Amino Acids (2013) 44:725–732
DOI 10.1007/s00726-012-1396-6

ORIGINAL ARTICLE

Isoleucine as a possible bridge between exogenous delivery and terrestrial enhancement of homochirality

Feng Li · Daniel Fitz · Bernd M. Rode

Received: 23 October 2011 / Accepted: 27 August 2012 / Published online: 12 September 2012

Abstract We report a highly enantioselective oligomerization of isoleucine stereomers in the salt-induced peptide formation reaction under plausibly prebiotic earth conditions. Up to 6.5-fold superiority in reactivity of L-isoleucine was observed, compared to its D-enantiomer, after 14 evaporation cycles in the presence of Cu^{2+} and NaCl. Since isoleucine is among the proteinogenic amino acids that were found enantioenriched in meteorites, this present work may further correlate the extraterrestrial delivery and endogenous production of biological homochirality by virtue of a protein constituent rather than the rarely occurring α-methylated amino acids.

Keywords Isoleucine · Allo-isoleucine · Homochirality · Salt-induced pepetide formation

Introduction

To probe the ultimate origin of life, extraterrestrial delivery of biomolecules with enantiomeric asymmetry has recently earned more popularity, since some amino acids with quite high enantiomeric excesses (*ee*s) were detected in meteorites like the Murchison (Cronin and Pizzarello 1997) and the Murray (Cronin and Pizzarello 1999) meteorites, and then were proven their non-terrestrial origin by isotope measurements (Engel and Macko 1997; Pizzarello and Huang 2005). Proponents argue the meteoritic enantioenrichment of organic molecules might be induced by asymmetric photolysis under irradiation of circularly polarized light (Bailey et al. 1998), and eventually transfer this chiral asymmetry to terrestrial biomolecules like amino acids (Levine et al. 2008) and sugars (Pizzarello and Weber 2004) on prebiotic earth after meteoritic bombardment. With a large probability, this tiny *ee* induced by circularly polarized light could also be amplified to a homochirality due to the solubility difference in enantiomers (Breslow and Levine 2006).

However, most of the enantioenriched amino acids in these meteorites are α-methylated and cannot be found in extant proteins, although the methylated α-carbon atom is believed to readily retain the chiral configuration from racemization during the tough travel to earth. The only exception in enantiomerically enriched protein constituents in extraterrestrial materials so far is the *ee* of L-isoleucine (L-Ile) in the GRA 95229 meteorite (Pizzarello et al. 2008), which was recovered in the ice sheet of Antarctica.

Ile, 2-amino-3-methylpentanoic acid, can be classified into two pairs of enantiomers due to its two chiral centers, namely L- and D-Ile (2*S*, 3*S* and 2*R*, 3*R*) and L- and D-*allo*-Ile (2*S*, 3*R* and 2*R*, 3*S*), of which only L-Ile occurs in terrestrial proteins. Distinct *ee*s of L-Ile (14 %) and of D-*allo*-Ile (12 %) have been identified in the GRA 95229 segment, which is considered a result of asymmetric distribution of chiral precursors like aldehydes in interstellar media (Pizzarello et al. 2008). Therefore, so peculiar is Ile in chemical evolution since it could be asymmetrically formed in extraterrestrial environments and keep its *ee* under the rampant conditions during its entry to the atmosphere and the impact on earth's surface, whereas other proteinogenic amino acids were found racemic in meteorites.

F. Li · D. Fitz · B. M. Rode (✉)
Faculty of Chemistry and Pharmacy, Institute of General, Inorganic and Theoretical Chemistry, University of Innsbruck, Innrain 52a, 6020 Innsbruck, Austria
e-mail: Bernd.M.Rode@uibk.ac.at

F. Li
Department of Earth Sciences, University of Oxford, Parks Road, Oxford OX1 3PR, UK

As a very plausible section of the classic primordial soup theory, the salt-induced peptide formation (SIPF) reaction is one of the most prebiotically relevant, feasible and facile pathways for condensation of amino acids under relatively crude conditions on pristine earth (Rode 1999). The SIPF reaction is intermediated by an octahedral complex, composed of a Cu^{2+} center, a monodental amino acid ligand, a bidentally chelated amino acid, a Cl^{-} ligand and two water molecules (Liedl and Rode 1992). In such complex, the nucleophilic attack that enables peptide bond formation is favored between the *N*- and *C*-termini of the coordinated forms.

