

Deracemization of Amino Acids by Partial Sublimation and via Homochiral Self-Organization

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Abstract Deracemization of a 50/50 mixture of enantiomers of aliphatic amino acids (Ala, Leu, Pro, Val) can be achieved by a simple sublimation of a pre-solubilized solid mixture of the racemates with a huge amount of a less-volatile optically active amino acid (Asn, Asp, Glu, Ser, Thr). The choice of chirality correlates with the handedness of the enantiopure amino acids—Asn, Asp, Glu, Ser, and Thr. The deracemization, enantioenrichment and enantiodepletion observed in these experiments clearly demonstrate the preferential homochiral interactions and a tendency of natural amino acids to homochiral self-organization. These data may contribute toward an ultimate understanding of the pathways by which prebiological homochirality might have emerged.

Keywords Amino acid · Enantioenrichment · Sublimation · Homochirality · Deracemization · Self-organization

The assumption that optically active compounds can serve as a resolving agent for racemic mixtures was made independently more than 130 years ago by Pasteur, Le Bel and Van't Hoff, the pioneers of the theory of molecular asymmetry and stereochemistry (Meyerhoffer 1906; Van't Hoff 1908). Although much less investigated than well known methods such as

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chromatography using chiral stationary phase carrier or resolution via formation of diastereomers, this approach presents a great interest because it can easily be correlated to the origin and evolution of biological homochirality on the Primitive Earth (Ávalos et al. 2010; Blackmond and Klussmann 2007; Blackmond 2010; Cintas 2008; Fletcher et al. 2007; Perry et al. 2007; Weissbuch and Lahav 2011) and that without the drastic conditions associated to racemization or deracemization processes.

Enantioenrichment, enantio-depletion or enantioresolution were observed during phase transitions such as fractional distillation (Koppenhoefer and Trettin 1989; Katagiri et al. 1996), partial crystallization (Kojo and Tanaka 2001; Kojo et al. 2004; Kojo 2010; Viedma 2001) or dissolution (Klussmann et al. 2006a, b; Blackmond and Klussmann 2007; Breslow 2011a). The changing of the enantiomeric excess in the partial sublimation of an enantioenriched compound was observed since 1959 (Pracejus 1959) and some examples were added in the two following decades (Kwart and Hoster 1967; Garin et al. 1977). In some cases a pure enantiomer can be isolated (Soloshonok et al. 2007). The behavior of the partial sublimation of a solid compound with an enantiomeric excess seemed to be resolved at the end of the 1970's by the hypothesis of the formation of a "eutectic" in this solid-gas phase transition (Garin et al. 1977; Jacques et al. 1981, pp 159–165; Eliel et al. 1994, p. 182). Recently several studies reported the changing of enantiomeric excess during sublimation of natural amino acids (AAs) or hydroxycarboxylic acids or their fluorinated derivatives (Nanita and Cooks 2006; Yang et al. 2006; Fletcher et al. 2007; Perry et al. 2007; Bellec and Guillemin 2010a, b; Soloshonok et al. 2007; Tsuzuki et al. 2010; Ueki et al. 2010; Yasumoto et al. 2010a, b, c; Viedma et al. 2011, 2012; see also a review of Han et al. 2011). Several results were non-consistent with the hypothesis of a eutectic formation and authors were looking for another explanation of the experimental data (Yasumoto et al. 2010b). We participated on these studies reporting the unambiguous formation of a eutectic in the slow partial sublimation of mandelic acid (Bellec and Guillemin 2010b) and, at the contrary, the evidence of the non-formation of a eutectic in the slow partial sublimation of (D + L) mixtures of leucine, probably for kinetic reasons (Bellec and Guillemin 2010a). All these recent publications show that we need a better knowledge of the sublimation of amino acids in various conditions to understand the possible role played by their sublimation on the Primitive Earth starting from mixtures of lightly enriched amino acids or of racemic mixtures in the presence of an enantioenriched compound.

As part of our continuing research on the enantioenrichment by partial sublimation without any chemical transformation, we studied the role played by a non-volatile enantiopure compound mixed with racemates. Among all proteinogenic amino acids there are just few that can form homochiral aggregates: racemic Asn and Thr crystallize from aqueous solutions as conglomerates (Jacques et al. 1981, p 58); a mixture of Asp and Glu gives enantiopure crystals under crystallization of supersaturated solutions (Viedma 2001). Racemic Ser is able to form homochiral ionic oligomers in the gas phase (Nanita and Cooks 2006) and gives almost enantiopure aqueous solution (>99 % ee) at the eutonic point (Klussmann et al. 2006a). The ability under far-from-equilibrium conditions of conglomerates to spontaneous resolution (Jacques et al. 1981, pp 4, 30 and chapter 4) is a unique property of racemic mixtures of this type.

