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A Short History of the Investigation of the Origin of the Terrestrial Biosphere's Homochirality (Some Personal Remarks, Recalling Historical Milestones)

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Résumé

A description of the history of the investigation of the origins of homochirality in our terrestrial biosphere over the last half decade is given here. It tells about the moment, when the discovery of the hitherto rather untold puzzle of the fact that biology is so biased in the structure of its chemical elements, in stark contrast to the laboratory experience, that whenever a (strictly abiotic) synthesis of the basic chemicals making up biology was undertaken– like in particular amino acids and sugars – it always resulted in the production of a racemic (exact 1:1) mix of left- and right-handed molecules. Is it due to an accident („Was God a lefthander?“), is it a physical law, and is it really necessary that living nature had to consist exclusively of the left- (or the right-) handed basic molecules to grow spontaneously from symmetric simple molecules during the evolution up to the subtle complex homochiral structures making up biochemistry today? The essay attempts to give credit to some of the pioneers of this scientific research, who quite often have been overlooked in spite of their essential contribution to the field. The importance of the tool of an analysis of homochirality in probing for traces of life beyond Earth is underlined and supports a further systematic investigation on extraterrestrial bodies in future space missions.

Mots-clés

chirality; enantiomers; homochirality; symmetry breaking

I THE VERY FIRST FLASH

It happened, I guess, very early in my scientific career, entering my university in München, - it was the Ludwig-Maximilian-Universität with its full name in the Department of Chemistry's

newly opened laboratories -, where I listened eagerly to my professors in my second semester in the year 1957. Personally almost everything was completely new for me, having left my “Gymnasium” focussing on old languages like Latin and Old Greek and neglecting pretty much the natural science courses like physics and chemistry. I had thought there must be more in it than the little boring things being taught there, so it was exactly my curiosity for this almost completely forgotten field chemistry that attracted me to learn more about it in my following university years. So in this fall 1957 it was the peculiar subject of Organic Chemistry when Prof. R. Huisgen lectured about the biological important group of amino acids. Among others he taught us that a (non-biological!) synthesis of these compounds was rather easily accomplished mixing the necessary ingredients together, such as water, acetaldehyde, cyanic acid (or ammonia), heating, - and finished was the product of alanine for instance, called after its inventor the famous *Strecker Synthesis* showing that the synthesis of biochemical compounds could be simply facilitated. The lecturer stressed however the point that principally the product of such an “abiotic” synthesis was always the production of 2 so called “enantiomer” amino acids, one of the geometrically *left*, one of the corresponding *right* conformation. Both forms, the L- (sometimes also S-) α -alanine and the D-(sometimes also R-) α -alanine appearing in the product at exactly equal stoichiometric concentrations or masses, being exactly identical except the fact they were the mirror images of each other making them non-superimposable (in spite of their otherwise identical properties), in a similar manner like our two hands behave to each other, being almost identical, but of course not superimposable. Alright that was accepted from us students in our first year. But at the very end of the semester, at the point when it came to the chapter “Natural Compounds”, the domain of Biochemistry, Huisgen drew a disturbing, yet fascinating conclusion to the basic course in Organic Chemistry: He claimed that the alanines, - the simplest amino acid to show this stereochemical enantiomer property of appearing in two antagonistic forms, a n-amino propionic acid – and all the other existing amino acid homologues identified in the biological tissue everywhere on Earth were surprisingly only of the (arbitrarily defined) L-form! Similarly, one could show that not only the amino acids making up living matter showed this strange property, but also all the other of the myriads of different specific biomolecules showed this very peculiar “Broken Symmetry”; next to the amino acids it is particularly the group of biological sugars which demonstrate this broken symmetry, - only the D-enantiomers of sugars show up in our terrestrial biology. This observation told to me in the audience struck me hard, - and never let me go. What could be or could have been the reason for this very confusing symmetry breaking in nature behind? Huisgen gave no answer as I remember, of course in these days a first-year-student did not dare to ask the professor at this desk in front of about 1000 students in the big auditory an obviously a bit critical question on a generally accepted textbook wisdom, which needed a special explanation.

