Energy Threshold for Chiral Symmetry Breaking in Molecular Self-Replication

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The homochirality of biological molecules – right-handed sugars and left-handed amino acids – is a signature of life. Extensive research has been devoted to understanding how enrichment of enantiomer over the other might have emerged from a prebiotic world. Here we use experimental data from the model Soai autocatalytic reaction system to evaluate the energy required for symmetry breaking and chiral amplification in molecular self-replication. One postulate for the source of the original imbalance is the tiny difference in energy between enantiomers due to parity violation in the weak force. We discuss the plausibility of parity violation energy difference coupled with asymmetric autocatalysis as a rationalization for absolute asymmetric synthesis and the origin of the homochirality of biological molecules. Our results allow us to identify the magnitude of the energy imbalance that gives rise to directed symmetry breaking and asymmetric amplification in this autocatalytic system.

Introduction

The homochirality of biological molecules is a key feature of the informational polymers – RNA, DNA, and proteins – on which life is based. Theoretical models and experimental studies aim to rationalize how one enantiomer of a molecule might have emerged over its mirror image in a prebiotic world, presumably in the absence of a chiral bias. The discovery in 1956 of asymmetry in the weak force[1-3] led to the concept that enantiomeric molecules, long considered to be identical in every respect except that their mirror images are non-superimposable, are in fact minutely different in energy due to parity violation. In analogy to the demonstration that charge parity violation allowed an imbalance between matter and anti-matter, parity violation energy difference (PVED) has been postulated to account for the original imbalance in enantiomeric molecules, leading to the observed biological enrichment towards L-amino acids and D-sugars (a *de lege* hypothesis).[4] This stands in contrast to the proposal that the direction of symmetry breaking may have occurred purely by chance (*de facto*) from random chemical fluctuations. Calculations of the energy difference between enantiomers continue to be refined and currently give estimates on the order of pico- to femtojoule/mol, but an experimental confirmation remains elusive.[5]

For more than six decades, theoretical proposals for the subsequent amplification of an initial imbalance of enantiomers have invoked asymmetric autocatalysis as a key mechanism to rationalize the emergence of biological homochirality.[6] An experimental proof-of-concept was not realized until 1995 with Soai's remarkable demonstration of asymmetric amplification in a reaction catalyzed by its own product.[7] More than two decades later, the Soai reaction remains the sole documented example in which amplification of a small difference in enantiomeric excess in the autocatalytic reaction product can lead asymptotically to a homochiral population.[8] In the current work, we exploit features of the Soai autocatalytic reaction as a model experimental system to provide an evaluation of the energy difference required for symmetry breaking and chiral amplification in molecular self-replication. We

compare this energy difference to the best current theoretical estimates for the energy difference between enantiomers due to parity violation and we discuss implications for asymmetric autocatalysis as a rationalization for absolute asymmetric synthesis and the origin of the homochirality of biological molecules.

Background

The mechanism of the Soai reaction (Figure 1), including the origin of the observed chiral amplification, was first elucidated in the experimental studies and kinetic modeling of Blackmond and Brown[8-11] for the reaction of **1a**. The model invokes stochastic (K = 4) formation of dimeric product species, where homochiral species ($\mathbf{D}^{RR}$ and $\mathbf{D}^{SS}$) serve as catalysts in their own formation and heterochiral ($\mathbf{D}^{RS}$) species form inactive reservoirs. The observed amplification of enantiomeric excess, *ee* (*ee* = (*R*-**2a** – *S*-**2a**)/(*R*-**2a** + *S*-**2a**)), is rationalized by the inactivity of the heterochiral species, which provides the inhibition mechanism required for asymmetric amplification in this irreversible closed system.[6,12,13] This model provides accurate temporal predictions of product *ee* solely from rate data in reactions of **1a** under a wide variety of conditions.[14] Tetramers have also been implicated as active catalysts,[15-19] leading to an identical mathematical model for reactions of substrate **1a**.[20] In more recent work on this reaction, reactions with pyrimidyl aldehyde substrates where R contains alkynyl groups have shown to give stronger asymmetric amplification than R=CH_3. More complex structures and mechanisms may need to be invoked in these cases,[18,20] where the formation of higher order species is biased towards inactive heterochiral species (K > 4). In all cases, however, once symmetry is broken, the enantiomeric excess of product **2a** inexorably approaches homochirality in an asymptotic manner with increasing reaction turnover.

In the absence of added reaction product or other chiral source in the Soai reaction of Figure 1, an uncatalyzed background reaction acts to recruit product molecules to form the dimers that ultimately catalyze their own formation. The outcome in the absence of added

product is expected to be racemic over a large enough set of reactions, although any given reaction is frequently observed to be "tipped" to amplify *ee* randomly in either direction. The stereochemical outcome of the Soai reaction may also be directed by a variety of chiral sources in addition to its own reaction product.[22,23] Intriguingly, Soai has demonstrated that enantiomers of isotopically chiral initiators – molecules exhibiting chirality only by virtue of an isotope such as $^{12}C/^{13}C$ or D/H – are able to direct the reaction faithfully towards opposite enantiomers of the reaction product. [24-28]

