Enantiomeric Enrichment

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Large Nonlinear Effect Observed in the Enantiomeric Excess of Proline in Solution and That in the Solid State**

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How homochiral amino acids and sugars arose out of a presumably prochiral prebiotic environment is a puzzling question,[1] to which many answers have been proposed: for example, absolute asymmetric synthesis with circularly polarized light, photoreactions in chiral crystals, and asymmetric automultiplication with asymmetric autocatalysis, as exemplified by the recent work of Soai et al.[2] There are reports describing a connection between the chirality of amino acids and sugars: Amplification of *ee* was observed in the α-aminoxylation of an aldehyde using proline as catalyst to generate a key intermediate of sugars,[3] while amino acids

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have been proposed as asymmetric catalysts for the synthesis of sugars.[4] Recently, Cordova et al. demonstrated the synthesis of a hexose (55% *ee*) from proline with low *ee* (20% *ee*), with a nonlinear effect.[5] Herein, we demonstrate experimentally a connection between an amino acid with low *ee* (10% *ee*) and a chiral sugar intermediate of high enantiomeric purity (96% *ee*).

Amino acids promote several organic transformations, and proline, a widely distributed amino acid, is one of the most effective organic catalysts.[6] In 1971, Hajos and Parrish, as well as Eder et al., reported an intramolecular aldol reaction catalyzed by proline.[7] Following the observation of the intermolecular version of this reaction by List, Lerner, and Barbas in 2000,[8] other highly enantioselective catalytic reactions using proline have been developed, including aldol[9] and Mannich[10] reactions, and α-amination[11] and α-aminoxylation[12] of carbonyl compounds.

We have observed that a solution of proline with high *ee* can be obtained from solid proline of low optical purity during the dissolution process. As proline is only very sparingly soluble in pure $CHCl_3$, and as the addition of EtOH increases its solubility therein, we employed $CHCl_3$ containing 1% EtOH as solvent. $CHCl_3$ stabilized with amylene (*not* with EtOH) was distilled from CaH_2 before use, and then EtOH was added (1%). At first, reproducibility of the solubility was poor. After several experiments, both the surface and the particle size of the solid proline were found to be important. Proline that had been recrystallized from EtOH and ground with a mortar under an Ar atmosphere was employed.

The experiment using 10% *ee* L-rich proline was performed as follows: $CHCl_3$ (20 mL) containing 1% EtOH was added to a mixture of L-proline (550 mg) and D-proline (450 mg; 10% *ee* combined) at 0°C, and the suspension was stirred for 24 h at this temperature under an Ar atmosphere. After filtration of the insoluble proline, a solution (17–19 mL) containing proline (40–65 mg) was obtained, the *ee* value of which was very high (85–99% *ee*). It is particularly unusual that a proline solution with very high *ee* was obtained from proline of low *ee*; this phenomenon is not observed in other solvents such as EtOH and dimethyl sulfoxide.

A solution of proline with very high *ee* (97–99% *ee*) was also obtained from proline with even lower *ee* (1.0% *ee*). Even by using nearly racemic proline (< 0.4% *ee*), which was prepared from L-proline (250 ± 1 mg) and D-proline (250 ± 1 mg), the optical purity of proline in solution was found to be very high. With the exception of phenylalanine (Phe), we were unable to demonstrate any enrichment of *ee* in $CHCl_3$ solution with other amino acids (Val, Met, and Tyr) because of their extremely low solubility in that solvent. In the case of Phe, although it is only very sparingly soluble in $CHCl_3$, a solution of Phe with 10% *ee* was obtained from a solid of 40% *ee*. This observation also indicates that there is something special in the dissolution of proline.

