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Emergence of Solution-Phase Homochirality via Crystal Engineering of Amino Acids

Martin Klussmann,[†,‡] Toshiko Izumi,[§] Andrew J. P. White,[†] Alan Armstrong,[†] and Donna G. Blackmond*[,†,§]

Contribution from the Department of Chemistry and the Department of Chemical Engineering and Chemical Technology, Imperial College London, London SW7 2AZ, U.K.

Received February 7, 2007; E-mail: d.blackmond@imperial.ac.uk

Abstract: The evolution of homochirality from a prebiotic environment has long intrigued scientists. Here we report how highly enantioenriched solutions may be produced by manipulation of amino acid phase behavior, a concept that has far-reaching implications for prebiotic chemistry. We demonstrate that the eutectic composition of aqueous mixtures of L and D amino acids may be tuned by the addition of achiral dicarboxylic acids that cocrystallize with chiral amino acids. We find that, in several cases, these systems yield new eutectic compositions of 98% ee or higher. This work suggests a forerunner of modern crystal engineering that provides a general and facile mechanism for the evolution of homochirality as well as a conceptual advance for the separation of enantiomers of molecules forming racemic compounds.

Introduction

The question of how biomolecular homochirality evolved from a prebiotic environment has intrigued scientists for more than a century. We recently introduced a new approach based on the equilibrium phase behavior of amino acids[1] that complements "far-from-equilibrium" models involving autocatalysis.[2−4] Here we report that highly enantioenriched solutions of amino acids may be prepared from nearly racemic mixtures by manipulation of amino acid phase behavior, a concept that greatly widens the scope of this model and has far-reaching implications for prebiotic chemistry. This work suggests a forerunner of modern crystal engineering that provides a general and facile mechanism for the evolution of homochirality. In addition, the concept of tuning the eutectic ee provides a wide scope for the enantiomeric separation of chiral molecules that form racemic compounds.

Background

Under isothermal equilibrium conditions, a nonracemic, nonenenantiopure chiral compound in water or other solvent generally exhibits a characteristic solution composition known as its eutectic, regardless of the overall ee of the chiral compound employed.[5] In our previous studies of the aqueous-phase behavior of amino acids,[1] we showed that strong asymmetric amplification of solution ee is obtained for some amino acids at their eutectic points; for example, serine at overall 1% ee exhibits a virtually enantiopure solution at 99% ee. Thus, high solution enantiopurity for samples with low overall ee would seem to be achievable, at least for a select few compounds with intrinsically high eutectic ee values. However, we recently reported results suggesting a general means of "tuning" the eutectic ee even for compounds that do not naturally exhibit high ee at the eutectic.[6] Hayashi found that unusually high solution ee is obtained for proline in chloroform compared to solvents such as ethanol,[7] and at the same time we found that a significant increase in the eutectic ee of proline is observed from ca. 50% in DMSO or alcohols to ca. 99% in $CHCl_3$.[6] We showed that this phenomenon is due to incorporation of one solvent molecule of $CHCl_3$ in the structure of the racemic D:L cocrystals of proline.[8] This result is in agreement with the model we developed for prediction of eutectic ee, ee^{eut}, based on the solubility ratio, α, for racemic (rac) compared to enantiopure (ep) amino acids and other compounds (eq 1; see ref 6).

$$ee^{eut} = \frac{1 - \left[\frac{\alpha^2}{4}\right]}{1 + \left[\frac{\alpha^2}{4}\right]} \qquad \alpha = \frac{[rac]}{[ep]} \tag{1}$$

While incorporation of solvent molecules to form solid structures known as solvates is well-known, the concept that this might affect eutectic composition had not been highlighted

[†] Department of Chemistry.
[‡] Current address: Max-Planck-Institut fuer Kohlenforschung, Kaiser-Wilhelm-Platz 1, 45470 Muelheim an der Ruhr, Germany.
[§] Department of Chemical Engineering and Chemical Technology.