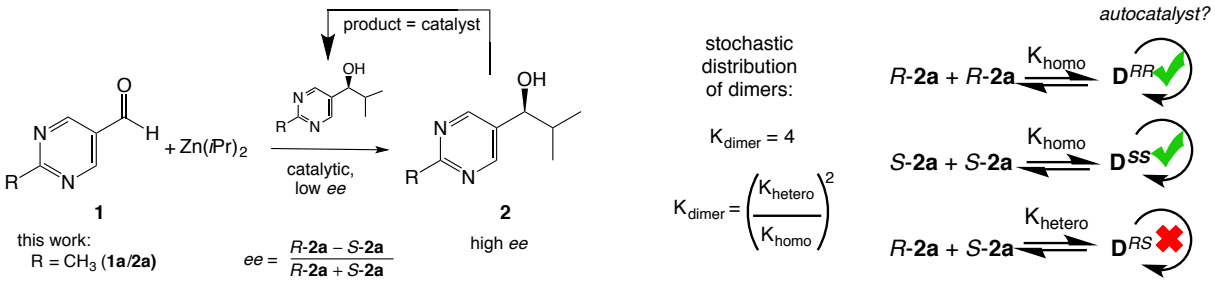

Figure 1. Left: the Soai autocatalytic reaction. Right: kinetic model[8,21] for asymmetric amplification in autocatalysis invoking stochastic formation of homochiral ($\mathbf{D}^{RR}$ and $\mathbf{D}^{SS}$) and heterochiral ($\mathbf{D}^{RS}$) dimers of product **2a**.

We recently reported mechanistic studies to probe the role of isotopically chiral molecule **3** added as an initiator in lieu of the reaction product in the Soai reaction of Scheme 1 to direct stereochemistry in this reaction network.[29] Surprisingly, our studies revealed that the initiator in fact acts as an *inhibitor* of the autocatalytic pathway. We helped to rationalize this behavior by expanding the dimer kinetic model in Figure 1 developed previously for the reaction of **1a** to include the role of the isotopically chiral initiator. Because the extensive experimental kinetic and modeling results available for **1a** provide a comprehensive and consistent mechanistic picture that is well-rationalized by the model of Figure 1 (as may be seen in the comparison to the data in the inset to Figure 2), we chose to carry out our studies using this system. Inhibition in the reaction employing the isotopically chiral initiator occurs when the first formed molecules of the reaction product **2a** arising from the uncatalyzed

background reaction are diverted into diastereomeric product-initiator complexes. We propose that the chiral bias induced by the initiator is caused by a small difference in stability between these complexes, given by K_{major} and K_{minor}, and characterized by the parameter **g** (Figure 2). Our work showed that the calculated difference is exceedingly small. Once this product-initiator binding approaches saturation, further molecules of **2a** produced in the background reaction are free to form the self-dimers that serve as catalysts in the autocatalytic regime. As a consequence of the difference between K_{major} and K_{minor}, a small imbalance in the free enantiomers of product **2a**, which is characterized by α_i, develops just at the point where the autocatalytic regime is instigated. This tiny imbalance gives rise to asymmetric amplification as reaction turnover increases. Thus the net action of the chiral initiator, after siphoning off a quantity of product **2a** into stable product-initiator complexes, is to provide a minute amount of nearly racemic free reaction product **2a** – smaller in quantity and at a lower *ee* value than could be accurately and reproducibly controlled directly by measurement – to "seed" the amplifying autocatalytic pathway. The current work is directed at identifying the magnitude of this imbalance.

The directing influence demonstrated by isotopically chiral **3**, remarkable in its own right, offers the further opportunity to develop a quantitative assessment of the fundamental property of symmetry breaking in autocatalytic self-replication. In our previous investigation, we employed the isotopically chiral initiator in highly enantioenriched form, as did Soai in his studies. We reasoned, however, that if **3** is instead employed at progressively *lower* enantiomeric excess values, its ability to effect symmetry breaking consistently in one direction will be compromised at some threshold initiator *ee* value where the reaction reverts to the stochastic behavior expected in the absence of a chiral directing force (Figure 3). Determination of this threshold initiator *ee* value will allow us to quantify the chiral bias in product **2a** that is required to direct the reaction faithfully towards one product enantiomer above background noise in the reaction of Scheme 1. In the current work, we employ reactions with scalemic

chiral initiator **3** to quantify the threshold reaction product *ee* and energy difference required to break symmetry in this autocatalytic self-replicating system.

