

# Possible Origins of Biohomochirality

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**Abstract:** The origin of biohomochirality and the reasons, why this form of homochirality (L-amino acids, D-sugars, etc.) and not the mirror image case was preferred have been the subject of numerous hypotheses, but a definitive solution to this riddle that might be strongly correlated with the origin of life itself has not been found to date.

In this paper different pathways trying to explain the origin of biohomochirality are reviewed, inter alia the chance hypothesis, spontaneous resolution mechanisms, preferential adsorption on chiral surfaces, the interaction of interstellar organic dust clouds with circularly polarised light and subsequent transport to earth via meteorites, and a possible preference for one enantiomeric form due to parity violation in weak nuclear interactions.

In the Salt-Induced Peptide Formation (SIPF) reaction a preference for the L-form of several amino acids was observed. The active complex in this reaction contains a central copper ion which, due to its high atomic number, provides larger parity violating energy differences (PVED's). In combination with the geometry of this complex that features a central chirality at this copper ion and further chiral influence through chiral amino acid ligands a completely new aspect to explain biohomochirality is presented.

**Keywords:** Biohomochirality, origin of life, L-amino acids, prebiotic chemistry, Salt-Induced Peptide Formation reaction.

## 1. INTRODUCTION

In all life forms on earth the essential biomolecules can be found almost only in one of the two enantiomeric forms. In the case of amino acids - with very rare exceptions - only the L-forms serve as building blocks of proteins, and in the backbone of DNA/RNA only D-sugars are incorporated. The origin and reason of this phenomenon, called biohomochirality, are still not known to date, although numerous hypotheses about this topic have been proposed and are vigorously discussed in the scientific community. Was it just by chance that life emerged as it is and not as its mirror image, or is this the result of local chiral influences, or was it even predetermined by the forces of nature? These are the three main directions of possible explanations concerning the origin of homochirality of biomolecules.

As structure and functioning of biomolecules were clarified in ever more detail within the last decades, it became evident that life as we know it is absolutely dependent on molecular asymmetry. The very specific conformations of biomolecules needed for their proper functioning and precise fitting together are achieved by consistent stabilised structural entities like  $\alpha$ -helices or  $\beta$ -sheets that can only be formed by enantiomerically pure building blocks. This gave a strong basis to the assumption that their handedness had to be selected at an early stage of evolution or that the availability of enantiomerically enriched chiral molecules was even a prerequisite for the advancement in chemical evolution leading to a system of constructively interacting classes of biomolecules.

However, even if one accepts that chirality had to be evolved to form proper biomolecules, this does not yet answer the question, why it had to be L-amino acids and D-sugars and not their mirror images that formed life on our planet. In the following we will try to outline the attempts to answer this riddle.

## 2. THE CHANCE HYPOTHESIS

### 2.1. Introduction

In the search for the origin of biohomochirality, the simplest - and most unsatisfactory - explanation is that it arose by chance. In chemical and biological evolution one crucial step could have been a single or at least a very rare incident on the way to life. If one of the bio-precursor molecules did the crucial step that led to autocatalytic effects so that only this biomolecule and its progeny underwent further chemical and biological evolution, and if by chance this specific molecule was a polypeptide that exclusively consisted of L-amino acids because it was formed in an enantiomorphous surrounding favouring enantioselective reactions with L-amino acids, this could have led to an early 'decision' in favour of one of two chemically equivalent forms. Having no clue about the type of molecule performing this 'first step' and its surrounding materials we will hardly be able to reproduce such a scenario, but we also cannot exclude this rather trivial explanation.

### 2.2. Spontaneous Resolution

Optical activity can be obtained out of an initially racemic mixture by various spontaneous resolution mechanisms like chiral autocatalysis in the process of crystallisation. While resolution of racemates by means of chiral reagents or catalysts is a common practice in chemistry, the spontaneous resolution of enantiomers upon crystallisation is a rather rare

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and somewhat unpredictable process [1]. Louis Pasteur was the first to detect enantiomorphous crystals that had formed by crystallisation of a tartrate salt whereby the enantiomers of the racemate spontaneously segregated into two different, mirror image like sets of crystals. In general, a crystallisation under equilibrium conditions of a racemic mixture of enantiomers that form enantiopure crystals (only ~5-10 % of all racemates crystallise as such conglomerates), which is a prerequisite for spontaneous resolution, will lead to a globally racemic precipitate. Enantiomeric enrichment in the solid sample can be reached either by seeding with a homochiral crystal of one enantiomeric form which favours the growth of new crystals of the same handedness (preferential crystallisation) or by stirring that disrupts locally-oscillating supersaturation and thereby setting the crystallisation under kinetic control and favouring the chirality of the first formed crystal. This may lead to an almost enantiopure crystalline precipitate with the disadvantage that the chirality sense cannot be predicted.

Another special case that may lead very elegantly to an almost homochiral product starting from a racemic mixture is spontaneous resolution under racemising conditions (SRURC). This process requires rapid equilibration of the enantiomers in solution and a slower autocatalytic reaction like crystallisation out of this solution. The distribution of the enantiomers in a racemic mixture in solution underlies statistical fluctuations which gives rise to the formation of small crystals. In this case, as the first crystal that by chance has one of the two chiral forms has formed, this chiral primary nucleation acts as a seed and rapidly catalyses its own growth and also causes other crystals nearby to be of the same handedness. As the disturbed equilibrium of the enantiomers remaining in solution is continuously re-established the crystallisation may lead to a globally enantiomerically pure precipitate.

Some chiral substances that normally do not form homochiral crystals have been shown to form conglomerates when specific achiral addends, so called conglomerators, like NaOH, NH<sub>3</sub>, or H<sub>2</sub>O are added [2]. Tartaric acid, for example, forms heterochiral crystals with equal numbers of mirror image molecules, whereas Pasteur's salt (sodium ammonium tartrate) in the presence of the before mentioned conglomerators crystallises as a conglomerate, for instance as tetrahydrate. If the conglomerator is added in deficiency the crystalline precipitate shows an overall enantiomeric excess in favour of the first formed crystal at the onset of crystallisation.

It has been argued [3] that whenever a racemate crystallises as conglomerate this would lead to highly enantioenriched micro-environments. When, for example, conglomerate crystals formed after evaporation of a puddle containing a racemic mixture are blown by the wind into several smaller other puddles stochastically a variety of different micro-environments with different enantioenrichment (of course globally racemic) is formed.