To clarify the reason for these phenomena, the crystal structures of L-proline[13] and DL-proline,[14] recrystallized from EtOH, were analyzed (Figure 1, Figure 2). The powder X-ray diffraction (XRD) method was used to confirm that the crystal forms of the powdered L- and DL-prolines were the same as those of the corresponding single crystals. Both

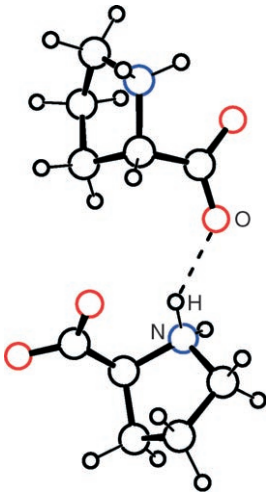


Figure 1. Hydrogen-bonded dimer structure formed between the columns in a crystal of L-proline.[13]

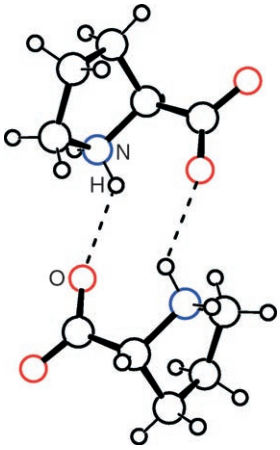


Figure 2. Hydrogen-bonded dimer structure formed between the columns in a crystal of DL-proline.

crystals have very similar structural motifs, forming a column structure with N–H···O hydrogen bonds. However, the joining modes of these columns are quite different: In the chiral crystal, the columns align antiparallel and each molecule in the column connects with those in two neighboring columns through hydrogen bonds to form a 2D sheet structure (Figure 3). On the other hand, in the racemic crystal each molecule in the column forms two centrosymmetric hydrogen bonds with the nearest neighboring enantiomeric column to form a ladder structure (Figure 4). These ladders are linked by rather weak C–H···O interactions. As the heterochiral molecules bind together with two hydrogen bonds, their association is energetically preferable to that of homochiral molecules, and this causes racemic crystals to be much less soluble than those of the pure enantiomers (0.01–0.09 $g\,L^{-1}$ versus 6.1–6.3 $g\,L^{-1}$ at 0°C in $CHCl_3$ containing 1% EtOH). Therefore, crystals of DL-proline precipitate preferably from a mixture of enantiomers in solution.

To shed more light on the reaction mechanism, the following experiments were performed: 1) $CHCl_3$ (100 mL)

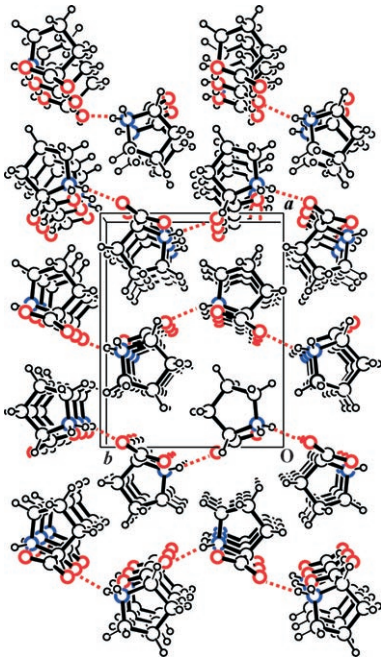


Figure 3. Crystal structure of L-proline.[13] O red, N blue, C black (large), H black (small).

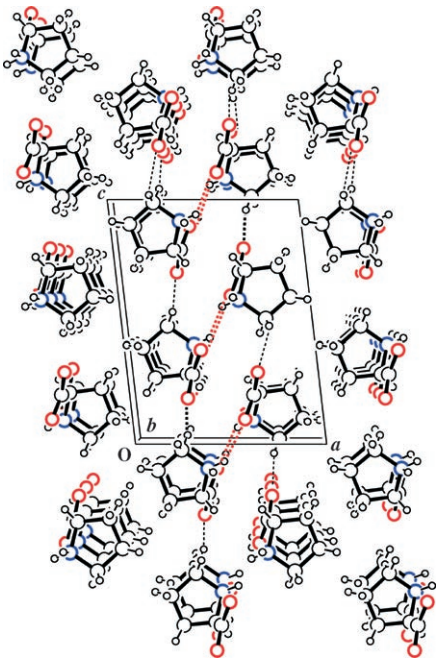


Figure 4. Crystal structure of DL-proline. O red, N blue, C black (large), H black (small).

