How did protein amino acids get left-handed while sugars got right-handed?

Term Paper for Physics 569*

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Abstract: Although chiral (mirror-symmetric) pairs of molecules are synthesized in equal proportion in laboratory experiments, life is partial to only one molecule of the pair. There have been many experimental and theoretical efforts to explain why living organisms have only L-amino acids in proteins and D-sugars in RNA and DNA, but it still remains an open question. I shall review some of the major efforts in explaining homochirality in living systems, and present my perspective on each of them. Finally, further directions in answering this question will be explored.

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1.Introduction

Looking down at our feet (or hands), it is easy to see that they are non-superimposable mirror images of each other. No matter how they are oriented relative to each other, we cannot superimpose all features of one foot (or hand) over the other. Molecules that cannot be superimposed on their mirror images are called chiral and the two mirror image forms are called enantiomers. Chirality (handedness) is a characteristic of molecules lacking a plane of symmetry. Since carbon has a valency of four, it can bind four different chemical groups in two different enantiomeric configurations (Fig. 1). Hence, many organic molecules are chiral. Enantiomers are classified according to their stereochemical arrangement as L- form or D- form (see Fig. 1 for example).

How can we tell chiral enantiomers apart in a laboratory? Enantiomers have similar physical properties and are identical with respect to ordinary chemical reactions. Differences arise only when they interact with other chiral molecules. For example, the antibiotic penicillin only binds to (and kills) D-alanine, which occurs in the cell walls of bacteria (but not in humans). Enzymes, which are chiral molecules, also distinguish between the two enantiomers of a chiral substrate. Chiral enantiomers are optically active and while one of them (d- form) rotates the plane of polarized light in a clockwise direction, the other (l-form) rotates it in the anticlockwise direction. Polarimetry is the oldest method to tell optical isomers (l- and d- forms) apart. Optical isomers also absorb left- and right-circularly polarized light to differing degrees. This is the basis of circular dichroism (CD) spectroscopy, which is now commonly employed to identify chiral enantiomers. Gas chromatography and crystallography are some of the popular methods used to separate configurational enantiomers (L- and D- forms). X-ray diffraction studies enable the direct determination of stereochemical arrangements of enantiomers.

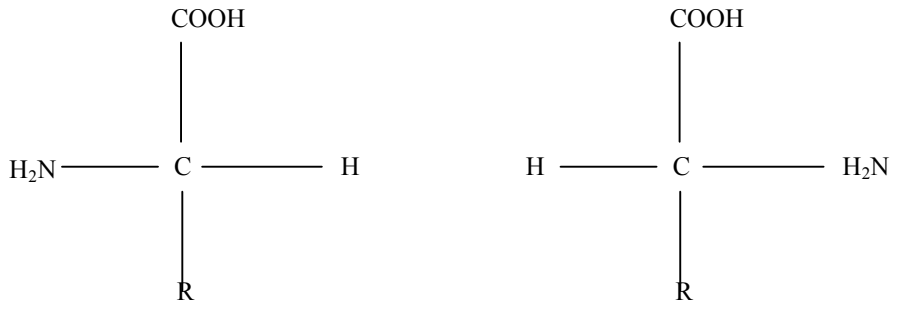

Fig. 1: **Configurational enantiomers of amino acids**. CORN rule : If looking into the plane of paper, the groups **CO**OH,H,**R** and **N**H_2 are arranged (in that order) around the C atom in a clockwise manner, the enantiomer is called L- form and D-form otherwise. L- and D- only refer to configurational arrangment around the carbon atom and do not refer to optical activity. While the L- and D- forms of a chiral molecule do rotate the plane of polarized light in different directions, some L-forms(or D-forms) rotate light to the left(levo or l- form) and some to the right(dextro or d- form). l- and d- forms are called optical isomers. Classifications by optical activity and by configuration are independent of each other. Nine of the nineteen L-amino acids commonly found in proteins are dextrorotatory (at a wavelength of 589 nm), and the rest are levo. Glycine (R=H) is the only achiral (non chiral) amino acid in proteins.