Also in homogeneous reactions without the involvement of crystallisation optical activity can be gained out of achiral reagents, like in the formation of the chiral product pyrimidylcarbinol out of 2-methylpyrimidine-5-carboxaldehyde and diisopropyl zinc. In the attempt to amplify random enantiomeric excess of one chiral form a strong dependence of the handedness of the product on extremely dilute chiral

impurities was detected [4]. This reaction, intensively investigated by Soai *et al.* with several related reactants, can almost lead to optical purity due to the asymmetric autocatalytic properties of the product that force its own reproduction. When starting from racemic reactants enantiomeric excesses of more than 99% with a stochastic distribution of the R and S configurations are achieved after a few reaction cycles [5]. When small amounts of marginally enantioenriched product are added [6], or in the presence of chiral influences like circularly polarised light [7] or chiral quartz surfaces [8] the chirality of the product can be affected and thereby, high enantiomeric excesses of one defined chiral form can be generated. Although the existence of the mentioned reactants can not be assumed for any scenario related to the origin of life, a similar mechanism might be able to produce and boost enantiomeric enrichment also with biologically more relevant molecules: a complex containing another transition metal will be discussed in the chapters 5 and 6, in connection with a possible amplification of an inherent chirality effect.

An interesting process somehow related to spontaneous resolution by crystallisation is the octamerisation of serine under positive ion electrospray conditions [9,10]. By spraying solutions of pure L- or pure D-serine larger amounts of hydrogen bond-stabilised homochiral octamers are formed while with enantiomerically impure mixtures much smaller octamer amounts are detected. These results show that homochiral octamers of serine are stabilised compared to heterochiral ones and present a process how homochiral amino acid conglomerates can be originated under specific conditions.

However, there is no indication again, why one of the enantiomeric forms should be preferred, and the spontaneous resolution mechanisms, therefore, only show possible ways, how the 'chance hypothesis' could have been realised. On the other hand such processes might be involved in the amplification of an enantiomeric excess that might be produced by the mechanisms in the following chapters.

### 3. LOCAL CHIRAL INFLUENCES

#### 3.1. Extraterrestrial Origin of Homochirality

The influence of circularly polarised light (CPL) can be used to induce enantioselectivity in various types of chemical reactions and to explain a preference for one enantiomeric form of biomolecules. After the first experiments proving this effect [11] in the 1930's, it has repeatedly been used for enantioselective photolysis or synthesis of chiral compounds [12]. Bonner and coworkers showed that by CPL photolysis of a racemic mixture of leucine an enantiomeric excess of 1.98% and 2.5% after 59% and 75% photolysis can be obtained [13]. The basis of these reactions is that one enantiomeric form of the molecules is photolysed and thus eliminated faster than the other one, depending on the sign of the polarisation. If the irradiation is stopped before the degradation is complete the result is an enantiomerically enriched mixture.

With the discovery of a potential strong IR circular polarisation source in a cloud of dust in the Orion OMC-1 constellation [14], a hypothesis of an extraterrestrial origin of homochirality got new impetus. This cloud, a region of high-

mass star formation similar to the region where our solar system was formed, contains many organic compounds. Circular polarisation can occur by scattering of light from non-spherical grains aligned in a magnetic field in this high-mass star forming regions, potentially leading to an excess of one enantiomeric form of biomolecules via photolysis. Model calculations have indicated that the presence of circularly polarised light implies the existence of the corresponding UV radiation, which is the essential part of the electromagnetic spectrum for inducing chiral asymmetry in organic molecules. Unfortunately, the high obscuration of this cloud of dust makes it impossible to measure the presence of UV light directly.

Binary star systems containing a magnetic white dwarf can also produce circularly polarised UV light (up to 50% circular polarisation), but such systems are only rarely associated with molecular clouds.

During chemical evolution on the primordial earth, in addition to the on-place formation of organic molecules, there have been many impacts of comets, interplanetary dust particles and meteors which led to a delivery of a huge amount of organic molecules to earth. Therefore it can be imagined that the earth was inoculated with compounds stemming from a region that was exposed to circularly polarised radiation.

For this reason carbonaceous chondrites, especially the Murchison meteorite that came down in Australia in 1969 have been investigated. This meteorite contains  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -amino acids [15], hydroxy [16], phosphonic and sulfonic acids [17] as well as further organic compounds like purines and pyrimidines [18]. To exclude terrestrial contamination and to avoid enantiomeric enrichment during the experiments,  $\alpha$ -branched amino acids that have never been reported to be part of the geosphere were analysed. Furthermore, the determination of isotopic distributions proved that the organic compounds are not of terrestrial origin [19-21], but brought to earth via the meteorite. These experiments showed an enantiomeric excess of up to 10% for a few  $\alpha$ -methylated amino acids [22]. Other experiments showed even a D/L ratio of 0.5 for alanine and of 0.3 for glutamic acid [23].

The excess of L-amino acids found in the Murchison meteorite, representing an extraterrestrial deliverer of organic molecules, could be a result of circularly polarised irradiation and could at least partially explain the origin of homochirality of biologically relevant molecules (Fig. 1).

An estimation of the amount of amino acids delivered to earth in its history postulated that  $6 \times 10^{13}$  tons of amino acids (taking in consideration that the concentration of amino acids in carbonaceous chondrites is 60 ppm and that 3% of all meteorites are carbonaceous chondrites) precipitated on earth so far,  $6 \times 10^{11}$  tons in a time period, where the earth had already sufficiently cooled down [24] to enable chemical evolution. However, the enriched chiral form of amino acids depends on the sign of CPL and on the wavelength, and if CPL had played an essential role in the origin of biomolecular homochirality on earth, in other regions of the universe, where circularly polarised light of opposite sign is predominant, molecules or even life-forms with opposite chirality would be more abundant.

### 3.2. Terrestrial Chiral Influences

Circularly polarised light also occurs on earth, although with rather small polarisations (<0.5%), in the solar radiation at twilight and from the interaction of sunlight with the terrestrial magnetic field [25]. However, even these small polarisation effects mostly compensate over the whole sky and over time which makes supplementary assumptions like unequal exposure to dawn and dusk skylight necessary. Unpolarised light in the presence of a parallel magnetic field can also induce enantioselective reactions [26] but the stereoselective effects of magnetochiral dichroism in the weak magnetic field of the earth ( $10^{-4}$  T) are very marginal.