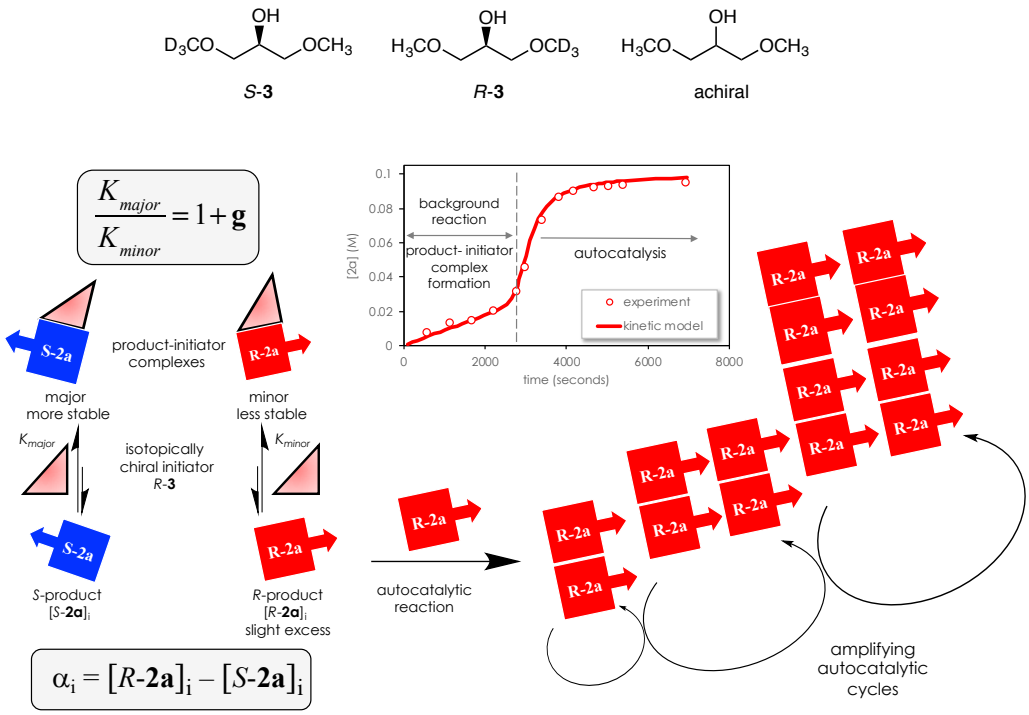

Figure 2. Simplified mechanism of asymmetric amplification in autocatalysis in the presence of chiral initiator *R*-**3** (light red triangles) that forms complexes with reaction product **2a** (blue and red block arrows) produced in the background reaction, defined by equilibrium constants K_{major} and K_{minor}. Slightly higher stability of the *R*-**3**/*S*-**2a** initiator complex (**g**>1) results in a slight excess in free *R*-**2**a ($\alpha_i > 0$) that becomes amplified in the autocatalytic cycles that begin to dominate as the product-initiator complexes saturate. Reactants **1a** and Zn(*i*Pr)$_2$ not shown for clarity. Inset graph compares an experimental kinetic profile (symbols) and kinetic model fit for the reaction under conditions: **1a** (110 mM), Zn(*i*Pr)$_2$ (261 mM), and 11 mM initiator *R*-**3** in toluene at -12 °C.[21,29]

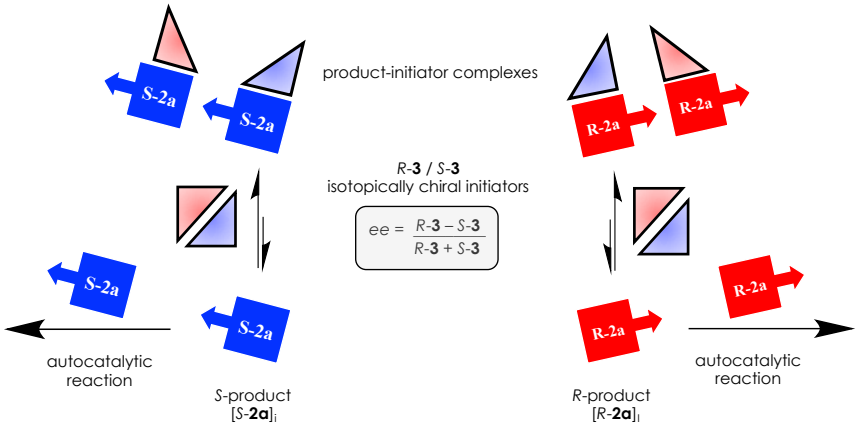

Figure 3. Mechanism from Figure 3 expanded to include both enantiomers of chiral initiator **3** (*R*-**3**, light red triangles; *S*-**3**, light blue triangles) forming complexes with reaction product **2a** (blue and red block arrows). Experiments were carried out at progressively lower *ee* values of **3** until the autocatalytic reaction of **2a** yielded stochastic results.[21]

Results

Reactions of aldehyde **1a** and Zn(*i*Pr)$_2$ were carried out in the presence of isotopically chiral alcohol **3** employed at successively lower *ee* values towards either *S*-**3** or *R*-**3**. Following the protocol first introduced by Soai in his studies, a small quantity of the initiator **3** and aldehyde **1a** are combined in stoichiometric amounts with excess Zn(*i*Pr)$_2$, followed by successive sequential additions of larger amounts of the reactants **1a** and Zn(*i*Pr)$_2$ to increase overall reaction turnover. Such a serial dilution procedure allows a catalytic amount of reaction product to be employed in each reaction in the sequence and helps avoid solubility considerations that can occur at very high concentrations.[30] The initiator **3** is employed only in the first reaction in the sequence. The sense of the enantiomeric excess observed at the end of such a sequence of consecutive reactions is in fact a reporter for the initial and decisive symmetry breaking event, much as PCR methods are used to replicate and amplify a particular segment of DNA.[31] At the point where the *ee* value of the initiator becomes too low to direct the reaction with fidelity, a stochastic result is expected in which the stereochemical outcome over a large number of trials is either racemic or is directed randomly towards one enantiomer or the other. Reactions using the achiral form of the initiator were carried out in identical protocols as a control for stochastic behavior and as a check for influence from cryptochiral impurities.

