A new twist in eutectic composition: deracemization of a racemic compound amino acid by Viedma ripening and temperature fluctuation.

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Abstract

Viedma ripening and temperature fluctuation are processes based on solution phase racemization and dissolution-growth of racemic or scalemic conglomerates resulting in solid-phase deracemization. The dissolution-growth process is performed by abrasive grinding in the first case and by the temperature fluctuation in the second. But both methods have the intrinsic drawback of being only applicable to conglomerates, accounting for only 10% of all chiral molecules and are not suitable for the 90% of chiral compounds that crystallize as racemic compound. Herein we show that the enantiomeric excess of the solution in the eutectic mixture formed by a racemic compound and one of its enantiomers in suspension changes dramatically by growth-dissolution of the crystals through grinding and temperature fluctuation, converting the racemic compound into the desired enantiomer. With this new finding the scope of Viedma ripening and temperature fluctuation could be significantly expanded and can shed new ideas about the origin of biological homochirality on earth.

Keywords: Amino acid, Chirality, Deracemization, Temperature Fluctuation, Viedma Ripening.

1 Introduction

The origin of biological homochirality, exclusively left-handed amino acids and right-handed sugars, remains as one of the most fundamental question and a fascinating aspect of prebiotic chemistry in the origin of life. [1]

Processes that can produce states of broken chiral symmetry are of particular interest to chemistry, physics, and biology. Several mechanisms that lead to an imbalance between enantiomers have been proposed together with an amplification mechanism as an explanation for the origin of biomolecular single handedness. [2-4]

On the molecular level, chirality has a profound impact on recognition and interaction events and is thus important to biochemistry and pharmacology. [5]

As a result of regulatory aspects, more and more efforts are put into bringing enantiopure medicines to the market. In many cases this can be achieved by an enantioselective synthesis. Nevertheless, in an increasing number of cases, this would involve too many synthetic steps for the process to be economically viable.

Chiral separation by crystallization can be an alternative. In addition, these protocols simplify enormously the production of enantiomerically pure substances and can be adapted to the pharmaceutical industry to obtain chiral drugs.

In 2005, Viedma demonstrated that continuous abrasive grinding of a racemic suspension of d- and l-NaClO3 conglomerate crystals under a near-equilibrium condition, transform the crystals in the suspension into an enantiopure state. [3]

Three years later (2008), Noorduin et al. were able to extend this process to intrinsically chiral organic compounds crystallizing as conglomerates and undergoing fast racemization of the pair of chiral molecules within the solution phase of the suspension. They showed that the process can be applied to amino acid derivatives, metal-organic complexes and isoindolinones. [6]

Currently, grinding-induced deracemization is being explored as a practical route to produce enantiomerically pure compounds that can be used in the manufacture of pharmaceuticals. It also provides a scenario for the evolution of single chirality as is found in nature.

In 2007, we suggested [7] that *"a growth-dissolution process enhanced by temperature fluctuation (or dry-wet cycles) in a system with a racemic suspension in solution of amino acids conglomerate crystals, or polymers, with racemization of molecules in solution could give complete chiral purity and may have played a key role in the origin of biological homochirality on Earth."*

One year later, 2008, we reported the first example of the evolution of total enantio-enrichment of the solid phase for the conglomerate of an essential amino acid mediated by solution-phase racemization in the absence of the attrition enhancement by a strong temperature gradient. [8]

In 2011, total deracemization by temperature fluctuation or temperature cycling was demonstrated by some of us in a suspension of NaClO3. [9] For this, a difference of 14ºC was established between the lower part and the upper part of a flask, initiating a dissolution-growth process of the crystals that finally generated a homochiral system (figure 1) It was shown that the homogenization of the temperature in the flask by strong agitation eliminated the deracemization phenomenon, demonstrating, unequivocally, that the driving force of the deracemization process was the fluctuation or cycles of temperature. Therefore, temperature fluctuation was shown to be a viable and efficient alternative to original attrition deracemization.

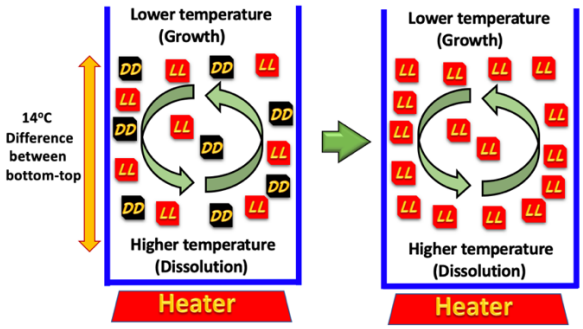

Figure 1

Deracemization by temperature fluctuation (14ºC) of a racemic suspension of NaClO3 crystals.

