

Article

Enantioselective Crystallization of Sodium Chlorate in the Presence of Racemic Hydrophobic Amino Acids and Static Magnetic Fields

María-Paz Zorzano ^{1,*}, Susana Osuna-Esteban ¹, Marta Ruiz-Bermejo ¹, Cesar Menor-Salván ¹ and Sabino Veintemillas-Verdaguer ²

¹ Centro de Astrobiología (CSIC-INTA), Carretera de Ajalvir km 4, Torrejón de Ardoz 28850, Madrid, 28850, Spain; E-Mails: osunaes@cab.inta-csic.es (S.O.-E.); ruizbm@cab.inta-csic.es (M.R.-B.); menorsc@cab.inta-csic.es (C.M.-S.)

² Department of Particulate Materials ICMM-CSIC, Sor Juana Inés de La Cruz 3, Cantoblanco 28049, Madrid, 28049, Spain; E-Mail: sabino@icmm.csic.es

* Author to whom correspondence should be addressed; E-Mail: zorzanomm@cab.inta-csic.es; Tel.: +34-91-520-6451; Fax: +34-91-520-2014.

Received: 27 March 2014; in revised form: 7 May 2014 / Accepted: 13 May 2014 /

Published: 5 June 2014

Abstract: We study the bias induced by a weak (200 mT) external magnetic field on the preferred handedness of sodium chlorate crystals obtained by slow evaporation at ambient conditions of its saturated saline solution with 20 ppm of added racemic (DL) hydrophobic amino acids. By applying the Fisher test to pairs of experiments with opposing magnetic field orientation we conclude, with a confidence level of 99.7%, that at the water-air interface of this saline solution there is an enantioselective magnetic interaction that acts upon racemic mixtures of hydrophobic chiral amino acids. This interaction has been observed with the three tested racemic hydrophobic amino acids: DL-Phe, DL-Trp and DL-Trp, at ambient conditions and in spite of the ubiquitous chiral organic contamination. This enantioselective magnetic dependence is not observed when there is only one handedness of added chiral amino-acid, if the added amino acid is not chiral or if there is no additive. This effect has been confirmed with a double blind test. This novel experimental observation may have implications for our view of plausible initial prebiotic scenarios and of the roles of the geomagnetic field in homochirality in the biosphere.

Keywords: astrobiology; prebiotic organic reactions in water; homochirality; magnetic field; racemic hydrophobic amino acids; geomagnetic field; terrestrial planets

1. Introduction

All the organic chemistry of terrestrial organisms is based on the L form of amino acids. Despite much effort devoted to elucidating how and why L-amino acids were preferentially selected with respect to the D-enantiomer no clear solution has yet been obtained [1]. Some enantioselective mechanisms have been proposed however none of them deal with organic molecules in plausible prebiotic Earth environments [2–4]. In the prebiotic chemistry research community, there is no unique, single, universal model of the prebiotic environmental conditions. There is no agreement as to what the exact conditions were regarding temperature, pH, red-ox potential, inorganic precursors, pressure (surface of liquid, gaseous phase, deep ocean) or even which were the first organic molecules that launched the auto-catalytic reactions. In practice, all plausible situations are explored experimentally because they may be representative of the present or past environmental conditions in the ancient Earth and also because they may be relevant to investigate the habitability of other outer Earth environments, such as Mars, Titan, Europa or even extra solar planets. Given that the prebiotic scenario has not yet been unequivocally identified, there is no agreement either as to what mechanism may have caused the original selection of one chiral handedness over the other. All scenarios are currently considered but to date no experiment has explored the plausible interaction of geomagnetic fields and primitive organic chiral molecules, such as amino acids, on the primordial ocean surface.

Simple symmetry arguments demonstrate that a static magnetic field cannot induce absolute enantioselection at thermodynamical equilibrium [5,6]. However, for far-from-equilibrium processes a magnetic field might induce enantioselection if the molecules are aligned since this constitutes a falsely chiral (*i.e.*, time-noninvariant enantiomorphous) arrangement [5,6]. According to this, a magnetic field alone might induce deracemization if the molecules are pre-aligned, as in a crystal or on the liquid-air surface and the process is far from equilibrium [5]. For this research, we shall investigate an out-of-equilibrium process where amino-acid arrangement is possible: the crystallization, at the air-liquid interface, of a sodium chlorate solution with racemic aminoacid additives. This experimental observations illustrates how the geomagnetic field, *i.e.*, a falsely chiral influence, can induce deracemization and enantioselection in interaction with racemic amino-acid mixtures.

To investigate this effect, one needs a technique that is sensitive to tiny amounts of chiral hydrophobic amino acids located only at the upper molecular layers of a saline water solution. We propose to use the crystallization of sodium chlorate NaClO₃ (a process which has proven to be particularly sensitive to tiny amounts of hydrophobic chiral amino acids) as a probe for plausible amino acid enantiomeric interactions at this interface. NaClO₃ is an achiral molecule but when several molecules join into small crystal nuclei they crystallize in an ordered spiral-like structure which can be either levo (l) or dextro (d) rotatory. A saturated NaClO₃ solution left at room temperature in a wide vessel crystallizes into an almost racemic mixture of crystals, *i.e.*, on average there are no preferences for (l) or (d) crystals [7]. The crystallization of NaClO₃ has shown to be a very convenient process to

detect tiny chiral effects that take place within the water-air interface as well as to detect the chiral sign of amino acid impurities [8–11]. We shall test for biases in the water-air induced crystallization of NaClO_3 crystallized with racemic additives (of the order of a few ppms) of hydrophobic amino acids (which will tend to be at the water-air interface of the solution) and an upwards or downwards external magnetic field. Three additional sets of control experiments, one without additives, one with the achiral hydrophobic amino-acid Gly additive and one with only one handedness of chiral hydrophobic amino acid (D-Trp), are investigated for comparison.

2. Experimental Section

In order to overcome the geomagnetic field at Madrid, where the experiments took place, and be able to control the direction of the local (downwards pointing) magnetic field we need to use an external magnetic field greater than 0.05 mT. We used a set of small Neodymium dipole magnets of about 5 mm height and 17 mm diameter. The magnetic field at the center has been measured and found to be 100 mT at a distance of 7 mm and 200 mT at 3 mm. This is the range of distances between the magnets and the water-air interface in our two different types of experimental setups. Which means that at the water-air interface which is right above the center of the magnets, where the maximal field is experienced, there is a magnetic field of 200 mT at most, *i.e.*, roughly 4000 times greater than the actual maximal geomagnetic field.

