Rationalization of Asymmetric Amplification via Autocatalysis Triggered by Isotopically Chiral Molecules

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ABSTRACT: Asymmetric amplification induced in the Soai autocatalytic reaction by chiral initiators that are enantiomeric only by virtue of an isotope, e.g. – CH$_3$ vs. CD$_3$ – is examined by spectroscopic, kinetic, and DFT modeling studies to help understand requirements for the emergence of biological homochirality. We find that the initiator inhibits the autocatalytic pathway at the outset of the reaction but ultimately provides the imbalance required for asymmetric amplification. This work provides clues in the ongoing search for prebiotically plausible versions of asymmetric autocatalysis.

The origin of homochirality in the chemical building blocks on which life is based is a question that has long intrigued scientists and laymen alike.$^1$ One of the most compelling proposals is that of asymmetric amplification via autocatalytic reactions, perhaps because such a process represents self-replication at its most basic molecular level and prefigures the self-replication of RNA. Theoretical models for autocatalysis in the emergence of homochirality were first presented in the mid 20$^{th}$ century,$^2$ but experimental proof-of-concept of asymmetric amplification by such a reaction process eluded researchers until Soai’s report of the autocatalytic alkylation of pyrimidyl aldehydes in 1995.$^3$ The reaction product feeds back to catalyze its own formation, and, beginning with a catalytic amount of product of low enantiomeric excess, product enantoienrichment increases with reaction turnover (Scheme 1).

Since the original report, Soai has gone on to demonstrate that the reaction may be initiated and directed with fidelity by a wide array of physical and chemical chiral sources in addition to its own reaction product, including circularly polarized light,$^4$ chiral crystals,$^5$ and most strikingly, isotopically chiral molecules.$^6$ In this latter case, molecules that are enantiomeric only by virtue of an isotope ($^{13}$C, $^2$H, $^{18}$O, and $^{15}$N, see Table 1) are shown to direct enantioselectivity in the Soai autocatalytic reaction with remarkable fidelity.

Table 1. Isotopic Enantiomers Employed as Enantioselective Initiators in the Soai Autocatalytic Reaction.

| Isotope | triggers $S$-pathway | triggers $R$-pathway | ref. |
|---------|----------------------|----------------------|------|
| $^{12}$C/$^{13}$C | H$_3$11COOHOCH$_3$ | H$_3$13COO$^{18}$CH$_3$ | 6 |
| H/D | $^2$H$_2$COOH | $^2$HCOO$^2$H$_2$ | 7 |
| $^{16}$O/$^{18}$O | $^{13}$H$_3$16COOH | $^{13}$H$_3$18COO$^{16}$H$_2$ | 8 |
| $^{14}$N/$^{15}$N | $^{14}$N$_2$ | $^{15}$N$_2$ | 9 |

The Soai reaction remains the sole documented experimental example of asymmetric amplification via autocatalysis. While the chemistry of this singular reaction has little prebiotic significance in its own right, understanding how such an exceedingly small difference between the two enantiomers of isotopically chiral initiators can dictate the stereochemical outcome of the Soai reaction may provide clues to the general requirements for asymmetric amplification via autocatalysis. Together with our knowledge of the Soai reaction mechanism accumulated from extensive experimental study over the past two decades,$^{13-20}$ such investigations may in turn help evaluate the feasibility of asymmetric autocatalysis as a mechanism for the emergence of biological homochirality beyond the specific case of the Soai reaction.

**Scheme 1. Chiral Amplification in the Soai Autocatalytic Reaction.**

![Scheme 1. Chiral Amplification in the Soai Autocatalytic Reaction.](image-url)
We report here the first mechanistic investigation probing the role of isotopically chiral initiators in selectively directing the Soai autocatalytic reaction, using initiator 3, which is chiral by means of methoxy –OCH₃ and –OCD₃ groups, as shown in Scheme 2. In the absence of added reaction product, 3 directs the outcome of the Soai reaction with fidelity as shown in Scheme 2. Kinetic, spectroscopic, and DFT computational studies are employed to identify key species and deconvolute their role in the complex reaction network. We show that diastereomeric 2:1 product:initiator complexes are formed that inhibit the autocatalytic reaction regime at the outset, producing a minute difference in product enantiomeric excess that ultimately enables asymmetric amplification via autocatalysis.