In 2013 Suwannsang et al. [10] reported another example on the effect of temperature fluctuations with racemization in solution, using programmed heating-cooling cycles, under near-ambient conditions, on the deracemization of a model compound, 1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-one.

A technical modification was introduced in this work and the entire system experienced intermittent temperature rises and falls, meaning that the temperature fluctuation occurred in time and not in space.

More recently, another modification returned to the fluctuation of temperature in space, although not within the same system but in two different intercommunicating systems with different temperatures. [11]

Obviously, deracemization through these two technical modifications take advantage of the same experimentally established principle, [8-9] i.e. homochirality can ultimately be achieved through a dissolution-growth process maintained by fluctuations in temperature or thermal cycles.

In some way, this new method could be considered as a derivative of the classic Viedma deracemization in which the dissolution-growth generated by grinding now occurs through temperature fluctuation.

Although being promising methods for deracemization, Viedma Ripening and the temperature cycling method have the intrinsic drawback of being only applicable to conglomerates. A rough estimate tells us that less than 10% of chiral compounds crystallize as conglomerates (the two enantiomers crystallize into separate crystals).[12] This restriction excludes the utilization of these deracemization methods for roughly 90% of the known chiral molecules since these compounds crystallize as racemic compounds, (both enantiomers crystallize in the same crystal).[12]

The deracemization of racemic compounds by grinding or temperature fluctuation, therefore, constitutes a great challenge and above all, considering prebiotic chemistry, the deracemization of the main chiral biological molecules

such as amino acids. Only 2 of the 20 proteinogenic amino acids are reported to form a racemic conglomerate (asparagine and threonine).

Here we report the deracemization in water of the proteinogenic amino acid aspartic acid, a racemic compound, using the combination of concepts behind Viedma ripening and temperature fluctuation. We show herein that the growth-dissolution of the crystals through grinding, temperature fluctuation and racemization in solution generates a drastic change in the enantiomeric excess (ee) in the eutectic of a crystalline suspension in which the racemic compound and one of its enantiomers are present in the solid phase. This alteration of the ee at the eutectic is the driving force behind the deracemization process and broadens the possibility of deracemization of racemic compounds by combining these two techniques.

2 Experimental

For the deracemization of aspartic acid, a suspension of L or D-aspartic acid crystals (1600 mg) and LD aspartic acid crystals (1600 mg) (1:1 ratio) were formed in a 25 mL flask with water (22 mL) in the presence of 3 mm glass beads (8 g.). The suspension was vigorously stirred (800 rpm) to uniformly homogenize the crystal size for 1-2 hours.

In a special experiment, a ratio between the phases of 1:1.5 was used (1066 mg L-aspartic acid and 1600 mg LD-aspartic acid)

After homogenization, solution phase racemization was initiated by adding sodium hydroxide (120-400 mg) and the flask was placed on a magnetic stirrer hot plate with gentle stirring (120 rpm) allowing the supernatant solution to appear. A temperature difference is generated between the bottom of the flask (in contact with the heating plate) and the top of the flask (in contact with the atmosphere at room temperature) of approximately 14-18°C. We have used a fan to aerate the top of the flask and cool this part of the system by establishing a suitable temperature fluctuation and cooling speed.

The temperature range of the experiments is of great importance in the deracemization time. We have chosen temperatures that allow deracemization in a few hours (78ºC-60ºC, 24 hours, 68ºC-50ºC, 48 hours). By increasing the racemization agent, the deracemization time could decrease significantly, but the yield is lower because more product remains in solution (with 120 mg of sodium hydroxide 2700 mg of aspartic acid are recovered and with 200 mg 2400 mg are recovered)

Samples of the solid phase were collected over time, dried immediately and enantiomeric purity was measured using chiral HPLC methods. The final product was verified by X-ray powder diffraction (XRPD) (see Supporting Information).