In this present work, four Ile stereomers were respectively subjected to the SIPF conditions, which refer to evaporation cycles of reaction solution containing amino acid, $CuCl_2$ and NaCl at 85 °C, although a L/D-mixed or racemic Ile sample, which was not included due to the complexity in analysis, may be of higher interest to investigate. Isotopic evidence has implied the possible temperatures of Precambrian oceans might have varied within 80–90 °C around 4 billion years ago (Robert and Chaussidon 2006), while the drying–wetting process could occur globally involving rainfalls, tides and evaporation in lakes and lagoons. It is, therefore, of great significance and credibility to investigate the oligomerization behaviors of Ile isomers and make a comparison of their reactivities under primordial earth scenarios in this experimental paradigm, since it could be delivered by meteorites with a convictive *ee*.

Results and discussion

General results

The maximal yield of Ile–Ile appears at $T = 14$ (T refers to reaction time/days, or the number of evaporation cycles) starting with 80 mM L-Ile, where the percentage yield is around 0.031 %, while *allo*-Ile shows less reactivity in dimerization with a peak value of 0.0089 % after 14 evaporation cycles of its L-enantiomer, as shown in Fig. 1. It can be clearly seen that Ile is most reactive at 80 mM and becomes less and less reactive along with the decrease of initial concentrations. Nevertheless, *allo*-Ile, which only differs in the chiral configuration of the β-carbon atom in the side chain, has yielded the least dipeptides within $T = 10$ at 80 mM, and shows its best reactivity at 40 mM even though still slightly lower than Ile. In the most dilute concentration of 20 mM *allo*-Ile seems slightly superior to Ile.

The same handed Ile and *allo*-Ile, namely L-Ile versus L-*allo*-Ile and D-Ile versus D-*allo*-Ile, have the same α-carbon conformation which dominates the "reactive plane" (see Fig. 6) for nucleophilic attack in the SIPF copper complex. Thus, the different reactivities of Ile and *allo*-Ile might be attributed to the properties (merely chirality here) of their side chains.

Significant enantioselectivity

All the values of enantioselectivity factors R, defined as the ratio of L-L-dipeptide yield to the D-D-yield, in Fig. 2, are

