

Thermodynamic control of asymmetric amplification in amino acid catalysis

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Ever since Pasteur noticed that tartrate crystals exist in two non-superimposable forms that are mirror images of one another—are left and right hands—the phenomenon of chirality has intrigued scientists. On the molecular level, chirality often has a profound impact on recognition and interaction events and is thus important to biochemistry and pharmacology. In chemical synthesis, much effort has been directed towards developing asymmetric synthesis strategies that yield product molecules with a significant excess of either the left-handed or right-handed enantiomer. This is usually achieved by making use of chiral auxiliaries or catalysts that influence the course of a reaction, with the enantiomeric excess (ee) of the product linearly related to the ee of the auxiliary or catalyst used. In recent years, however, an increasing number of asymmetric reactions have been documented where this relationship is nonlinear¹, an effect that can lead to asymmetric amplification. Theoretical models^{2,3} have long suggested that autocatalytic processes can result in kinetically controlled asymmetric amplification, a prediction that has now been verified experimentally^{4–6} and rationalized mechanistically^{7–14} for an autocatalytic alkylation reaction. Here we show an alternative mechanism that gives rise to asymmetric amplification based on the equilibrium solid-liquid phase behaviour of amino acids in solution. This amplification mechanism is robust and can operate in aqueous systems, making it an appealing proposition for explaining one of the most tantalizing examples of asymmetric amplification—the development of high enantiomeric excess in biomolecules from a presumably racemic prebiotic world.

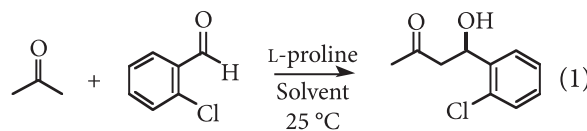
Recent investigations have rekindled interest in exploring nonlinear effects in amino acid catalysis^{15,16} despite experimental and theoretical work¹⁷ that has effectively discounted earlier reports¹⁸ of nonlinear behaviour in these systems. Our measurements on the proline-catalysed aldol reaction (1) (see Supplementary Information for experimental details) reveal behaviour more complex than was found in either the earlier or later work: two regimes exist with different relationships between the ee of the proline catalyst and the ee of the aldol product. If the reaction is carried out using proline concentrations low enough that the catalyst is fully dissolved, the relationship is linear (Fig. 1a, squares). When using higher catalyst concentrations, so that dissolved proline is in equilibrium with solid proline, the product ee is largely independent of proline ee (Fig. 1a, circles). This latter, unusual relationship cannot be explained by any of the existing models for nonlinear effects in asymmetric catalysis¹⁹. However, this phenomenon mirrors the trend in the ee of dissolved proline as a function of total proline ee (Fig. 1b), with the proline solution ee found to remain constant at about 50% ee even though the total proline ee is varied from near racemic to near enantiopure.

The solid-solution phase behaviour of enantiomers and their

racemates was the focus of extensive study at the turn of the last century²⁰ and is generally well-understood²¹, but non-enantiopure, non-racemic mixtures (particularly those of free amino acids) have received less attention. We therefore explored the phase behaviour of proline and found that when the two enantiomers (L and D) of proline are present in unequal proportions above their solubility limit in dimethyl sulphoxide (DMSO), two separate solid phases are formed at equilibrium. Fourier-transform infrared (FTIR) spectroscopic analysis of the solids confirmed that these are a racemic compound (that is, crystals in which L:D = 1:1), and an enantiopure solid of the excess enantiomer. The ternary phase diagram for the system D-proline/L-proline/DMSO constructed from our data is shown in Fig. 2, where pure DMSO is represented at the apex, and pure D and L proline at the left and right vertices, respectively.

The low solubility of proline means that the part of the diagram that is of interest for our solution phase system is located very near to the apex, and this portion is expanded in the main part of Fig. 2. The diagram is symmetric: racemic mixtures are represented along the vertical line intersecting the DMSO vertex, and the phase behaviour of D-proline to the left side of this line reflects the L-proline phase behaviour on the right. The phase rule dictates that at constant temperature the composition of a solution of proline in equilibrium with the two solid phases is fixed, and this composition is given in Fig. 2 by the eutectic at E' (or E). Only proline compositions with very low ee (where the excess of the major enantiomer is too low to establish its solid phase) or very high ee (where the minor enantiomer concentration is insufficient to form the solid racemic compound) will show a variation of solution ee with overall proline ee, dictated by the lines E'R (or ER) and E'A' (or EA), respectively, in Fig. 2.

