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Theoretical Studies of Glutamate Enantiomers at Different pH Conditions

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Abstract. Life, at least as we know it, is invariably linked to homochirality. Here, we present a new approach to understanding the homochirality using glutamic acid as a model. Glutamic acid is the key amino acid in living organisms. It was probably formed abiotically under conditions prevailing in the early Earth's atmosphere and its two enantiomers have been found in meteorites. The two enantiomers, however, do not exhibit biological activity to the same extent. The D-enantiomer usually has either very little or no activity as compared to the L enantiomer. In order to find an explanation for the differences, we have undertaken theoretical studies with classical and semi-empirical force field models on solvated L- and D-glutamate. Our results show strong interactions between charged atoms of the D-enantiomer that determine a very rigid spatial configuration resulting in a low capacity to adapt to different environmental conditions and to respond to energy perturbations. Our results might provide a possible explanation for the natural selection of the L-glutamate enantiomer as a basic constituent of living matter.

1. Introduction

One of the landmarks of terrestrial biochemistry is its homochirality, being based predominantly upon the L-amino acids and D-sugars to the virtual exclusion of their infrequent enantiomers D-amino acids and L-sugars. For the presence of racemic and scalemic mixtures of amino acids enantiomers in extraterrestrial materials, as meteorites, three main hypotheses were considered: a) they are products of extraterrestrial life, b) they are abiotic compounds from early stages of the solar nebula, and c) they are result of terrestrial contamination. The last hypothesis, the terrestrial contamination, will be discarded based on the data of concentrations identified in several of the meteorites and chondrites. McDonald & Bada (1995) based on the detection of amino acids, mainly of L-enantiomers in the Martian meteorite EETA 79001, considered these as terrestrial contaminants. The relative order of abundance found in this samples was $Glu > Ser > Gly > Ala > Asp$, considering only the amino acids commonly found in proteins. Brinton et al. (1998) studying Antarctic micrometeorites (AMMs) identifies an excess of L-enantiomers of amino acids and by this fact considered as terrestrial contamination. The abundance order found in these AMMs is: $Ala > Glu > Gly > Ser > Asp$, again presenting only the protein most common in amino acids. Similar results appears after the analysis of other samples, such as Allan Hill carbonaceous chondrite, meteorite 77306 (Kotra et al (1979)), where the abundance order found was: $Gly > Ala > Asp > Ser > Val > Thr > Leu > Ile$, in Martian meteorite Nakhla the abundance order is $Gly > Glu > Ser > Asp > Ala$. At last, as examples, in Murchinson carbonaceous meteorite the relative abundance order is: $Gly > Ala > Glu > Val > Ile > Leu > Ser$ (Glavin et al. (1999)).

From the previous examples, selected from all the reported data, emerge some clear facts: 1) the abundance of amino acids is not the same in all samples, 2) a significant number of amino acid not common on Earth are found in meteorites, and 3) the amounts of amino acids in extraterrestrial samples differ completely to the frequencies found in terrestrial samples for common amino acids in proteins. Based on the reports of SwissProt data bank these abundances are: $Leu > Ala > Gly > Ser > Val > Glu > Ile > Thr > Arg > Asp$. The only common fact is the presence of the same amino acids but the frequencies are different. A question that emerges is: does this fact depend on the modernity of the samples reported by the databank ? The data cited by Kvenvolden (1975) is useful for addressing this hypothesis. The order of amino acid abundances in Middle Middle Miocene (Mercenaria) and modern fossils are also different from the SwissProt and from the extraterrestrial samples, these are: for Miocene $Ala > Pro > Glu > Val > Leu > Lys$, and for modern fossils: $Asp > Gly > Ala > Ser > Glu > Leu > Pro$. With this data, it seems that the amino acids, in almost all extraterrestrial samples, belong to extraterrestrial sources and do not constitute terrestrial contamination. Following this, a new question arises:- How do these amino acids resist not only the different extreme conditions in space but also those inside Earth's atmosphere ? The studies of Abelson (1954, 1959) suggest a plausible answer. Abelson determined a stratification of stability to temperature by heating amino acid solutions at different times. It was found that the amino acids Ala, Gly, Glu, Leu, Ile, Pro and Val are relative stable molecules. Abelson found that Asp, Lys, and Phe were intermediary stable amino acids and the relatively unstable amino acids were Ser, Thr, Arg, Tyr. With this data Abelson concluded that these molecules are sufficiently stable to remain intact for more than a billion years. It is important to note the identified extraterrestrial amino acids consists mainly of the relative resistant group. This fact was confirmed in studies of the oxidative conditions (Khan & Souden (1971)), which showed the same stable amino acids. A further interesting fact in identifying meteoritic amino acids with having an extraterrestrial origin is the presence of amino acids that are uncommon on Earth's surface, such as norvaline, isovaline, norleucine, 2-methylnorvaline, pseudoleucine,amongst the amino acids identified in the Murchinson carbonaceous meteorite. It is still not easy to select the correct hypotheses presented above. However, the presence of these amino acids in the Murchinson meteorite, which is 3.4 billion years old, appears to suggest that abiotic extraterrestrial syntheses is the likeliest possibility but we can not exclude the possibility that extraterrestrial life is the origin (Cronin, Gandy & Pizzarello (1981)).