All glass material was steamed for sterilization during 2 h. The experiments were run at room temperature. We used NaClO_3 from Sigma-Aldrich S.A. (ACS quality) and ultrapure water (Millipore Milli-Q Q. Gard). The NaClO_3 used has a 99.91% purity. The ferromagnetic metals Fe, Mn and Co are present as impurities in amounts of maximum 5 ppm each. These metallic impurities are unavoidable and constantly present as a background together with the salt ions and ambient impurities. For each crystallization process 50 mL of a NaClO_3 aqueous solution (48.8 g NaClO_3 in 100 g of dissolution, saturated 51.2%, 9.86 molal saturated at 30 °C) was prepared at 45 °C under vigorous stirring (500 rpm) together with a small amount (4 ppm for the aerosol experiments, and 20 ppm for the steady crystallization ones) of one or several amino acid forms, depending on the experiment. All the amino acids were obtained from Sigma-Aldrich. The hot solution was filtered, through a 0.2 μm -pore size sterile nylon.

To guarantee the independence of the observations with respect to the specific details of the experimental setup we propose two different techniques where the interface between the bulk liquid and the air is relevant. The solution described above is put to crystallize under two possible setups: an aerosol-liquid cycle (see Figure 1-Left) or a steady crystallization (see Figure 1-Right). For the aerosol-liquid setup, a round-bottom flask, with 5 mL of the liquid solution, was placed within a bath of the ultrasonic aerosol generator (a commercial, small ultrasound generator of 1'8 MHz, 30 W) [10]. After 2 min, the ultrasonic device induces the formation of an optically thick cloud of aerosols generated from the water-air interface solution. There is a continuous recycling process, from the aerosol phase to the liquid bulk interface and back. Because of the intense evaporation in the aerosols, this is the first environment to reach supersaturation and crystal nucleation of NaClO_3 is initiated at the aerosol phase roughly after 2 h. This method induces in about 4 h the homochiral crystallization of NaClO_3 for the steady crystallization setup we have crystallized 1 mL of the solution described above

in a small open crystal vessel, of about 2 cm diameter and 4 cm height under different experimental conditions. The first crystal nuclei are formed on the liquid pool surface which, due to the evaporation, is the first place to reach supersaturation. These crystals then grow further at the bottom of the vessel, reducing the supersaturation in NaClO_3 and preventing the formation of new crystals nuclei. After between one and three days there are observable crystals of few mm size within the remaining liquid solution.

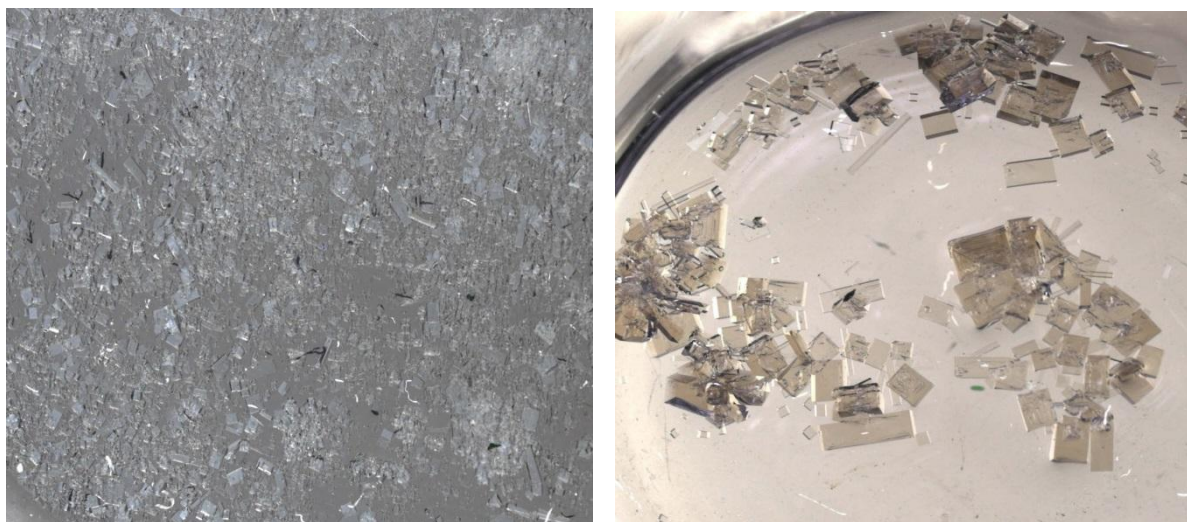
Figure 1. Water-air induced crystallization setups. **(Left)** Aerosol experimental setup. Here the flask has a magnet within the crystal vessel with the South pole facing upwards. **(Right)** Steady crystallization experimental setup, one vessel with magnetic field and one without.



Both crystallization setups share an essential feature: they initiate the crystallization with constituents of the solution at the interface between air and the liquid bulk and are very sensitive to water-air surface impurities [10,11]. As we will show later the final preferred handedness of NaClO_3 may be biased by chiral impurities (such as amino acids) located within this environment and is independent of the procedure used. Once this is confirmed and to run a larger number of experiments in parallel improving the statistics, the steady crystallization procedure will be the only one used.

The chirality of the crystals obtained under either crystallization setup was determined from their optical activity using a stereomicroscope and two linear polarizers [12–15]. See Figure 2 for two examples of homochiral crystallization distributions. We count the experiments that lead to (l) homochiral experiments $n_{(l)}$, and (d) homochiral experiments $n_{(d)}$ for a set of experimental cases. If all (either one or many) the crystals in the vessel are levorotatory the result is marked as (l), if all are dextrorotatory the result is (d). The rest which have both (l) and (d) crystals in arbitrary rate are classified as (r) [14,15].

Figure 2. Examples of NaClO_3 homochiral distributions of crystals obtained from an aerosol crystallization experiment (**Left**) and a steady crystallization experiments (**Right**). The biggest crystals are a few mm size. (**Left**) Dextro homochiral NaClO_3 crystals (here seen as light crystals) obtained from the co-crystallization with 4 ppm of DL-Phe and an external upwards pointing magnetic field (B_{\uparrow}). (**Right**) Levo (here seen as dark crystals) homochiral crystals obtained from the co-crystallization with 20 ppm of DL-Phe and an external downwards pointing magnetic field (B_{\downarrow}).