and EtOH (1 mL) were added to a mixture of L-proline (2.25 g) and D-proline (2.75 g; total 10% *ee* D-rich), and the reaction mixture was stirred for a certain time. 2) D-proline (2.75 g, total 10% *ee* D-rich) was added to a $CHCl_3$/EtOH suspension (100 mL:1 mL) of L-proline (2.25 g) at 0 °C, and the reaction mixture was stirred for a certain time. In these two reactions, the *ee* of proline in solution was measured while the content of DL-proline in the solid was analyzed by XRD (the results are summarized in Table 1 and Table 2, respectively). Both experiments show an increase in DL-proline content of the solid with reaction time. After 12 h,

Table 1: The effect of time on the *ee* of proline in solution and on the content of the DL isomer in the solid.[a]

Entry	*t* [h]	Solubility [mg mL^{-1}][b]	*ee* [%][c]	Content [%][d]
1	0.17	2.9	7[e]	7
2	1	0.3	57[e]	46
3	6	1.3	96[e]	77
4	12	3.5	95[e]	84

[a] $CHCl_3$ containing 1% EtOH was added to a mixture of L- and D-proline; see text for details. [b] Solubility of proline in $CHCl_3$/EtOH (100:1). [c] *ee* of proline in solution. [d] Content of DL-proline in solid. [e] D-enantiomer rich.

Table 2: The effect of time on the *ee* of proline in solution and on the content of the DL isomer in the solid.[a]

Entry	*t* [h]	Solubility [mg mL^{-1}][b]	*ee* [%][c]	Content [%][d]
1	0	6.7	−100[e]	0
2	0.17	3.9	76[f]	33
3	1	0.65	68[f]	54
4	6	2.6	90[f]	83
5	12	3.7	92[f]	92

[a] D-proline was added to a suspension of L-proline in $CHCl_3$ containing 1% EtOH; see text for details. [b] Solubility of proline in $CHCl_3$/EtOH (100:1). [c] *ee* of proline in solution. [d] Content of DL-proline in solid. [e] L-enantiomer rich. [f] D-enantiomer rich.

most of the L- and D-proline had been converted into DL-proline and the *ee* of proline in solution was very high (>90% *ee*). In the second experiment, the proline isomer in excess in solution changed from the L to the D enantiomer within 1 h, with the formation of solid DL-proline. Thus, the highly selective dissolution of one enantiomer of proline is caused not by a simple extraction of the excess enantiomer but by the following dissolution and crystallization mechanism: Soluble L- and D-prolines dissolve in the solvent, and the less-soluble racemic DL-proline, but not a conglomerate, precipitates.

The dissolution and crystallization process is also different from that of standard recrystallization. Although the recrystallization of proline from EtOH or from EtOH/Et_2O[15] has been known for a long time, there are no reports describing a change in the *ee* value of proline by recrystallization, either in solution or in the solid state. We thus examined the recrystallization of proline (10% *ee*) in different solvents and analyzed the *ee* of proline in the filtrate (Table 3). $CHCl_3$ could not be used as solvent owing to the low solubility of proline in this solvent alone. No increase in *ee* was observed in H_2O. Though a substantial increase in *ee* was observed in EtOH or in *i*PrOH and water (1:1), it was not so large relative to that observed with $CHCl_3$ containing 1% EtOH.