3 Results and Discussion

We observed in all the systems that the continuous nucleation-growth-dissolution of the crystals through temperature fluctuation and racemization in solution leads to a rise in ee of solid phase in time. (Figure 2)

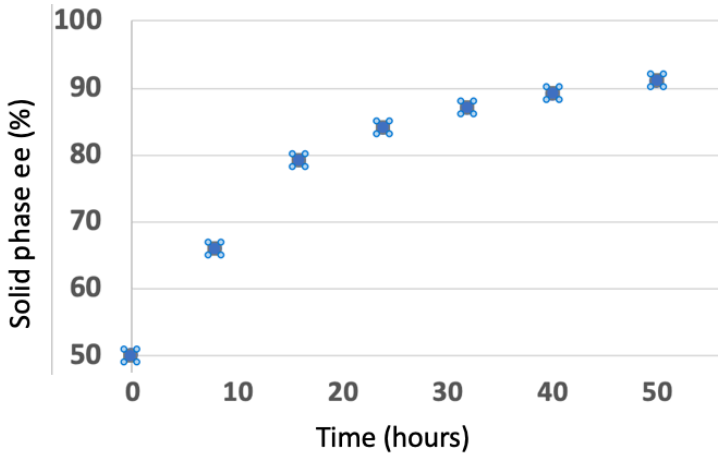

Figure 2

Evolution of solid-phase ee for racemic compound aspartic acid and one enantiomer with solution-phase racemization.

Furthermore, under the same conditions, experiments were carried out, in which suspensions of equal amounts of racemic compound and now, with both enantiomers present in the system, were stirred for two days. The final solid phase was characterized by X-ray powder diffraction (XRPD), corresponding to the racemic compound. The incorporation of the two enantiomers in the racemic phase shows that the aspartic acid, racemic compound, is in fact the most stable phase at these temperatures and conditions, which contrasts with the result obtained when only one enantiomer is present (Figure 3).

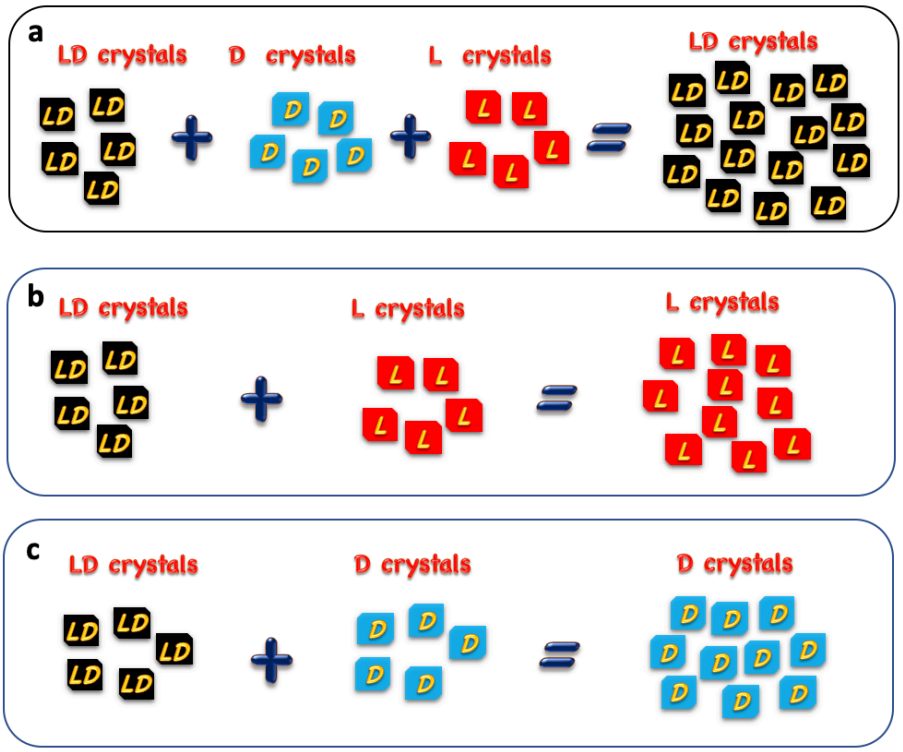

Figure 3

Evolution of three experiments following the protocol established in the text.
(a) the two enantiomers are present in the system with the racemic compound. Finally, the entire product is a racemic compound, that is, the stable phase is the racemic compound
(b) Only the L enantiomer is present in the system with the racemic compound. Finally, all the product belongs to the enantiomer L.
(c) Only the D enantiomer is present in the system with the racemic compound. Finally, the entire product belongs to the enantiomer D.

At the eutectic point, where the saturated solution and the two enantiomeric crystals coexist in equilibrium, the composition of the solution of the chiral substance is fixed at a given temperature [12]. That is, initially even if the D and L chiral crystals differ in amount, the solution phase will contain identical concentrations of each chiral molecule. For a racemic conglomerate, thermodynamics predicts that the supernatant solution phase is racemic for any solid composition. Therefore, at equilibrium a conglomerate always exhibits a solution ee of 0% (Figure 4).

During the process of Viedma ripening a slurry of the conglomerate crystals in a saturated solution is intensively ground to promote breakage and dissolution as well as growth of the crystals. The grinding increases the number of small clusters, and enhances the cluster incorporation to crystals of the same phase.