Table 1 shows the results from multiple trials of sequential reactions of substrate **1a** using initiator **3** prepared as isotopically chiral *R*-**3** (96.5% *ee*), *S*-**3** (87.0% *ee*), and decreasing *ee* values down to 0.1% *ee*. All reactions yielded alcohol **2a** quantitatively upon workup of the reaction mixture. Each hand of the initiator faithfully directs the reaction towards one product, with *S*-**3** giving *S*-**2a** and *R*-**3** giving *R*-**2**a. The isotopically chiral initiator retains its stereochemical influence at 1% *ee* but loses its consistent direction at ca. 0.1% *ee* of **3**. We conclude that the point where symmetry breaking loses its correlation to the handedness of the chiral initiator occurs between 1% and 0.1% *ee* of **3**.

Table 1. Autocatalytic reaction of Scheme 1 with isotopically chiral initiator **3** added in lieu of product **2a**.[a]

ee of initiator 3	sense of stereochemical outcome of 2a
97% (*R*) ↑	↑↑↑↑↑↑↑↑↑↑↑
85% (*S*) ↓	↓↓↓↓↓↓↓↓↓↓
52% (*R*) ↑	↑↑↑↑↑↑↑↑↑↑↑
16% (*R*) ↑	↑↑↑↑↑↑↑↑↑↑↑
5% (*R*) ↑	↑↑↑↑↑↑↑↑↑↑↑↑
1% (*R*) ↑	↑↔↑↑↑
0.16% (*R*) ↑	↓↓↑↓↑↓↓↑↑↑
0.1% (*R*) ↑	↔↑↔↑↔↓↑↑↑↑↓↓
0.1% (*S*) ↓	↑↔↑↑↔↓↓↓↓↓↓↑

[a] Each arrow represents the stereochemical outcome for a given reaction under the sequential reaction protocol. (*R*) = ↑; (*S*) = ↓ denotes the stereochemical sense of the initiator **3** and product **2a**. ↔ denotes a failure to break symmetry (i.e., an outcome measuring lower than 1% ee (*R*-**2a**) or (*S*-**2a**).[21]

To rationalize the results of Table 1, we modified the model we previously developed[8] to describe reactions with isotopically chiral initiator **3** added in lieu of chiral reaction product **2a** and to include binding between initiator **3** and product **2a** formed in the uncatalyzed background reaction. Experimental kinetic profiles of reactions at different initiator **3** concentrations such as that shown in the inset to Figure 2 were fit to the model to determine the kinetic parameters.[29] However, neither this deterministic kinetic model nor high-level DFT calculations of the product-initiator complexes were able to determine accurately the exceedingly small stability difference, **g**, between K_{major} and K_{minor}.[29] We thus turned to stochastic modeling[30] which includes the effects of microscopic fluctuations on the macroscopic behavior of this autocatalytic reaction. The system was modeled using the chemical Langevin equation by incorporating a fluctuation term and a normally distributed white noise process associated with it into each reaction step in the kinetic model. Our treatment closely follows that of Kondepudi and Nelson, who constructed stochastic models of a simplified Frank-type autocatalytic system more than a decade before Soai autocatalysis was reported experimentally.[31-33] At any given time during the reaction, the absolute imbalance between the *R* and *S* products is defined by the parameter α. For each simulation, a series of random processes are generated that lead to random fluctuations in α over the course of the reaction. Repeated simulations result in different reaction trajectories, and analysis of the results over a large number of simulations gives a probability distribution of the final reaction outcome for α under any set of reaction conditions. The term α_{crit} represents the maximum magnitude of these random microscopic fluctuations in α before the system commits decisively to one stereochemical direction.

The value for α_{crit} = ca. 5.8 x 10^{-11} M is determined from stochastic simulations of the reaction network from experiments carried out in the presence of achiral initiator **3**. In reactions employing chiral initiator *S*-**3** or *R*-**3**, if the initial imbalance in product **2a** created by the stability difference (K_{major}/K_{minor} = 1+**g**) is greater than the inherent statistical fluctuations

($\alpha_i > \alpha_{crit}$), symmetry will be broken consistently in favor of the stereochemical outcome dictated by the major enantiomer of the initiator **3**. If the imbalance is less than the critical concentration ($\alpha_i < \alpha_{crit}$), then the reaction will revert to stochastic behavior.

Figure 4 shows the results of stochastic reaction simulations to determine the value of the parameter **g** for which the reaction gives stereochemically directed behavior with initiator **3** at 1% *ee* and stochastic behavior with initiator **3** at 0.1% *ee*. These calculations reveal that values lower than $\mathbf{g} = 1 \times 10^{-7}$ yield stochastic behavior for both values of initiator **3** *ee*, but reactions with 1% *ee* begin to be stereochemically directed by the chirality of the initiator for the increased value of $\mathbf{g}$ = ca. 2×10^{-6}, a miniscule difference of 0.0002% between K_{major} and K_{minor} that helps to explain our difficulty is assessment either by DFT calculations or by deterministic kinetic modeling.