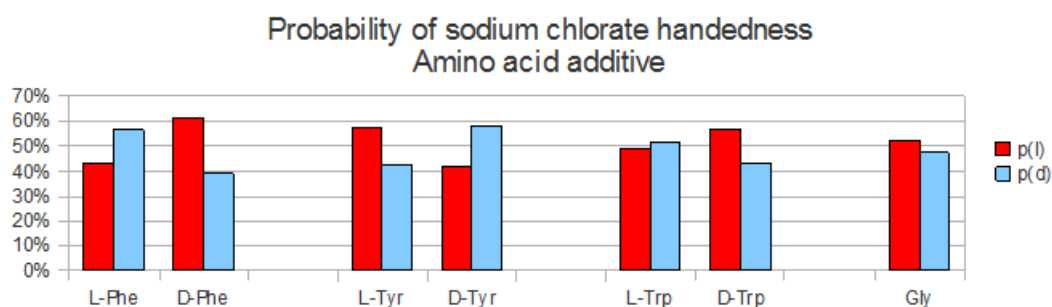


In order to detect chiral effects at the interfaces, we first need to confirm that the handedness of NaClO_3 can be biased by the only presence of a chiral amino acid, the results are summarized in Table 1. We performed an approximately equal number of experiments with each setup using solutions with D- and L-Phe, D- and L-Tyr. To guarantee the independence of the observations with respect to the specific details of the experimental setup (aerosol cycle *vs.* steady crystal vessel), the observations of both crystallization setups were added. The biased is observed in both experimental setups equally, confirming that the water-air interface is the place where the final handedness is determined. Roughly $69\% \pm 4\%$ of the experiments with homochiral amino acid additives lead to pure homochiral NaClO_3 crystallizations, the rest which have both (l) and (d) crystals are discarded in this analysis. In addition, experiments with D- and L-Trp have been performed however using only the steady crystallization process. In this later case, roughly $54\% \pm 12\%$ of the experiments are homochiral. A control experiment (using only the steady crystallization process), has been run adding an achiral amino acid instead, Gly, see Table 1. In this case roughly $55\% \pm 9\%$ of the experiments are homochiral. Using the experimental data of Table 1 we derive, with a confidence level of 95%, the true proportion p of each handedness of NaClO_3 which varies depending upon the added amino acid. These probabilities are plot in Figure 3.

Table 1. Amino acid effect on the crystallizations of NaClO₃ with amino acid impurities under water-air induced crystallization conditions. The addition of chiral hydrophobic amino acids favors a preferred handedness in the sodium chlorate crystallization, the achiral hydrophobic amino acid Gly does not induce a significant bias.

Chiral Amino Acid Impurity	n(l)	n(d)
(a) L-Phe	31	41
(b) D-Phe	38	24
(c) L-Tyr	31	23
(d) D-Tyr	18	25
(e) L-Trp	69	73
(f) D-Trp	66	51
(g) Gly	90	82

Figure 3. True proportion or probability of sodium chlorate handedness, (l) vs. (d), as a function of added hydrophobic amino acid. Both handedness are equiprobable, up to the statistical error, in the case of Gly, whereas with chiral amino acids (L or D) Phe, Tyr and Trp additives, there is a significant bias in the proportion towards one or other handedness that depends on the chiral sign of the amino acid and on the amino acid type.



The crystallization of sodium chlorate is exposed to ambient contaminants and also to the unavoidable salt and additive impurities. To assess if the handedness of an amino acid additive alone drives a non-random biased output, we apply Fisher's exact statistical test to pairs of experiments with opposing conditions. In each pair we shall evaluate if the variation of one single factor induces a significantly different output from one setup to the other, keeping in mind that all background effects (ambient, solute and solvant impurities) are common in both situations. By applying Fisher test to the results in Table 1, we conclude that the handedness of NaClO₃ is biased by the presence of one or other enantiomer of the amino acid, with a level of confidence of 98.89% for Phe (rows a and b), with a level of confidence of 95.81% for Tyr (rows c and d) and with a level of confidence of 91.61% for Trp (rows e and f). In the case of Gly, the outcome is almost equally probable (52% ± 8% for (l) handedness) for one and other handedness showing that an achiral amino acid additive does not bias the crystallization of sodium chlorate. These tests confirm that these experimental setups can be used to detect the footprint of the organization of chiral hydrophobic amino acids at the water air interface.

3. Results and Discussion

3.1. Results

Next we shall elucidate if, at the water-air interface of a saline solution and starting from a racemic situation, a magnetic field can strengthen the influence of one chiral form of the amino acid. Again, other background influences such as ambient and salt contaminants, ferromagnetic metallic impurities, or enantiomeric excesses of commercial racemic DL amino-acids cannot be discarded a priori. In order to avoid this systematic and unavoidable influence the experiments are always tested using the same solution simultaneously under different configurations, namely upwards pointing and downwards pointing field, to apply the Fisher test to a common situation with disregard of background common effects. We have therefore performed experiments with racemic amino acid mixtures and an external magnetic field pointing upwards (B_{\uparrow}) or downwards (B_{\downarrow}). Roughly $52\% \pm 4\%$ of the experiments with magnetic field and racemic amino acid mixtures lead to pure homochiral distributions, the rest which have both (l) and (d) crystals in arbitrary rates are discarded. The results are summarized in Table 2. To determine if the variation of the magnetic field orientation alone can bias the output we apply Fisher's exact statistical test to pairs of experiments where only this factor is changed. The hypotheses tested are summarized in Table 3.

Table 2. Magnetic field orientation effect (upwards B_{\uparrow} or downwards B_{\downarrow} pointing external field) on the water-air induced crystallization of NaClO_3 with racemic mixtures of hydrophobic amino acids impurities, DL-Phe, DL-Tyr and DL-Trp.

Magnetic Field Orientation	$n_{(l)}$	$n_{(d)}$
(I) DL-Phe B_{\uparrow}	25	49
(II) DL-Phe B_{\downarrow}	49	45
(III) DL-Tyr B_{\uparrow}	25	14
(IV) DL-Tyr B_{\downarrow}	25	32
(V) DL-Trp B_{\uparrow}	39	54
(VI) DL-Trp B_{\downarrow}	58	38

Table 3. By applying the Fisher test to pairs of experiments of Table 2 where only one condition is changed all hypotheses with confidence level above 95% are accepted.

Fisher Test: Hypothesis Tested Using $n_{(l)}$ and $n_{(d)}$	Confidence Level	Pair of Experiments
The orientation of the external magnetic field has an effect on the preferred handedness of NaClO_3 crystallized with DL-Phe.	99.46%	I and II
The orientation of the external magnetic field has an effect on the preferred handedness of NaClO_3 crystallized with DL-Tyr.	98.49%	III and IV
The orientation of the external magnetic field has an effect on the preferred handedness of NaClO_3 crystallized with DL-Trp.	99.30%	V and VI
The bias induced by B_{\uparrow} on the preferred handedness of NaClO_3 crystallized with racemic additives depends on the type of amino acid added (Phe vs. Tyr).	99.95%	I and III
The bias induced by B_{\downarrow} on the preferred handedness of NaClO_3 crystallized with racemic additives depends on the type of amino acid added (Tyr vs. Trp).	99.19%	IV and VI

These tests indicate that the output does depend on the orientation of the field, *i.e.*, that there is an enantioselective interaction where the magnetic field orientation is relevant, and that the enantioselective effect of the field is dependent on the type of amino-acid. This suggests that the chiral amino-acids and the static magnetic field mediate the enantioselective deracemization.