Clusters or subcritical crystals of the major enantiomer in the solution are less likely to dissolve than those of the minor enantiomer, because they are more likely to be rescued from dissolution by fusing with the more prevalent and abundant crystals of the same hand while the clusters of the minority solid phase have a lower probability of encountering a crystal of the same handedness [13]

In this way the clusters of the minor enantiomer are more abundant relatively in solution and therefore dissolve, then the solution ee is found to be enriched in the enantiomer that forms the minor population in the solid phase.

This was demonstrated experimentally, and without racemization during the grinding of a slurry, the solution contains an enantiomeric excess that is the inverse of that in the solid phase. [13]

In the presence of a racemization catalyst, this inverted enantiomeric excess in the solution phase erodes, becoming the driving force for the deracemization process by means of a net flux of molecules from crystals of the minor handedness to crystals of the handedness that forms the major population in the solid phase. In this way, an enantiopure crystalline final state can be achieved, in up to 100% yield. [13]

But the experiments in this work are different, they start from systems in which the racemic compound and one of its enantiomers are present in the solid phase, coexisting in equilibrium with the saturated solution from the beginning, this mixture have received little attention until Blackmon's seminal work [14]. Before the addition of the basic racemization catalyst the minor enantiomer can be present in solution only via dissolution from the solid racemic compound.

Therefore, the ee in the solution at equilibrium is controlled by the relative solubility of the racemic compound and the enantiopure crystals and this ee can fall between 0 and 100% ee. [15] In the case of aspartic acid, the chromatographic measure of the eutectic ee of solution has a value of approximately 28% of the common chiral molecule in the two phases, the L-molecule in this experiment. (Figure 4)

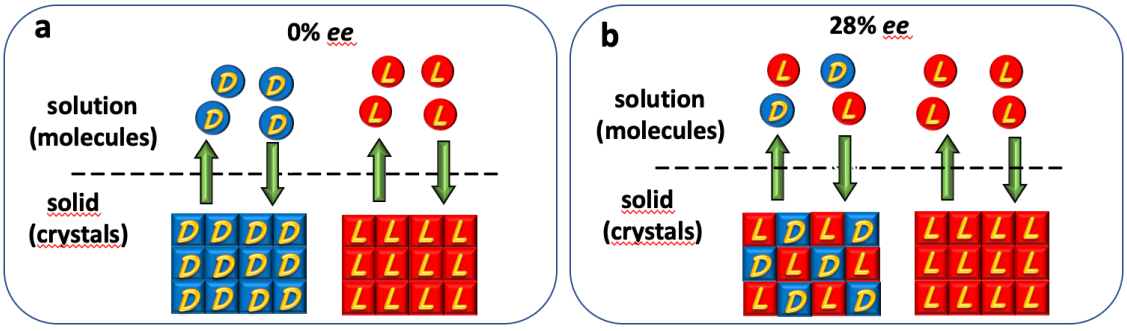

Figure 4

(a) For a racemic conglomerate the supernatant solution phase is racemic (0% ee at the eutectic). (b) For a racemic compound coexisting in equilibrium with one of its enantiomers, the ee in solution is controlled by the relative solubility of the racemic compound and the enantiopure crystals (28% for aspartic acid was measured by chromatography)

It is evident, in the present case, that the eutectic composition formed by the enantiomer and the racemic compound is always thermodynamically unfavorable for the deracemization of the racemic compound in a racemizing medium. In stagnant conditions there would be a permanent flow of molecules L towards D

(entropy) and therefore the enantiomer crystals would disappear feeding the racemic compound.

However, in our experiments the racemic compound disappears feeding the enantiomeric crystals.

To try to explain this behavior, additional experiments were performed under the same experimental conditions but without racemization. Samples of the solution were taken a few hours after the start of the experiments to measure ee. We found that the liquid phase surprisingly approaches 0% ee when at the beginning of the experiment the enantiomeric excess of the eutectic measure was 28% (Figure 5).