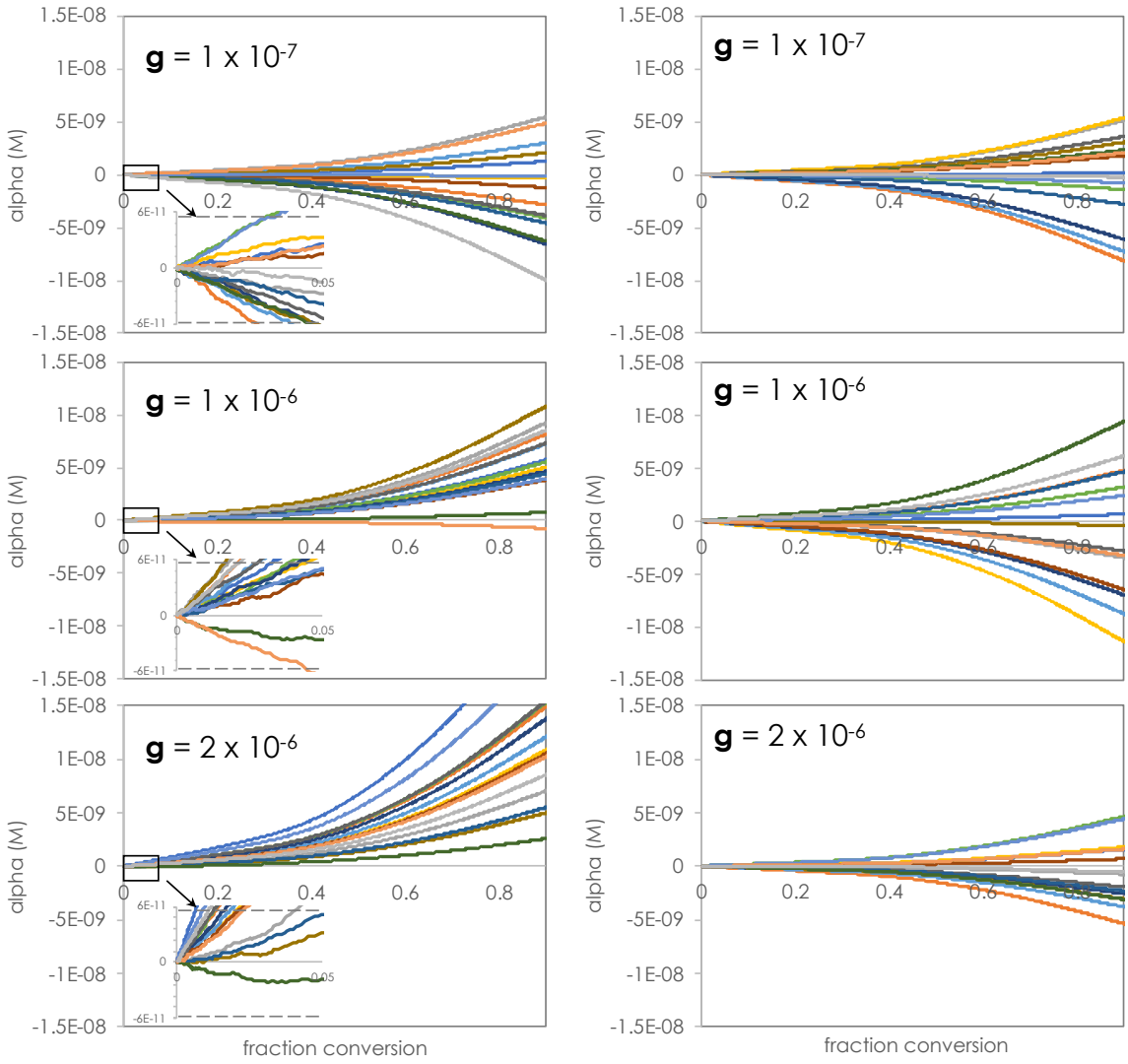

Figure 4. Stochastic simulations of the kinetic model of the Soai reaction in the presence of initiator **3** at 1% *ee* (left) and 0.1% *ee* (right) for different values of **g**. Plots show α vs. fraction conversion for the reaction sequence subsequent to the initial reaction including initiator **3**. Insets in the plots of 1% *ee* **3** expand the region at low conversion, with dashed lines showing the boundaries of α_{crit}. Simulated over 100,000 time steps with 15 out of a total of 40 trajectories shown.[21]

Discussion

The calculated stability difference between the diastereomeric product-initiator complexes coupled with our autocatalytic reaction conditions allow us to estimate that the threshold value for *ee* of reaction product **2a** that is required to break symmetry consistently towards one enantiomer lies between the values of 3.5 x 10^{-7} – 3.5 x 10^{-8} %*ee*. This may be compared to Soai's experimental report[36] that the system began to show inconsistent results when the reaction product was employed at values below 10^{-5} %*ee*, presumably hampered either by inaccuracies in the measurement or by the presence of cryptochiral impurities. It is worth noting that attempts to produce stochastic results in the absence of an added chiral source in the Soai reaction have been notoriously fraught with difficulties attributed to cryptochiral contamination.[37-38] Our indirect measurement of a threshold autocatalyst *ee* value that is two to three orders of magnitude *lower* than that of the most reliable experimental measurement highlights the challenges inherent in accurately and reproducibly carrying out experiments employing such small differences in enantiomer concentrations.[39] Indeed, our calculations imply that a direct experimental measurement of the threshold for symmetry breaking under our conditions would involve an absolute excess enantiomer concentration roughly equivalent to 20 mg of the autocatalyst in an Olympic-size swimming pool.