An important fact is that, in almost, all extraterrestrial samples there is a scalemic mixture between the two amino acids enantiomers. In general, the D/L enantiomers ratio in most samples range from 0.021 up to 0.670 (Botta & Bada (2002)). The origin of the enantiomers mixtures has several hypotheses. Bonner & Rubenstein (1987) suggest that ultraviolet circularly polarized light (CPL), of a particular handedness, emitted by neutron start could lead to either preferential syntheses or degradation of specific enantiomers of chiral compounds either in the interstellar molecular clouds or in planetary bodies. This is known as Rubenstein-Bonner hypotheses. Recently, it has been shown that amino are formed by irradiation of interstellar/circumstellar ice analogs with ultraviolet (UV) CPL, produced by a synchrotron radiation beamline, and thus allowed us to quantify the effect of such polarized light on the production of amino acids (Nuevo et al. (2007)). These results can be compared to the enantiomeric excesses measured in primitive meteorites such as Murchison. Another hypothesis of chirality is the parity violation energy differences (PVED's) that represents an essential property of particle and atomic handedness used to cope with the complex phenomenon of asymmetry in the universe. This proposal is based on the findings of Lee & Yang , in 1956, who proposed that parity is not always conserved. Their classical paper 'Question of parity conservation in weak interactions' analyzed numerous cases of strong and weak interactions and showed that the evidence for parity conservation applies only to the first interaction and not to the weak one. The absolute magnitudes of such PVED's are too small to be measured experimentally by our current available instruments, but they can be evaluated by theoretical calculations. This origin and the amplification of the effect by the living matter could explain the fact of the stereoselectivity in living matter on Earth (Avalos et al. (2000)).

Studies of D- and L- enantiomers of amino acids have added new dimensions to our understanding of the occurrence and fate of amino acids in terrestrial and extraterrestrial samples. The present study was developed with glutamic acid as example. Glutamic acid was chosen for a number of reasons. Practically all other amino acids can be metabolically derived from it (Morowitz (2002)), and following the results of Breslow & Levine (2006), it appears likely that there is partial transfer of enantioselective chiralities to all molecules derived from it. As mentioned previously, it is present in several meteorites and other extraterrestrial sources (Engel & Macko (2001)), and glutamate chronology studies show racemization in foraminifers fossils (Takano et al. (2006)). Moreover, it is present as scalemic mixtures in meteorites (Engel & Macko (2001)), which apparently is one of Earth's prebiotic amino acids (Miller (1974); Delaye & Lazcano (2005)), and was demonstrated recently to play an important role in the evolution of the hominoid brain (Burki & Kaesmann (2004)). It is also a key excitatory neurotransmitter in higher organisms (Dingledine et al. (1999)) and is utilized by the food industry as one of the major food additives and it is responsible for the umami flavor (McCabe & Rolls (2007)). In addition, just as with other amino acids, it occurs as two enantiomers: L and D-Glu; both enantiomers present different interaction capacities with their receptor and transporters (Moriyoshi et al. (1991)), and there are binding differences with calcium and other minerals by the two enantiomers (Hazen Filley & Goodfriend (2001)). Thus, using glutamic acid, and through theoretical and computational processes, we present a proposal that (in conjunction with other hypotheses) CPL and PDVE's explain the Earth living matter stereoselectivity for L- type enantiomers.