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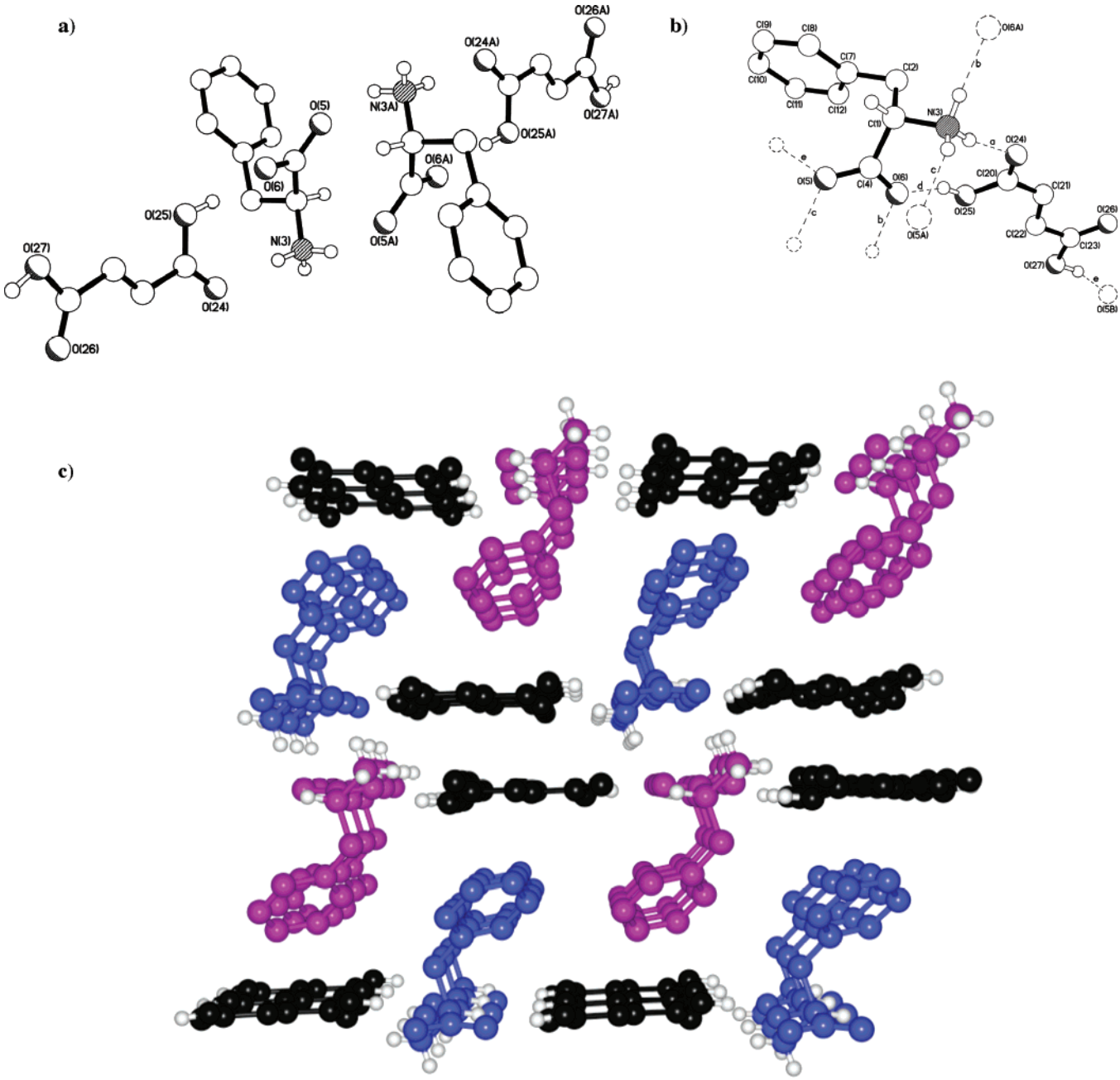

Figure 1. Structure of racemic phenylalanine cocrystallized in a 1:1 ratio with fumaric acid: (a) the contents of the unit cell; (b) the asymmetric unit showing the five unique hydrogen bonds; (c) extended structure showing opposite enantiomers in blue and magenta and fumaric acid in black (see Supporting Information).

previously. We reasoned that such hydrogen-bonding interactions that lead to solvate formation might provide a means to alter the solubility ratio, with a concomitant change in solution ee. This led us to the current study to examine the potential of this concept to widen the scope of solution-phase enantioenrichment made possible by hydrogen bonding of additives in solid-phase structures of amino acid cocrystals.

Results and Discussion

We decided to explore the behavior of small, achiral molecules of potential prebiotic relevance that might be capable of undergoing solid-phase hydrogen-bonding interactions with amino acids in aqueous systems. Interestingly, we found that neither urea nor thiourea, nor any of a series of heterocyclic bases (cytosine, guanine, thymine, or uracil) had any effect on amino acid eutectic ee values in aqueous solution. Next we screened dicarboxylic acids, several of which are known to incorporate into the solid phase of some amino acids, although to our knowledge eutectic compositions for such cases have

(7) Hayashi, Y.; Matsuzawa, M.; Yamaguchi, J.; Yonehara, S.; Matsumoto, Y.; Shoji, M.; Hashizume, D.; Koshino, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 4593−4597. Hayashi also suggested that the high solution ee was due to a difference in solubilities between the enantiopure and racemic solids, but his work did not uncover the cause of this difference. See also: Kellogg, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 494−497.