For proline in DMSO at 25 °C, the eutectic composition corresponds to a solution enantiomeric excess of about 50% ee. Any saturated solution of scalemic proline in equilibrium with a sufficient excess of solid will exhibit this ee, regardless of the overall ee of the proline employed. Given that the proline-catalysed aldol reaction (1) occurs in the solution phase, this finding readily explains the relationships between proline ee and the product ee illustrated in Fig. 1a; that is, the ee of the aldol product appears to depend linearly on the ee of the accessible proline catalyst.



Phase diagrams such as the one shown in Fig. 2 are useful for optimizing crystallization conditions, where the aim is typically the production of a solid of high enantiopurity by depleting the solution

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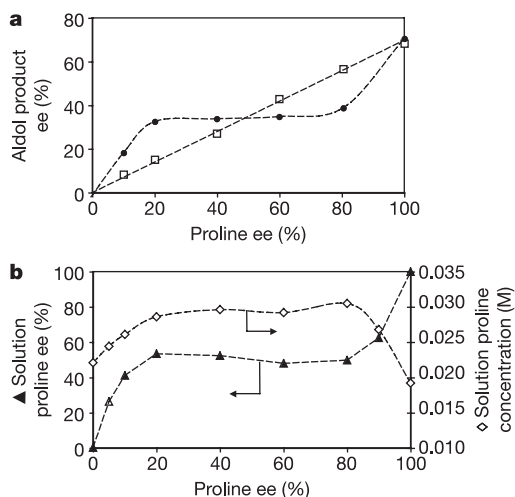


Figure 1 | Reaction and solution behaviour as a function of the overall proline enantiomeric excess. **a**, Product enantiomeric excess versus proline enantiomeric excess for the aldol reaction of equation (1). Squares, proline concentration 0.025 M; circles, total proline concentration 0.1 M (corresponding to ~4 mol% solution proline). **b**, Solution proline enantiomeric excess (left axis, triangles) and solution proline concentration (right axis, diamonds) as a function of the overall enantiomeric excess for proline at 0.1 M; all experiments in DMSO with 0.8 wt% H₂O at 25 °C.

enantiomeric excess. However, our findings suggest that aiming for the opposite effect—high solution enantiomeric excess and lower solid ee—provides a means of realizing asymmetric amplification in solution. This prompted us to examine the phase behaviour of a number of amino acids, to explore their potential for higher asymmetric amplification than is possible with proline and its eutectic positioned at about 50% ee. Although all but two of the twenty proteinogenic amino acids are known to form racemic compounds (crystals with a 1:1 ratio of the D and L enantiomers) and not conglomerates (a mixture of pure D and pure L crystals), we were unable to find literature values for the eutectic composition of any ternary phase system of free amino acids that form racemic

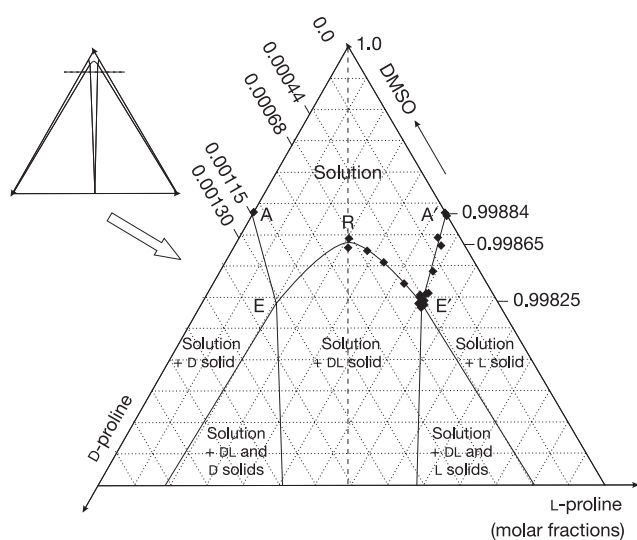


Figure 2 | Ternary phase diagram of D-proline, L-proline and DMSO at 25 °C. The main figure expands the area above the dashed line in the inset (not to scale). Saturated solutions exist along the line A'E'R and AER. E and E' represent the eutectic mixture composition where three phases (solid enantiopure proline, solid racemic compound, and liquid phase) exist in equilibrium.

Table 1 | Solution enantiomeric excess at the eutectic point in water at 25 °C for selected amino acids

Amino acid	ee of solution at eutectic (%)	Amino acid	ee of solution at eutectic (%)
Threonine	0	Methionine	85
Valine	46	Leucine	87
Alanine	60	Histidine	93
Phenylalanine	83	Serine	>99

compounds in water or other solvent. The two amino acids that form conglomerates, threonine²² and arginine, exhibit 0% ee at the eutectic. Our own measurements collated in Table 1 reveal that several of the proteinogenic amino acids that form racemic compounds in fact exhibit high eutectic ee values. Serine, with its eutectic at >99% ee, provides a virtually enantiopure solution from a nearly racemic sample under solid–liquid equilibrium conditions.