**Scheme 2. Soai Autocatalytic Reaction Initiated by Isotopically Chiral Alcohol 3.**

Initiator 3 offers the unique opportunity to employ both ¹H and ²H NMR spectroscopy to probe simultaneously the methoxy –OCH₃ and –OCD₃ groups of the molecule interacting with Soai reaction components. We investigated interactions between these molecules by titrating initiator 3 with increasing amounts of product 2. Under reaction conditions in the presence of (iPr)₂Zn, both product 2 and initiator 3 alcohols are present as Znalkoxides, denoted as 2' and 3' respectively, and their interactions are shown in Figure 1. ¹H spectra (top panel) and ²H spectra (bottom panel) of initiator S-3' are shown in the presence of 2 equivalents of reaction product S-2' (in blue) and R-2' (in magenta). The initiator S-3' methoxy groups appear between 3.1-3.3 ppm in both ¹H and ²H NMR spectra. In the presence of product 2’, these peaks shift significantly, and the spectra simplify to a single peak at the point where 2 equivalents of product are added.²¹ Shifts of different magnitude for S-3' in the presence of S-2' compared to R-2', along with collapse to a single peak at 2 equiv. product, imply formation of distinct diastereomeric product-initiator species that reach saturation at a 2:1 ratio between product and initiator.

Interestingly, Fig. 1 reveals that the magnitude of chemical shift for 3' is greater for the mismatched (S-3' + R-2') species in the ¹H-NMR spectrum (which monitors the initiator –OCH₃ group), but greater for the matched (S-3' + S-2') species in the ²H-NMR spectrum (which monitors the initiator –OCD₃ group). These differences between matched vs. mismatched interactions in the –OCD₃ and –OCH₃ regions are consistently observed with either hand of initiator 3 and either hand of product 2.²¹

**Figure 1.** Titration of initiator S-3’ with 2 equiv. product R-2 (90% ee, magenta) or S-2 (90% ee, blue) in the presence of 10 equiv. (iPr)₂Zn at 0 °C monitored by ¹H-NMR (top) and ²H-NMR spectroscopy (bottom).²¹

¹H DOSY measurements provide further support for formation of a 2:1 product:initiator complex. DOSY spectra of the species formed at 25 °C from [(iPr)₂Zn] = 92 mM, [S-3] = 8.7 mM and [S-2] = 21 mM in d₈-toluene gave a diffusion coefficient D = 7.20 x 10⁻¹⁰ m².s⁻¹. Applying a calibration curve developed in our previous work for estimating molecular weights of Soai reaction intermediates to our current DOSY measurement for the complex formed at a 2:1 product:initiator ratio yields a prediction that falls within 12% of the molecular weight of a 2:1 product initiator complex.²¹
Plausible structures for putative 2:1 product:initiator matched and mismatched complexes from S-3 may be proposed based on these considerations and on prior studies of Soai reaction structures as well as known dialkylzinc chemistry. We hypothesized that such complexes would contain the stable Zn-O-Zn-O square core that has been observed for many alkylzinc structures, including the square product dimer first proposed by Brown and coworkers as the resting state in this system,\textsuperscript{15,17,18} drawing on the Noyori-Kitamura model for Zn alkylations.\textsuperscript{23} DFT calculations identified the matched (S-3'/S-2'/S-2') and mismatched (S-3'/R-2'/R-2') complexes shown in Figure 2 as lowest energy structures. Since the tetrahedral Zn adds an additional chiral center, 32 diastereomeric structures are possible. These other potential structures and their relative energies are shown in the Supporting Information.\textsuperscript{21}

![Matched and Mismatched Structures](image)

**Figure 2.** Lowest energy structures for 2:1 product:initiator complexes determined by DFT calculations.\textsuperscript{21} Left: matched (S-3'/S-2'/S-2') complex. Right: mismatched (S-3'/R-2'/R-2') complex. Protons are removed for clarity. Ball-and-stick models shown with the CD$_3$ group designated by the green ball. Matched and mismatched designations are defined in Ref. 22.