While any chiral molecules synthesized in laboratories are always made in racemic (equal proportions of both enantiomers) mixtures, the active organic molecules in all living organisms are found to always comprise of one enantiomer of a chiral pair. This is a distinguishing feature of life. In particular, protein amino acids are found to occur only in L-form while sugars in DNA and RNA are found to occur only in D-form. ***Why is the L/D mirror-symmetry broken by life?***

The reason for homochirality in living systems has been long researched, and while many studies yield useful insights, the question remains unanswered. Most hypotheses can be divided into two broad categories: those of biotic origin and those of abiotic origin [1]. According to the hypothesis of abiotic origin, asymmetry is a result of environmental conditions or sheer accident at the time of evolution. The asymmetry got amplified and propagated over time. The hypothesis of biotic origin assumes that chiral purity is necessary for biological function and the asymmetry is the result of struggle for existence or some other biological selection mechanism. In the following sections, some of main hypotheses (summarized in Fig. 2) are discussed in brief, presenting the main points of each hypothesis and results of experiments performed to test their validity. Many people use a combination of some of these hypotheses to explain homochirality.

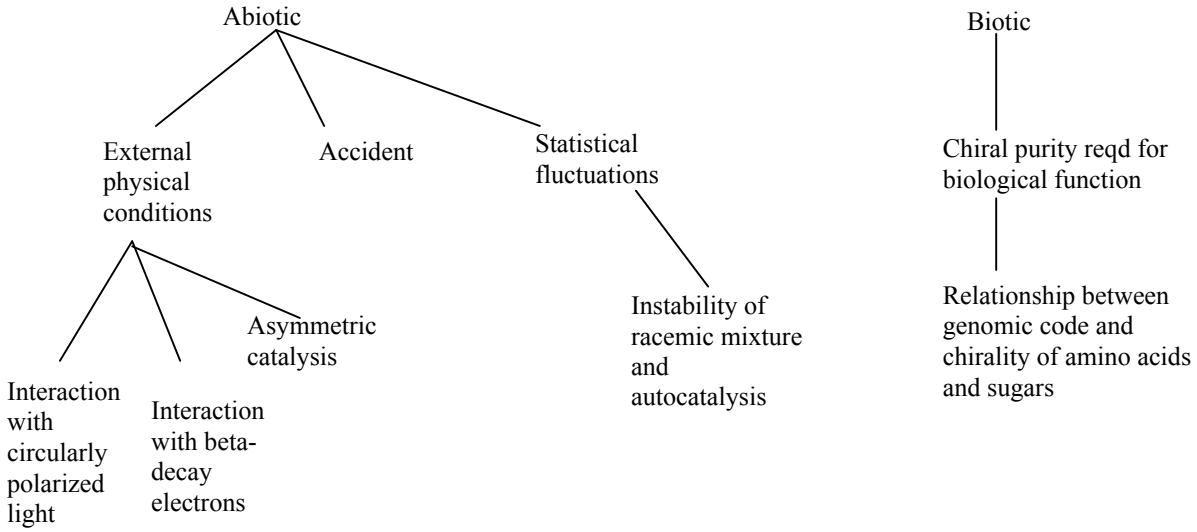

Fig. 2 : **Summary of various hypotheses on origin of homochirality in living systems.**

I would like to emphasize here that this term paper is not a comprehensive or exhaustive review of this widely researched topic. This paper aims to provide a broad overview of the problem and enumerate different methods used so far to address it. After discussing the main points of each approach and the experiments performed to check them, I will give my perspective of the feasibility of each approach. For more detailed accounts, the interested reader may want to refer to the bibliography.

2.Hypotheses, Experiments and Discussions

Most theories to explain homochirality in living systems usually break up the problem into these steps:

a. Mirror symmetry breaking: The pre-biotic soup is believed to be a warm soup consisting of racemic mixtures of amino acid enantiomers (and sugars). How did this homogenous phase separate into chirally pure components? ***How did an asymmetry (assumed to be small to start with) arise in the population of both enantiomers?***

b. Chiral amplification: Assuming that the mirror-symmetry was broken in the early stages of chemical evolution, ***how did the small difference in population of the enantiomeric forms get amplified***?

c. Chiral transmission: ***How did the preference*** of one chiral form over the other, ***propagate so that all living systems are made of 100 percent optically pure components?***

The answer to the last question is common across theories. Since molecules act as substrates for their own replication, if the substrate molecules were chirally pure, chiral purity would propagate through the mechanism of translation. Almost all the hypotheses of abiotic origin attempt to answer (a) and (b). The biotic hypotheses take a different approach to the problem that will be discussed later within their framework.