Double Blind Study and Control Experiments

Given the relevance of this assertion, the experiment has been re-run with an specifically designed double-blind test using two amino acids and a control solution to elucidate with no preconceived expectations if there is a selective interaction of the magnetic field with the added chiral amino acids. Three solutions with either DL-Phe additive, DL-Tyr additive or none additive (control group) were prepared as described above by one of the team members. The solutions were blindly labeled as “RED”, “GREEN” and “BLUE” by one of the authors. The blind test consists in the statistical investigation of the chiral sign of the crystallization products of amounts of 1 mL of each one of these solutions in crystallization vessels (the aerosol setup was not used in this test) with a magnet below in either orientation. After 1 or 3 days there were observable crystals. Again, images of the vessels bottom with the NaClO₃ crystals were taken using the two polarized filters and the microscope. The images were inspected a posteriori by a second investigator who was physically away from the laboratory during the months that the experiments took place. Each image file, corresponding to one crystallization experiment, was assigned to one of the three possible options (l), (d) or (r). All the results (images) of this double-blind test as well as their classification are accessible for the reader as Supporting Information. The results are summarized in Table 4, allowing the reader to perform the full classification exercise.

Table 4. Double blind experiment: statistical analysis of the crystallization products of a solution of NaClO₃ (control test) and of the same solution with chiral racemic impurities under an external magnetic field. The three solutions are labeled RED, GREEN and BLUE.

Magnetic Field Orientation	n _(l)	n _(d)
(i) RED B _↑	46	26
(ii) RED B _↓	34	46
(iii) GREEN B _↑	26	18
(iv) GREEN B _↓	24	17
(v) BLUE B _↑	32	45
(vi) BLUE B _↓	49	29

The crystallization of solutions with magnetic fields (in either north or south configuration) is homochiral (*i.e.*, either (l) or (d)) in 26% ± 4% of samples in the solution labeled as GREEN, 42% ± 4% for solution RED and 44% ± 4% for solution BLUE. The difference in the homochiral ratio suggests that solution GREEN is of different nature. Furthermore, we observe that solutions RED and BLUE do have a differentiated crystallization preference when exposed to a magnetic field and that the pattern is opposite, whereas solution GREEN has no differentiated response to upwards or downwards magnetic field and in both cases the preferential homochiral crystallization is (l). By comparison of the double-blind test (with crystallization vessels only) with the ones summarized in Table 2 (which include both

aerosol and crystallization vessels) we conclude first that the solution can be unequivocally identified according to its behavior when exposed to a magnetic field. We therefore identify solution GREEN as the one with no additive. Notice that the GREEN solution shows the crystallization of NaClO_3 exposed solely to ambient contaminants (organic and inorganic ambient impurities that deposit slowly at the air/water interface during the 1–3 days of crystallization) or inner contaminants (impurities in the salt solute or mili-Q water) with an external upwards or downwards magnetic field and that both experiments favor (l) vs. (d). Finally by comparison with Table 2 we identify RED as the one having DL-Tyr additive and BLUE as the one having DL-Phe additive. This identification is correct. The above summarized results can be easily obtained by the reader by reviewing and classifying all the images in the Supporting Information. Table 5 summarizes the hypotheses tested using of experiments of the double blind test. The level of confidence of acceptance of the hypotheses has increased because of the greater sample size of the homochiral observations.

Table 5. By applying the Fisher test to pairs of experiments of Table 4 where only one condition is changed all hypotheses with confidence level above 95% are accepted.

Fisher Test: Hypothesis Tested Using n(l) and n(d)	Confidence Level	Pair of Experiments
The orientation of the external magnetic field has an effect on the preferred handedness of NaClO_3 crystallized with DL-Tyr.	99.80%	i and ii
The orientation of the external magnetic field has an effect on the preferred handedness of NaClO_3 crystallized with DL-Phe.	99.80%	v and vi
The bias induced by B_{\uparrow} on the preferred handedness of NaClO_3 crystallized with racemic additives depends on the type of amino acid added (Phe vs. Tyr).	99.82%	i and v
The bias induced by B_{\downarrow} on the preferred handedness of NaClO_3 crystallized with racemic additives depends on the type of amino acid added (Phe vs. Tyr).	99.68%	ii and vi

In summary, according to Fisher test, the results are statistically significant with a confidence level of 99.7% or greater for all the study cases. This means that it is unlikely that these field dependent and amino acid dependent results occurred by chance alone. We conclude that “there is an enantioselective interaction at the water-air surface of a NaClO_3 saline solution with racemic amino acid additives induced by the magnetic field and the amino-acids.”

To elucidate the relevance of the chiral character of the additive, the same investigation is performed in a control experiment, using as additive the achiral amino acid Gly. For the experiments with Gly additives roughly $56\% \pm 6\%$ of the results are homochiral. In the case of Gly, the two results are not significantly different, but differ from the ones obtained without additives (Green solution of the double blind test), see Table 6. We can then state that this enantioselective effect of the field is only present if the amino acid which is acting as seed is chiral.

Table 6. Control experiment with an achiral additive: Gly.

Magnetic Field Orientation	$n_{(l)}$	$n_{(d)}$
(a) Gly B_{\uparrow}	90	89
(b) Gly B_{\downarrow}	75	94

To weight the relevance of the competition between either handedness a test is performed using only one chiral additive, namely 20 ppm of D-Trp, roughly $49\% \pm 7\%$ of the results are homochiral, see Table 7. The result with either orientation of the magnetic field are equivalent.

Table 7. Control experiment with only on chiral amino acid: D-Trp.

Magnetic Field Orientation	$n_{(l)}$	$n_{(d)}$
(a) D-Trp B_{\uparrow}	23	36
(b) D-Trp B_{\downarrow}	12	19