Prediction of enantioselectivity in solution catalysis from knowledge of these eutectics was confirmed in the results of the aldol reaction of equation (1) carried out using instead of L-proline several of the amino acids listed in Table 1 at varying levels of enantiopurity, with three-phase equilibrium established before reaction. Figure 3 shows how the product enantiomeric excesses obtained with catalysts of varying ee values compare with the product ee that is obtained when using enantiopure amino acid catalyst. The data indicate that the position of the eutectic dictates the product selectivity for this amino-acid-catalysed transformation. Product enantioselectivities for aldol reactions carried out in aqueous media using these amino acid catalysts were also found to correlate well with the position of the eutectic, although yields and chemo- and enantioselectivities were in general lower (see Supplementary Information). As expected from the eutectic values listed in Table 1, serine offers the most significant chiral amplification: nearly racemic (1% ee) serine gives a product enantioselectivity virtually indistinguishable from that obtained using enantiopure serine.

Here we have established that the coupling of solid–liquid phase behaviour and amino acid catalysis results in an efficient and robust mechanism for asymmetric amplification. This finding suggests that amino acids may have played a role in the evolution of biomolecular

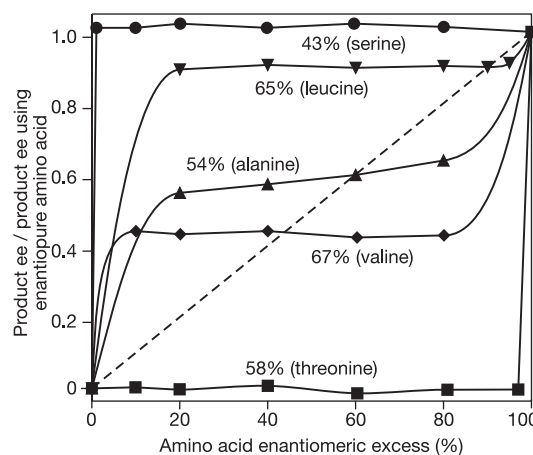


Figure 3 | Nonlinear effects in an amino-acid mediated aldol reaction. Product enantiomeric excess expressed as a fraction of that obtained using enantiopure amino acid versus amino acid enantiomeric excess in the aldol reaction of equation (1) carried out in *N,N*-dimethylformamide (DMF) at ambient temperature using serine (circles), leucine (down triangles), alanine (up triangles), valine (diamonds), and threonine (squares). Percentage product ee values found for the enantiopure catalysts are given.

homochirality²³. One can easily imagine the coexistence of solid and dissolved amino acids under a range of environmental conditions in a prebiotic landscape, and high ee could then evolve from an initially small imbalance in ee if (1) the amino acid forms a racemic compound rather than a conglomerate; (2) the amino acid exhibits high enantiopurity at its eutectic; and (3) the amino acid acts with high selectivity as an asymmetric catalyst in solution phase transformations. In fact, our studies show that serine meets the first two criteria, and recent work²⁴ has demonstrated that serine catalyses certain asymmetric aldol reactions with high enantioselectivity; it has also been highlighted as one of a small number of amino acids likely to have played an important role in prebiotic chemistry^{25,26}.

A scenario for homochiral evolution based on our amplification mechanism was hinted at some years ago²⁷, and it also shares some similarities with the suggestion that chiral enrichment may result from the selective dissolution of only one (enantiopure) crystal of a conglomerate in a microenvironment²⁸, but seems ultimately much simpler. A particularly appealing feature of the scenario we outline here is that it is based on an equilibrium mechanism, in contrast to the far-from-equilibrium environments invoked in kinetically induced amplification via autocatalytic reactions or crystallizations^{29,30}. Finally, we note that further enhancements in solution-phase enantiomeric excess may be discovered in more complex multi-component systems with a greater number of equilibrium solid and liquid phases. This possibility and the influence of interactions between different amino acids and between amino acids and other molecules such as sugars is the subject of ongoing investigations by our group, as is the search for high selectivity in amino-acid-catalysed reactions of potential biological relevance.