2.1 Abiotic Hypotheses

2.1.1 Accident/Chance theory

The first guess towards solving the problem would be to suppose that chiral purity had formed as a result of chance. By chance, the initial mixture of enantiomers contained an excess of one of the enantiomers, and this casual asymmetry was amplified and propagated through generations due to self-replication of the enantiomers. This assumes that the rate of production of new enantiomers in nature was small compared to the rate of self-replication of enantiomers in living systems. What could be the cause of the initial accident leading to asymmetry? All kinds of speculations are equally plausible, but the most interesting one involved a suggestion that the source of this asymmetry was extra-terrestrial. In 1969, the Murchison meteorite crashed into Australia and various groups studied the chemistry of its contents and deposits. The meteorite was shown to have contained an excess of the "right" kind of amino acids and some people proposed that this meteorite brought in enough of the "right" kind of enantiomeric excess needed to explain the chiral purity we see today [2]. Many people opposed the conclusions of this finding, arguing that the meteorite could be contaminated with terrestrial amino acids. Apart from this, comets and meteorites were also perhaps too hot for any sort of chemistry to take place in them. And while the extra-terrestrial bit does seem fascinating, I would doubt that chirally pure enantiomers were produced in meteorites and comets! If chiral purity was indeed an accident, how would we ever know? It might be fruitless to speculate on possible accidents, as there would be no way to check our hypotheses.

2.1.2 Statistical Fluctuations

According to this hypothesis, under certain conditions, a homogenous and symmetrical distribution of enantiomers may become unstable and one of the enantiomers would eventually gain over the other due to physical system dynamics. F. C. Frank provided the first detailed mathematical explanation of this hypothesis [3]. Frank showed that if enantiomers of one kind acted as catalysts for their own production and as antagonists for the production of the enantiomers of the other kind, then the racemic mixture would become unstable after a period of time t_1. After some time scale t_2, one of the enantiomers (either) would increase in number and eventually outdo the other. Either enantiomer could win at random, and only an initial excess could determine the survivor definitively. Frank thus showed that chiral symmetry is broken spontaneously by chemical reactions that do not have a specific preference for any of the enantiomers. The most was promising element in Frank's theory was that the amplification depended exponentially on the time scales t_1 and t_2. Hence, it predicted experimentally observable time scales (t_1 and t_2). Various experiments were done to test this theory [4,5,6]. A recent experiment [6] reports an asymmetry in the enantiomeric population obtained from the crystallization of an isothermal, saturated solution of sodium chlorate in racemic form. The sodium chlorate solution was stirred to promote autocatalysis (growth of crystals on a seed crystal through nucleation) and small glass beads were introduced into the solution that crushed large crystals formed in the solution and recycled them by making them part of the solution again. Thus their experiment had an interesting nonlinear mechanism, whereby both autocatalysis and suppression were introduced. When glass balls were not introduced, but there was an initial single enantiomeric excess of about 5 percent, they found that over 8 hours this initial excess was amplified to almost 100 percent. When there was no initial enantiomeric excess, but glass balls were introduced to recycle enantiomers, one of the enantiomers (at random) wholly dominated after about 24 hours. Introducing glass balls along with initial enantiomeric excess ensured that only the enantiomer in initial excess won in the end. Data from their paper is presented in Figs. 3 and 4.