In our studies we used these five AAs able to local symmetry breaking to demonstrate that they can serve as chiral templates for other natural AAs, showing in this way that the phase transitions of mixtures of amino acids present a plausible path towards homochirality—the prerequisite and requirement for the origin and evolution of life. We report here that aliphatic AAs (Ala, Leu, Pro, Val) can be deracemized by simple sublimation of a pre-solubilized solid mixture of racemates with less-volatile optically active AAs (Asn, Glu, Asp, Ser, Thr) (Fig. 1). The correlations between the composition of the starting mixtures and the results are detailed.

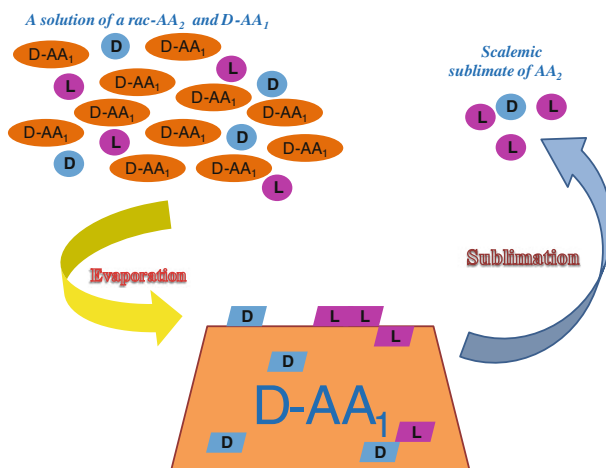


Fig. 1 Sublimation of a co-precipitated mixture of DL-aliphatic AA₂ and non-volatile D-AA₁

To perform the experiments, an aqueous solution containing about 1 g of an enantiopure AA (Asn, Asp, Glu, Ser, Thr) and several tens of milligrams of one or several racemic AA (Ala, Leu,

Table 1 Partial sublimation of simple mixtures of racemic aliphatic AA with an enantiopure amino acid

Entry	Starting mixture ^a	ee of the sublimate, % (amount, mg ^b)
1	L-Asn + DL-Ala	11.3–13.6 D (1–2) ^c
2	D-Asn + DL-Ala	10.9–15.2 L (1–2) ^c
3	L-Asn + DL-Val	7.6 D (3)
4	L-Asn + DL-Leu	5.5±4 D (1.5) ^c
5	L-Asn + DL-Pro	10.7±2.0 D (6) ^c
6	D-Asn + DL-Pro	10.8±4.7 L (9) ^c
7	L-Ser + DL-Ala	18.5 D (4.9)
8	L-Ser + DL-Val	1.1 D (5)
9	L-Ser + DL-Leu	0.4 D (3.5)
10	L-Ser + DL-Pro	8.9 D (12)
11	L-Thr + DL-Ala	38.2 D (<1)
12	D-Thr + DL-Ala	45.7 L (<1)
13	L-Thr + DL-Val	13.9±3.6 D (2) ^c
14	L-Thr + DL-Leu	47.1 D (<1)
15	L-Thr + DL-Pro	8.1 D (6)
16	L-Glu + DL-Ala	14.0 D (<1)
17	L-Glu + DL-Val	13.4 D (4)
18	L-Glu + DL-Leu	9.4 D (2)
19	L-Glu + DL-Pro	3.7 D (9)
20	D-Asp + DL-Ala	51.5 L (2)
21	L-Asp + DL-Val	44.0 D (5)
22	L-Asp + DL-Leu	51.2 (~1)
23	L-Asp + DL-Pro	12.8 D (10)

T~100–105 °C, *t*=14 h.^aThe mass of enantiopure AA—975 mg, of the racemic ones—25 mg in each case^bHydrochlorides^cBased on 2 or 3 experiments

Table 2 Partial sublimation of mixtures of L/D-Asn and non-racemic Ala

Entry	Starting mixture ^a	Starting ee (Ala), %	ee of the sublimate (Ala), %
1	L-Asn + Ala	12 D	24.8±1.0 D ^b
2	L-Asn + Ala	35 D	63.2 D
3	D-Asn + Ala	35 L	60.8 L
4	L-Asn + Ala	62 D	69.9 D
5	L-Asn + Ala	64 D	71.0±1.9 D ^b
6	D-Asn + Ala	64 L	72.0±3.0 L ^b
7	L-Asn + Ala	80 L	89.8 D
8	L-Asn + Ala	13 L	4.3 L
9	L-Asn + Ala	25 L	12.4±3.0 L ^b
10	L-Asn + Ala	80 L	72.9 L

Conditions: 100 – 105 °C,
14 h^a975 mg of Asn, 25 mg of
Ala^bBased on 2 or 3 experiments

Pro, Val) was evaporated to dryness. The solid residues were ground and then partially sublimed during 14 h at a controlled temperature and pressure. The first sublimate was collected from the cold finger and analyzed by GC analysis using a chiral column (see ESI). The partial sublimation gave a non-racemic sublimate of the aliphatic AAs.