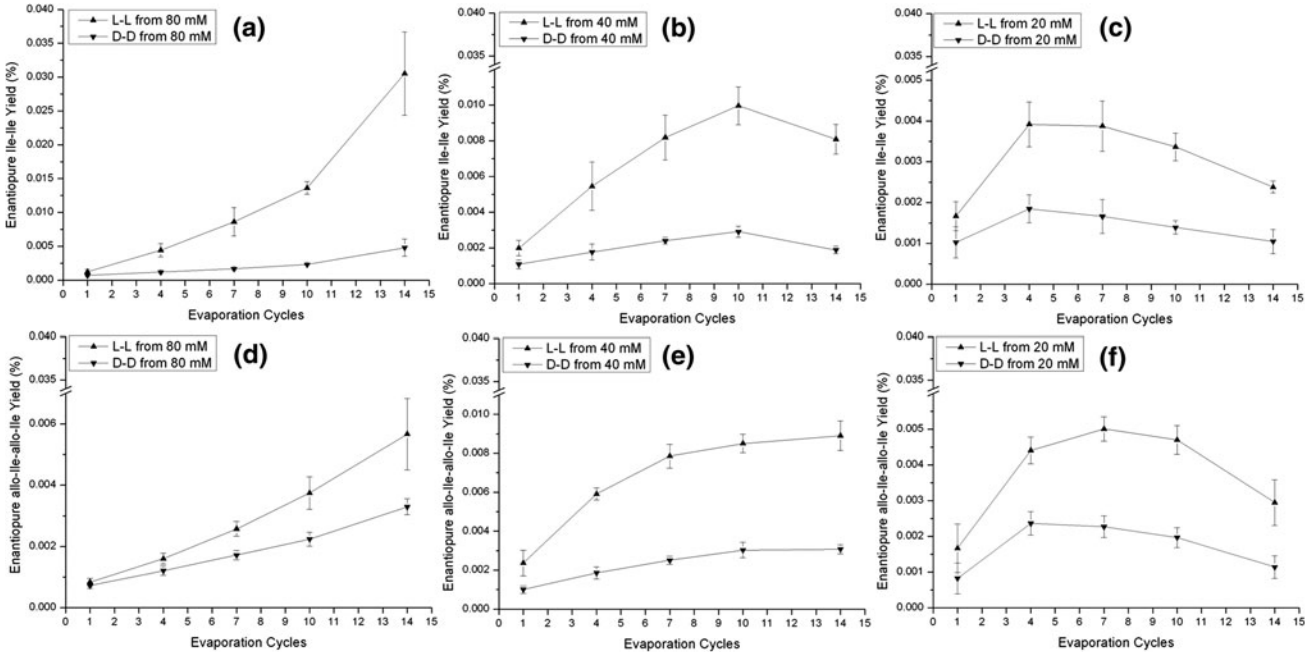

Fig. 1 Dipeptide yields of Ile (**a–c**) and *allo*-Ile (**d–f**) with standard deviations calculated from five independent repeats of experiment

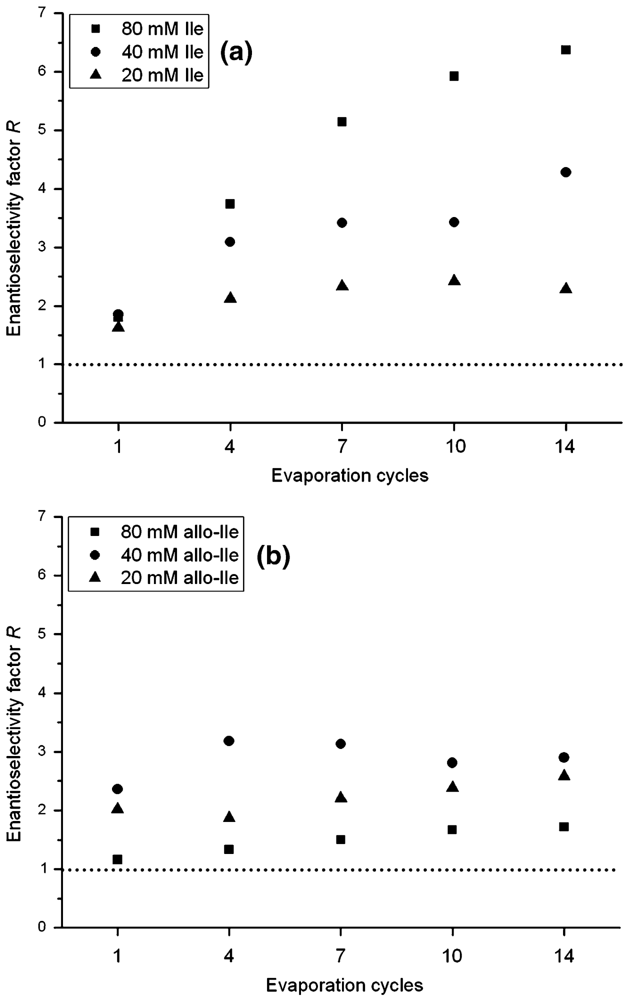

Fig. 2 Enantioselectivity factors R (the L-L/D-D ratio of dipeptide yields) of isoleucine (**a**) and *allo*-isoleucine (**b**)

distinctly higher than 1, which indicates a consistent preference for L-handed both Ile and *allo*-Ile in dimerization by the SIPF mechanism. The most outstanding enantioselectivity in Ile dimerization is observed for the 80 mM case where the L-form is around 6.5 times as reactive as the D-form. The highest R value for *allo*-Ile appears at 40 mM with an almost threefold superiority for the production of the L-L-dipeptide. These distinct asymmetries in peptide synthesis can be directly seen in the corresponding peak areas in the chromatograms in Fig. 3. Coincidently, both 80 mM Ile and 40 mM *allo*-Ile have achieved the highest dipeptide yields in addition to their highest enantioselectivity under the same conditions. This positive correlation between reactivity and enantioselectivity implies that the notable L-preference for the Ile stereomers is a significant property under the SIPF conditions rather than a tiny and random bias provoked by low dipeptide peaks within the range of the detection limit.