3.2. Discussion

In the absence of perturbations, the homochiral crystallization of sodium chlorate follows a binomial distribution, that is there are only two mutually exclusive possible outcomes, (l) and (d), and p is the probability of one outcome and $(1 - p)$ the probability of the other one. The shape of a binomial distribution is symmetrical when $p = 0.5$. Under the presence of chiral effects the distribution of sodium chlorate homochiral crystals is asymmetric and the probability of the favored state p deviates from 0.5. This work is not aimed at calculating the exact probability or proportion p of one handedness over the other one. It is clear from all the tests in this manuscript that the true proportion, for this experimental setup, shows very weak deviation from equiprobability (has a value of circa 60%). To assert with a significant confidence level that the p value in one experiment differs from 50% knowing that this deviation is small, we need much bigger sample sizes. However a few conclusions may be derived by a quick analyzes of the reported data. In particular, using the results of all the experiments (shown in Tables 2 and 4), we can state with a confidence level of 95% (*i.e.*, there is one chance in twenty that the true population proportion falls outside the range that is given) that the true proportion of the preferred handedness is $p_{(l)} = 57\% \pm 7\%$ for experiments with DL-Phe and B_{\downarrow} , $p_{(d)} = 62\% \pm 8\%$ for DL-Phe and B_{\uparrow} , $p_{(l)} = 64\% \pm 9\%$ for DL-Tyr and B_{\uparrow} and $p_{(d)} = 57\% \pm 8\%$ for DL-Tyr and B_{\downarrow} , $p_{(l)} = 58\% \pm 10\%$ for DL-Trp and B_{\uparrow} and $p_{(d)} = 61\% \pm 9\%$ for DL-Trp and B_{\downarrow} . In either case, p shows a significant deviation towards one handedness, (l) or (d), and the two outcomes are not equiprobable. See the chart in Figure 4 summarizing the probabilities for the cases with DL amino acids. As for the control experiments the results are summarized in Figure 5. For the solution with Gly additive (Table 6) then for B_{\uparrow} $p_{(l)} = 50\% \pm 8\%$ and for B_{\downarrow} $p_{(l)} = 56\% \pm 9\%$ showing in either case no significant deviation from equiprobability. In the case of solutions with D-Trp, both for B_{\uparrow} $p_{(l)} = 61\% \pm 11\%$ and for B_{\downarrow} $p_{(l)} = 61\% \pm 15\%$, show a preferred (d) handedness. For the GREEN control experiment (saline solution without amino acid additive) the sample size is too small and the error margin of the p estimate is therefore big. However, if we evaluate the p value of the solutions with no amino acids, exposed solely to ambient contamination and with the action of a magnetic field (named GREEN in the double-blind test) in Table 4, then for B_{\uparrow} $p_{(l)} = 59\% \pm 15\%$ and for

$B_{\downarrow} p_{(l)} = 59\% \pm 15\%$, that is both experiments show a preferred (l) handedness which is attributed to the influence of ambient contaminants which act after the long time exposure required for evaporation. In the case of solutions with Gly, the achiral additive that is present from the beginning in the solution, serve as seed to launch quickly the scalemic nucleation of crystals and the outcome is therefore equiprobable. This internal additives act earlier as nucleation seeds than ambient in-falling contamination. The unavoidable salt metallic ferromagnetic impurities are present at levels of 15 ppm, which are comparable with the concentration of the added amino acids (20 ppm). These metallic impurities may serve as ordering structure where racemic amino acids arrange in one or another preferred scalemic arrangement depending on the magnetic field orientation. What is significant and common to all the control experiments is that when there are no racemic chiral additives in the solution then the preferred crystal handedness does not vary with the magnetic field orientation. There is no enantioselective effect unless the two handedness of a chiral amino acid are present. See the chart in Figure 5 summarizing the probabilities of these control experiments.

Our experimental work shows the existence of an interaction at the water-air interface that depends on the magnetic field orientation and that strengthens the influence of one or other handedness when there are racemic mixtures of chiral amino acids in the saline solution.

Figure 4. True proportion or probability of sodium chlorate handedness, (l) vs. (d), as function of the magnetic field orientation, for different racemic chiral additives, DL-Phe, DL-Tyr and DL-Trp, in the initial solutions and for opposing magnetic field orientation. The preferred handedness varies with magnetic field orientation suggesting that there is an interaction of the field with chiral amino acids.

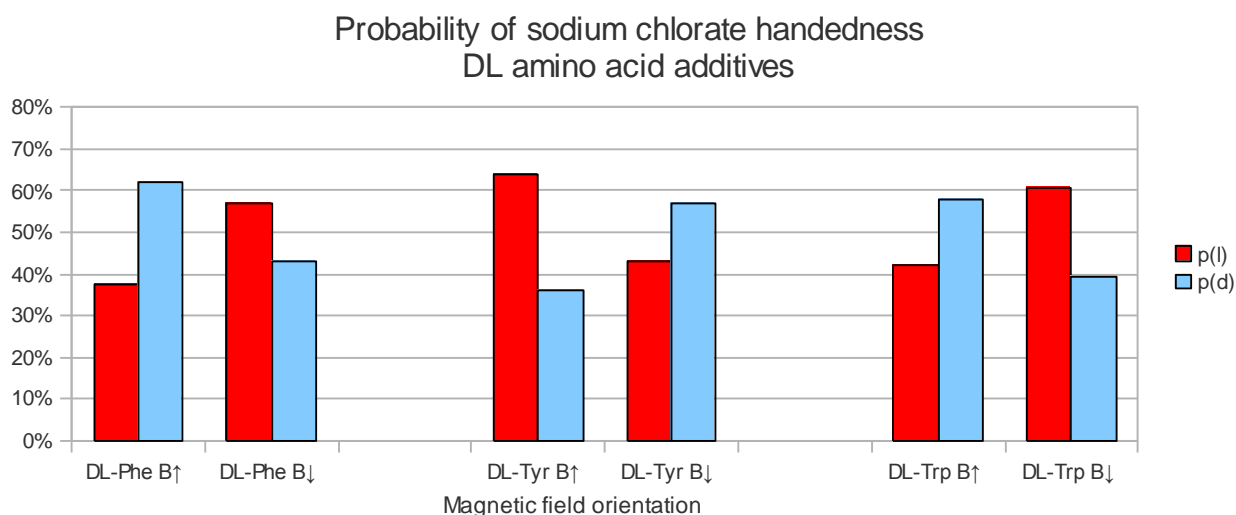
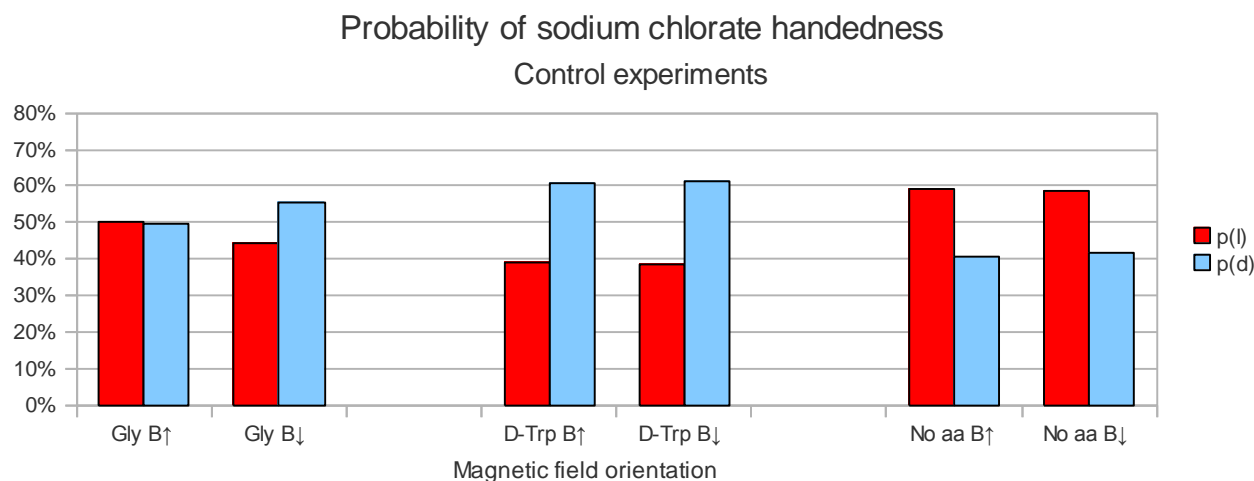