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- Girard, C. & Kagan, H. B. Nonlinear effects in asymmetric synthesis and stereoselective reactions: ten years of investigation. *Angew. Chem. Int. Edn* **37**, 2923–2959 (1998).
- Frank, F. C. Spontaneous asymmetric synthesis. *Biochim. Biophys. Acta* **11**, 459–463 (1953).
- Calvin, M. *Molecular Evolution* (Oxford Univ. Press, Oxford, UK, 1969).
- Shibata, T., Morioka, H., Hayase, T., Choji, K. & Soai, K. Highly enantioselective catalytic asymmetric automultiplication of chiral pyrimidyl alcohol. *J. Am. Chem. Soc.* **118**, 471–472 (1996).
- Shibata, T., Choji, K., Hayase, T., Aizu, Y. & Soai, K. Asymmetric autocatalytic reaction of 3-quinolylalkanol with amplification of enantiomeric excess. *Chem. Commun.*, 1235–1236 (1996).
- Soai, K., Shibata, T., Morioka, H. & Choji, K. Asymmetric autocatalysis and amplification of enantiomeric excess of a chiral molecule. *Nature* **378**, 767–768 (1995).
- 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).
- Blackmond, D. G. Description of the condition for asymmetric amplification in autocatalytic reactions. *Adv. Synth. Catal.* **344**, 156–158 (2002).
- Buono, F. G. & Blackmond, D. G. Kinetic evidence for a tetrameric transition state in the asymmetric autocatalytic alkylation of pyrimidyl aldehydes. *J. Am. Chem. Soc.* **125**, 8978–8979 (2003).
- Buono, F. G., Iwamura, H. & Blackmond, D. G. Physical and chemical rationalization for asymmetric amplification in autocatalytic reactions. *Angew. Chem. Int. Edn* **43**, 2099–2103 (2004).
- Blackmond, D. G. Asymmetric autocatalysis and its implications for the origin of homochirality. *Proc. Natl. Acad. Sci. USA* **101**, 5732–5736 (2004).
- Gridnev, I. D. & Brown, J. M. Asymmetric autocatalysis: novel structures, novel mechanism? *Proc. Natl. Acad. Sci. USA* **101**, 5727–5731 (2004).
- 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. Edn* **43**, 4884–4887 (2004).
- 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).
- Mathew, S. P., Iwamura, H. & Blackmond, D. G. Amplification of enantiomeric excess in a proline-mediated reaction. *Angew. Chem. Int. Edn* **43**, 3317–3321 (2004).
- Iwamura, H., Mathew, S. P. & Blackmond, D. G. In situ catalyst improvement in the proline-mediated α -amination of aldehydes. *J. Am. Chem. Soc.* **126**, 11770–11771 (2004).
- Hoang, L., Bahmanyar, S., Houk, K. N. & List, B. Kinetic and stereochemical evidence for the involvement of only one proline molecule in the transition states of proline-catalyzed intra- and intermolecular aldol reactions. *J. Am. Chem. Soc.* **125**, 16–17 (2003).
- Agami, C. Mechanism of the proline-catalyzed enantioselective aldol reaction. Recent advances. *Bull. Soc. Chim. Fr.*(3), 499–507 (1988).
- Blackmond, D. G. Kinetic aspects of nonlinear effects in asymmetric catalysis. *Acc. Chem. Res.* **33**, 402–411 (2000).
- Roozboom, H. W. B. Solubility and melting-point as criteria for racemate compounds, pseudoracemic mix-crystals and inactive conglomerates. *Z. Phys. Chem. Stoechiometrie Verwandtschaftslehre* **28**, 494–517 (1899).
- Jacques, J., Collet, A. & Wilen, S. H. *Enantiomers, Racemates and Resolution* Ch. 3 (John Wiley, New York, 1981).
- Rodrigo, A. A., Lorenz, H. & Seidel-Morgenstern, A. Online monitoring of preferential crystallization of enantiomers. *Chirality* **16**, 499–508 (2004).
- Podlech, J. Origin of organic molecules and biomolecular homochirality. *Cell. Mol. Life Sci.* **58**, 44–60 (2001).
- Cordova, A. et al. Acyclic amino acid-catalyzed direct asymmetric aldol reactions: alanine, the simplest stereoselective organocatalyst. *Chem. Commun.* 3586 (2005).
- Borsenberger, V. et al. Exploratory studies to investigate a linked prebiotic origin of RNA and coded peptides. *Chem. Biodivers.* **1**, 203–246 (2004).
- Takats, Z., Nanita, S. C. & Cooks, R. G. Serine octamer reactions: indicators of prebiotic relevance. *Angew. Chem. Int. Edn* **42**, 3521–3523 (2003).
- Morowitz, H. J. A mechanism for the amplification of fluctuations in racemic mixtures. *J. Theor. Biol.* **25**, 491 (1969).
- Welch, C. J. Formation of highly enantioenriched microenvironments by stochastic sorting of conglomerate crystals: a plausible mechanism for generation of enantioenrichment on the prebiotic earth. *Chirality* **13**, 425–427 (2001).
- Kondepudi, D. K. & Asakura, K. Chiral autocatalysis, spontaneous symmetry breaking, and stochastic behavior. *Acc. Chem. Res.* **34**, 946–954 (2001).
- Kondepudi, D. K., Kaufman, R. J. & Singh, N. Chiral symmetry breaking in sodium chlorate crystallization. *Science* **250**, 975–977 (1990).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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