This episode of my first year in chemistry haunted me over the years, WHY for heaven’s sake was the nature around us so symmetry-biased in view of the beautifully symmetrical laws in physics and chemistry. It does not matter at all whether we believe in creation of the world around us by a sort of GOD, such as the conventional wisdom of our Bible, Koran or Thora tries to teach us or whether we rather believe in Darwin’s Evolution Theory, which of course is much more widely accepted among scientists, but it is difficult to assume a left-handed creator or “intelligent design” or any sort of physical law which threw life into existence with this preference of one side only, the so called “homochiral” feature...

II HOW IT WENT ON WITH THE STRANGE PUZZLE OF MY OWN BEGINNINGS IN THE VAST FIELD OF CHEMISTRY

It took me in fact almost 11 years more of waiting when a unique chance opened for me in the year 1968 to focus my scientific curiosity on the study of the origins of biochemical Homochirality. I joined the recently opened Nuclear Research Institute (KFA, now

Forschungszentrum FZ) Jülich in Germany, my assignment was “to do some innovative research of strictly non-nuclear character” of my own choice...what an opportunity, the 60’s were a period of economic boom, young and ambitious scientists were offered a lot of careers in research areas, without too many limitations, not being forced to guarantee economic profits at all stages of their projects without being haunted by the prospect of getting fired after the end of time-limited research grants like the situation appears for young scientists momentarily. One allowed me to build a small working group devoted exclusively to the subject of Homochirality. Of course I learned quickly,- searching the available literature - that I was by no means not alone in the field. There were great people before me who occasionally devoted their interest to this peculiar topic, but their number was small and they left the subject quite often far from any final solution. Actually it was the great *Louis Pasteur* of course who opened the field by observing that the basic organic element in grapes was tartaric acid (or rather the salts of the acid), existing in 3 different forms, a mixture of the L- and the D-enantiomer – distinguishable by their unique property to rotate a linearly polarized beam of light either to one or the other direction – and a meso-tartaric form, which showed no such phenomenon of any such rotation. As we know Pasteur believed in a special “force de vie”, a particular “force of life” reserved to biochemical matter which could not be synthesized in the laboratory. In the long time after Pasteur’s fundamental discovery it was firmly established that this phenomenon of Homochirality – that is the break of molecular symmetry – holds in general for (almost!) all the organic elements in biology, of which all the amino acids and carbohydrates are the most pronounced examples.

The very nice thing in this project – we are talking here about the late 60’s to 70’s, where there was not even the slightest idea of a world wide web enabling one to search the literature in fractions of a second! – was the fact, that obviously the number of living researchers working in this peculiar, rather exotic field of chemistry, was rather small. A few names to be remembered may suffice at this point only: *G. Wyrouboff, P. Curie, Y. Yamagata, W. Bonner, T.L.V. Ulbricht, F. Vester, A. S. Garay, F. F. Seelig, C. Ponnampereuma, G. Spach, A. Brack, and some others* were among those, who devoted their energy to study this outstanding phenomenon of Homochirality enthusiastically.