Figure 5. True proportion or probability of sodium chlorate handedness, (l) vs. (d), in the control experiments, as function of the magnetic field orientation and initial solution, with only 20 ppm of the achiral amino acid Gly, with 20 ppm of the chiral amino acid D-Trp or without amino acid and exposed solely to ambient chiral. The preferred handedness does not vary with magnetic field orientation indicating that in these control experiments there is no enantioselective interaction.



4. Conclusions

4.1. Review of Plausible Effects

Providing a detailed physicochemical molecular model of the water-air interface processes and energy differences associated to a plausible interaction between amino acids, water molecules, solvent saline ions, unavoidable metallic impurities and external magnetic field is beyond the scope of this experimental work. For completeness, we review here some mechanisms that have been put forward in the literature and may be relevant to interpret and contextualize these observations.

Ordered packing and self-assembling processes at the water interface between neighboring amino acids may induce the formation of ordered clusters and conglomerates with segregated enantiomers. For example, it is known that racemic mixtures of amphiphilic molecules at water interfaces can self-assemble into 2D crystallites of three types: (I) enantiomerically disordered solid solutions, (II) enantiomorphous conglomerates with segregated enantiomers and (III) racemic compounds in which both enantiomers are packed together. In this latter case, it has been experimentally shown that α -activated amino acids derivatives polymerize in oligopeptides with an enhanced homochiral sequence [16]. In this context we may postulate that, in this saline solution, the external magnetic field may exert some influence on the effectiveness of chiral amino acid self-assemblies at the water-air interface or on the metallic impurities of the salt and that these enantiopure clusters would in turn favor the nucleation of one handedness of the probe, *i.e.*, the NaClO₃ crystals.

Alternatively, a severely debated proposal suggested in 1985 by Gilat [17–20] postulated the existence of what he named “chiral interaction” between chiral biomolecules (such as amino acids and proteins) and a polar solvent (such as water) in the presence of an interface, that leads to the formation of a magnetic moment of opposite direction for each enantiomer (μ_{\uparrow} for L and μ_{\downarrow} for D). In this model,

under an external magnetic field the L and D enantiomers located at the water-air interface would have different energies and this would in turn induce (according to Boltzmann law) different population within this interface. This hypothesis has been seriously questioned because, as pointed out first in 1986 by Barron, who formulated the symmetry requirements for a reaction to yield a chiral result, enantioselectivity induced by magnetic fields per se is not allowed [5]. However this work also emphasized that this conclusion only applies at thermodynamic equilibrium, and goes on to say that for far-from-equilibrium processes a magnetic field might induce enantioselection if the molecules are aligned since this constitutes a falsely chiral (*i.e.*, time-noninvariant enantiomorphous) arrangement [5,6]. Thus, for a magnetic field to genuinely induce preferential deracemization of sodium chlorate crystals aided by racemic amino acids, this would require a process well away from equilibrium, with the amino acids aligned at the surface. Here the far from-equilibrium conditions are driven by the phase transition process of the crystallization of a sodium chlorate solution. Similarly Micali *et al.* [21] have recently shown how a falsely chiral influence (consisting of combined rotational and magnetically tuned effective gravity, together with the magnetic orientation of the self-assembled helical supramolecular aggregates) induces enantioselection in a far-from-equilibrium process involving the formation of chiral J-aggregates. These external forces were only applied during the nucleation step of the aggregation and it was shown to be sufficient to achieve chiral selection. The experimental results reported here describe the effect of the static magnetic fields on the chiral crystallization of sodium chlorate which deracemizes in the presence of racemic amino acids providing an example of enantioselection in a far-from-equilibrium process (namely the crystallization of a super saturated solution) under a falsely chiral influence. The statistical analysis shows that the orientation of the magnetic field influences the absolute configuration of the crystals.

Other experimental works have explored the induction of enantioselectivity by magnetic fields due to changes in the optical properties of materials. Among them the one on chemical synthesis in 1994 by Zadel *et al.*, which was later on publicly declared as fraudulent [22,23]. An example of other successful and relevant results are those regarding the study of a static magnetic field collinear with an unpolarized light beam [24]. Also other experiments have explored the interaction of extremely intense magnetic fields (of the order of 5T) with the crystal growth rate of L-alanine in aqueous solution [25]. Regarding crystallization rates it is also important to emphasize that in the presence of an appropriate chiral impurity primary and secondary nucleation processes may be affected. For example if DL-Glu is crystallized in the presence of L-Lys, D-Glu crystallizes first followed by L-Glu. However, the nucleation effects reported here required very intense magnetic fields y contrast to the weak magnetic field of circa 200 mT used in this work.

4.2. Conclusions and Plausible Implications

We have presented a novel technique, the statistical investigation of the chiral sign of the crystallization products of NaClO₃ solutions with chiral additives, to detect tiny enantiomeric excesses of chiral impurities located at the water-air interface. The technique is robust and all the steps involved are relatively simple and require inexpensive and common equipment and products which makes this test accessible for standard laboratories. Furthermore, since there are two differentiated experimental steps (solution and crystallization preparation *vs.* images and sample counting) this procedure can be

easily used for double-blind tests. This technique provides promising results and this work will be further extended to explore the interactions of other amino acids, peptides, mixtures of amino acids and other chiral compounds.

We have shown that the orientation of the magnetic field does have a significant effect on the preferred handedness of the NaClO₃ crystallized with a few ppm of racemic mixtures of chiral hydrophobic amino acids such as DL-Phe, DL-Tyr and DL-Trp and that this effect is due to an enantioselective interaction with the chiral amino acids in the solution. This enantioselective effect is not observed when there is only one handedness of added chiral amino-acid (for instance D-Trp), if the added amino acid is not chiral (Gly) or if there is no additive (and thus the solution is exposed solely to ambient and solute impurities), confirming that the magnetic field interacts only with the few ppms of added DL-amino acids. It is unclear what the exact role of the metallic ferromagnetic impurities and salt ions in the solution may be in this enantioselective print of the DL amino acids, however they are constantly present in all compared cases and show no enantioselective interaction with the magnetic field in the absence of racemic amino acids. This enantioselective interaction is robust, observed in the three cases tested (Phe, Try and Trp), and biases the handedness of the output in spite of the small amount of amino acids (20 ppm's) and the unavoidable constant background chiral contamination of laboratories. The experiments have been run systematically using the same solution in parallel under different conditions as control. According to our observations, the orientation of the magnetic field can strengthen the influence of one chiral form of the amino acid at the water air interface. Let us remark that the three type of hydrophobic amino acids chosen, are similar in structure and have one (Phe and Tyr) or two (Trp) aromatic rings. The presence of the aromatic ring may be important for the interaction with the magnetic field. In the future, these tests shall be extended to other classes of amino acids, other pHs and other metallic additives to elucidate the role of other intermediate factors in the formation of chiral structures.

