Angewandte
Chemie

DOI: 10.1002/ange.200602520

Rationalization and Prediction of Solution Enantiomeric Excess in Ternary Phase Systems**

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In memory of Hendrik Willem Bakhuis Roozeboom
(1854–1907)

The evolution of homochirality from a prebiotic environment has intrigued scientists for more than a century. Theoretical models[1,2] have suggested how an initial small imbalance in enantiomeric excess (*ee*) could be amplified by autocatalytic processes. Proof of concept of such "far-from-equilibrium" models was realized in the case of the Soai autocatalytic reaction,[3] but the reaction itself is unlikely to have been viable in a prebiotic environment. Most recently, we introduced a fundamentally different model for the origin of homochirality based on the equilibrium phase behavior of proteinogenic amino acids.[4] At the eutectic point, where saturated solution and two different solid phases coexist in equilibrium,[5–7] the solution composition of a chiral substance is fixed at a given temperature, regardless of its overall *ee* value. This leads to strong asymmetric amplification of solution *ee* values for amino acids exhibiting a high eutectic *ee* value; for example, we showed that serine at overall 1% *ee* exhibits a virtually enantiopure solution at more than 99% *ee*. However, the eutectic composition for any particular chiral compound is not known a priori, and it would appear that high enantiopurity in solution is achievable for only a select few chiral compounds by this approach.

Here we present a model based on simple solubility concepts that rationalizes and predicts the eutectic composition of chiral compounds in terms of the relative solubilities of racemic versus enantiopure mixtures. In addition, we explore the role of solvent in dictating the solution *ee* value under equilibrium conditions. We reveal a case where the eutectic *ee* value of an amino acid is significantly enhanced by incorporation of a small achiral molecule in the structure of the racemic D:L cocrystals. This implies that it may in fact be possible to produce enantiopure solutions for chiral com-

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[**] We are grateful for generous support from the EPSRC and Astra Zeneca. We thank Richard Sweeney for X-ray powder diffraction analysis.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

pounds of lower eutectic values, a concept that may have implications for prebiotic chemistry.

The concentration at the eutectic was first described for conglomerates (compounds that form separate crystals of the two enantiomers) by "Meyerhoffer's double solubility rule",[8] and is now commonly expressed using the solubility ratio α [Eq. (1)], with [*rac*] and [ep] representing the solubility of racemic and enantiopure mixtures, respectively. Ideal conglomerates exhibit an α value of 2, and their eutectic composition is identical with the racemate.[9]

$$\alpha = \frac{[rac]}{[\mathrm{ep}]} \tag{1}$$

The magnitude of the solubility ratio (α) and the eutectic composition for substances that form racemic compounds (compounds in which the enantiomers cocrystallise in 1:1 ratio in the elementary cell) is less straightforward. In our earlier studies of the ternary phase behavior of amino acids,[4] we noticed that the concentration of the excess enantiomer at the eutectic closely corresponds to the solubility of the pure enantiomer, whereas the concentration of the minor enantiomer at the eutectic is generally lower than in the case of the pure racemic compound. With these observations in mind, and assuming ideal behavior, for example, no association/dissociation or other interaction between enantiomers in solution, we derived relationships that describe the composition of a chiral compound X at the eutectic. In the following, the excess enantiomer of X is arbitrarily assigned as (+), the minor one as (−). Our derivation is based on the concept of a constant solubility product (SP) of enantiomers in solution for mixed enantiomer cases.[10] The solubility product may be written either for the racemic system or for any scalemic system of enantiomers, and these descriptions may be equated [Eq. (2)].

$$\mathrm{SP} = [(+)]_{rac}\,[(-)]_{rac} = [(+)]_{\mathrm{eut}}\,[(-)]_{\mathrm{eut}} \tag{2}$$