The R values in most cases, especially at the most reactive concentrations, keep an increasing trend along with the reaction cycles, from 1–2 at $T = 1$ to 3–6 after $T = 7$. That means that the enantioselectivity is gradually amplified and the L-enantiomers are more and more preferred in dimerization with the reaction time going by. Furthermore, the increasing enantioselectivity avoids a possible disturbance by systemic errors shown in Fig. 1, which presents the range of standard deviations (SDs) for each dipeptide yield calculated by the data of five independent repeats of each experiment. In these diagrams, all the fringes of SDs for the data after $T = 1$ in all systems are not overlaid with each other, and therefore, we can conclude a very reliable significance in different reactivities of the Ile and *allo*-Ile enantiomers in the SIPF reaction.

Occurrence of racemization?

The α-hydrogen of 20 natural amino acids can be abstracted by H_2O or basic ions, which subsequently results in a planar rearrangement of the residual three functional groups on the α-carbon (Ebbers et al. 1997). Such an intermediate planar carbanion can rebind a hydrogen ion but from both sides of the plane with almost equal probability, and, in turn, the trend to an eventual racemate is therefore established (Cohen and Chyba 2000). However, experimental measurement indicates a pH value of SIPF solution containing Ile around 2.8 due to hydrolysis equilibrium of Cu^{2+}. In such an acidic environment Ile may not readily racemize.

The experimental samples were further analyzed through a chiral HPLC column Phenomenex Chirex 3126 D-penicillamine (4.6 × 150 mm), which can directly separate amino acid enantiomers by the chiral ligand selector. This measurement gives a convincing result on possible appearance of the other enantiomer during reaction. As shown in Fig. 4, all four isomers in 80 mM were not racemized to a detectable extent after 14 cycles. For diluter concentrations, whose chromatographs are not displayed here, racemization was not detected, either. In addition, previous research (Smith and Evans 1980) has also documented the relatively slow racemization rate of Ile compared to other amino acids, because of the retarding effect of the bulky alkyl side chain group (Smith and Evans 1989).

Comparison with its isomer leucine

Leucine (Leu) is isomeric to Ile and only differs in its methylated γ-carbon atom instead of the β-carbon, which results in the non-chiral side chain of Leu and makes Leu a little bit more hydrophilic but less soluble than Ile. In Fig. 5, Leu (Li et al. 2010) appears less reactive in the SIPF