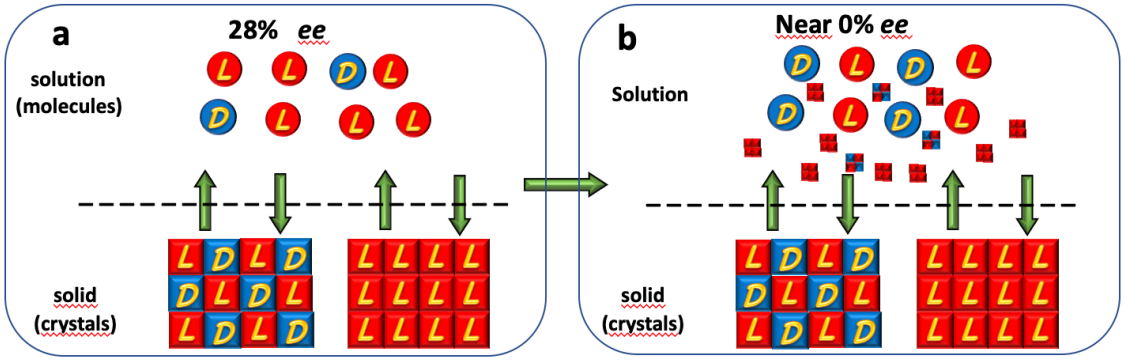

Figure 5

(a) Racemic crystals of aspartic acid coexisting in equilibrium with one of its enantiomers have an ee in solution of 28%.
(b) Racemic crystals of aspartic acid coexisting with one of its enantiomers under experimental conditions show an ee close to zero.

This shows that the rapid formation or growth of crystals, depletes the initial excess population of molecules in the solution. Populations tend to equalize.

We suggest that there is an easier and faster incorporation to the crystals of the common molecules to the enantiomer and the racemic compound due to the greater number of equivalent positions on the surface of crystals. [16] On the other hand, initial minority D molecules in solution are less likely to find the proper structural position in crystals already present, [16] so they could have a longer residence time in solution.

It has been postulated that the racemization catalyzed by the surface of the crystals could help to explain some data during the Viedma ripening [17] and recent experimental evidence of the participation of the crystal-solution interface in the racemization during this process has been found.[18] This effect could be influencing the racemization of the chiral molecules in our experiments.

Therefore, the ratio between the two solid phases present in suspension could be important and for compounds with a thermodynamically more unfavorable enantiomeric excess in the eutectic, a special ratio would be necessary.

The crystals of the racemic compound serve as a gradual feeding to crystals of the enantiomer present in the system in contrast to the original Viedma ripening in which only the opposite enantiomer serves as food.

This is so, in this case, not only when the quantity of the enantiomer-racemic compound is the same in solid state (1:1), but in a higher proportion for the racemic compound (1:1.5) the system advances towards homochirality, although much slower (Figure 6).

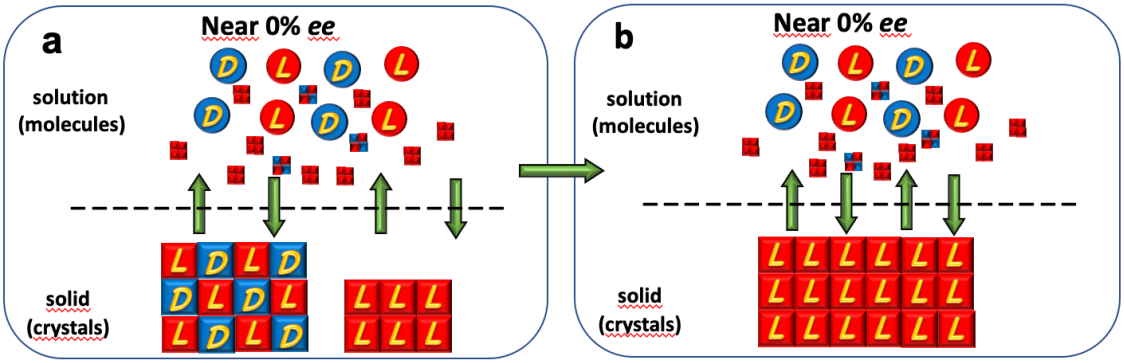

Figure 6

(a) Racemic aspartic acid crystals that coexist in equilibrium with crystals of one of its enantiomers under experimental condition present an ee close to zero, not being necessary an equality in the quantity of both solid phases.
(b) The crystals of the racemic compound serve as a gradual feed for crystals of the enantiomer present in the solution.

With this new finding, deracemization of a solid phase might not be restricted to 10% of chiral molecules that crystallize as a conglomerate since this method could be extended to some other racemic compounds. For this, it would be necessary to choose the appropriate solvent, racemization agent, temperature fluctuation, ratio between the two phases, stirring and cooling rate and hope that both phases are not separated by too large an energy gap.

When a compound is stable as racemic compound, deracemization could still be achieved, in some cases, using the perpetual struggle between thermodynamics and kinetics.

Competing interest statement: Complutense University of Madrid has applied for a patent (P202130729) on the use of this deracemization method with C.V. as inventor

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