Our *Jülich Approach* consisted of a model to be described as such: Consider theoretically that the mirror images of the D- and the L-enantiomers of any amino acids making up the body of proteins in the biological organisms were not exactly identical, but only *nearly* identical, the energy of formation perhaps distinguishable perhaps by an extremely minute amount only, so small that hitherto available analytical instruments were not able to prove such a difference of perhaps the L- and the D-alanine; then it may have been overlooked so far that in the end of a slowly evolving biosphere from the beginning some few billion years ago on Earth the L-alanine had an extremely slight, yet definite advantage of growing finally into a self-reproducing cell, overriding ultimately the energetically inferior D-alanine in the end. The phase of the Chemical Evolution on early Earth could be imagined as an amplification process which eventually would enrich the energetically favoured enantiomer up to an endproduct of 100 % homochirality. Such an amplification – enriching a favoured enantiomer, small as the initial advantage over the competing compound would be - could be simulated in our laboratory. We had at this point yet no idea whatsoever from which such a minute difference of the enantiomer compounds would eventually stem. We at Jülich designed 2 different experimental lines, the first one using crystallization as the amplification machine, with the aim to prove or disapprove such an energetic preference.(1) We looked at the potassium ammonium salt of the tartaric acid, the very same compound which lead *L. Pasteur* to the discovery of chirality. A racemic mixture was crystallized from its saturated aqueous solution, for several times repeatedly collecting the solid crystals and dissolving freshly the mixture at higher temperatures and letting it crystallize at lower temperatures again. At the end of some

cycles we could measure a small optical rotation by a sensitive measurement, which allowed us to calculate the “elementary energy difference of L- and D-tartrates.

The second experimental procedure was as follows: We subjected some exactly racemized mixtures of D- and L-alanine, -lysine, and alpha-aminobutyric acids to a several weeks-lasting polycondensation reaction resulting in the production of the corresponding polymers. These polymers were investigated for any preferred optical rotation, below or above zero. Our hypothesis was that, if the rotation of the polymer showed any significant rotation, then the inherent energetic bias – expressed in the kinetic preference in building the polymer – would be amplified to such a value, which could be measured by our conventional spectropolarimeter. Logic? And the final result published by us, together with my co-worker, the late *W. Darge* (2) was in fact a confirmation of a very small, but significant excess of the D-enantiomers of the investigated amino acids. The problem was of course that the re-calculated energy difference of the monomeric enantiomers was too big – in the order of a few per mille in relation to the total value of x kJ or so, that the work was generally not well received in the scientific community. The even more important point of criticism was the open question how such a hypothetical energy difference of enantiomer carbon compounds should be explained at all; such a statement was in stark contrast to all hitherto common knowledge, published in textbooks of chemistry as a sort of well-established principal dogma of its kind.

III A TRUE REVELATION

There were two things discovered in literature which appeared like a sort of a true enlightenment supporting our experimental design: First it was a fundamental short essay by *Y. Yamagata* (3) in distant Japan, who apparently argued along similar lines like we in Jülich, small energy differences in enantiomer molecules could be “amplified” in the process of chemical evolution to such an excess that we observe today in our 100 % pure homochiral biosphere. Very short contribution helped strengthening our peculiar view. And it was him, who pointed to a so called “Parity Violation in the Weak Interaction”, a fundamental statement for which formulation 2 Chinese-born US-scientists, namely *T. D. Lee and C. N. Yang* (4) were awarded the Nobel Prize in Physics in 1957. They reported a peculiar phenomenon in physics, quite revolutionary at this point more than 60 years back in time from now: The normally less known so called Weak Interaction showed a strong “Asymmetry”, like the same phenomenon in our electromagnetic forces ruling biochemistry, where we observe only one enantiomer, the other one missing... In *Lee and Yang's* Weak Interaction –experimentally confirmed by *Ms. Wu* – we see that the atom leaving β -particle possesses only one spin along the path, while the other mirror image of this spin cannot be observed. Strange! Immediately it comes to one's mind, that perhaps the one phenomenon in physics could be related to the other one in biochemistry, could it? The first researchers following this idea directly after *Lee and Yang's* discovery were *T. L. V. Ulbricht and F. Vester* (5) attempting a link between these phenomena and proving a connection by some experimental set-up. The second part of enlightenment was a personal meeting in Moscow with my late friend and excellent scientist *Andras Garay*, (6) who was also busy proving the basic idea of transferring the parity violation of the weak interaction to the realm of chemistry, - could it be that the fact that life is so much biased goes back to the nuclear property of the atom basically?

