phase behavior of chiral compounds can rationalize and predict amplification of solution enantiomeric excess in systems of enantiomers in solvent.

$$ee^{eut} = \frac{1 - \frac{\alpha^2}{4}}{1 + \frac{\alpha^2}{4}} \cdot 100\%; \qquad \alpha = \frac{[rac]}{[ep]} \tag{5}$$

If the solution *ee* is dictated by the eutectic *ee*, it would appear that high enantiopurity in solution is achievable by this phase behavior approach only for chiral compounds that happen to exhibit high eutectic *ee* values. That is, are we stuck with the hand that Nature has dealt us? Or can we find a means for engineering the phase behavior in order to enhance eutectic *ee* values? The case of proline is interesting in this respect. For proline in DMSO, MeOH, and EtOH, the eutectic ee values are 50, 54, and 58% ee, respectively, while in $CHCl_3$, the eutectic ee rises to >99%, as was first noted by Hayashi and coworkers.[29] Such solvent-dependent ternary phase behavior clearly could not be predicted from study of the binary system of enantiomers alone, although it has been suggested that such predictions should be possible from binary system melting point measurements.[28,30] By contrast, the model based on the solubility ratio α does indeed successfully

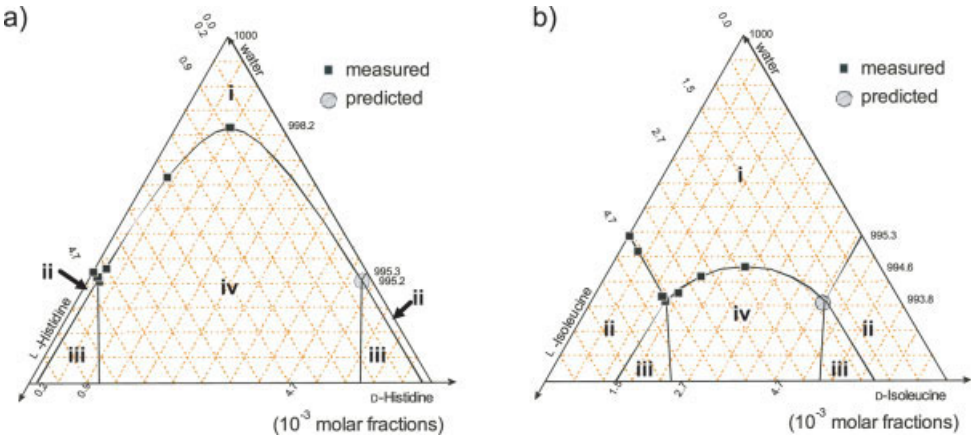


Figure 1. Ternary phase diagrams for two representative amino acids in water at 25°C, with pure H_2O at the apex, and the pure D and L enantiomers at the left and right corners of the (truncated) triangle.

(a) histidine (ee^{eut} = 94%; α = 0.38, and (b) isoleucine (ee^{eut} = 52%; α = 1.16). Regions depicted are as follows: i. solution (D + L + water); ii. enantiopure solid + solution; iii. enantiopure solid + DL solid + solution, and iv. DL solid + solution. Diagrams are truncated near the apex (>0.95 molar fraction water) to focus on the portion showing three-phase equilibrium. For additional phase diagrams of other amino acids and other chiral compounds. See Ref. 11.

predict the eutectic for proline in both DMSO (α = 1.155), and in $CHCl_3$ (α = 0.002).

What is the role of the solvent in altering the relative solubilities of the enantiopure and racemic solids to give such a significant enhancement of the eutectic ee in $CHCl_3$? If we can understand this behavior, might it then be possible to manipulate the eutectic ee of other chiral compounds, enabling an approach to enantiopure solutions for a wider range of compounds?

The answers to these questions were revealed when a crystal structure obtained for the 1:1 D:L proline cocrystal crystallized from $CHCl_3$, was compared to the racemate crystallized from EtOH. While enantiopure proline crystallized from $CHCl_3$ gives a powder X-ray pattern identical to that published[31] for anhydrous proline crystallized from ethanol, the solid racemate crystallized from proline in $CHCl_3$ exhibits a novel structure differing from all those previously published for the proline racemate in other solvents. Figure 2a shows that cocrystals of the racemic compound are formed incorporating one molecule of $CHCl_3$ per pair of proline molecules. The proline molecules exist in zwitterionic form in an extensive hydrogen bonding network that includes the chloroform C—H proton. The structure contains two independent molecules of proline in the asymmetric unit, one with the carboxylate unit in a pseudo-equatorial position with respect to the pyrrolidine ring, and the other as pseudo-axial. These proline molecules are linked to each other and neighboring symmetry-related molecules in the racemic crystal by a series of

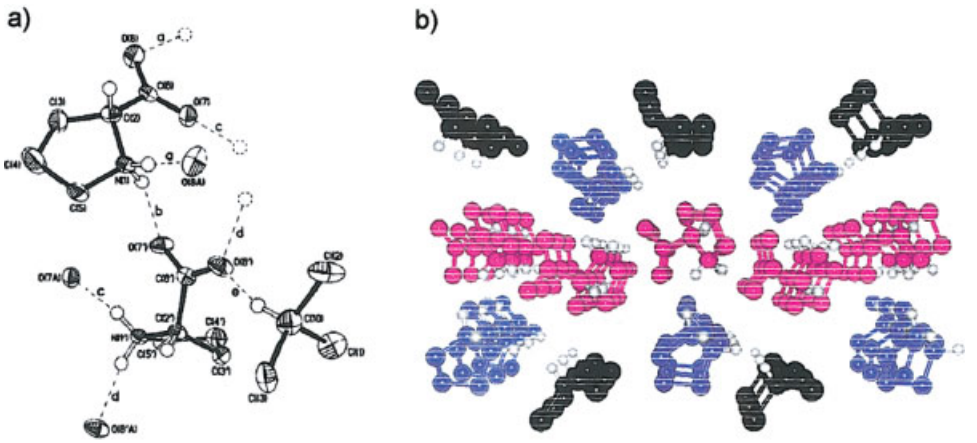


Figure 2. Crystal structure of the racemate of proline crystallized from $CHCl_3$ (CDCC 612603).