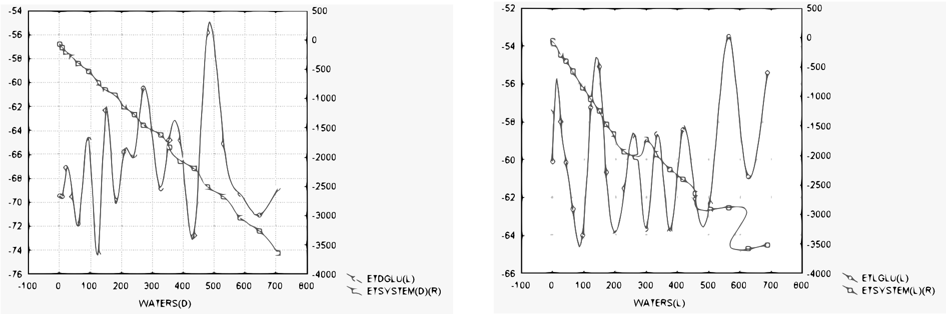

Figure 1. Energy landscapes, for the total system and for different D- and L-Glu in all established conformations.

2. Methods

Initially, L and D-Glu structures *in vacuo* was derived from Biopolymer program from InsightII, then the charges where modify, at different pH values, with charged hydrogen option. These molecules where solvated with layer from 0 to 21 Å that is from *in vacuo* up to about 500 water molecules by glutamate molecule, using Solvation program from InsightII. Using the program Discover 3 the total, potential and kinetic energies from the system, the glutamate and the solvent was calculated by, both, energy minimization and molecular dynamics following Charmm parameterizations. Once the structures where optimized, we calculated other molecular parameters as dipolar moments, using the program MM2, from classical molecular mechanics. For this optimized structures also all the internal variables where calculated with the program MOE. Using the same program partial atomic charges where calculated following the method of Gasteiger (PEOE). With these optimized structures was generated all the possible hydrogen bonds for each conformer and the same conformers where compared by the VMS programs using the RMS as spatial similarity indicator. All the statistical analysis where developed with the program Statistica 6.0 and graphs where developed with the programs Statistica 6.0 and Mathematica 3.0.

3. Results

Glutamic acid has partial charges, even if the total net charge is null, at different pH values. This fact has a strong effect on the results of conformers' calculation. Though the results shown here belong to the neutral pH value, the most abundant form in biological samples on earth, the trend is similar for all the pH range evaluated. The evaluation of energetic values, potential, kinetic and total energy, for all the conformations that both enantiomers can acquired reports the existence of different energy landscape for D- and L-Glu as appears represented in Figure 1. For L-Glu the minima has similar values, unlike the values found for D-Glu.

The potential energy values are -74.267 ± 3.061 and $-81.540 \pm 4.379 KJ/mol$ for L- and D-Glu respectively. The, roughly, 10% difference in mean values implies a possible major stability for D-enantiomer against L-Glu. This appears confirmed using a simple model as: $\Delta E(L - Glu, D - Glu) = E_t(L - Glu) -$