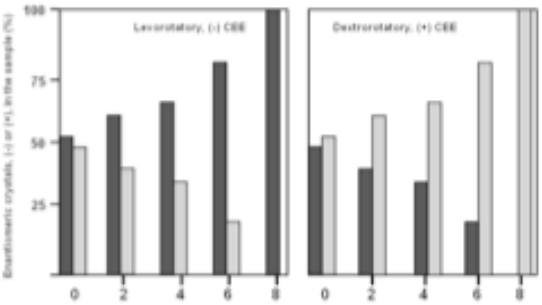

Fig. 3 [3] : Solutions with initial excess of L-/D- by 5 percent show complete L-/D- dominance after 8 hours (600 rpm stirring).

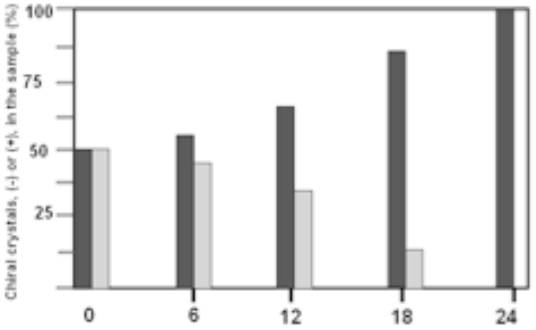

Fig. 4 [3] : Solutions with initial racemic mixture of L-/D- form with glass balls introduced, show total symmetry breaking and optical purity after 24 hours(600 rpm stirring). The selection of D- or L- in the end was found to be random.

While this is a smart demonstration of a very beautiful and mathematically precise idea and yields 100 percent optical purity, we should be careful in applying this idea to our homochirality problem. First, we are lead back to the problem of explaining the initial specific enantiomeric excess. If we assume that the initial mixture was racemic with no bias, we do not know a precise suppression and recycling mechanism as used in the experiment that might have existed during chemical evolution. Again, if one existed, it would mean that the choice of D- or L- forms we see in living systems today was completely random. Also, such processes can only ensure local breaking of symmetry in separate areas of the system over separate time scales and the choice of the winning enantiomer being random, these processes may preserve the global symmetric distribution of enantiomers [1]. Hence, such processes may have little relevance in explaining biological homochirality.

2.1.3 External physical conditions leading to homochirality

One of these mechanisms involved the presence of asymmetric inorganic catalysts that would lead to synthesis of only type of amino acid. However, all inorganic catalysts exist in both chiral forms in almost equal proportions and they could not have produced global homochirality. The other two more carefully worked out mechanisms are elaborated below.

2.1.3.1 Parity Violation theories

Since the famous theory of parity violation in weak interactions proposed by Lee and Yang [7], there have been various theories proposing that the origin of homochirality in living systems had to do with this fundamental asymmetry in particle interactions in nature. The processes proposed are mainly those that involve interaction of polarized particles with amino acids/sugars (Vester-Ulbricht processes [8]), and those in which parity violating effects induce differences in the energies of the two enantiomers of amino acids/sugars, making one more stable than the other (Yamagata- processes [9]).

Vester-Ulbricht: Electrons emitted through radioactive beta-decays are left handed, i.e., their spin is most likely to point in a direction opposite to their velocity. This happens due to the parity non-conserving nature of weak interactions involved in beta-decay. Such an electron (spin-polarized) interacts with chiral substances just as plane-polarized light would. It has been shown that such spin-polarized electrons preferentially ionize one type of enantiomer over the other [10]. In fact, such processes destroy D-amino acids (and L-sugars) preferentially over L-amino acids (and D-sugars). Various attempts were made to calculate the asymmetry induced by such processes [11,12]. Hegstrom [13] demonstrated that the relative asymmetry induced in the cross section for ionization of D-amino acid over that for L-amino acid was not greater than 10^{-2} to 10^{-3}. Experiments by Gidley [14] report asymmetries ranging between 10^{-3} to 10^{-6} for three kinds of amino acids. Whether these experiments provide good numerical estimates of what could have happened during biochemical evolution, and whether these asymmetries could override statistical fluctuations and get amplified to result in a 100 percent dominance of one enantiomer has been the topic of much debate [15,16,17]. Various estimates give conflicting values, so I

shall not list them here. In brief, this process might be a likely one that caused imbalance in the enantiomeric population, but it is not likely that it can result in complete homochirality.