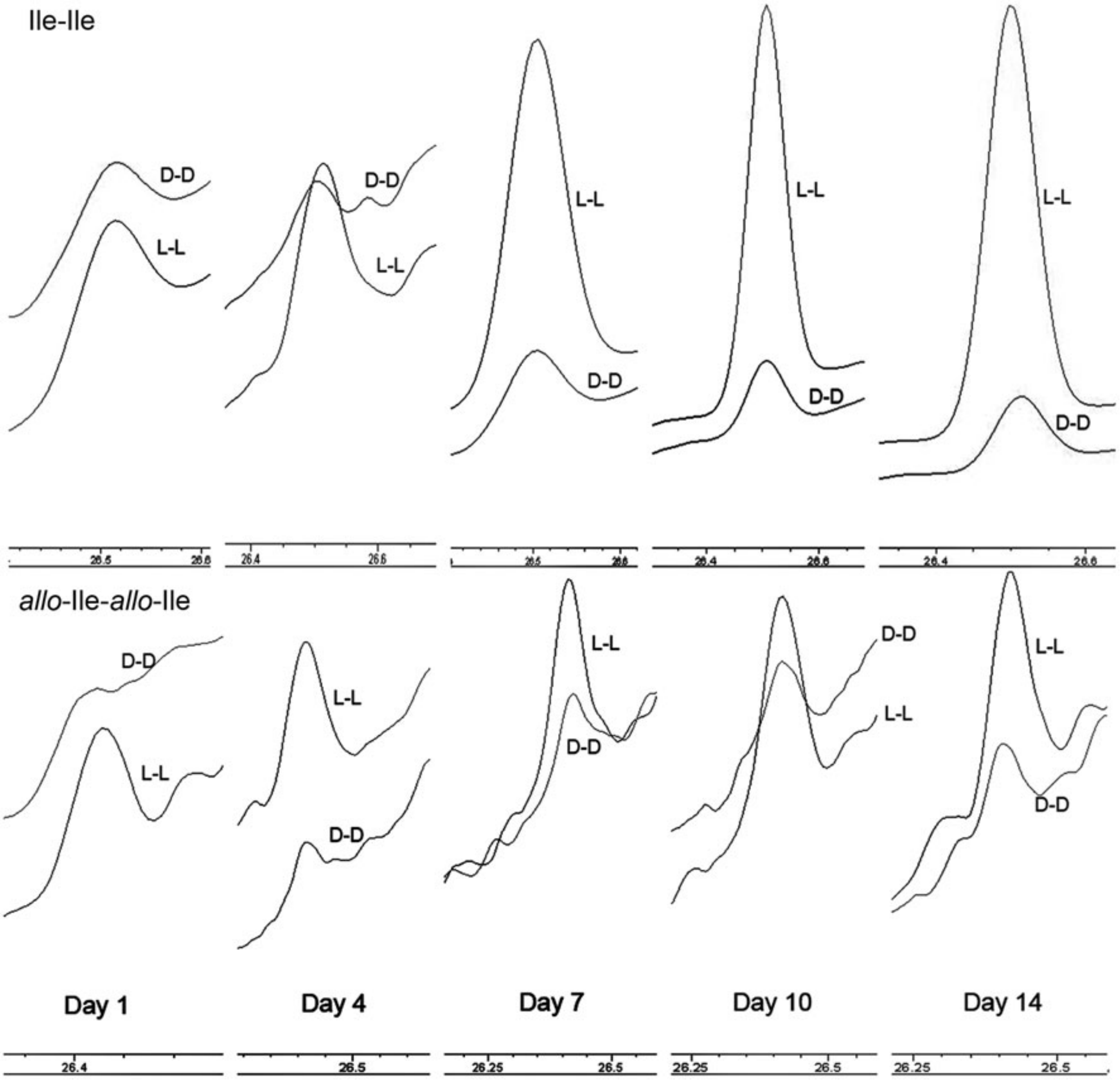

Fig. 3 Chromatograms of enantiopure L-L- and D-D-dipeptide produced by the enantiomers of 80 mM Ile (*upper row*) and 80 mM *allo*-Ile (*lower row*), respectively, where the L-form preference is most pronounced

mechanism than the Ile stereomers in the 80 and 40 mM cases but more productive at 20 mM than Ile and *allo*-Ile. This phenomenon might be ascribed to the co-effect of solubility and hydrophilicity. In the experiments with high initial concentrations, the less soluble Leu might not be completely redissolved in the refilled water after each evaporation cycle, and this lack of free Leu in the reactive solution probably results in the relatively low yields in these cases. At 20 mM one could believe that all these three amino acids would be easily redissolved after every cycle, and under these circumstances with equal supply of reactants, Leu with its higher hydrophilicity would be more active in the aqueous environment.

Figure 5 also reflects that Leu only shows an L-preference in the 80 and 20 mM cases, which is quite inferior to Ile. However, in the 20 mM case, where Leu shows its highest reactivity, the enantioselectivity is correspondingly on top. Such a positive reactivity–enantioselectivity correlation is the same as those of Ile and *allo*-Ile, indicating a common feature of these isomeric amino acids when exposed to the SIPF reaction.

Theoretical approach to the enantioselectivity

Ab initio calculations of the optimized geometry of the active SIPF complex at Hartree–Fock level were performed with the basis sets as follows, 6-31G(d) for Cu, 6-311++G(2d, 2p) for Cl and 6-311++G(d, p) for H, C, N, and O with the Gaussian 03 package. The influence of the aqueous environment was simulated by the polarizable continuum model.