### 3.3. Preferential Adsorption on Chiral Surfaces

Quartz is a widespread mineral and the most common form of silica ( $\text{SiO}_2$ ) on earth. Its crystals are asymmetric and appear in a left- and right-handed form. Adsorption or reaction processes on such chiral surfaces are enantioselective. Theoretical investigations about adsorption of alanine on quartz [27] and also kaolinite [28] surfaces showed these energetic differences for the interaction of one amino acid enantiomer with the two mirror image crystals. On a chiral surface one chiral form of amino acids or peptides might be adsorbed preferentially and therefore stabilised against hydrolysis or activated for chain elongation or further reactions. It was shown that on L-quartz L-alanine hydrochloride is adsorbed preferentially [29] with an enantiomeric excess of a few percent and that aspartic acid adsorbs slightly enantioselective on chiral calcite surfaces [30]. However, extensive studies about the distribution of quartz enantiomers on earth [31] show equal occurrence of both forms which restricts a possible influence of chiral minerals to the origin of

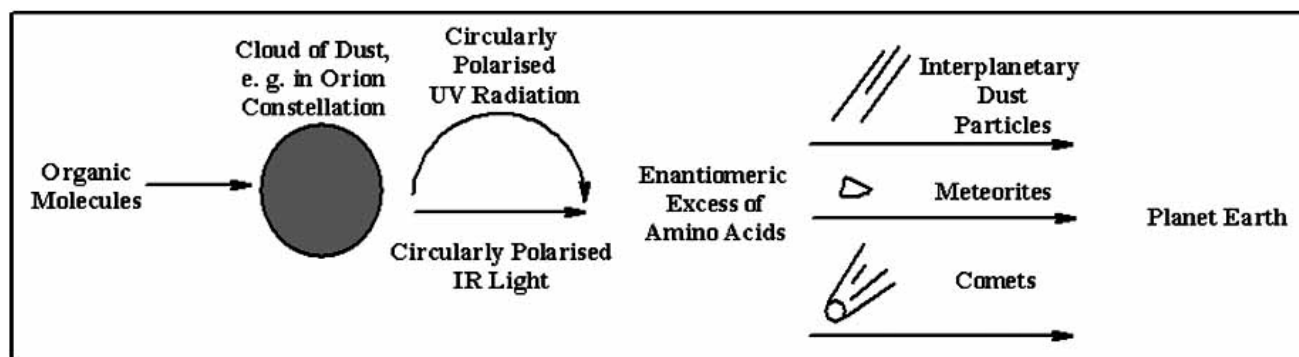


Fig. (1). Possible pathway for extraterrestrial origin of homochirality on earth.

homochirality to local environments where one chiral surface form might have predominated.

## 4. PARITY VIOLATION IN WEAK INTERACTIONS

### 4.1. Introduction

In 1956 two Chinese physicists, Lee and Yang, analysed some discrepancies in the decay of meson particles and proposed a non-conservation of parity in weak interactions as possible explanation [32], which gained them the Nobel prize in physics in 1957. This effect of parity violation was shortly later experimentally proven by showing that electrons ejected together with antineutrinos from unstable  $^{60}\text{Co}$  nuclei in the process of  $\beta$ -decay are predominantly left handed [33,34].

These fundamental discoveries overthrew the former idea that the forces of nature could not 'distinguish between left and right'. Conservation of parity had long been a basic dogma in the description of structure and interactions of macroscopic as well as microscopic objects.

Later the weak nuclear and the electromagnetic forces could be unified in the Electroweak Theory [35-37], showing that all atoms are inherently chiral due to parity violation in the weak nuclear force and the inherent chirality of the electrons. This inherent optical activity is approximately proportional to  $Z^5$ ,  $Z$  being the atomic number of an element [38]. For lighter atoms like H, C, O or N this effect is extremely small (smaller than molecular optical activity of enantiomers by a factor of  $10^{-9}$  to  $10^{-12}$ ) but for heavier atoms (Pb, Cs, ...) a slight optical activity can be measured by optical rotatory dispersion (ORD).

Furthermore, right and left handed enantiomers in the world of matter do not have exactly the same energy [39,40]. The overall optical activity of a molecule is the sum of electroweak and electromagnetic contributions, so the values for two enantiomers will be opposite in sign but have different magnitudes. The immediate consequence of parity violation in weak interactions in chiral molecules is a parity-violating shift of the electronic binding energy,  $E_{pv}$ , which is positive for one enantiomer and negative for its mirror image. The total parity-violating energy difference (PVED) between two enantiomers is, therefore, twice  $E_{pv}$ .

Earlier theoretical approaches proposed that L-amino acids are stabilised compared to their D-enantiomers by PVED values of  $10^{-20}$  to  $10^{-17}$  hartrees [38,41,42] ( $10^{-17}$  to  $10^{14}$  kT at room temperature). More recent calculations, however, have produced values that are strongly depending on the conformation of the molecules [43,44] but the L-form is mostly the stabilised one. This energy difference would lead to an enantiomeric excess of only  $10^{-16}$  to  $10^{-13}$  %, so it would have to be greatly amplified by an appropriate mechanism to lead to a substantive homochirality.

### 4.2. The Accumulation Hypothesis

Yamagata [45] proposed in 1966 that due to parity violation in weak interactions the two enantiomeric forms of a chiral molecule would have slightly different energies and therefore different equilibrium populations and probabilities of chemical reaction. As these small differences are operative at each step in the formation of homochiral biopolymers

they would, after a large number of polymerisation steps, provide a distinctive advantage in favour of the PVED-stabilised enantiomeric form. There are a number of problems that let this hypothesis appear unrealistic as an individual chiral amplification mechanism [46]. First, it was assumed that the polymerisation steps were irreversible and second, it was not considered that in any realistic prebiotic peptide formation reaction mixed polypeptides consisting of enantiomerically impure monomers would be the prevailing products. Even though it seems possible that homochiral polypeptides appear more frequently than statistically predicted, due to stabilisation in forms of  $\alpha$ -helix secondary structures and thus increased stability against hydrolysis (this stabilisation effect would equally apply to either L- or D-enantiomers).

The accumulation principle was also postulated for crystallisation processes. In enantiomorphic crystals the PVED-favoured form was assumed to be markedly preferred due to the large number of units in a crystal which would lead to an imbalanced population between the two chiral forms of crystals. However, the number of enantiomorphous L- and D-crystals in any thermodynamically controlled crystallisation is equal within statistical fluctuations, as demonstrated by comprehensive studies about the distribution of (+)- and (-)-quartz on earth [31] showing an almost perfect equivalence of the two chiral crystal forms (49.83% (-)- and 50.17% (+)-quartz).

It seems that accumulation of the small parity-violating energy differences between enantiomers alone, at least between organic mirror images consisting only of lighter atoms, is not an appropriate mechanism to lead to considerable enantiomeric enrichment or even to homochirality. For this reason, the accumulation hypothesis has gradually disappeared from the debate about the origin of biohomochirality.