Yamagata processes: The weak neutral currents (mediated by the Z^0 boson) originating in matter (due to the weak force) have been shown to result in a preferential stabilization of the L-amino acids (and D-sugars) over the D-amino acids (and L-sugars) [18,19]. Precise electroweak calculations predict a value of the order of 10^{-30} for the difference in energy between the two amino acid enantiomers. Kondepudi and Nelson [20,21] used the fact that the rate constants for autocatalysis of amino acids and sugars are different because of the above energy difference induced by weak interactions. By solving equations for chiral symmetry breaking similar to those by Frank [3], they reported that it would take an order of 10^4 years for the L-amino acids to outnumber the D- amino acids in large bodies such as oceans. While this seems compelling, some authors [22] pointed out that the electroweak advantage in the Kondepudi-Nelson scheme takes place by repetitive steps of the order of 10^{17} and the earth must have contained at least 10^{34} chiral molecules to take proper account of statistical fluctuations. It seems difficult to beleive that such a large number of molecules would be reacting at the same time. Kondepudi and Nelson argued that this need not be the total number reacting at any time, but that it would be the number that is fluxed through the system over 10^4 years. Experiments to validate this are impossible due to the time scale involved and experiments to measure the energy difference predicted by electroweak calculations work close to their sensitivity limit due to the small numbers involved.

Both the theories listed above are still being debated over and it is not clear if one mechanism or both together would cause the observed 100 percent chiral purity in living forms. However, these theories are attractive as they use unified electroweak interactions and it would be a perfect dream come true if macroscopic effects such as mirror-symmetry breaking and homochirality could be predicted using the theory of elementary particles.

For the sake of completeness, it might be worth mentioning here that there have been theories of phase transitions in amino acids [23,24]. Abdus Salam [23] suggested that a phase transition occurred during biochemical evolution that amplified the asymmetry in the enantiomeric populations. This hypothesis uses ideas from BCS theory and calculates a critical temperature of about 250K for such a phase transition in amino acids. It further predicts that eventually all D- amino acids will be converted to L- amino acids. Many people argued that the earth was too hot for this phase transition to have occurred in the pre-biotic era. To circumvent this problem, Salam and some others postulated that life could have originated extra-terrestrially. Even if a phase transition is theoretically possible, it is hard to believe that the amino acids remained at their critical point (for these phase transitions) long enough, so that the initial asymmetry in enantiomeric population (due to electroweak interactions or other causes) could be amplified to a significant extent. Recent experiments [25] to corroborate the Salam hypothesis do not validate the existence of the phase transition; they only show that the D- and L- amino acids exhibit different transitional behaviour at the predicted critical point. They also

deny the existence of any transition from D- to L- forms in amino acids swept through the whole critical region.

2.2 Biotic hypothesis

The biotic hypothesis says that features of biochemical evolution cannot explain the origin of homochirality in living systems and an insight into the metabolic and reproductive processes in living systems is necessary to understand this asymmetry. These theories are attractive from a biological standpoint, as they do not explain homochirality as a purely physio-chemical phenomenon, but link it to something unique to living systems, i.e., transmission of information. Various aspects of the biotic hypotheses are linked and I shall organize this section in a logical sequence of questions that these efforts attempt to answer instead of by the different approaches themselves.

2.2.1 Why is chiral purity necessary for life?

2.2.1.1 Factors eliminating "chiral contamination" in living systems

In many investigations carried out in vitro, it has been found that macromolecular systems resist the inclusion of racemic elements into them. Joyce et al. [26] reported highly efficient replication of chiral molecules by oligomerisation when both the template and monomer were of the same handedness. When these reactions were carried out with racemic mixtures of monomers, the inclusion of enantiomers of opposite handedness served as a terminator for the replication. Biosynthesis (maybe as a result of evolution) is organized so that metabolism is protected against possible errors in chirality of molecules.