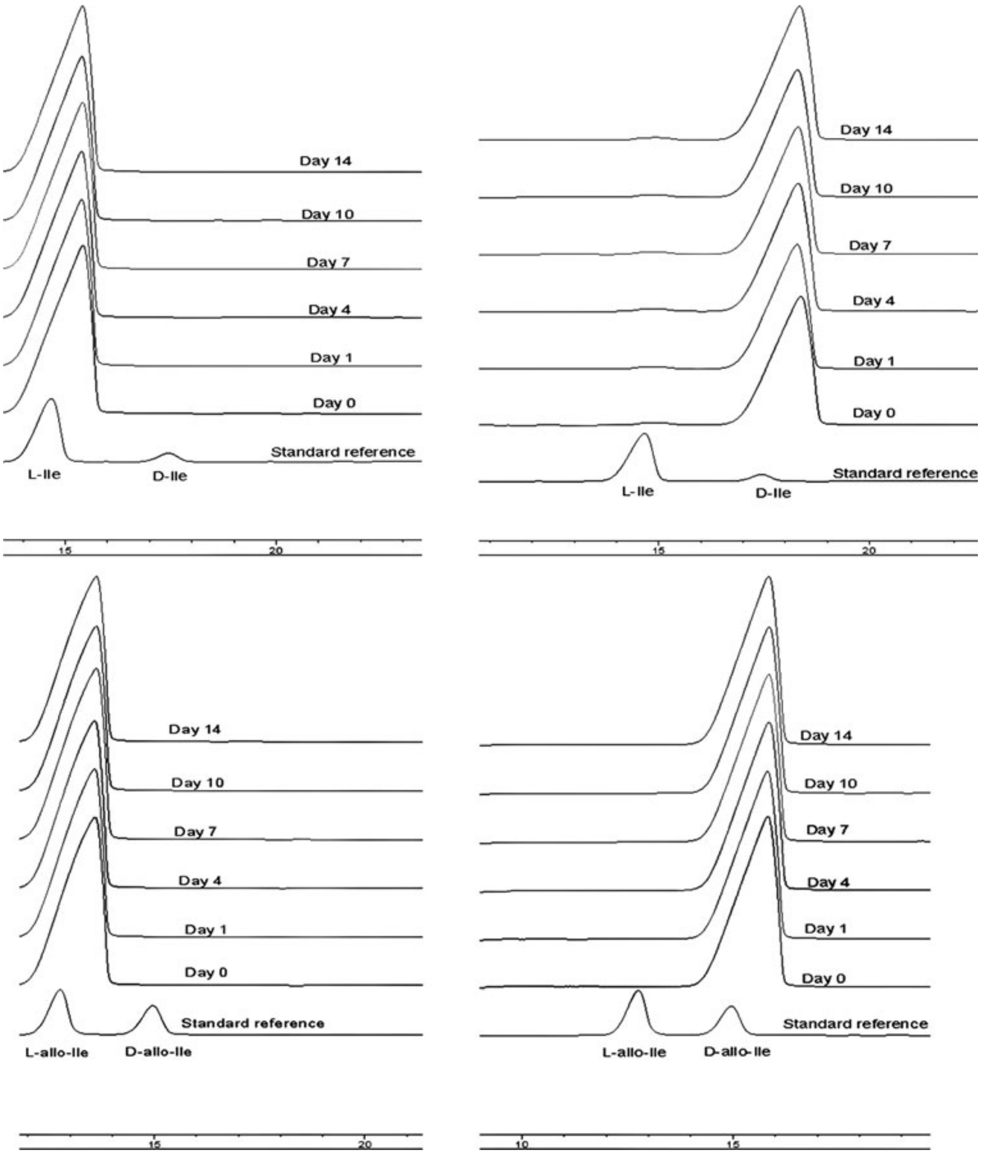

Fig. 4 Chromatographs showing the retained enantiomeric conservation of 80 mM Ile and allo-Ile in the samples after the SIPF processes

The geometry optimization result of the Ile–Cu complex is visualized in Fig. 6a, where the equatorial plane composed of four coordinated atoms (Cl, O, N and O) around the Cu center is slightly distorted toward a tetrahedral structure. Such a distorted structure lack of central asymmetry may evoke an inherent chirality (Lahamer et al. 2000), which is overlaid with the intrinsic chirality of the Cu atom (Feinberg 1978; Hegstrom and Kondepudi 1990) and the parity violating energy difference (PVED) between amino acid enantiomers (Mason and Tranter 1984, 1985; Tranter 1985), which are ascribed to parity violation in weak interactions (Lee and Yang 1956). Therefore, these distorted complexes containing enantiomeric ligands may possess different physicochemical properties just like diastereomers, and higher distortion will stand for more chiral induction.

The magnitude of geometric distortion can be measured by two dihedral angles, which are formed by the O–Cu (O atom of the monodental amino acid ligand) and Cl–Cu beelines with the Cu–N–O plane (N and O atoms of the chelated amino acid). In the Ile complex, the O–Cu distortion angle is 3.9°/176.1° together with a Cl–Cu angle of 4.1°/175.9°, which is quite a distorted structure.