In September 1973 we decided to call these rather few colleagues in the field around the world to Jülich to exchange the latest ideas and results touching this fascinating subject of “Broken Symmetry in Chemistry” in order to stimulate a much broader interest. With “we” I mean it was the prominent scientist *A. S. Garay* (Director of the Biology Research Center Szeged, Hungary), *C. Ponnampereuma* (Prof. Univ. Maryland and Director of the Laboratory of Chemical Evolution USA), *K. Wagener* (Inst. Phys. Chemistry, KFA Jülich) and *W. Thiemann* who succeeded to assemble about 40 participants to discuss and argue about the latest ideas,

theories and experiments on this subject. The whole Symposium was published (7) – a total of 540 pages inclusive the accompanying completely recorded discussions; the central contributions to this Jülich Conference were published in the Journal of Molecular Evolution. Another international symposium on “Generation and Amplification of Chirality in Chemical Systems” was organised in 1980 at the University of Bremen by the author jointly with *C. Ponnampereuma*, published as a separate issue (8), promoting even a greater interest in this topic within the international scientific community.

IV A NEW AREA OPENED, WHICH SUPPORTED EARLY HYPOTHESES

At the University of Oxford there arose a group of theoretical chemists, who calculated *ab-initio* the effect, which the “Parity Violation of the Weak Interaction” would have on the molecular energy content of chiral organic chemicals, in particular on amino acids. And, - alas, they found that this nuclear property would indeed, as speculated by several authors before, affect the entire molecular structure in such a way, that the L-enantiomer would have a small difference to its corresponding enantiomer, the D-enantiomer. The Oxford group around *S. F. Mason* consisted of *A. J. MacDermott*, *G. E. Tranter*, *P. A. Hegstrom*, (9) who confirmed our earlier thesis, that enantiomers are distinguishable by a certain difference, called from then on the PVED – the Parity Violation Energy Difference. We first celebrated this new knowledge supporting our earlier starting point, the textbook wisdom, “Enantiomer compounds are just the exact mirror image of each other” was not true anymore: We learned that the true enantiomer of the L-alanine for example was the D-alanine from antimatter! And vice versa! Yet our joy was a bit too early: First of all the PVDifference was so immensely small, something in the order of 10^{-14} eV, that most people doubt whether this extremely small effect could ever have directed chemical evolution into the 100 % result of D-amino acids in the present biosphere at all. Any advantage of the L-amino acids would have been overruled in the billions of years of evolution on Earth by a simple randomizing racemisation (induced by heat, radiation, pressure, etc.). Another results lead to the general scepticism that the surprisingly found energetic advantage of the “right”, namely the L-amino acids depended on the conformation of the 3-dimensional structure of the compound, rather variable in its structure in vacuo, solid state, or dissolved (in water) state. Anyway the activities around the value of the effect of parity violation in weak interactions inducing possibly the geometrical structure of the compounds triggered an enormous interest throughout academic institutions around the world. The problem is now discussed at many occasions, throughout international conferences, papers in respected journals and monographs.

V THE SEARCH FOR HOMOCHIRALITY’S ORIGINS EXTENDED AT AN ENORMOUS RATE

In all these years since our first cautious hesitating steps in the early 70’s into this rather novel research field of searching for the rather mysterious origin of the chemical homochirality gained an enormous scientific popularity: My own rough guess would be that around the globe a few hundreds of scientists have been engaged investigating this problem. And with the number of scientists the number of theories and more or less weird theories have accumulated attempting to demonstrate numerous physical reasons for the rise of this truly striking phenomenon of our terrestrial biological homochirality. Earth rotation was simulated in the laboratory influencing the outcome of stereoselective chemical products, Earth magnetic field operating jointly with specific electric fields were tested to affect the stereoselective product, even Earth gravitation as well as sunlight being broken through the Earth early atmosphere was proposed to have influenced the first asymmetric prebiotic molecules of the early Earth some billion of years ago during the chemical evolution.