(a) The asymmetric unit showing the hydrogen bonding interactions between the two independent proline molecules and the included chloroform solvent molecule (50% probability ellipsoids). (b) Extended structure. The blue proline molecules are all of one enantiomer, while the magenta proline molecules are all of the opposite enantiomer. Chloroform molecules are shown in black.

N–H···O hydrogen bonding interactions that extend to form a two-proline-thick corrugated two-dimensional (2-D) sheet (Figure 2b). The chloroform molecules, and its symmetry-related counterparts, are located on the top and bottom faces of this sheet, and are held in place by a C–H···O hydrogen bond to one of the carboxylate oxygens of the pseudo-axial proline molecule. The 2-D sheet is particularly notable in that the axial and equatorial prolines are comprised of opposite enantiomers, and these conformations switch in the adjacent centrosymmetrically-related sheets.

The extensive hydrogen-bonding network within the structure of the D:L proline-chloroform co-crystals may help to explain the significant reduction in solubility of DL-proline-chloroform compared to racemates crystallized from DMSO, EtOH, or water.[‡] This may be thought of as an analogy to clathrate compounds, although here we may consider that it is the proline enantiomers themselves that are "trapped" in the solvate-racemate structure, causing them to dissolve much less readily than does the enantiopure compound from its crystal form.

The finding that the enantiomeric excess of an amino acid in solution may be significantly enhanced by incorporation of a small, achiral molecule into its solid racemate suggests a general and facile route to homochirality that may have prebiotic relevance. Cycles of rain and evaporation establishing solid-solution equilibrium in pools containing amino acids with appropriate hydrogen-bonding partner molecules could yield enantiopure solutions from a small initial imbalance of amino acid enantiomers. These molecules might serve as efficient asymmetric catalysts or as building blocks themselves for construction of the complex molecules required for recognition, replication and ultimately for the chemical basis of life. Ongoing work in our group focuses on determining eutectic ee values for proteinogenic amino acids in the presence of a wide range of potential hydrogen bonding partners comprising small molecules of prebiotic relevance.

Summary

How molecules developed the complexity and single-handedness found in living organisms today is one of the great scientific questions of our time. The two models presented here for rationalizing the origin of homochirality are as disparate as can be imagined: in one case, an initial small imbalance of enantiomers is shown to propagate through an autocatalytic mechanism that incorporates a means for suppressing production of the minor enantiomer. This "far-from-equilibrium" scenario has been demonstrated by a reaction that is itself not relevant to prebiotic chemistry.

The second case presents a route invoking the distortion between solid and liquid phase compositions for two enantiomers in solvent under thermodynamic equilibrium conditions. Proof of concept demonstrating enhanced solution ee was presented in this case for amino acids in aqueous systems, much more likely chemistry for the prebiotic soup. An advantage of this equilibrium model is that it has time on its side; once the distortion between solid and solution ee is established, eons could pass while the assembly of appropriate molecules occurs, allowing the production of enantioenriched molecules of greater complexity. It is appealing that this problem may have been solved by a combination of physical and reaction chemistry, as is the idea that Nature might have helped engineer the approach to homochirality by manipulation of phase behavior.

In summary, the models presented here provide plausible pathways for the evolution of homochirality in Nature.[§] These investigations were inspired by classical chemistry—the language of molecules, their structures, reactions, and interactions—and may now be combined with studies from fields, such as synthetic biology, complex systems research, and chemical engineering. This new field of "systems chemistry" aims to uncover the chemical roots of biological organization ultimately to enable the engineering of novel biological functions and systems. Building systems that can self-recognize and self-assemble, that can process information, transport material and energy, and undergo reactions, may impact far-ranging applications and future technology in areas including material science, synthetic biology, and pharmaceuticals.

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[‡]Hayashi and coworkers (see previous footnote) were the first to suggest that the greater stability of D:L cocrystals helps rationalize the amplification of solution ee for proline in $CHCl_3$, but their work did not uncover the role of $CHCl_3$ in promoting this stability.

[§]A final comment on the use of the term "homochirality". Coined by Lord Kelvin in 1884, the word was first used as an adjective: "two equal and similar right hands are homochirally similar. Equal and similar left hands are heterochirally similar." (Lord Kelvin, Baltimore Lectures, C.J. Clay and Sons, London, 1904, p. 619). It has been suggested that this word should not be used with the meaning of "enantiomerically pure" (see G. Helmchen in Houben-Weyl, Methods of Organic Chemistry, 4th Ed., vol. E21a, Thieme-Verlag: Stuttgart, 1995.p. 73). It seems in keeping with the spirit of the original definition that l-amino acids and d-sugars may each, as a group, be termed "homochiral". DGB is grateful to Prof. Dr. Dieter Seebach (ETH) for providing the reference to Lord Kelvin's work.

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