However, the Leu–Cu complex in Fig. 6b seems even more distorted with the O–Cu and Cl–Cu distortion angles of 6.8°/173.2° and 3.6°/176.4°, respectively. Theoretically, the Leu–Cu complex should have induced a higher enantioselectivity than Ile, but Ile has another chiral center at the β-carbon which might lead to a higher PVED value between its enantiomers and eventually behave more enantioselectively.

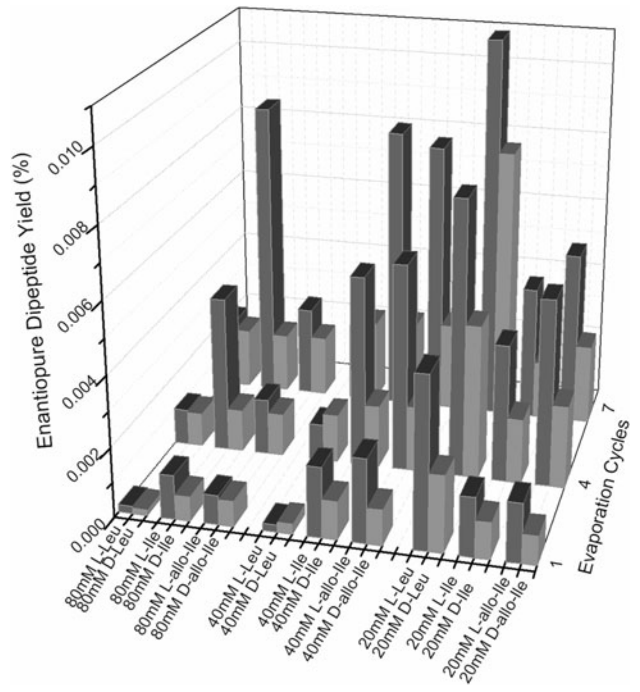

Fig. 5 Summary of enantiopure dipeptide yields produced from the isomeric amino acids Leu, Ile and *allo*-Ile in the SIPF reaction starting with different concentrations

Concluding remarks

This work investigates the subsequent oligomerization behavior of the isoleucine and *allo*-isoleucine enantiomers in primordial earth scenarios, since their enantiomeric balance could have been broken in extraterrestrial environments and then delivered to prebiotic earth by meteorites. L-Ile as the only biologically relevant amino acid has achieved the best reactivity in dimerization, around 6.5 times higher than its D-enantiomer. L-Ile is consistently favored in the SIPF reaction with significant superiority to its D-counterpart. The L-preference also occurs with *allo*-Ile, indicating that the enantioselectivity in the SIPF reaction is mainly determined by the configuration of the α-carbon atom, while the other chiral center in the side chain may influence the magnitude of enantioselectivity but cannot reverse it. In addition, a positive correlation between dipeptide productivity and enantioselectivity was observed, which has further approved that the significant enantioselectivity does derive from different reactivities of intermediate complexes with different enantiomeric ligands, rather than a random imbalance standing out of the low values.

Ile is unique among all 20 proteinogenic amino acids for its enantiomeric asymmetry in extraterrestrial materials, and is now found the highest L-preference in the SIPF mechanism known so far. This finding lines out an evolutionary route for a protein monomer, from initial symmetry breaking in extraterrestrial environments, subsequent delivery to the earth with the enantiomeric excess conserved, till the eventual oligomerization to a homochiral L-peptide in primitive earth scenarios, and most importantly, for first time correlates the extraterrestrial delivery hypothesis with the indigenous "soup theory" in terms of origin of homochirality. Therefore, it is rational to hypothesize that life with a chiral consensus might be a cooperative outcome of multiple sources (Chyba and Sagan 1992).