### 4.3. The Vester-Ulbricht Hypothesis

Shortly after the discovery of parity violation in weak interactions Vester and Ulbricht postulated that longitudinally polarised electrons emitted in the process of  $\beta$ -decay after impinging on matter subsequently generated circularly polarised bremsstrahlung photons which would induce asymmetric reactions in initially racemic mixtures due to different synthetic or degradative interactions with the two enantiomers [47-49]. They carried out numerous organic reactions in the presence of several different  $\beta$ -emitting radionuclides at different temperatures and for different time intervals but all the results of these initial attempts to confirm this hypothesis experimentally showed no or just minimal optical activity within the experimental error range. They concluded that more powerful radioactive sources and longer irradiation times would be required for unambiguous stereoselective enrichment. In spite of these initial negative results a wide variety of subsequent similar experiments and theoretical investigations followed over the next decades.

First positive results regarding the Vester-Ulbricht hypothesis were published in 1968 by Garay [50]. He reported that D-tyrosine dissolved in alkaline aqueous ethanol containing ca. 0.35 mCi of  $^{90}\text{SrCl}_2$  as the  $\beta$ -radiation source underwent radiolysis faster than L-tyrosine under the same conditions. However, these findings and the experimental design were doubted some years later and advanced experi-

ments with several amino acids and a stronger  $\beta$ -ray/bremsstrahlung source (61,700 Ci of  $^{90}\text{Sr}$ - $^{90}\text{Y}$ ) could not confirm any enantioselective radiolysis [51,52].

Quite similar was the fate of experiments with  $^{32}\text{P}$  as  $\beta$ -ray source. Darge *et al.* [53] investigated the radiolysis of DL-tryptophan in frozen aqueous solution ( $-25^\circ\text{C}$ ) containing  $^{32}\text{P}$ -labelled phosphate and found 19% enrichment of D-tryptophan in the undecomposed residue after a gross radiolysis of 33%. Bonner *et al.* [54,55] repeated these experiments with DL-tryptophan and also DL-leucine, but found no stereoselective radiolysis due to  $^{32}\text{P}$   $\beta$ -radiation and its bremsstrahlung at all.

Experiments with  $^{14}\text{C}$ -labelled crystalline racemic and optically active amino acids were performed [56] as well, and again no enantioselective degradation during the internal  $^{14}\text{C}$ - $\beta$ -radiolysis occurred.

A possible explanation for the consistent failure to detect enantioselective radiolysis in all the above mentioned experiments could be that the continuous energy spectrum of bremsstrahlung has its maximum in the low energy region [57]. The low energy bremsstrahlung photons, which would be capable of inducing photochemical changes in organic molecules show much less circular polarisation than the high energy photons [58-60], while the highly polarised high energy bremsstrahlung photons are of insufficient intensity and unsuitable wavelength to enable enantioselective interactions. Another effect that exerts a detrimental influence on the efficiency of the Vester-Ulbricht mechanism is that high energy ionising radiation also causes radoracemisation [56,61,62], even boosted in the presence of silica [63] or clay minerals [64], which diminishes an eventually generated stereoselective enrichment or even nullifies it. This could even be the main reason that no convincing experimental evidence supporting the validity of the Vester-Ulbricht hypothesis for the origin of biohomochirality has been produced to date.

Further investigations in this direction were experiments about direct enantioselective effects of chiral particles like polarised electrons, protons, muons, and positrons produced in linear accelerators and cyclotrons. These types of experiments offer a number of advantages over the radiation from natural radionuclides, inter alia variable energies and variable degrees of polarisation including also reversed chiralities of the particles, thus providing a "symmetry check" for any observed enantioselective effects. Furthermore, the interactions can be studied directly on target substrates, thereby minimising the effects of accompanying bremsstrahlung.

Especially the irradiation of enantiomers with polarised positrons was extensively investigated, both experimentally and theoretically, but none of these experiments could provide convincing and reproducible results [65,66].

Although no evidence has been obtained so far that the Vester-Ulbricht hypothesis might be able to explain the way to homochiral biological systems, one strongly related experiment also implying  $\beta$ -radiation should not stay unmentioned. It was reported [67] that when crystallising a racemic mixture of ammonium sodium tartrate in the presence of  $\beta$ -emitting potassium phosphate (containing the  $^{32}\text{P}$  isotope) an excess of L-tartrate crystals is found whose amount depends on the level of radioactivity of the system.

This finding could indicate another, more indirect way, how chirality caused by parity violation could have been propagated to biomolecules.

#### 4.4. Salam Phase Transition

A completely new way to arrive from small parity violating energy differences to homochirality was suggested by Salam in 1991 [68]. He speculated that quantum mechanical cooperative and condensation phenomena could give rise to second-order phase transitions below a critical temperature  $T_C$  in terms of a Bose-Einstein condensation including Cooper-pairing. Beneath this generally rather low temperature that, according to rough calculations should be around 250 K, amino acids would transform to the PVED-stabilised L-form. This suggestion could be experimentally tested by cooling a racemic mixture of amino acids and looking for a possibly occurring optical activity if this kind of phase transition takes place and transforms the initially racemic mixture into the enantiomerically pure form with lower ground state energy. One obvious difficulty of this phase transition from one enantiomer to its mirror image is how to overcome the large activation energy that would inhibit the phase transition in the racemic mixture as chemical bonds would have to be broken in the process.

Experimental efforts to find evidence for a phase transition in amino acids were made by Figureau who cooled down cysteine molecules from 77 to 0.01 K but without observing any change in the optical activity [69]. More extensive investigations were performed by Wang *et al.* [70,71] with single crystals of D- and L-alanine and valine. They too did not detect a configuration change from D- to L-amino acids but they found a phase transition in which L- and D-enantiomers exhibit different transition behaviour. The observed anomalies in the temperature dependence of specific heat at a temperature of around 270 K imply a significant difference between D- and L-valine of  $10^{-4}$  eV per molecule ( $\sim 10^{-2}$  kJ/mol) at this transition point. As this value coincides well with the electron coupling energy they propose that the specific heat jump of D-valine might be attributed to the electron coupling while forming a Cooper pair. Further temperature dependent X-ray diffraction data of D- and L-alanine show that beneath the transition temperature the dihedral angle of the carboxy group differs by about  $2^\circ$  between the enantiomers (D-alanine:  $43.97^\circ$ , L-alanine:  $45.72^\circ$ ) whereas it is almost identical above the transition temperature.

Albeit no experimental evidence for a Salam phase transition from one enantiomer to another is available to date, the data of Wang can at least prove that phase transitions within enantiomers display parity-violating properties. The question remains if these unequal transitions could have a direct influence on chemical evolution on earth as the transition temperature of 270 K seems too low for any favourable evolutionary scenario.