The enantiomers $(+)_{rac}$ and $(-)_{rac}$ are present in equal concentrations in the racemic system [Eq. (3)]. On the basis of the experimental observations mentioned above, the concentration of the excess enantiomer at the eutectic, $[(+)]_{\mathrm{eut}}$, is assumed to be equal to [ep], the solubility of the enantiopure substance [Eq. (4)].

$$[(+)]_{rac} = [(-)]_{rac} = \frac{[rac]}{2} \tag{3}$$

$$[(+)]_{\mathrm{eut}} = [\mathrm{ep}] \tag{4}$$

Using these relationships, the solubility product (SP) from Equation (2) may be rewritten as in Equation (5), from which we may derive a relationship for $[(-)]_{\mathrm{eut}}$ in terms of racemic and enantiopure solubilities [Eq. (6)].

$$\mathrm{SP} = \frac{[rac]^2}{4} = [\mathrm{ep}]\,[(-)]_{\mathrm{eut}} \tag{5}$$

$$[(-)]_{\mathrm{eut}} = \frac{[rac]^2}{4\,[\mathrm{ep}]} \tag{6}$$

The total solution concentration of compound X, $[\mathrm{X}]_{\mathrm{eut}}$, can then be expressed by combining Equations (1), (4), and (6) to give Equation (7). Equally, the eutectic *ee* value is described by a simple expression based solely on the solubility ratio (α) [Eq. (8)].

$$[\mathrm{X}]_{\mathrm{eut}} = [(+)]_{\mathrm{eut}} + [(-)]_{\mathrm{eut}} = [\mathrm{ep}]\left(1 + \frac{\alpha^2}{4}\right) \tag{7}$$

$$ee_{\mathrm{eut}} = \frac{[(+)]_{\mathrm{eut}} - [(-)]_{\mathrm{eut}}}{[(+)]_{\mathrm{eut}} - [(-)]_{\mathrm{eut}}}\,100\,\% = \frac{1 - \frac{\alpha^2}{4}}{1 + \frac{\alpha^2}{4}}\,100\,\% \tag{8}$$

These relationships hold true for $\alpha \leq 2$, because otherwise the assumption of Equation (4) would no longer be valid. As for the special case of $\alpha = 2$, the calculated ee_{eut} value is 0% and thereby the "double solubility rule" for ideal conglomerates is also accommodated by this model.[11]

To examine the predictive capability of Equations (7) and (8), we measured the eutectic compositions of a variety of amino acids along with their solubilities of racemate and enantiopure substance in solvents to determine α values for each case (Table 1). Figure 1 shows a plot of eutectic *ee* values versus the solubility ratio (α) for both our own experimental data as well as for a compilation of published data[12] in comparison with the prediction made by Equation (8).

Figure 1 shows very good agreement between experimental and theoretical eutectic *ee* values from our own experimental data. In addition, our solubility data of amino acids in water generally agrees well with reported values, where available.[13,14] The data we compiled from the literature for other chiral compounds also follow the trend of the model for ee_{eut} values, but in some cases deviation is observed in the region of higher α values. Two notably interesting cases are mandelic acid[12d] and benzylidene camphor[12c] (highlighted with a dotted and dashed box, respectively, in Figure 1), for which α and eutectic *ee* values have been reported as a function of temperature. Clearly, a different model than the one presented in this work must be invoked to account for a eutectic *ee* value that does not change with changing α value, and indeed for any behavior resulting in $\alpha > 2$. A possible explanation for this could be temperature-dependent association/dissociation or agglomeration phenomena that would not be accounted for in our model. However, in the cases where we have studied temperature effects on eutectic *ee* values for amino acids, we found that the values for α change with temperature and the eutectic *ee* changes in accordance with the model of Equation (8) (methionine: entries 5 and 6 in Table 1, + in Figure 1; valine: entries 13 and 14 in Table 1, × in Figure 1).