The results are collected in Tables 1, 2 and 3. Table 1 concerns the sublimation of simple mixtures of one enantiopure AA (Asn, Asp, Glu, Ser, Thr) with one racemic AA (Ala, Leu, Pro, Val). All the sublimate show an enantiomeric excess (*ee*) which can reach up to 51 %. This *ee* is strongly dependent on the nature of the starting material: solids prepared from a L-non-sublimable AA always gave a D-enriched sublimate of aliphatic-AAs and, respectively, with one of the 5 non-sublimable D-AAs, the L enantiomer is always dominant in sublimate. These results clearly show that homochiral affinities (L-L or D-D) between different amino acids are favored to D-L affinities. The lowest *ee*'s were obtained for Ser mixtures with Val and Leu (entries 8, 9) and the best ones for threonine mixtures with Ala and Leu (38–47 %, entries 11,12,14) and, with Asp with Ala, Leu and Val (44–51.5 %, entries 20–22).

The preferential homochiral interactions observed here are in agreement with the former work of Lahav et al. where resolution of conglomerates were performed with the assistance of tailor-made impurities (Addadi et al. 1982a, b, 1985; Weissbuch et al. 2005; Weissbuch and Lahav 2011). Nevertheless, to make more obvious the assumption of the preferential homochiral interaction, we conducted a series of experiments on the sublimation of non-racemic mixtures of Ala and enantiopure Asn (Table 2). When one sublimes the mixtures of L-Asn and D-enriched Ala (entries 1,2,4,5,7) or D-Asn and L-enriched Ala (entries 3,6), enantioenriched sublimate were obtained. The increase of the *ee* ranges between 7 and 28 %. On the contrary, depletion took

Table 3 Partial sublimation of mixtures of amino acids

Entry	Starting mixture ^a	ee of the sublimate, % (amount, mg ^b)
1	L-Asn, DL-Ala, DL-Val, DL-Pro	D-Ala 17.8±1.5, D-Val 12.2±1.9, D-Pro 4.2±0.5 (8–10) ^c
2	D-Asn, DL-Ala, DL-Val, DL-Pro	L-Ala 28.3±10.0, L-Val 12.1±1.8, L-Pro 5.8±2.0 (8–10.5) ^c

Conditions: 100–105 °C, 14 h

^a Otherwise specified the mass of enantiopure AA—975 mg, of the racemates—25 mg

^b Hydrochlorides

^c The range of values obtained in 3–4 parallel experiments

place when subliming the “homochiral mixtures” of L-Asn with L-enriched Ala (entries 8–10); in these cases, the residues are enantioenriched.

What is the behavior of much more complex starting mixtures? A series of experiments were conducted and two examples are reported in Table 3 (for all data see ESI). Similar conclusions can be deduced from these results but no synergetic effect was observed.

In conclusion, the deracemisation of natural amino acids via sublimation has been accomplished for the first time. The deracemisation, enantioenrichment and enantio-depletion observed in these experiments clearly demonstrate the preferential homochiral interactions. The choice of the handedness correlates with the one of the enantiopure AA (Asn, Asp, Glu, Ser, and Thr).

A lot of mechanisms have been proposed for enantioenrichment on the Primitive Earth (see for example Ávalos et al. 2010; Blackmond and Klussmann 2007; Blackmond 2010, 2011; Brandenburg et al. 2005; Breslow 2011b; Goldberg 2013; Klabunovskii 2012; Weissbuch et al. 2005; Weissbuch and Lahav 2011), but up to now the sole experimentally detected sources of the initial enantiomeric imbalance are enantioenriched AA's in the meteorites falling on the Earth (see for example Cronin and Pizzarello 1997), what has been proposed as a trigger for homochirogenesis. The recent data on enantiomeric composition of Tagish Lake C2-type carbonaceous meteorite revealed a large enantiomeric excess of aspartic and glutamic acids (Glavin et al. 2012) that we used in our studies. On the other hand, the role played by sublimation on the Primitive Earth and in frozen planets has already been discussed (Viedma et al. 2012).

Our results suggest an *endogeneous alternative* based on the sublimation due to the presence of enantiopure materials. In both cases, the presence of an enantioenriched AA in the starting mixture leads to a changing of enantiomeric excess during a phase transition that might have a prebiotic significance. Consequently, studies devoted to the use in sublimation of organic or inorganic chiral compounds or crystals abundant as enantiopure on the Primitive Earth are currently under progress in our lab.

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