These experimental results describe the effect of the static magnetic fields in the chiral crystallization of sodium chlorate which deracemizes in the presence of racemic amino acids providing a plausible example of enantioselection in a far-from-equilibrium process under a falsely chiral influence.

We believe that the experimental results reported in this manuscript, namely the existence of an enantioselective interaction with racemic mixtures of chiral amino acids at the water-air interface in a saline solution, may have far-reaching implications. In particular, and regarding the prebiotic Terrestrial environment, it suggests that the geomagnetic field of the Earth could have played a significant role on the prebiotic origin of homochirality acting enantioselectively on all hydrophobic chiral amino acids and peptides participating in the ocean-atmospheric chemistry of the primordial aerosol cycle [26–30]. Of the four solar terrestrial planets the Earth is the only one that has an atmosphere such that the pressure and temperature conditions below it can sustain liquid water. The huge water pool of saline water in the oceans, which covers approximately 71% of the Earth's surface, and the atmosphere are connected through an evaporation, condensation and rain cycle. Furthermore, the Earth has a geomagnetic field [31] (which now ranges between 0.03 mT and 0.06 mT, depending on the location) which is the strongest of the four terrestrial planets. It has been recently shown that an appreciable magnetic field had developed as early as 3.45 billion years ago (Ga), right after the Late Heavy Bombardment (3.90 Ga) and before the development of an oxygen-rich atmosphere (2.30 Ga) suggesting that the magnetic field may have predated the

establishment of life [32]. The measured field strengths, while less than that of the present-day Earth, may have been sufficient to shroud the Earth in a protective shield that prevented ionization and erosion by the charged particles in the solar wind. The geomagnetic field orientation has changed in intensity and orientation [31]. However, prebiotic chemistry, must have had one location where there was a higher concentration of products or where the transport processes were more intense or where plausible intermediate factors (such as solvated metallic ions) may be available. This place had one given magnetic field orientation. As shown in this experimental work every amino acid type (hydrophobic, less hydrophobic, achiral, *etc.*) may have a different interaction with a magnetic field orientation. Therefore, the final outcome of the selection process (*i.e.*, L or D) shall depend on the overall interaction of a network of organic molecules at the location where organic chemistry reactions take place, with the local magnetic field and the global water transport cycle. Other effects, such as fluctuations or chiral interactions with minerals such as clays, may have induced also local enantiomeric excesses, but this geomagnetic effect must have been ubiquitous, constantly present over time, unavoidable for all the water-based prebiotic chemistry and relevant for all amino acids and peptides. Furthermore, the existence of such a magnetic interaction may help explaining the role of metals in the prebiotic origin of life. We believe that this experiment shall provide interesting experimental information for those elucidating what may have been the initial prebiotic conditions.

Acknowledgments

We acknowledge an anonymous referee for the well documented comments and suggestions for improvement, as well as by urging the team to double-blind test the experiment to validate the robustness of this result. We acknowledge the interest of Meir Lahav and his suggestion for further control experiments. We acknowledge both Meir Lahav and Laurence David Barron for reviewing this work and pointing out the possible role of magnetic impurities. We also are thankful to all the participants of the Working Group meeting “Chirality in Systems Chemistry”, Toledo, Spain, 8–10 April 2011 of the COST CM0703 “Systems chemistry” project, where the members of this work were invited to present this work for open discussion. The authors acknowledge Rafael Pérez del Real and the Optoelectronics Laboratory of INTA, for the measurements of the magnetic field generated by the magnets used in the experiments. The pictures of the experimental setup have been taken by Rogelio Sánchez Verdasco from CAB at INTA. We also thank the scientific support and advice of David Hochberg. This research was supported by the Instituto Nacional de Técnica Aeroespacial (INTA) and by the grant AYA2006-15648-C02-02 of the Ministerio de Educación y Ciencia (Spain) and by the project grants AYA2011-25720 and AYA2012-38707 of MINECO (Spain).

Author Contributions

María-Paz Zorzano has designed the experiments, analyzed the data and written the manuscript. Susana Osuna-Esteban has performed the experiments and analyzed the data. Susana Osuna-Esteban, Marta Ruiz-Bermejo and Sabino Veintemillas-Verdaguer have helped in the contextual interpretation of the results. Cesar Menor-Salván has performed an independent analysis of the data.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Avalos, M.; Babiano, R.; Cintas, P.; Jimenez, J.L.; Palacios, J.C.; Barron, L.D. Absolute asymmetric synthesis under physical fields: Facts and fictions. *Chem. Rev.* **1998**, *98*, 2391–2402.
2. Rikken, G.L.J.A.; Raupach, E. Enantioselective magnetochiral photochemistry. *Nature* **2000**, *405*, 932–935.
3. Szabó-Nagy, A.; Keszthelyi, L. Demonstration of the parity-violating energy difference between enantiomers. *Proc. Natl. Acad. Sci.* **1999**, *96*, 4252–4255.
4. Kojo, S.; Tanaka, K. Enantioselective crystallization of D,L-amino acids induced by spontaneous asymmetric resolution of D,L-Asparagine. *Chem. Commun.* **2001**, *19*, 1980–1981.
5. Barron, L.D. Can a magnetic field induce absolute asymmetric synthesis? *Science* **1994**, *266*, 1491–1492.
6. Barron, L.D. True and false chirality and absolute enantioselection. *Rend. Lincei* **2013**, *24*, 179–189.
7. Kondepudi, D.K.; Kaufman, R.J.; Singh, N. Chiral symmetry breaking in sodium chlorate crystallization. *Science* **1990**, *250*, 975–976.
8. Veintemillas-Verdaguer, S.; Osuna-Esteban, S.; Herrero, M.A. The effect of stirring on sodium chlorate crystallization under symmetry breaking conditions. *J. Cryst. Growth* **2007**, *303*, 562–567.
9. Viedma, C. Selective Chiral Symmetry breaking during crystallization: Parity violation or cryptochiral environment in control? *Cryst. Growth Des.* **2007**, *7*, 553–556.
10. Osuna-Esteban, S.; Zorzano, M.P.; Menor-Salvan, C.; Ruiz-Bermejo, M.; Veintemillas-Verdaguer, S. Asymmetric chiral growth of micron-size NaClO₃ crystals in water aerosols. *Phys. Rev. Lett.* **2008**, *100*, doi:10.1103/PhysRevLett.100.146102.
11. Viedma, C. Chiral symmetry breaking during crystallization: Complete chiral purity induced by nonlinear autocatalysis and recycling. *Phys. Rev. Lett.* **2005**, *94*, doi:10.1103/PhysRevLett.94.065504.
12. Kondepudi, D.K.; Crook, K.E. Theory of conglomerate crystallization in the presence of chiral impurities. *Cryst. Growth Des.* **2005**, *5*, 2173–2179.
13. As the light travels toward the observer, the plane of polarization of a linearly polarized beam is rotated by the crystal either to the left (counterclockwise) or to the right (clockwise) relative to its plane upon entry. If the former holds, the crystal is levorotatory (l); if the latter, it is dextrorotatory (d) [14]. To observe the optical activity using a microscope place the first linear polarizer at the exit of light, below the crystals, to create a linearly polarized light beam. Then place the second polarizer at the end of the microscope above the crystals and rotate to left or right until the brightness of the crystals is maximized. If we must rotate the second polarizer to the right of the first one, to maximize the brightness of the crystals, this means that they are dextrorotatory. If for the same second polarizer position, the crystals are seen dark, this means that