The eutectic *ee* values found for amino acids span the whole range from 0 to almost 100% *ee*. Intriguingly, serine exhibits a eutectic *ee* value of over 99% *ee*, while threonine, which differs in structure by one methyl group (to create a second stereogenic center), is known to be a conglomerate with 0% *ee* at the eutectic.[5]

Table 1: Solubility ratio (α) and eutectic *ee* values of selected amino acids and chiral ligands **1**–**4**.[a]

Entry	Compound	Symbol[b]	Solvent	[rac][a]	[ep][a]	α[c]	$[X]_{eut}$[a]	calcd $[X]_{eut}$[d]	ee_{eut}[a] [%]	calcd ee_{eut}[e] [%]
1	alanine	◆	water	34.11	33.39	1.021	n.d.	42.10	60.4[f]	58.6
2	histidine	◆	water	1.78	4.66	0.383	4.79	4.83	93.7[f]	92.9
3	isoleucine	◆	water	5.38	4.66	1.155	6.15	6.21	51.8	50.0
4	leucine	◆	water	1.36	3.19	0.426	3.31	3.33	87.9[f]	91.3
5	methionine	+	water	3.88	7.33	0.529	7.72	7.85	85.3[f]	86.9
6[g]	methionine	+	water	8.26	11.02	0.750	12.10	12.57	78.8	75.3
7	phenylalanine	◆	water	1.56	2.97	0.525	3.22	3.18	83.2[f]	87.1
8	proline	◆	DMSO	1.33	1.15	1.155	1.73	1.53	49.6[f]	50.0
9	proline	◆	$CHCl_3$	0.001	0.51	0.002	0.36	0.51	99.2	100.0
10	proline	◆	$CHCl_3$/MeOH[h]	1.21	31.49	0.038	36.07	31.50	96.4	99.9
11	proline	◆	$CHCl_3$/MeOH[i]	7.92	63.84	0.124	67.90	64.08	93.6	99.2
12	serine	◆	water	8.64	63.74	0.130	64.81	64.03	>99.0[f]	99.1
13	valine	×	water	11.27	9.15	1.231	n.d.	12.62	46.7[f]	45.1
14[g]	valine	×	water	16.52	12.49	1.323	16.95	17.95	36.8	39.1
15	binol (**1**)	▲	toluene	2.29	5.66	0.405	5.95	5.89	91.0	92.1
16	binap (**2**)	●	toluene	0.29	5.95	0.048	5.91	5.95	99.9	99.9
17	taddol (**3**)	■	toluene	0.25	28.63	0.009	28.89	28.63	99.8	100.0
18	salen (**4**)	▼	hexane	7.03	3.42	2.057	6.97	7.03	0.0	−2.8

[a] Data measured at 25 °C unless noted otherwise. Concentrations in 10^{-3} molar fraction. [b] See Figure 1. [c] According to Equation (1). [d] According to Equation (7). [e] According to Equation (8). [f] From Ref. [4]. [g] At 60 °C. [h] 1.0 M MeOH. [i] 2.0 M MeOH. n.d. = not determined.

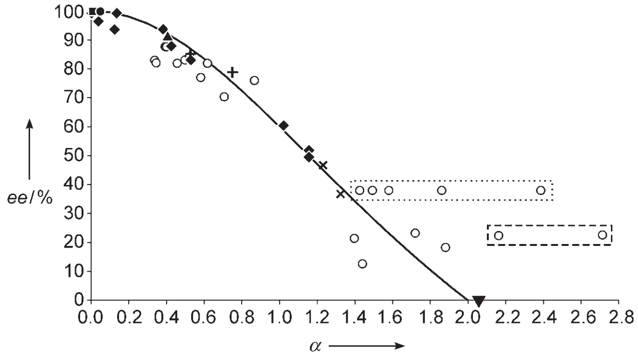

Figure 1. Experimental eutectic *ee* values for chiral compounds as a function of the solubility ratio α. Black line: Equation (8); black symbols (◆, ■, ●, ▲, ▼, +, ×): this work (see Table 1); ○: literature data (see Supporting Information); dotted and dashed boxes: data measured at different temperatures (see text for details).