For the Soai reaction of Scheme 1 under our conditions, we calculate that the energy required to break symmetry with a consistent chiral bias lies between 1.5 x10^{-7} – 1.5 x 10^{-8} kJ/mol. Our work offers the first comparative assessment of symmetry breaking energy based on experimental results, giving a value that is five to seven orders of magnitude *larger* than current best estimates for parity-violation energy difference, PVED, caused by asymmetry in the weak force.[5] This result may appear to cast doubt on whether chiral selection could arise from PVED under such conditions, and indeed previous authors have suggested such a conclusion based purely on theoretical kinetic models.[40] However, our result neither rules out this possibility nor directly contradicts the computational model of Kondepudi and Nelson,[33-

[35] who set conditions under which an autocatalytic network could break symmetry based on PVED. They argue from their reversible Frank-type model that the extreme sensitivity of autocatalysis as the system passes through the critical point in a bifurcating system could permit the dominance of one enantiomer to emerge, even when the level of random noise fluctuation is larger than the critical concentration. They assert that a constant signal, however small, can be detected by a signal averaging process even when embedded within large noise, given enough time. The conditions that they estimate would be required for this process may give pause, however, including geologic time scales (10,000-100,000 years) and homogeneous volumes on the order of large lakes (equaling the volume of more than one thousand perfectly mixed Olympic swimming pools) that enjoy an uninterrupted, well-mixed influx of reactant molecules and no side reactions. Their estimate of the plausibility of PVED in symmetry breaking via autocatalysis thus depends on the validity of a number of assumptions concerning magnitudes of rate constants, racemization rates, diffusive/macroscopic mixing, the availability of temporally increasing reactant concentrations, the reversibility of the reaction, and the absence of intervening chemistry. Our work can add to this discussion in that we have the benefit of results from studying an experimental system to help constrain parameters, which may help to provide context for an autocatalytic reaction scenario invoking PVED in symmetry breaking. What our experimental results tell us is that it is likely, on the time and volume scales of feasible laboratory experiments, this energy difference will be lost in the stochastic noise of any autocatalytic network exhibiting the kinetic features of the Soai reaction. The key conclusion is that any hope of making an experimental observation of PVED-induced symmetry breaking in Soai autocatalysis is thus exceedingly small. While we cast doubt on the plausibility of making an experimental assessment of the role of PVED, it is important to note that our work cannot formally discount the intrinsic energy difference between enantiomers as the original source of macroscopic molecular asymmetry.

The Soai reaction remains the only confirmed case of chiral amplification in autocatalysis, and its chemistry is not amenable to an origin of life scenario. No reactions involving chiral molecules under prebiotically plausible conditions have yet been discovered. In the absence of such a mechanism, the propagation of an imbalance of enantiomers from PVED may be seen as an even more challenging proposition. Further, contrary to early calculations suggesting that the weak neutral force would favor the terrestrially dominant L-amino acids, Quack has concluded from extensive later work that theoretical calculations provide no direct relation between PVED and the hand of the chiral molecule, as would be expected, for example, from the *de lege* postulate for an L-amino acid world.[41,42]

It is important to note that the Soai reaction is itself a singularity, not only in its (to date) unique capacity to amplify *ee* but also in its stunning autocatalytic efficiency and persistence; the irreversible nature of this reaction allows it to exhibit true exponential kinetics rather than the parabolic kinetic profile characteristic of the product inhibition that ultimately suppresses template-directed self-replication in non-enzymatic nucleic acid or peptide replicators as reaction turnover increases.[43-45] *Persistence* is key to the sustained propagation and amplification of a small initial imbalance of enantiomers, a problem related to that of proposed pre-genetic metabolic cycles that must remain stable against inexorable decay from inapposite side reactions.[46,47] Starting from the threshold product *ee* that we determined in the Soai reaction, the gradient for chiral amplification is slow, reaching less than 5% *ee* even after 10 million reaction turnovers. To achieve a virtually homochiral product at 99% ee, the system must persist without losses or diversion for ten billion turnovers. Conceptualizing a prebiotically relevant autocatalytic system with these characteristics of selectivity, efficiency, and persistence under plausible concentration conditions remains an elusive challenge in attempts to rationalize the emergence of biological homochirality.

Conclusions

We have determined the energy required for symmetry breaking and chiral amplification in the Soai autocatalytic reaction as a model experimental system for molecular self-replication. This work examines the likelihood that parity violation energy difference of enantiomers is implicated in the origin of chiral symmetry breaking and highlights the challenges inherent in a search for a prebiotically plausible version of Soai autocatalysis. We may conclude, as has been suggested previously,[48] that the rationalization of biological homochirality might involve not a single reaction or event but rather a series of persistent chemical and physical processes that act synergistically and stepwise in the emergence of life on earth.

Data Availability Statement

All relevant data supporting the findings of this study are available within the paper and its Supplementary Information files. Additional data, including the Mathematica code used in this stochastic modeling, are available upon request from the authors.

References and Notes:

1 Lee, T.D.; Yang, C.N. Question of parity conservation in weak interactions. *Phys. Rev.* **104**, 254–258 (1956). erratum: *Phys. Rev.* **106**, 1371 (1957).