Some kind of relationship can be seen between these phase transitions in crystals and structural transitions in polypeptides that have been investigated recently [72]. Homochiral polyglutamic acid and polylysine, each consisting of 24 identical residues, are water soluble polypeptides whose secondary structure depends on the degree of ionisation of their side chains. In their neutral state they assume a

well characterised  $\alpha$ -helical structure while in the ionised state they are in an equilibrium between fluctuating unstructured conformations ('random coil'). The transition energy between these two states was found to differ markedly between the poly-L- and poly-D-peptides when the transition was performed in water whereas the differences were greatly diminished in 80% deuterium oxide. These findings are interpreted on the basis of slightly higher solvation energies of the poly-L-peptides, as their PVED induced magnetic component interacts with magnetic ortho-H<sub>2</sub>O (75% of the bulk water, the rest is non-magnetic para-H<sub>2</sub>O) [73] which leads to different behaviour in H<sub>2</sub>O but much less in D<sub>2</sub>O where the spin isomers are of a much lower distinction.

## 5. THE SALT-INDUCED PEPTIDE FORMATION (SIPF) REACTION

### 5.1. Introduction

The Salt-Induced Peptide Formation (SIPF) reaction [74-76] supplies the simplest and most plausible explanation for peptide formation under prebiotic conditions on earth known to date. Dipeptides and higher peptides are formed in aqueous solution containing sodium chloride, copper ions, and amino acids at temperatures of 60 - 90°C.

In 1985, some theoretical studies with the Monte Carlo method about the solute structures in NaCl solutions have shown that at concentrations higher than 3M the first hydration shell of the sodium ions is unsaturated [77,78]. The coordination number of 6 water molecules for every sodium ion cannot be maintained any longer which leads to a strong dehydrating effect providing the thermodynamical basis for condensation of amino acids to peptides in aqueous solution.

Other theoretical investigations in the early 1980s had shown that divalent cations can facilitate reactions between glycine and ammonia to form an amide bond by complexing and activating them [79].

Based on these theoretical findings several alkaline earth and transition metal ions M(II) were experimentally investigated. Solutions containing amino acids, NaCl, and a small amount of M(II)Cl<sub>2</sub> were kept at temperatures of 80°C and monitored for the eventual formation of peptides [74]. Cu(II) was found to be the by far most suitable catalyst for this purpose, leading to the formation of considerable amounts of pure and mixed oligopeptides starting from solutions containing glycine and alanine [80,81]. This reaction was named 'Salt-Induced Peptide Formation' (SIPF) reaction.

These first experiments were performed as constant volume experiments with rather high NaCl concentrations up to 5M but the required high concentrations of sodium chloride can also be reached through evaporation of water. More recent experiments are carried out as evaporation cycles [82], starting with a sodium chloride concentration of 0.5M which is similar to the salt concentration of today's oceans and probably not much different from the salt concentration of the oceans on the primordial earth. This kind of experiments mimics a scenario likely to have occurred in lagunas, puddles or salt lakes on the primordial earth which after heat-induced evaporation of water were regularly refilled due to tidal rise or by rain.

### 5.2. Plausibility of This Scenario

Recently it was shown that amino acids can be formed in atmospheric processes in a neutral or even slightly oxidising atmosphere consisting of N<sub>2</sub>, CO<sub>2</sub>, and water vapour as it is proposed for the primitive earth by geochemists today [83,84]. In Miller type experiments with this atmosphere and electric discharges as an energy source several amino acids (glycine, alanine, valine, serine, proline, histidine, lysine) were detected within a few days or weeks [85,86].

The presence of sodium chloride in primitive oceans, lagoons, and even lakes is most likely and the so called 'green zones' in precambrian rock formations, consisting mainly of copper minerals, give strong evidence that sufficient Cu(II) was available in the early chemical evolution ~3.8 billion years ago [87-89]. The presence of even very small amounts of oxygen keeps Cu(II) in its divalent state [90]. In the cooling process of earth after the first formation of a hydrosphere temperatures between 80 and 100°C are most likely, and at such temperatures regular drying/wetting cycles have to be assumed, taking into consideration day/night cycles, heavy rainfalls, and tidal fluctuations.

Following these considerations the environment on the primordial earth seems to have provided very suitable conditions for the SIPF reaction.

### 5.3. Properties of the SIPF Reaction

Several properties of the SIPF reaction let it appear very favourable for the production of the first peptides in the process of chemical evolution. First of all it shows a preference for the biologically relevant  $\alpha$ -amino acids over  $\beta$ - and  $\gamma$ -amino acids [91] due to their better complex formation ability with the catalytic Cu(II) ion. Another important feature, especially when we think about a possible involvement of this reaction in the origin of bihomochirality, is its good conservation of optical purity of the involved amino acids, especially when starting from lower concentrations of amino acids (<40 mM) [92,93]. As a third point the universal applicability of this reaction should be mentioned. It is the only known peptide forming reaction that, under prebiotic conditions, works with all amino acids investigated so far, although in varying yields.

A larger number of amino acids as single and binary systems was investigated and a preferential formation of certain dipeptides and consequently a bias for certain amino acid sequences was obtained. These sequence preferences were compared to the composition of membrane proteins of archaea and procaryota [94,95], which are among the oldest and most 'primitive' organisms that we still can find on earth. The basic idea behind that comparison was that any later developed replication mechanism for peptides and proteins would most likely reproduce peptide units already existing instead of 'inventing' completely new amino acid sequences. Accordingly it should be possible to find some kind of 'fingerprint' of the reaction responsible for the formation of the first peptides on earth in the proteins of the oldest organisms still existing on earth now. The dipeptides of the amino acids Asp, Glu, Gly, Pro, Lys, His, Ala, Leu, and Val in single and binary systems in the SIPF reaction were analysed and the relative yields of the formed dipeptides AB and BA were

compared to the relative occurrence of these amino acid pairings in the membrane proteins of these monocellular organisms, showing an astonishing accordance, that by chance would only occur with a statistical probability of  $10^{-16}$  in the case of archaea and  $10^{-14}$  for prokaryota.

#### 5.4. Reaction Mechanism of the SIPF Reaction

Both experimental and theoretical methods were employed to obtain detailed information about the reaction mechanism. Several attempts to perform the SIPF reaction with other compounds than NaCl and Cu(II) demonstrated the dependence on these three ions [96]. The crucial SIPF complex responsible for the reaction consists of a central Cu(II) ion that is chelated by one deprotonated amino acid via its amino group and one oxygen of the carboxyl group, while a second, protonated, amino acid (or peptide) binds end-on via the carboxyl group. One chloride ligand is also bound to Cu(II) and two water molecules are attached to the central ion, but at a larger distance [76,97] (Figs. 8,9).