Out of all these rather phantastic ideas a strong factor, competing seriously with the PVED effect as the initiator of the stereoselective result of terrestrial evolution of the biosphere,

stands out as an influence: A circularly polarized UV radiation – called “stereoselective photochemical reaction” – may have pushed the origin of the first (pre-) biological cells during or after the Hadrosphere phase of Earth into the homochiral direction as we observe it today. It is well known, since the 30’s of the last century by *W. Kuhn and E. Knopf*, (10) that a circularly polarized UV beam could interfere with a suitable chemical reaction in such a way, that the resulting product would contain an excess of one or the other enantiomer, depending on the direction left or right of the polarized light beam. In the last 30 or so years it has been speculated that the young planet Earth got in contact with some strong UV light sources emitted from a nearby neutron star, which was to some extent polarized. And this strong light source interacted with the chemical reagents forming the first organic compounds of some biological use in a stereoselective way. Principally we must assume though that there might have existed only one strong event of such an influence from outside Earth, and this must have been of the preferred polarisation direction forming preferentially the first excess of L-amino acids and D-sugars as we see them in the biosphere. Numerous are laboratory experiments and theories supporting this speculation apparently confirming such a theory on the origin of the fitting homochiral products. Among the first authors was the group of *W. A. Bonner*(11) reporting such effects in the late 70’s and early 80’s. During the times up to the present, it was in particular the *Nice University Group of U. Meierhenrich*,(12) who published a great number of results supporting the polarized UV-radiation-directed origin of homochirality in an impressive amount of papers published in numerous international journals and monographs. I must admit that the vast material so far published up to today would likely support convincingly the process of polarized UV radiation having induced stereoselectivity on Earth. Although of course the above discussed PVED effect is up to this date not yet completely out of the game. (To my personal opinion the PVED, being a universal property of matter, would work in the same way throughout the whole universe, while on the other hand, the direction of the polarized UV radiation would be restricted to our own Solar System only! So in the end, the PVED effect would deserve the attribute of possessing more “beauty” in its own...)

Moreover, not to be forgotten at all, there are of course many scientists around the world, who do not believe in a sort of “directed” chiral decision, a number of arguments could be raised, too, in favour of a random choice of the presently preferred L-amino acids and the D-sugars: Pure accident could have stirred the evolution as we see its products today, an accidentally chosen beginning of the “first dissymmetric cell” could have triggered the homochiral solution, leaving the other enantiomer design no chance anymore, once self-reproduction, etc. had started in favour of the L-amino acid and D-sugar world at a considerable rate. “God creating the living world must have been a left-hander...” as somebody had expressed this scenario in a beautiful short way.

As this essay should focus on the first steps into the realm of the studies on the origin of homochirality only, not ever only attempting to describe the enormously expanding (hi-)story of the field up to today, a few thoughts should be devoted here to highlight the very latest developments and applications of the field.

VI RECENT CONSEQUENCES OF OUR STUDIES INTO THE FIELD OF HOMOCHIRALITY

Wherever the true origins of the homochirality of our terrestrial biosphere lie and whether any “Eureka” claims could ever be proven at all, the whole scientific community seems to agree that homochirality is perhaps the unique mark of anything which claims to be “living/biological” on Earth and beyond Earth. A biological entity built up from basically symmetrical chemical elements seems to be extremely implausible at all. All the complex macromolecular structures of our biochemistry can be designed by nature only on the ground of homochiral elements, just imagine a DNA, RNA, or a polymer carbohydrate like