they are levorotatory. In this case, by rotating the second polarizer the same angle to the left, the crystals shall be seen bright.

14. Donald Bloss, F. *Crystallography and Crystal Chemistry*, Mineralogical Society of America; Monograph Series; Holt, Rinehart and Winston: Washington, DC, USA, 1994.
15. We have chosen to evaluate the handedness of the crystallization per vessel, considering each vessel output as one single experiment. We chose this method instead of counting individual crystals and weighting their contribution through crystal surface size or mass (*i.e.*, measuring the excess fraction per experiment) because of the following reason. For both experimental set-ups the crystal size usually ranges between 1 and 2 mm, many crystals are superposed forming aggregates and in some cases there are in addition multiple smaller crystals induced by secondary nucleation (in this case the handedness of secondary nuclei is inherited from the mother crystal). Separating and weighting these 1–2 mm sized of the crystal distribution and distinguishing secondary crystals for the circa 2000 experiments that have been run for this test is not a practical experimental approach for this setup. Furthermore, our approach, namely saving the images of each crystallization experiment and defining the output as either homochiral—(l) or (d)—or arbitrary rate (r), allows the non-expert reader or any other parties outside the laboratory team, to evaluate the outcome of the experiments easily and independently.
16. Zepik, H.; Shavit, E.; Tang, M.; Jensen, T.R.; Kjaer, K.; Boldbach, G.; Leiserowitz, L.; Weissbuch, I.; Lahav, M. Chiral amplification of oligopeptides in two-dimensional crystalline self-assemblies on water. *Science* **2002**, *295*, 1266–1269.
17. Gilat, G.; Schulman, L.S. Chiral interaction, magnitude of the effects and application to natural selection of L-enantiomer. *Chem. Phys. Lett.* **1985**, *121*, 13–16.
18. Gilat, G. Chiral interactions in biomolecules. *Chem. Phys. Lett.* **1985**, *121*, 9–12.
19. Gilat, G. On the Biological Advantage of Chirality. Available online: <http://arxiv.org/abs/physics/9911041v1> (accessed on 18 November 1999).
20. Gilat, G. Chiral interaction and thermodynamics. *Chem. Phys. Lett.* **1987**, *137*, 492–494.
21. Micali, N.; Engelkamp, H.; van Rhee, P.G.; Christianen, P.C.M.; Monsú, S.L.; Maan, J.C. Selection of supramolecular chirality by application of rotational and magnetic forces. *Nat. Chem.* **2012**, *4*, 201–207.
22. Zadel, G.; Eisenbrdun, C.; Wolff, J.; Breitmaier, E. Enantioselective reactions in a static magnetic field. *Angew. Chmi. Int. Ed. Engl.* **1994**, *33*, 454–456.
23. Breitmaier, E. Letter to the Editors: No enantioselective reactions in a static magnetic field. *Angew. Chmi. Int. Ed. Engl.* **1994**, *33*, 454–456.
24. Rikken, G.L.J.A.; Raupach, E. Enantioselective magnetochiral photochemistry. *Nature* **2000**, *405*, 932–935.
25. Zhao, B.; Guzman, L.A.; Ogawa, K.; Shimizu, K. Effect of magnetic field on the growth rate of L-Alanine crystal. *J. Chem. Eng. Jpn.* **2003**, *36*, 959–962.
26. Armstrong, D.W.; Kullman, J.P.; Xianghong, C.; Rowe, M. Composition and chirality of amino acids in aerosol/dust from laboratory and residential enclosures. *Chirality* **2001**, *13*, 153–158.
27. O'Dowd, C.D.; Facchini, M.C.; Cavalli, F.; Ceburnis, D.; Mircea, M.; Decesari, S.; Fuzzi, S.; Yoon, Y.J.; Putaud, J.P. Biogenically driven organic contribution to marine aerosol. *Nature* **2004**, *431*, 676–680.

28. Nilsson, E.D.; Mårtensson, E.M.; VanEkeren, J.S.; de Leeuw, G.; Moerman, M.; O’Dowd, C. Primary marine aerosol emissions: Size resolved eddy covariance measurements with estimates of the sea salt and organic carbon fractions. *Atmos. Chem. Phys. Discuss.* **2007**, *7*, 13345–13400.
29. Dobson, C.M.; Barney Ellison, G.; Tuck, A.F.; Vaida, V. Atmospheric aerosols as prebiotic chemical reactors. *Proc. Nat. Acad. Sci.* **2000**, *97*, 11864–11868.
30. Ruiz-Bermejo, M.; Menor-Salvan, C.; Osuna-Esteban, S.; Veintemillas-Verdaguer, S. Prebiotic microreactors: A Synthesis of purines and dihydroxy compounds in aqueous aerosol. *Orig. Life Evol. Biosph.* **2007**, *37*, 123–142.
31. Valet, P. Time variations in geomagnetic intensity. *Rev. Geophys.* **2003**, *41*, 1–44.
32. Tarduno, J.A.; Cottrell, R.D.; Watkeys, M.K.; Hofmann, A.; Doubrovine¹, P.V; Mamajek, E.E.; Liu, D.; Sibeck, D.G; Neukirch, L.P.; Usui, Y. Geodynamo, solar wind, and magnetopause 3.4 to 3.45 billion years ago. *Science* **2010**, *5*, 1238–1240.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).