The continuous presence of one chloride ligand in the complex is supported by Monte Carlo simulations and by neutron diffraction studies [98]. Other ions than  $\text{Cl}^-$  drastically reduce the formation of peptides indicating that the chloride ligand provides the necessary steric and electronic preconditions for the formation of the peptide linkage. Because of the Jahn-Teller effect and due to the different nature of the ligands attached to the central copper ion the complex should be considered a strongly and irregularly distorted octahedron, which was confirmed by calculations at ab initio level [97] that also showed a particular stability of this species. The partial charges in this active complex favour a nucleophilic attack of the amino group of the chelated amino acid at the other amino acid's carboxyl carbon [75].

Two main reasons seem to be responsible for the varying reactivity of different amino acids. On the one hand the donor/acceptor properties of the amino acid's side chain influences the charge distribution at the reaction centres, and on the other hand voluminous side chains make larger amino acids relatively immobile due to steric hindrance.

The concentrated solutions have a rather low but for this reaction favourable pH between 2 and 3 because of hydrolysis of copper and due to complex formation of amino acids with copper. The dehydrating effect of the Na ions could be achieved by divalent ions like Mg(II), Ca(II) or Ba(II) even more easily but their disadvantage is a shift of the pH to even lower values, which increases proton-catalysed peptide hydrolysis rates and also shifts the Cu(II) complex species distribution away from its optimum [99].

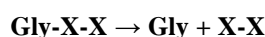
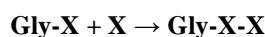
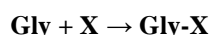
Whenever one of the two reacting partners is a peptide and the second one an amino acid, the amino acid will form the chelate ligand and the peptide will be coordinated via carboxyl oxygen only, providing a certain 'guideline' for the growth of oligopeptides. One side reaction under the specific conditions of the SIPF reaction also plays an important role in the preferentially produced amino acid sequences. The dehydrating environment leads to a substantial formation of cyclic anhydrides of dipeptides, e.g. diketopiperazine (DKP) in the case of diglycine. Subsequent hydrolysis of these anhydrides can lead to a sequence inversion of the original dipeptide according to the nucleophilicity of the partners.

The preferentially formed mixed dipeptide from a mixture of glycine and alanine in the SIPF reaction is Gly-Ala; after longer reaction times, however, most of it is converted to the slightly more stable Ala-Gly [92].

Hydrolysis of formed peptides occurs under SIPF conditions and reduces yields in some cases after longer reaction times. Hydrolysis rates have been shown to be notably lower in the presence of clay minerals which stabilise formed peptides and promote chain elongation [100].

The combination of peptide formation and hydrolysis delivers another, more welcome effect. In the course of extended investigations on the formation of peptides from binary amino acid mixtures, it was found that some amino acids and peptides catalyse the formation of peptides from other amino acids. This effect was called 'mutual amino acid catalysis'. Several possible catalysts were tested, and glycine, diglycine, DKP (diketopiperazine), and histidine turned out to be the most effective ones [101-104], but diketopiperazine acts as a catalyst only after hydrolysis to diglycine.

The reaction mechanism for the catalytic effect of glycine, for example, can be explained as follows, X being another amino acid:



Higher oligopeptides containing the catalyst are formed first. A subsequent hydrolysis breaks the catalyst-X bond and leads to the dipeptide X-X and the catalyst molecule, which can then act as a catalyst for other X molecules. To obtain the best yields of homo-dipeptides, a ratio catalyst / amino acid of about 1 : 8 turned out to be ideal.

## 6. STEREOSELECTIVITY OF THE SIPF REACTION

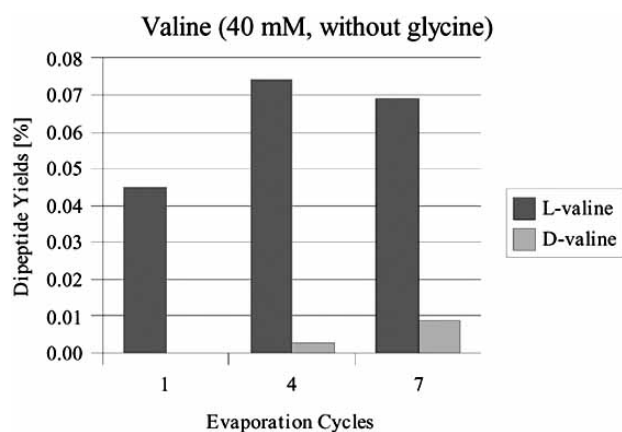
### 6.1. Experimental Findings

First indications towards a stereoselectivity of the Salt-Induced Peptide Formation reaction were already detected in 1992 [82] but at that time were just a surprising by-product of general investigations about the SIPF reaction. The yields obtained for L-alanine were perceptibly higher than for the D-enantiomer. At that time no reasonable argument for this preference could be found so it was just interpreted as insignificant statistical fluctuations or as a consequence of the different production methods of L- and D-amino acids and hence, some impurities remaining even in analytical grade substances, but it animated to comprehensive investigations into this direction. Systematic investigations about the formation of homodipeptides from L- and D-alanine [93] showed an L-L over D-D preference of about 10%. By means of spectrophotometric and potentiometric titrations the complex formation constants for the Cu(II)-L-alanine and Cu(II)-D-alanine complexes were determined. The obtained data show that (i) the complexation with two homochiral amino acid ligands is preferred over a complexation with two amino acids of different chirality and (ii) the complexation with L-alanine is slightly favoured over a complexation with the D-form. Circular dichroism measurements were also performed and these spectra provided again no

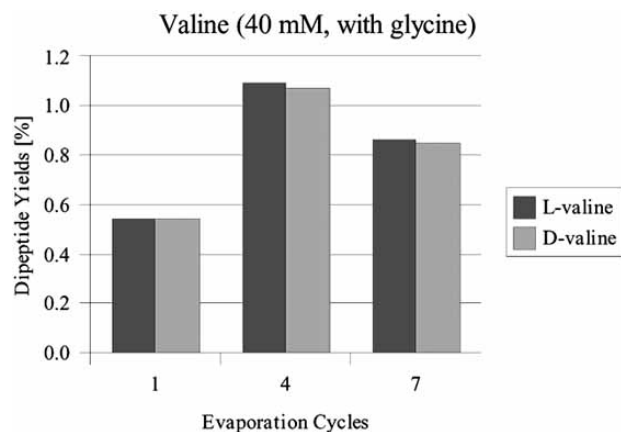
ticeable differences in the wavelength of the adsorption maxima at around 240 nm between the complexed L- and D-alanine, disappearing in measurements without Cu(II) ions.