(8) Racemic proline crystallizes as what is known as a racemic compound, a 1:1 cocrystal of D and L proline. While the majority of racemic chiral compounds crystallize in this fashion, approximately 15% crystallize as what are known as conglomerates, forming separate enantiopure D and L crystals. More rarely, compounds crystallize as less well defined solid solutions known as pseudoracemates.

Table 1. Solution ee at the Eutectic for Amino Acids in Aqueous Solution in the Presence of Carboxylic Acid Additives[a]

enantiomeric enrichment | enantiomeric depletion

additive \ amino acid	% ee at the eutectic						
	thr	val	ile	met	phe	leu	his
none	0	47	52	85	88	88	94
oxalic acid	**50**	**66**	**82**	43	23	**98**	63
malonic acid	**11**	50	51	69	86	91	91
succinic acid	2	**93**	52	42	79	89	56
maleic acid	**34**	**69**	**71**	55	7	81	82
fumaric acid	**24**	**99**	59	72	**99**	92	98
adipic acid	0	48	51	66	94	92	77

[a] Values for eutectic ee for amino acids in the absence of additives were reported in refs 1 and 6. The value for phe was reported to be 83% ee in those studies; further detailed studies gave the value of 88% ee shown here, in agreement with ref 10.

never been reported. Table 1 shows that the eutectic ee can change significantly in the presence of some acids, with solution enantioenrichment observed in many cases, except for methionine and histidine. Threonine, which forms a conglomerate[8] in water with $ee^{eut} = 0$, becomes a racemic compound with ee^{eut} increasing up to 50% ee in the presence of oxalic acid.[9] The most striking enhancements of amino acid aqueous solution ee occur with fumaric acid. In the case of valine, the eutectic ee rises from 47% to 99% ee. Similarly for phenylalanine, a virtually enantiopure solution is obtained in the presence of fumaric acid.

While the formation of solvates is well-known and solid crystal structures have been reported in a myriad of cases, the concept that solvate formation may influence solubility, and therefore eutectic composition, has not previously been discussed. This provides a general mechanism for solution enantioenrichment for equilibrium nonracemic mixtures of compounds. Additionally, a deeper understanding of the relationship between crystal structure and solution composition will contribute to research in areas such as crystal engineering, where the rational synthesis of materials with defined properties is the ultimate goal.[11]

Solid cocrystals are formed via hydrogen bonding in a 2:1 ratio of valine to fumaric acid for both enantiopure and racemic valine.[12] Fumaric acid cocrystallizes with phenylalanine in a 1:1 ratio for both the enantiopure[13] and racemic cases. Figure 1 shows the crystal structure of the racemic crystal of phenylalanine and fumaric acid, a structure that has not been reported previously. The three N−H protons of the zwitterionic phenylalanine form hydrogen bonds to the two nonprotonated oxygen atoms in a second phenylalanine and to one oxygen in fumaric acid. The two O−H protons of fumaric acid also form hydrogen bonds to the two nonprotonated oxygen atoms in phenylalanine, giving a total of five unique hydrogen bonds in the unit cell. These hydrogen bonds serve to form two-molecule-thick sheets, bounded on their upper and lower faces by the pendant phenyl rings (see the Supporting Information for a detailed crystal structure analysis).

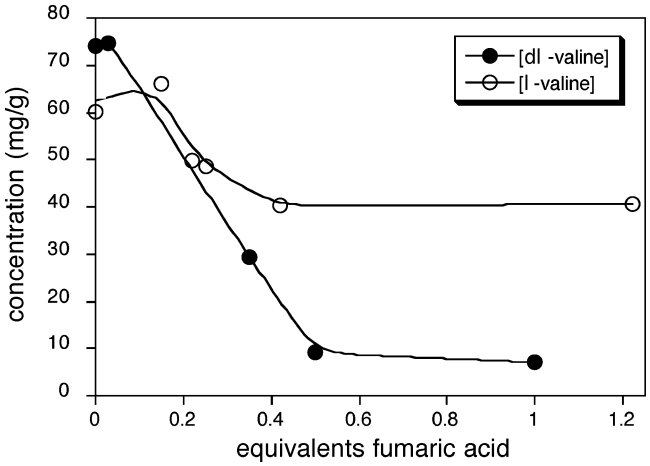

Figure 2. Solution concentration of valine as a function of the number of equivalents of fumaric acid added: (○) L-valine; (●) DL-valine.

Solution phase enantioenrichment occurs in these cases because the solubility of the racemate is strongly suppressed relative to that of the enantiopure system, as confirmed by experimental measurements and in accord with eq 1. Figure 2 shows the solution concentration of the L-valine and DL-valine systems as a function of fumaric acid equivalents. While the presence of fumaric acid decreases the equilibrium solution concentration of valine for both the enantiopure and racemic cases, the suppression is much greater (a factor of 10) for the racemate. This effect of the additive on relative solubility is shown schematically in Figure 3.