2 Wu, C.S; Ambler, E.; Hayward, R.W.; Hoppes, D.; Hudson, R. Experimental test of parity conservation in beta decay. *Phys. Rev.* **105**, 1413-1415 (1957).

3 Garwin, R.L.; Lederman, L.M.; Weinrich, M. Observations of the failure of conservation of parity and charge conjugation in meson decays: the magnetic moment of the free muon. *Phys. Rev.* **105**, 1415-1417 (1957).

4 Yamagata, Y. A hypothesis for the asymmetric appearance of biomolecules on earth. *J. Theor. Biology* **11**, 495-498 (1966).

5 Quack, M. How important is parity violation for molecular and biomolecular chirality? *Angew. Chemie Int. Ed*. **41**, 4618-4630 (2002).

6 Frank, F.C. On spontaneous asymmetric synthesis. *Biochim. Biophys. Acta* **11**, 459-463 (1953).

7 Soai, K.; Shibata, T.; Morioka, H.; Choji, K. Asymmetric autocatalysis and amplification of enantiomeric excess of a chiral molecule. *Nature* **378**, 767-768 (1995).

8 Blackmond, D.G.; McMillan, C.R.; Ramdeehul, S.; Schorm, A.; Brown, J.M. Origins of asymmetric amplification in autocatalytic alkylzinc additions. *J. Am. Chem. Soc*. **123**, 10103–10104 (2001).

9 Blackmond, D.G. Description of the condition for asymmetric amplification in autocatalytic reactions. *Adv. Synth. Catal.* **344**, 156–158 (2002).

10 Blackmond, D.G. Asymmetric autocatalysis and its implications for the origin of homochirality. *Proc. Natl. Acad. Sci. USA* **101**, 5732–-5736 (2004).

11 Gridnev, I.D.; Serafimov, J.M.; Brown, J.M. Solution structure and reagent binding of the zinc alkoxide catalyst in the Soai asymmetric autocatalytic reaction. *Angew. Chem. Int. Ed.*, **43**, 4884–4887 (2004).

12 Jafarpour, F.; Biancalani, T.; Goldenfeld, N. Noise-Induced Mechanism for Biological Homochirality of Early Life Self-Replicators, *Phys. Rev. Lett.* **115**, 158101 (2015).

13 Stich, M.; Ribó, J.M.; Blackmond, D.G.; Hochberg, D. Necessary conditions for the emergence of homochirality via autocatalytic self-replication. *J. Chem. Phys*. **145**, 074111 (2016).

14 Blackmond, D.G. Mechanistic study of the Soai reaction informed by kinetic analysis. *Tetrahedron: Asymm*. **17**, 584-589 (2006).

15 Buono, F.G.; Blackmond, D.G. Kinetic evidence for a tetrameric transition state in the asymmetric alkylation of pyrimidyl aldehydes. *J. Am. Chem. Soc.* **125**, 8978-8979 (2003).

16 Gridnev, I.D.; Serafimov, J.M.; Quiney, H.; Brown, J.M.; Reflections on spontaneous asymmetric synthesis by amplifying autocatalysis. *Org. Biomol. Chem.* **1**, 3811-3819 (2003).

17 Quaranta, M.; Gehring, T.; Odell, B.; Brown, J.M.; Blackmond, D.G. Unusual inverse temperature dependence on reaction rate in the asymmetric autocatalytic alkylation of pyrimidyl aldehydes. *J. Am. Chem. Soc.* **132**, 15104-15107 (2010).

18 Gridnev, I.D.; Vorobiev, A.Kh. Quantification of sophisticated equilibria in the reaction pool and amplifying catalytic cycle of the Soai reaction. *ACS Catal.*, **2**, 2137-2149 (2012).

19 Matsumoto, A.; Abe, T.; Hara, A.; Tobita, T.; Sasagawa, T.; Kawasaki, T.; Soai, K. Crystal structure of the isopropylzinc alkoxide of pyrimidyl alkanol: mechanistic insights for asymmetric autocatalysis with amplification of enantiomeric excess. *Angew. Chemie Int. Ed.* **54**, 15218-15221 (2015).

20 Schiaffino, L.; Ercolani, E. Amplification of chirality and enantioselectivity in the asymmetric autocatalytic Soai reaction. *ChemPhysChem*, **10**, 2508-2515 (2009).

21 See Supporting Information for details.

22 Shibata, T.; Yamamoto, J.; Matsumoto, N.; Yonekubo, S.; Osanai, S.; Soai, K. Amplification of a slight enantiomeric imbalance in molecules based on asymmetric autocatalysis: the first correlation between high enantiomeric enrichment in a chiral molecule and circularly polarized light. *J. Am. Chem. Soc.* **120**, 12157-12158 (1998).

23 Soai, K.; Osanai, S.; Kadowaki, K.; Yonekubo, S.; Shibata, T.; Sato. I. D- and L-Quartz-promoted highly enantioselective synthesis of a chiral organic compound. *J. Am. Chem. Soc.* **121**, 11235-11236 (1999).

24 Kawasaki, T.; Matsumura, Y.; Tsutsumi, T.; Suzuki, K.; Ito, M.; Soai, K. Asymmetric autocatalysis triggered by carbon isotope (13C/12C). *Science* **324**, 492-495 (2009).