Further experiments were then carried out with the amino acids valine, proline, tryptophan, lysine, and serine [105]. The yields of homodipeptides for the systems containing one of the chiral forms with different starting concentrations were analysed and then compared, looking for a possible preference for one enantiomer. Similar experiments were performed with systems containing also glycine as a catalyst which boosts dipeptide yields in some cases in the process of 'mutual amino acid catalysis' and simulates a prebiotically more realistic scenario, as the simplest amino acid glycine was most probably present in any peptide forming process.

The most striking result is that D-valine (without the catalytic effect of glycine) hardly forms any dipeptides whereas L-valine produces multiple yields as can be seen in Fig. 2. Glycine has a very strong catalytic effect in the valine systems. The L-form of valine generates higher dipeptide yields in any case, but the D-form comes closer in this scenario (Fig. 3).

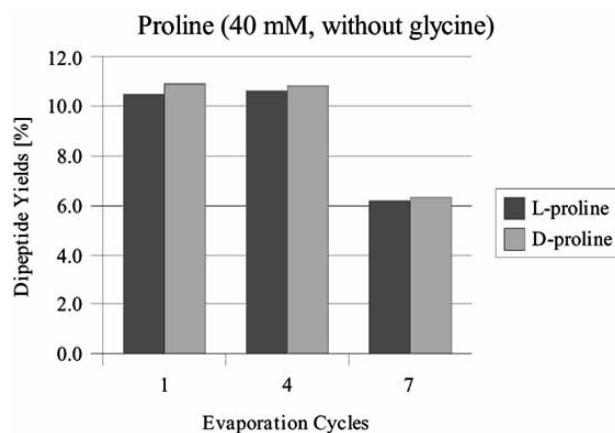


**Fig. (2).** Dipeptide yields for L- and D-valine with a starting concentration of 40 mM without glycine.

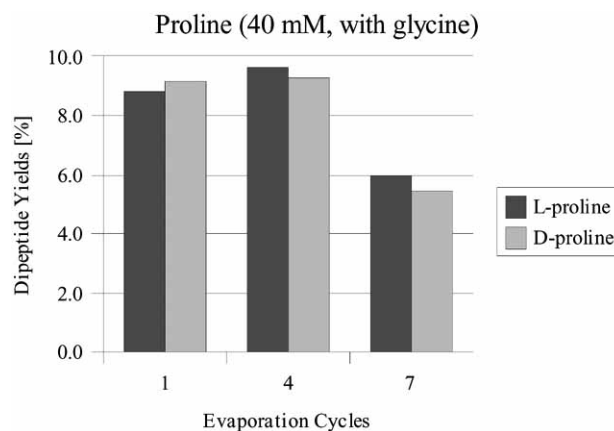


**Fig. (3).** Dipeptide yields for L- and D-valine with a starting concentration of 40 mM and with glycine as a catalyst.

The data obtained for the proline systems don't show a really consistent pro for one enantiomeric form, neither with nor without glycine as a catalyst (Figs. 4,5).

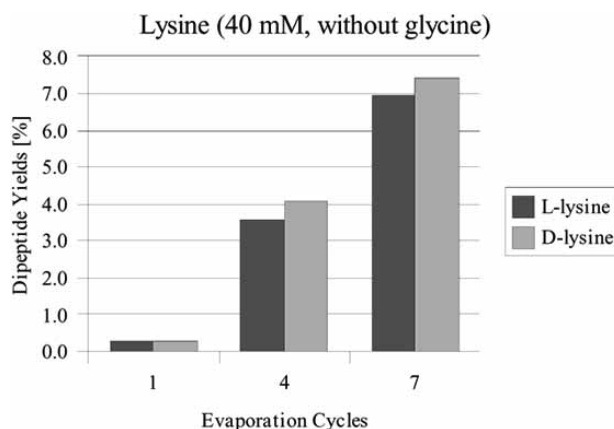


**Fig. (4).** Dipeptide yields for L- and D-proline with a starting concentration of 40 mM without glycine.

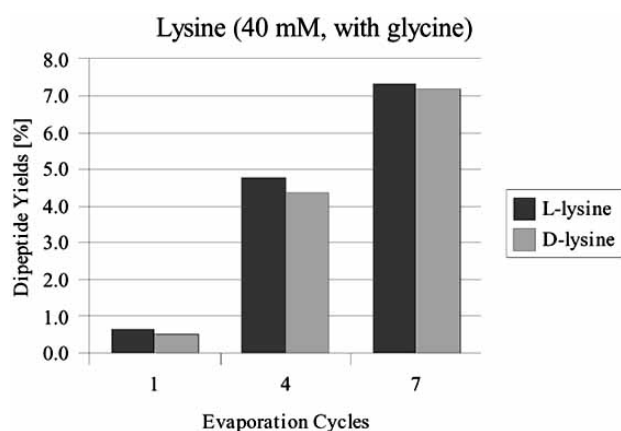


**Fig. (5).** Dipeptide yields for L- and D-proline with a starting concentration of 40 mM and with glycine as a catalyst.

For lysine and serine even a preference for the non-biological D-form was found when no glycine was added (see Fig. 6 for the case of lysine). In the presence of glycine, however, the situation is reversed and the L-forms come slightly on top (Fig. 7).



**Fig. (6).** Dipeptide yields for L- and D-lysine with a starting concentration of 40 mM without glycine.



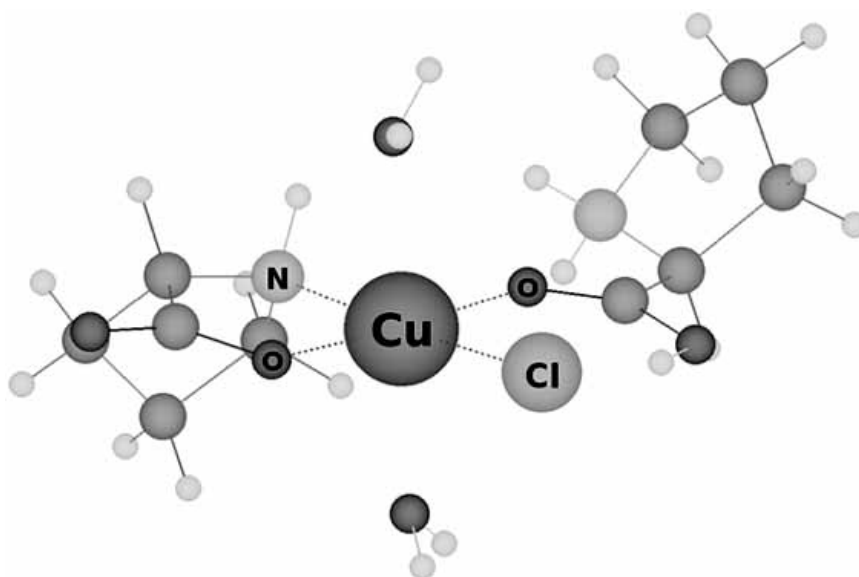
**Fig. (7).** Dipeptide yields for L- and D-lysine with a starting concentration of 40 mM and with glycine as a catalyst.