This "tuning" of solution-phase composition by the presence of additives[14] provides a conceptual advance in the application of phase behavior to chiral separations. Since more than 80% of known chiral compounds crystallize as racemic mixtures

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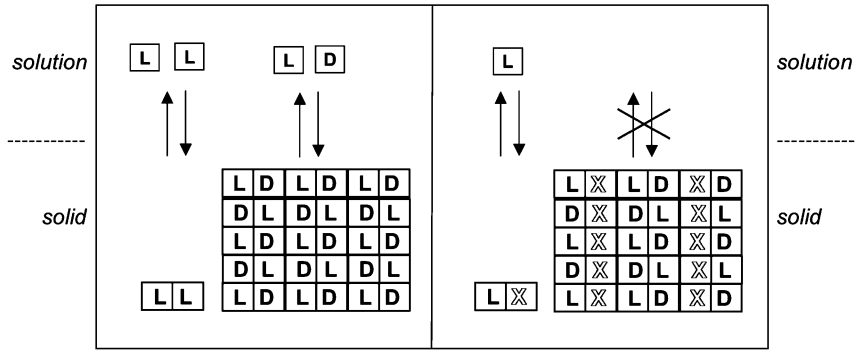

Figure 3. Pictorial representation of the solution enantioenrichment caused by an additive (shown as an open X) by incorporation into the structure of solid enantiopure and racemic compounds (enantiomers shown as **L** and **D**). The left picture shows a nearly racemic mixture of a compound exhibiting a eutectic ee of ca. 50% ee. The right picture shows that if the additive suppresses the solubility of the racemic compound more than that of the enantiopure compound, the solution ee at the eutectic will be enhanced.

rather than as separate enantiopure solids, the resolution of a chiral compound typically requires either the use of chiral acids or bases to form diastereomers with differing solubilities or the employment of methods to inhibit crystallization of the racemic compound.[5] When this is not practical, separation may be achieved for the limited number of compounds that have intrinsically high eutectic points via repetitive decanting and capturing of the solution phase.[15] The possibility of enhancement of the solution-phase ee by judicious choice of a small achiral molecule that incorporates in the racemate structure greatly broadens the scope of this latter methodology.

Our finding that the solution enantiomeric excess of an amino acid may be significantly enhanced by incorporation of a small, achiral molecule into its solid racemate thus expands the scope of candidates for potential solution enantioenrichment beyond that suggested by our original model.[1] These results demonstrate not only that the evolution of single chirality in solution from compounds present in low overall ee is not limited to those few amino acids—such as serine—that happen to exhibit intrinsically high eutectic ee values in the absence of additives but also that many amino acid/additive combinations may provide highly enantioenriched solutions from nearly racemic samples. This suggests a general and facile route to molecular homochirality that may have prebiotic relevance. Cycles of rain and evaporation establishing solid−solution equilibrium in pools containing amino acids and appropriate hydrogen-bonding partner molecules could yield enantiopure solutions from a small initial imbalance of amino acid enantiomers. This initial asymmetry could have been delivered to the young earth, as amino acids with an ee of up to 15% have been found in carbonaceous chondritic meteorites.[16] Multicomponent systems such as those we are studying mimic the complex mixtures extracted from meteorites, including relatively high levels of not only oxalic acid but also all other diacids in Table 1.[17] Our model also addresses the viability of amino acids as candidates for the evolution of homochirality in biomolecules, which has been questioned because of their tendency toward racemization. This work shows that as long as solid−liquid equilibrium is maintained, a mixture of amino acid enantiomers will continue to exhibit its eutectic solution composition even in the face of slow racemization. Thus, systems such as phenylalanine/fumaric acid or valine/fumaric acid could afford highly enantioenriched aqueous solutions over an extended period of time. Such solutions might serve as efficient asymmetric catalysts or as homochiral building blocks themselves for construction of the complex molecules required for recognition, replication, and ultimately for the chemical basis of life.

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Supporting Information Available: Text, tables, and figures giving details of materials, experimental methods, and crystal studies and a CIF file giving crystal data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) The results in Table 1 are from initial screening experiments, and slight changes are likely to occur upon a more detailed inspection. The results given for the systems valine/fumaric acid, valine/succinic acid, and phenylalanine/fumaric acid, however, have been studied in detail and were reproduced within 1% of the original screening result. All cases shown in Table 1 where a strong change of eutectic occurs are most likely caused by a change of the amino acid solid phase, with the additive cocrystallizing with the racemic compound, the enantiopure amino acid, or both.

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