Figure 2 shows that a “double square” fused Zn-O core incorporates one Zn alkoxide from each of the two product molecules and one from the initiator, with the center oxygen arising from the initiator. The two squares are situated nearly perpendicular to one another. The matched and mismatched structures are not measurably different in energy.\textsuperscript{21} However, the position of the –OCD$_3$ group in the matched complex compared to that in the mismatched complex may now be considered in light of our observation of the different chemical shift for each species in the $^1$H vs. $^2$H NMR spectrum (Figure 1). The relative magnitude of the shift may correlate with the degree of flexibility of the –OCH$_3$ or –OCD$_3$ group in the structure. If a larger shift indicates less flexibility, or closer interaction between initiator and product, then our NMR results predict that the –OCD$_3$ group may be interacting more closely for the matched species while the –OCH$_3$ group is interacting more closely for the mismatched species. This is indeed suggested by the structures in Fig. 2.

The NMR clues about the nature of the interaction between the initiator 3 and the reaction product 2 led us to explore the role of the initiator under reaction conditions, where the initiator is introduced at the outset in lieu of added reaction product, as in Soai’s studies of isotopically chiral initiators.\textsuperscript{5-9} Temporal progress of the reaction of Scheme 2 was monitored by reaction calorimetry in the presence of increasing amounts of initiator S-3.\textsuperscript{21} Figure 3 reveals that in the presence of 3, the reaction exhibits a significant induction period of zero-order kinetics and low reactivity prior to onset of the characteristic autocatalytic profile. In the absence of the initiator, autocatalysis commences within a few minutes; however, the length of the induction period increases with increasing inhibitor concentration, demonstrating that autocatalysis is in fact inhibited by the presence of chiral initiator 3. Figure 3 (bottom) shows that the maximum rate decreases and shifts to higher conversions as inhibitor concentration increases. The longer background reaction period exhibited at higher inhibitor concentration results in greater depletion of the aldehyde concentration driving force prior to the onset of autocatalysis.

![Kinetic Profiles](image)

**Figure 3.** Kinetic profiles of the reaction of Scheme 1 from reaction calorimetry. 110 mM 1, 275 mM (iPr)$_2$Zn in toluene at -12 °C with concentrations of initiator S-3 as shown. Top: fraction conversion vs. time. Bottom: reaction rate vs. fraction conversion. Inset in top plot shows $^1$H spectra of the initiator 3$^+$–OCD$_3$ group at reaction time points noted for 17 mM.\textsuperscript{21}
In the absence of initiator, the reaction produces racemic product 2. Racemic product was also obtained at the end of the reactions using either S-3 or R-3, but, as has been demonstrated by Soai,\textsuperscript{6,12} sequential amplifying cycles subjecting the reaction mixtures to additional reactants consistently produces high (> 95% ee) enantioselectivity (towards S-2 using S-3 and R-2 using R-3) for reactions at all levels of initiator.\textsuperscript{21} Figure 3 also shows \textsuperscript{1}H-NMR spectra taken at three time points during a reaction employing high initiator concentration. Comparison with the \textsuperscript{1}H-NMR spectrum in Figure 1 reveals that the spectrum observed as the autocatalytic regime begins to dominate (point c) resembles that of the initiator-product interaction at 2 equiv. product 2.

The observed delay in the onset of the autocatalytic profile as a function of increasing initiator concentration along with our NMR observations of a 2:1 product:initiator interaction leads us to propose a plausible rationale both for how the initiator inhibits the autocatalytic pathway and how it imparts selectivity in the reaction. Our earlier study of the Soai reaction,\textsuperscript{13} where we developed a modified Kagan ML\textsubscript{2} model\textsuperscript{24,25} for autocatalytic asymmetric amplification, demonstrated that in the case of substrate 1, product 2 formed in the reaction is driven strongly toward stochastic dimer formation. The fact that only the homochiral species D\textsubscript{SS} and D\textsubscript{SS} are active as autocatalysts provides the inhibition mechanism that is key to autocatalytic amplification of enantiomeric excess. Scheme 3 pictorially summarizes the proposed role of the initiator for reactions carried out in the absence of added product 2, overlaid onto a simplified Soai reaction network.