The results for tryptophan show an overall preference for the L-enantiomer, although the values for this rather unreactive amino acid should be handled with care as the dipeptide yields are not so far above the detection minimum.

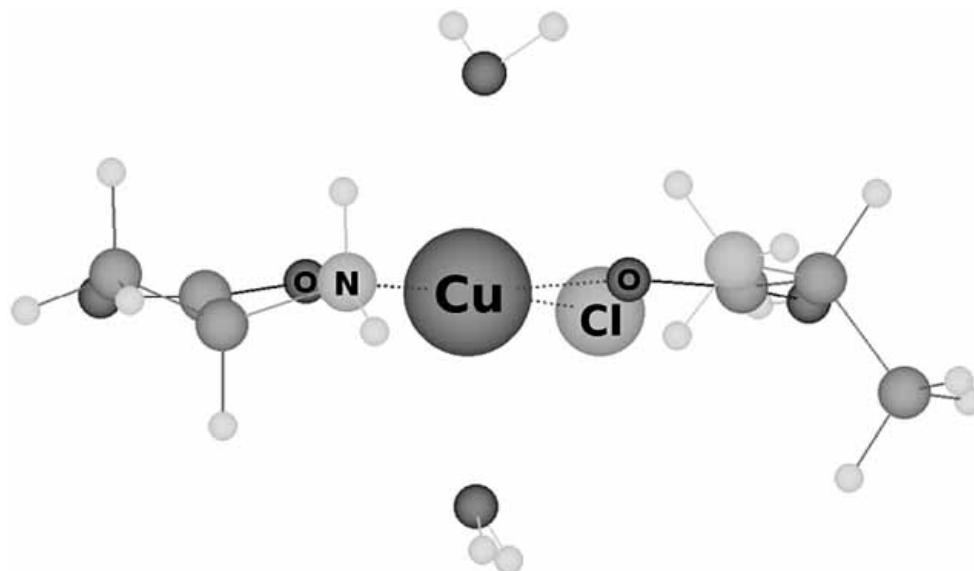
Valine and alanine are the only two amino acids investigated so far for which a persistent and substantial preference for the L-enantiomer in the SIPF reaction was detected. This leads to the assumption that amino acids with aliphatic side chains might have played a key role in the origin of homochirality of peptides built from L-amino acids.

## 6.2. Possible Explanation

A possible explanation for the stereoselectivity of the SIPF reaction in some cases can be found based on the geometry of the active complex. Since the two axial water ligands are at an elongated distance due to Jahn-Teller distortion the equatorial 'plane' consisting of one chelating amino



**Fig. (8).** Tilted view onto the 'plane' of a SIPF complex with two L-proline ligands.



**Fig. (9).** View directly into the distorted 'plane' of a SIPF complex with two L-alanine ligands.

acid, one end-on amino acid or peptide, and one chloride ligand can be easily distorted towards a tetrahedron-like conformation. This leads to central chirality at the Cu(II) ion in addition to its relatively high inherent optical activity provided by parity violation due to its high atomic number ( $Z = 29$ ). Chiral ligands like most amino acids or peptides induce an even stronger chirality at the copper centre. The Cu(II) complex, therefore, seems to act as a chemical 'amplifier' for the inherent small chirality due to parity violation.

The stronger the distortion of the 'plane' towards a tetrahedron is, the higher is the central chirality at the Cu(II) centre and the more this can lead to enlarged parity violating energy differences between the L-amino acid complex and its D-counterpart. This means that the high PVED value provided by the copper centre should have more effect in discriminating between an L-amino acid and a D-amino acid complex when this 'plane' is more distorted.

To support this hypothesis the complex geometry was investigated in detail by performing several *ab initio* geometry optimisations at Hartree Fock level with different basis sets (6-31G(d) for Cu, 6-311++G(2d,2p) for Cl and 6-311++G(d,p) for H, C, N, and O) using the Gaussian 03 package. The influence of the surrounding water was simulated by the Polarizable Continuum Model (PCM).

First of all the SIPF complex with the only achiral proteinogenic amino acid, glycine, was optimised as a kind of reference system. In this case an almost perfect 'plane' is formed by the copper centre, the chloride ligand, and the two glycine molecules with a dihedral angle between the end-on bound glycine and the plane formed by the copper ion and the bidentally bound glycine of just  $1.14^\circ$  and an angle between this plane and the chloride ligand of  $-1.7^\circ$ .

The SIPF complex containing two proline ligands was then examined. For proline no consistent preference for one chiral form had been observed in the experimental investigations which, following our hypothesis, should be indicated by a not so much distorted complex and, therefore, less pronounced central chirality at the Cu(II) centre. The calculated geometry supports our assumption. Almost no distortion towards a tetrahedral conformation occurs in the proline complex, as can be seen in Fig. 8. The equatorial 'plane' is just a little more twisted than in the glycine case and shows a dihedral angle for the end-on bound proline of only  $0.51^\circ$  and  $-6.0^\circ$  for chloride.

The next calculation featured alanine, which shows an obvious preference for the L-form in the SIPF reaction. The geometry optimisation of the corresponding complex showed indeed a much more distorted system. The dihedral angle to the single bound amino acid is  $6.5^\circ$  and  $-2.4^\circ$  to the chloride ligand. This distortion towards a tetrahedral conformation can be seen in Fig. 9.

## CONCLUSION

The Salt-Induced Peptide Formation reaction was most probably the main source of peptides on our primordial earth at a very early stage of chemical evolution. The stereoselective properties of the reaction preferring the usage of L-amino acids in some cases also provides a new indication towards the reason of bihomochirality. Although the mechanism for this preference is not fully understood yet, a reasonable hy-

pothesis for this phenomenon could be proposed and has to be further investigated by experiments with other amino acids and by additional theoretical investigations of SIPF complex species.

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