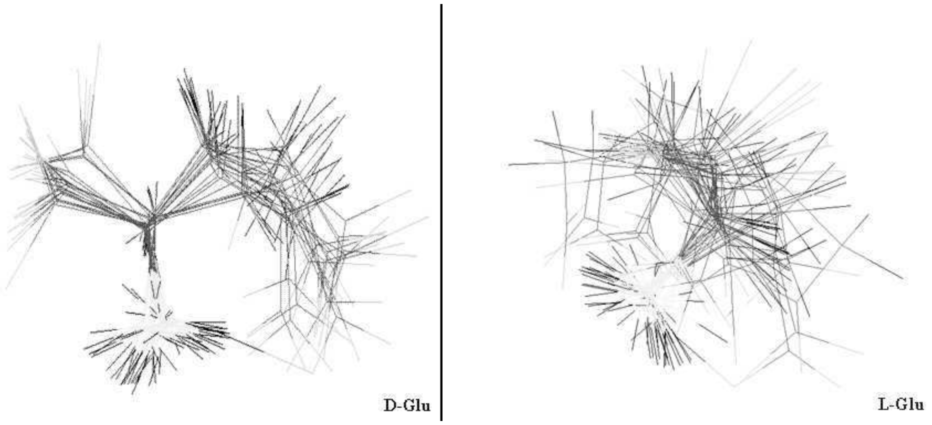

Figure 2. Representation of all found D- and L-Glu conformations with identical solvation levels for both enantiomers.

$E_t(D-Glu)$, where if $\Delta E(L-Glu, D-Glu) > 0$ then D-Glu is the more stable structure, and the average for all identical conditions is $\Delta E = 7.344 \pm 5.766$. The representation of all the conformations acquired by D- and L-Glu in identical environmental conditions are shown in Figure 2.

From this study, it is apparent that L-Glu is a more flexible molecule than D-Glu. After the calculation of several molecular features, with the goal understanding the factors that rule their behavior, we found that the partial charges of possible charged atoms at different environmental pH's is useful for generating a multiple correlation model that shows this as a major source of D-enantiomers low flexibility, implying low resilience and consequently it is significantly more fragile with respect to L-Glu. L-Glu is a more flexible and resilient molecule that can support strong environmental changes and energy stress, absorbing the energy differences thus changing the conformations by the flexible lateral chain. The equations describing these findings are: For L-Glu $Q_p(N) = -1.31 - 0.10Q_p(OXT) - 0.32Q_p(O) - \underline{1.30}Q_p(OE1) - \underline{1.60}Q_p(OE2)$, $R^2 = 0.7145$; $p = 0.0003$, and for D-Glu $Q_p(N) = -0.49 - \underline{0.33}Q_p(OXT) - 0.18Q_p(O) - \underline{0.70}Q_p(OE1) - \underline{0.73}Q_p(OE2)$, $R^2 = 0.5317$; $p = 0.0121$, where Q_i are the partial charges of the charged atoms in the molecules. Underlined values are statistically significant at $p = 0.05$ level.

4. Conclusions

From the present study, with glutamate as example, it is possible to present the following general conclusions: the D-Glu enantiomer is more stable, less flexible and it presents lower resilience than the L-Glu enantiomer. The lower flexibility of D-Glu limits its possibilities to interact with different ligands, while at the same time the higher stability of the D-Glu enantiomer makes it more susceptible to degradation by external energetic stress such as thermal perturbations and photolysis. If stability is related to energy values, the D-Glu enantiomer requires more energy for its synthesis, since it shows higher energy values. This trend is independent of pH due to the fact that in the entire pH range the glutamic acid exhibits partial charges distributed in specific regions of the molecule. The higher D-Glu enantiomer stability might explain living matter's "preference" for a more adaptable molecule such as L-Glu. This feature might have been transmitted to all other amino acids that could have been derived from it. The global proposal to explain the living matter stereoselectivity present two phases: enantiomeric excess (ee) following the Rubenstein-Bonner hypothesis for selective synthesis along with the parity violation of energy differences and latter the low flexibility of one of the enantiomers facilitates the degradation of this kind of molecules allowing the excess of the other type. The parity violation energy differences will be reflected, using the molecular orbitals theory, in subtle but significant differences in the overlapping matrix of bonding electrons among atoms belonging to each type of enantiomers.

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