Figure 1 shows that the predictive capability of our simple model of Equation (8) extends beyond amino acids for a number of common chiral ligands (Table 1, entries 15–18: binol (**1**), binap (**2**), taddol (**3**), and salen (**4**); see Supporting Information for structures). This has implications for studies of nonlinear effects in asymmetric catalysis involving these ligands. If catalyst preparation and reaction procedures are not carried out under homogeneous conditions, the *ee* value of the species present during catalytic turnover may be influenced by solid–liquid phase behavior.

The case of proline deserves special comment. Proline was found to exhibit very different eutectic *ee* values in different solvents: 50 % *ee* in DMSO compared to 99 % *ee* in $CHCl_3$, spanning a 500-fold difference in α values. Recently, Hayashi et al. reported a similar enhancement in solution *ee* values for proline in $CHCl_3/CH_3CH_2OH$, which they rationalized in terms of preferential precipitation of D:L cocrystals.[15,16] However, the D:L structure they presented was obtained through crystallization from ethanol, in which the eutectic *ee* value is significantly lower than it is in $CHCl_3$ (≈54 % *ee*, see Supporting Information), and is similar to a D:L structure reported previously crystallized from methanol.[17] The role of $CHCl_3$ in enhancing solution *ee* values is difficult to rationalize from these results.

Enantiopure proline crystallized from $CHCl_3$ gives a powder X-ray pattern identical to that published[18] for anhydrous proline crystallized from ethanol (see Supporting Information). We found, however, that the solid racemate crystallized from proline in $CHCl_3/CH_3OH$ exhibits a novel structure differing from all those previously published for the proline racemate.[17,19] Figure 2 shows that cocrystals of a 1:1 D:L compound are formed, incorporating one molecule of $CHCl_3$ per pair of proline molecules (see Supporting Information). The proline molecules exist in zwitterionic form in an extensive hydrogen-bonding network that includes the proton from chloroform. The structure contains two independent molecules of proline in the asymmetric unit, one with the carboxylate unit in a pseudo-equatorial position with respect to the pyrrolidine ring, and the other in a pseudo-axial position. These proline molecules are linked to each other and neighboring symmetry-related molecules in the racemic crystal by a series of N−H···O hydrogen-bonding interactions that extend to form a two-proline-thick corrugated two-dimensional sheet (Figure 3). The chloroform molecule, and its symmetry-related counterparts, are located on the top and bottom faces of this sheet and are held in place by a C−H···O hydrogen bond to one of the carboxylate oxygen atoms of the pseudo-axial proline molecule. The 2D sheet is particularly notable in that the axial and equatorial proline units are comprised of opposite enantiomers, and these conformations switch in the adjacent centrosymmetrically related sheets.

As predicted by our model, the reduced solubility of this novel racemate compared to enantiopure proline causes a significant enhancement of the eutectic *ee* value of the system in $CHCl_3$, thus providing a virtually enantiopure solution. The