**Scheme 3. Soai Reaction Network with Uncatalyzed Pathway (Green), Initiator-Product Pathway (Magenta), and Autocatalytic Pathway (Blue).**

The fact that higher initiator concentrations cause the autocatalytic regime to be slower to take off, and that autocatalytic behavior appears to be triggered at a 2:1 product:initiator ratio, supports the proposal that molecules of product 2' slowly formed in the background uncatalyzed reaction (green pathway in Scheme 3) react preferentially with the initiator (magenta pathway in Scheme 3) instead of immediately forming self-dimers. After the reaction product has fully saturated the available initiator in forming highly stable matched and mismatched complexes, subsequently formed product 2'' is free to self-react to give the active homochiral dimers D\textsubscript{RR} and D\textsubscript{SS}, which instigate the autocatalytic regime. Scheme 3 rationalizes selectivity in the autocatalytic pathway by suggesting that that a minutely stronger mismatched interaction pulls a slightly greater amount of R-2' out of the active catalyst network and thus induces a slight enantiomeric excess towards product S-2'', allowing the S-2' autocatalytic cycle to dominate.

Soai and coworkers have carried out extensive synthetic studies demonstrating the remarkable fidelity with which \textsuperscript{13}C, \textsuperscript{15}O, \textsuperscript{13}N, and \textsuperscript{1}H isotopically chiral alcohols or amino acids are able to direct enantioselectivity in the Soai autocatalytic reaction.\textsuperscript{6,12} While they did not report mechanistic studies, they proposed that the initiator directly induces an enantiomeric excess in the product of the reaction of the pyrimidyl aldehyde with (Pr)\textsubscript{2}Zn, with this slightly enantioenriched product ultimately allowing amplifying autocatalysis. However, this hypothesis cannot account for the inhibiting role of the initiator observed in Fig. 3 or its strong interaction with product shown in Fig. 1. Our mechanistic studies suggest that the role of the isotopically chiral initiator may not be in acting directly as a chiral catalyst or chiral auxiliary in the Soai reaction itself,\textsuperscript{26} instead, by selectively and strongly binding to chiral product 2', the initiator modulates the concentration and enantiomeric excess of 2' available at the point where the autocatalytic reaction begins to dominate the network. The net result is akin to seeding the reaction with an immeasurable amount of slightly enantioenriched product 2 at that point in time. In support of this proposal, the parameters for the kinetic model developed from a fit to the reaction progress data at different initiator concentrations based on the mechanism in Scheme 3 also successfully predict both rate and temporal enantiomeric excess for reactions carried out with added product 2 as catalyst.\textsuperscript{21}

The fidelity with which the isotopically chiral initiator is able to direct the Soai reaction product is based on two requirements: 1) a minute difference between the stability of the matched and mismatched product-initiator species; and 2) a greater affinity of the product for the initiator than for its self-interaction.

The Soai reaction remains a singular, striking example of amplification of enantiomeric excess in an autocatalytic reaction and a manifestly efficient means of approaching homochirality. The fact that the system can be
directed with fidelity by chiral sources with the slight differences exhibited by isotopically chiral molecules such as 3 is a testament to the sensitivity of this reaction at the point where it enters the autocatalytic regime. In general, our work suggests that for all the myriad different physical and chemical means through which chiral induction has been demonstrated in this reaction, 27 autocatalytic amplification of enantiomeric excess is unleashed in all cases by selective product interrogation that creates a tiny enantiomeric imbalance in the Soai reaction product itself. The identity of the directing force may thus be seen as incidental; the autocatalytic power of the active Soai product species itself accounts for both the efficiency and the remarkable chiral amplification characteristic of this reaction.

Autocatalysis with asymmetric amplification is a far-from-equilibrium reaction system that is defined by two events: 28 i) a symmetry breaking transition that is highly sensitive to small asymmetric influences; and ii) a reaction that exhibits a higher order burst of autocatalytic activity. The first criterion is necessary to obtain selectivity; the second criterion is required for two reasons: first to maintain this selectivity above stochastic noise, and second to propagate the selective pathway at the expense of that of its enantiomer. Our work reveals that the Soai reaction meets these requisites when any given chiral directing force becomes large enough to overcome the stochastic noise level of the racemic background reaction just at the point where the reaction enters its autocatalytic regime. This elegant solution to the question of homochirality will help fuel the ongoing search to discover an autocatalytic reaction that exhibits these features and exemplifies prebiotically plausible chemistry.

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Supporting Information. Details of the experimental protocols, analytical procedures, kinetic and spectroscopic studies, kinetic modeling, DFT calculations.

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Synopsis. Isotopically chiral initiators inhibit the autocatalytic pathway of the Soai reaction at the outset. Amplifying autocatalysis is triggered upon saturation of a 2:1 product:initiator complex.