25 Kawasaki, T.; Shimizu, M.; Nishiyama, D.; Ito, M.; Ozawa, H.; Soai, K. Asymmetric autocatalysis induced by meteoritic amino acids with hydrogen isotope chirality. *Chem. Commun.* 4396-4398 (2009).

26 Kawasaki, T.; Okano, Y.; Suzuki, E.; Takano, S.; Oji, S.; Soai, K. Asymmetric autocatalysis triggered by chiral isotopomer arising from oxygen isotope substitution. *Angew. Chemie Int. Ed.* **50**, 8131-8133 (2011).

27 Matsumoto, A.; Ozaki, H.; Harada, S.; Tada, K.; Ayugase, T.; Ozawa, H.; Kawasaki, T.; Soai, K. Asymmetric induction by a nitrogen $^{14}N/^{15}N$ isotopomer in conjunction with asymmetric autocatalysis. *Angew. Chemie Int. Ed.* **55**, 15246-15249 (2016).

28 Sato, I.; Omiya, D.; Saito, T.; Soai, K. Highly enantioselective synthesis induced by chiral primary alcohols due to deuterium substitution. *J. Am. Chem. Soc.* **122**, 11739-11740 (2000).

29 Hawbaker, N.A.; Blackmond, D.G. Rationalization of asymmetric amplification via autocatalysis triggered by isotopically chiral molecules. *ACS Central Sci.* **4**, 776-780 (2018).

30 Buono, F.G.; Iwamura, H.; Blackmond, D.G. Physical and chemical rationalization for asymmetric amplification in autocatalytic reactions. *Angew. Chemie Int. Ed.* **43**, 2099-2103 (2004).

31 Saiki, R.; Scharf, S.; Faloona, F.; Mullis, K.; Horn, G.; Erlich, H.; Arnheim, N. "Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia" *Science* **230** 1350–1354 (1985).

32 Schnoerr, D.; Sanguinetti, G.; Grima, R. Approximation and inference methods for stochastic biochemical kinetics—a tutorial review. *J. Phys. A: Math. and Theoret.* **50**, 093001 (2017).

33 Kondepudi, D.K.; Asakura, A. Chiral autocatalysis, spontaneous symmetry breaking, and stochastic behavior. *Acc. Chem. Res.* **34**, 946-954 (2001).

34 Kondepudi, D.K.; Nelson, G.W. Weak neutral currents and the origin of biomolecular chirality. *Nature* **314**, 438-441 (1985).

35 Kondepudi, D.K.; Nelson, G.W. Chiral symmetry breaking in non-equilibrium systems. *Phys. Rev. Lett.* **50**, 1023-1026 (1983).

36 Sato, I.; Urabe, H.; Ishiguro, S.; Shibata, T.; Soai, K. Amplification of chirality from extremely low to greater than 99.5% ee by asymmetric autocatalysis. *Angew. Chemie Int. Ed.* **42**, 315-317 (2003).

37 Singleton, D.A.; Vo, L.K. Enantioselective synthesis without discrete optically active additives. *J. Am. Chem. Soc.* **124**, 10010-10011 (2002).

38 Singleton, D.A.; Vo, L.K. A few molecules can control the enantiomeric outcome. Evidence supporting absolute asymmetric synthesis using the Soai asymmetric autocatalysis. *Org. Lett.* **5**, 4337-4339 (2003).

39 Maioli, M.; Varadi, G.; Kurdi, R.; Caglioti, L.; Pályi, G. Limits of the classical concept of concentration. *J. Phys. Chem. B*, **120**, 7438-7445 (2016).

40 Lente, G. The effet of prity violation on kinetic models of enantioselective autocatalysis. *Phys. Chem. Chem. Phys.*, **9**, 6134-6141 (2007).

41 Quack, M. On biomolecular homochirality as a quasi-fossil of the evolution of life. *Adv. Chem. Phys.* **157**, John Wiley & Sons, New York 249-290 (2015).

42 Berger, R.; Quack, M. Electroweak quantum chemistry of alanine: parity violation in gas and condensed phases. *ChemPhysChem* **1**, 57-60 (2000).

43 von Kiedrowski, G. A self-replicating hexadeoxynucleotide. *Angew. Chemie Int. Ed. Engl.* **25**, 932-935 (1986).

44 Plöger, T.A.; von Kiedrowski, G. A self-replicating peptide nucleic acid. *Org. Biomol. Chem.* **12**, 6908-6914 (2014).

45 Bag, B.G.; von Kiedrowski, G. Templates, autocatalysis, and molecular replication. *Pure. Appl. Chemistry* **68**, 2145-2152 (1996).

46 Orgel, L.E. The implausibility of metabolic cycles on the prebiotic earth. *PLOS Biology,* **6**, e18, 0005-0013 (2008).

47 Blackmond, D.G. An examination of the role of autocatalytic cycles in the chemistry of proposed primordial reactions. *Angew. Chemie Int. Ed*. **48**, 386-390 (2009).

48 Budin, I.; Szostak, J.W. Expanding roles for diverse physical phenomena during the origin of life. *Annu. Rev. Biophys*. **39**, 245–263 (2010).

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