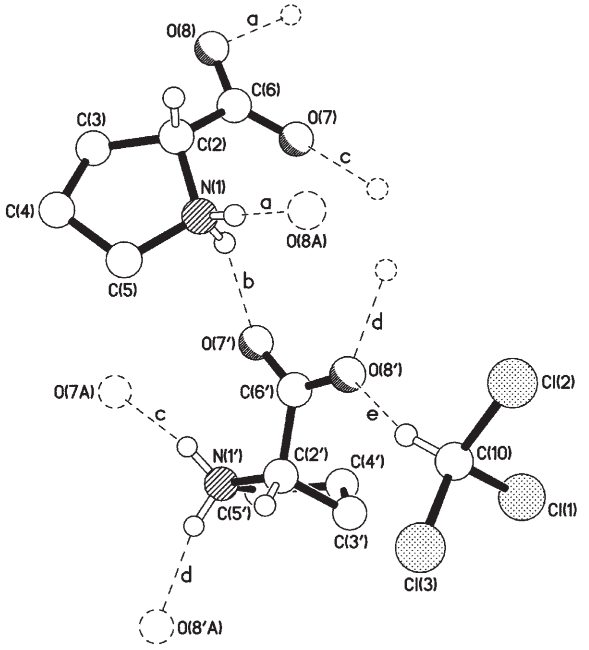

Figure 2. The asymmetric unit of DL-proline/chloroform showing the hydrogen-bonding interactions between the two independent proline molecules and the included chloroform solvent molecule (ellipsoids shown at 50% probability). The hydrogen-bonding geometries (X⋯O [Å], H⋯O [Å], X–H⋯O [°]) are a) 2.708(2), 1.81, 178; b) 2.826(3), 2.01, 150; c) 2.809(2), 2.00, 148; d) 2.761(2), 1.88, 165; e) 3.098(3), 2.22, 152.

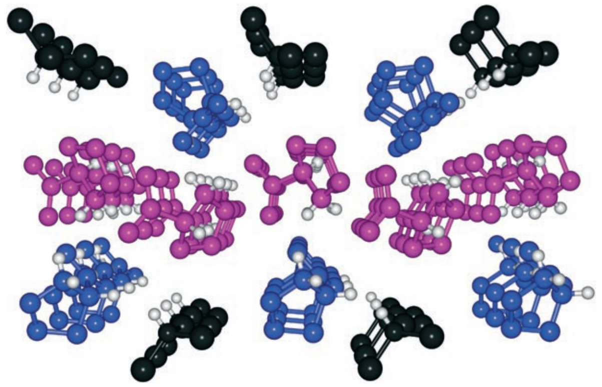

Figure 3. Structure of racemic proline crystallized from a $CHCl_3$/CH_3OH mixture, with one $CHCl_3$ molecule (black) incorporated per pair of proline molecules. The blue proline molecules correspond to one enantiomer, while the magenta proline molecules correspond to the opposite enantiomer. These conformations switch in adjacent sheets.

extensive hydrogen-bonding network within the structure of the DL-proline/chloroform cocrystals may help to explain the significant decrease in the solubility of DL-proline in chloroform compared to racemates crystallized from DMSO, CH_3CH_2OH, or water. Hayashi et al. have also suggested that the greater stability of D:L cocrystals helps to rationalize the amplification of solution *ee* values for proline, but their study did not uncover the role of $CHCl_3$ in promoting this stability.[15,20]

The case of proline also addresses the question of whether a single binary phase diagram of enantiomers may be used to predict ternary phase behavior. It has been shown in some cases that the enantiomeric composition at the eutectic in the ternary phase diagram is closely related to that in the binary phase diagram.[12c–e,21] However, a similar number of examples are known which show that this is not always a reliable basis for the direct prediction of ternary phase behavior; while binary phase diagrams necessarily provide a eutectic only at the melting temperature, the eutectics of ternary phase diagrams can be temperature-dependent.[12a–c] Clearly a single binary phase diagram could not have predicted the solvent-dependent ternary phase behavior that we observed with proline, while our model based on the solubility ratio (α) does. With the solvent composition of the eutectic, the enantiopure and racemic case thus known, the key points of a ternary phase diagram are already delineated (see Supporting Information for phase diagrams of most compounds in Table 1).

In summary, we have developed a model based on simple solubility concepts that successfully rationalizes and predicts solution *ee* values for chiral compounds in solid–solution equilibrium. In addition, our finding that the enantiomeric excess of an amino acid in solution may be significantly enhanced by incorporation of a small, achiral molecule into its solid racemate suggests a general and facile route to homochirality that may have prebiotic relevance. Such enantiomerically enriched solutions may provide asymmetric catalysts or synthetic building blocks for construction of the complex molecules required for recognition, replication, and ultimately for the chemical basis of life.

Received: June 22, 2006
Revised: August 8, 2006

Keywords: amino acids · chirality · enantioselectivity · phase diagrams · solvent effects

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