starch/lignin built from monomer racemic molecules alone, one would never ever find any complex, functional, and beautiful structures like specific helices and the various well-defined tertiary and quaternary structures which make up our biochemical architecture. This unique property of “life” seems to distinguish living from non-living materials. It looks as this homochiral precondition would even hold for any imagined living entities built from some alien non-carbon based chemicals. It would exceed here to roam a bit on the thermodynamics of a living cell in sharp contrast to a sort of mineral (non-living) cell. The majority of researchers would allow a living entity to break for a limited amount of time and space the law of entropy production, the so called Third Law of Thermodynamics, stating that in any natural and spontaneous process the total entropy of the system is forced to increase. The lone exception from the rule is the territory of life (13): a living entity is allowed to break this law for a certain amount of time and space only, - in other words, biology alone allows to reduce the entropy of a limited system for a certain time regime! Examples of this phenomenon is the well accepted theory of (Darwinian) evolution, where we observe that systems of higher order evolve spontaneously from systems of lower order, - as well as the simple observation of the development of a cell or a multicellular organism from the fertilization of a rather simple egg, truly a process from less to higher order, or from larger to lower entropy of the system, proceeding without any help from outside the system!

From here it follows logically that in the search for life outside planet Earth we should focus our attention to systems of a - more or less - homochiral nature, independent of the problem, whether the observed system is of a familiar carbonaceous nature (like ours! Alas, we have no other living system than our own terrestrial, built from carbonaceous material, to compare with!) or not. This line of argumentation has long since taken up into the design of space vehicles exploring the universe around us for traces of non-terrestrial lives. Many years ago a fundamental paper was published along this lines of thoughts by a consortium of researchers proposing such an ambitious project SETH (stands for Search for Extraterrestrial Homochirality), cf. *A. J. MacDermott et al.* (14) Spectacular was the rather recent success of the ESA sponsored space mission ROSETTA/PHILAE, when a module landed on the comet 67P/Churyumov-Gerasimenko, which aimed at analysing the surface for organic compounds. (15) For the first time the vehicle carried - among many other instruments - onboard a gas chromatograph coupled with a mass spectrometer equipped to identify (homo-?)chiral organic chemicals like enantiomer amino and other acids. While the identification of some 20 organic compounds by mass spectrometer succeeded, unfortunately the gas chromatograph instrument was not able to do its job so far properly, there were no results satisfying our curiosity for the potential existence of chiral amino acids on this comet. Yet, other projects aiming on the analysis of chiral compounds on solid objects within our solar system are well under way, such as the ExoMars mission sponsored by the European Space Agency ESA having on board similar analytical equipments as on our ROSETTA mission. Japanese researchers are aiming to study within a return mission potentially chiral compounds on some asteroids, the rovers CURIOSITY and DISCOVERY from the last Mars mission organized by NASA have similar analytical tools on board and have reported already some, yet hitherto not confirmed, data produced by some similar chirality detecting devices like ours.

Of course the run for detecting any living features beyond Earth seems to bear fascinating results on the existence of life in the Universe in the next future. Moreover the ever increasing number of newly discovered exoplanets outside our own Solar System is the target of interesting speculations on a potential biological habitat farther away from our home solar system. Will be ever able to discover living species there? Perhaps an answer could lie in the more systematic search for homochirality there; we do not necessarily have to go there and pick up some samples, which seems rather hopeless for the next future due to the immense distances separating us from these targets. But we could try to get some information through

spectroscopic investigations looking for some alien biospheres emitting or reflecting some polarized light telling us about possible homochiral structures on their home planets.

As a hopeful young scientist, attending as a humble guest a NASA sponsored meeting back in the early 70's preparing the 2 spectacular VIKING missions to planet Mars, I dared to propose cautiously to carry a simple polarimeter (measuring the optical rotation of a chiral compound) onboard the mission to look for any(homo-)chirality of organics, and was not surprised – of course! - that my remark was not taken seriously, on the contrary I earned a rather arrogant smile from the audience. This very first idea of applying a polarimeter searching for life anywhere in this Universe giving us perhaps some hints for receiving an answer to our question “Is there anybody out there?” as to where exactly to direct our sensors, was possibly after all not so naïve...

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