In light of the above, it is interesting to note the role played by an enzyme such as D-amino acid oxidase. All organisms contain certain stereospecific enzymes that oxidize D-amino acids and serve no other biological function known to date. These enzymes may be remnants of evolution that favored chiral purity. Also, a frequent occurrence of D-amino acids was found in the cell walls of bacteria [27]. Some scientists believe that by utilizing D-amino acids in cell walls, the bacteria prevent these amino acids from participating in metabolism. There are various findings, which relate chiral "contamination" with diseases. Kogl [28] reported to have found an abnormal content of D-amino acids in malignant tumors and some others have shown that racemisation of certain amino acids occurs in the human brain and eye with ageing and cataract formation [29,30].

2.2.1.2 Informational meaning of chiral purity in biomolecules

Information in biological systems directs self-reproduction. The information contained in proteins, enzymes and DNA regulate the assembly of daughter molecules. If a regulator of biosynthesis (like an enzyme) is chirally pure, all its active centers are built of homochiral monomers. When interacting with biomolecules, these regulators can then distinguish chiral forms by selectively binding only the "correct" chiral form. In the event that the chirality of a regulator molecule is contaminated, its selectivity is reduced and

hence function is impaired. A similar argument can be made about chiral contamination of protein monomer units leading to "errors" in protein folding, so that a protein with a particular amino acid code need not fold into the same structure every time. For organisms to preserve the ordering of molecular information for existence and proliferation, their enzymes, proteins and DNAs should be chirally pure [1].

2.2.2 Would they win by turning left or right?

This question is unfortunately not one for political speculation, and although various theories about how one chiral form was preferred over the other (assuming that chiral purity is necessary) exist in the literature, sufficient experimental confirmations do not exist.

Ageno [31] proposed that life might have originally existed in two forms, based on either D- or L- form of amino acids. The eventual domination of the L- form was attributed to evolution through natural selection and competition. No survival advantage of the L-form over D- form has ever been proposed or tested in experiments. Proposals involving competitive advantages of the L- amino acids or D-sugars are unfortunately not testable, although plausible. Some authors have suggested that codons on the genome display a preference for binding to L-amino acids [32,33,34,35]. Some bioinformatics based studies show that the genetic code is correlated with the production of D-sugars and L-amino acids [36]. Definite experimental evidence is still lacking.

3. Conclusions and future directions

This term paper reviewed the origin of homochirality in living systems from various standpoints. Hopefully, the reader is now familiar with various approaches employed so far to address this problem, their successes and shortcomings. Before concluding, I would like to summarize my perspective on the issue of homochirality, and hence give a coloured outline for future directions in answering this question.

While the abiotic hypotheses are attractive from a physics point of view, the numbers they predict are so tiny and require so many stringent physical conditions, that it is hard for me to believe that homochirality could be explained on their basis. First, so little is known about pre-biotic conditions, that it might never be known if the environmental conditions assumed in the abiotic hypotheses involving statistical fluctuations and weak interactions ever existed. Moreover, a reliable estimate of the time scale over which such mechanisms would have amplified chiral asymmetry to result in homochirality is not available and if ever one will be, no experiment would be able to test it. Again, it is not obvious why such local asymmetries could lead to a globally uniform effect. Accident based theories suffer from the same glitches and further offer no explanation of why one chiral form would be preferred over the other. Theories involving phase transitions are nearly improbable because of the time that a large and rapidly evolving chemical system would have to wait at the transition point for the effect of the transition to be observably large. Moreover, none of these theories unequivocally predict the 100 percent chiral purity observed in life forms.

In my opinion, it seems unlikely that a complex system such as life evolved to have homochiral units purely due to accident or physical conditions. Complete chiral purity seems to be indicative of a biological advantage rather than chance. Perhaps there is a reason why L-amino acids in proteins and D-sugars in DNA (and not the other way round) are required for biological function. Are these facts somehow correlated? Does having L-amino acids require having D-sugars? While Crick was of the opinion that any connection between codons and amino acids was completely fortuitous, Woese [37] argued that there is a specific stereochemical relationship between amino acids and the genomic code. Does the genomic sequence indeed code for L-amino acids and D-sugars as suggested by Woese and others [34,35,37]? Experiments in this direction are feasible today and are likely to throw some light on our problem. In conclusion, the problem of homochirality is still open and although rich with many different hypotheses and ideas, there is a lot of room for fresh thinking and experimentation.

Acknowledgements

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