For some compounds, homochiral crystals have been obtained from solutions of low *ee* by the preferential

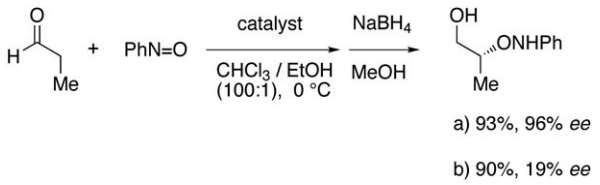
Table 3: The effect of solvent on the *ee* of proline in solution through recrystallization.[a]

Entry	Solvent	*ee* [%]
1	H_2O	14
2	EtOH	43
3	*i*PrOH/H_2O (1:1)	39

[a] Proline (10% *ee*) was recrystallized from the indicated solvent, and the *ee* of proline in the filtrate was determined.

crystallization of conglomerates,[16] a process that is distinct from the present phenomena in which a solution with very high *ee* is obtained from a solid of low *ee*. Though several examples are known for which a chiral crystal is more soluble than the corresponding racemic one, there is only one report describing the enrichment of an enantiomer in solution during recrystallization, a special case in which polymorphic transformation occurs during crystallization.[17] In the present case, a solution of proline with very high *ee* is obtained from a solid of low *ee* during the processes of dissolution and crystallization, a mechanism which is completely different to that of any other known resolution.

As optically pure proline is known to promote several asymmetric reactions, one possible application of the solution of proline of high *ee* obtained from proline solid of low *ee* is α-aminoxylation of propanal.[12a,b,c] As shown in Scheme 1, the



Scheme 1. α-Aminoxylation of propanal using proline as catalyst. A solution of proline prepared from solid proline (10% *ee*) used a) after filtration gave the product with 96% *ee* and b) without filtration gave the product with only 19% *ee*.

product was obtained in 96% *ee*, demonstrating that this proline solution can be applied to other useful asymmetric transformations. When proline with 10% *ee* was employed in α-aminoxylation without filtration, the *ee* of the product was low (19% *ee*) and a slight nonlinear effect was observed, as reported by Blackmond and co-workers.[3] This slight increase in *ee* can be explained in some part as follows: The initial *ee* of proline in solution is very high, but as the reaction proceeds the *ee* of proline in solution begins to decrease because the generated product acts as a polarized solvent to bring both D- and L-proline from the solid into the organic phase. That is, while the *ee* of proline in the initial solution is extremely high, this *ee* in solution decreases as the reaction progresses owing to the increased solubility of both L- and D-proline in the mixture of solvent, propanal, and forming products.[18,19]

The fact that a solution of proline of high *ee* was obtained from solid proline with low *ee* may be involved in the origin of chirality on Earth. Proline has been used as the catalyst in a short synthesis of sugars[4,5] and in the synthesis of α-hydroxyaldehydes, key molecules for sugar synthesis, by reaction of aldehydes with molecular oxygen under photoirradiation—plausible prebiotic conditions.[20] As an enantiomerically enriched solution of proline might be separated from solid proline by filtration through strata, one can speculate that in the prebiotic era a similar mechanism involving proline of very low *ee* was involved in the generation of other biologically important homochiral organic molecules.

Some amino acids have been found in meteorites with significant enantiomeric excess.[21] Though proline is scarcely present in meteorites, it was found in the room-temperature residue of an interstellar ice analogue that had been irradiated with UV light under high vacuum at 12 K, which indicates that proline may have been produced in the prebiotic era.[22] Therefore, it seems possible that asymmetric photolysis in interstellar clouds may produce optically active proline. Under certain circumstances the imbalance thus generated could be amplified into an optically enriched solution of proline by selective dissolution. Such a solution can promote many organic transformations and generate important intermediates of sugar synthesis with very high optical purity, as demonstrated in the present report. Though the present reaction is only successful under certain limited conditions, it may indicate a possible mechanism by which an amino acid of low *ee* generated homochirality in biologically important organic molecules in the prebiotic environment.[23]

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[23] *Note added in proof (June 12, 2006)*: Since this manuscript was accepted for publication, a paper by Blackmond and co-workers has appeared that describes a similar phenomenon (M. Klussmann, H. Iwamura, S. P. Mathew, D. H. Wells, Jr., U. Pandya, A. Armstrong, D. G. Blackmond, *Nature* **2006**, *441*, 621). The results in our present Communication explain the greater stability of crystals of DL-proline over those of the separate enantiomers and provide a rationale for the nonlinear effects observed in the *ee* of proline in solution and hence in the aldol reaction.