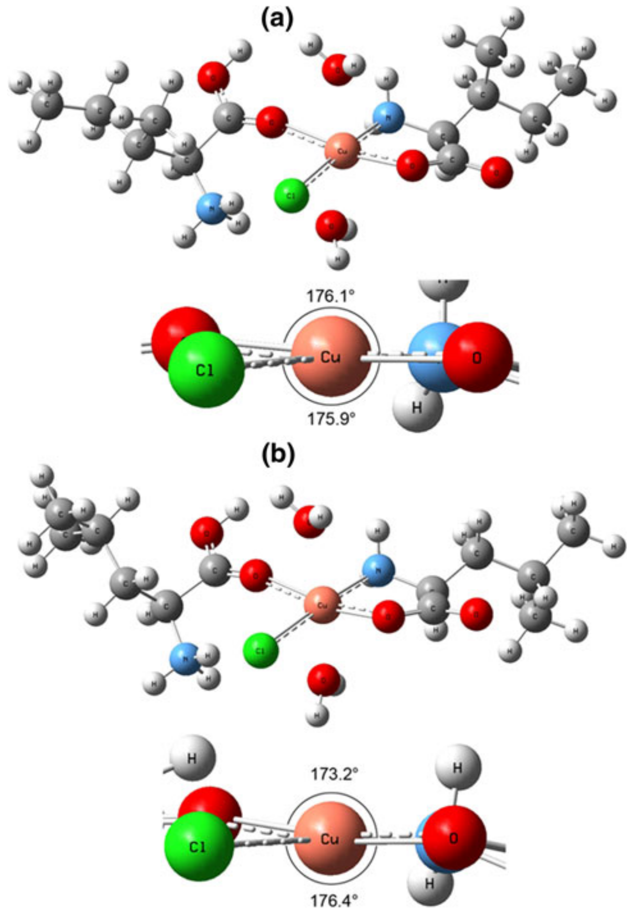

Fig. 6 Geometrically optimized structures of SIPF complexes with two L-Ile (**a**) and two L-Leu (**b**) ligands, respectively, including the panoramic view (*upper*) and the zoomed-in snapshot horizontal to the distorted equatorial plane (*lower*) for each subfigure

Materials and methods

Materials

All enantiopure Ile and *allo*-Ile, as well as L-Ile–L-Ile, of analytical grade were purchased from Bachem AG, Bubendorf, Switzerland. Rare peptide standard of L-*allo*-Ile–L-*allo*-Ile and Thr–Thr was synthesized by Science

Peptide Biological Technology Co. Ltd., Shanghai, China. NaCl and $CuCl_2{\cdot}2H_2O$ were manufactured by Merck, D-Darmstadt. Sodium *n*-hexanesulfonate monohydrate as the ion-pairing reagent and acetonitrile (super-gradient grade for HPLC) were obtained from Sigma-Aldrich GmbH, Germany. KH_2PO_4 and concentrated phosphoric acid were provided by Fluka Chemie AG, Switzerland. The ultrapure water used for solution preparation is the Rotisolv® HPLC gradient grade water by Carl Roth GmbH, Karlsruhe, Germany.

Evaporation cycles

The synthesis of peptides by the SIPF mechanism was performed in drying-and-wetting cycle experiments that simulate the relevant scenarios on primordial earth. The SIPF solution including 500 mM NaCl and 40 mM $CuCl_2$ was used to dissolve enantiopure amino acid reactant at different starting concentrations of 80, 40 and 20 mM. After the mixing procedure, 1 mL aliquots of the reacting solutions were distributed into 2 mL HPLC sample vials and then placed in a thermostatic oven at 85 °C to start the first evaporation cycle within 24 h, which provided a slow and favorable increase of solute concentration for peptide formation. After each cycle, 1 mL of ultrapure water was refilled to the residue for next cycle under the same conditions. The evaporated samples after 0, 1, 4, 7, 10 and 14 cycles were collected and frozen dry at −20 °C till analyses with high performance liquid chromatography (HPLC).

HPLC analysis

The samples for HPLC analysis were first redissolved with 1 mL water, filtered through 0.22 μm hydrophilic PVDF syringe filters (Carl Roth GmbH, Germany), and then analyzed by reversed-phase ion-pairing HPLC on the Agilent 1100 series system with a diode array UV–Vis detector.

For quantitative identification of dipeptide products, 2 μL of a sample solution was injected into a reverse-phase Phenomenex Luna C18(2) (3 μm, 2.0 × 150 mm) column. The UV–Vis detector was set at 200 nm, 4 nm bandwidth and with a reference wavelength at 550 nm, 100 nm bandwidth. Solvent A was prepared with 50 mM KH_2PO_4 and 7 mM $n\text{-}C_6H_{13}SO_3Na$ in ultrapure water, adjusted to pH 2.3 with concentrated H_3PO_4, while solvent B was pure acetonitrile. The elution gradient parameters were 0 min 1 % B, 10 min 5 % B, 15 min 10 % B, 20 min 20 % B, 25 min 20 % B, 28 min 25 % B, 30 min 5 % B, 33 min 1 % B, stop time 35 min, flow rate 0.25 mL/min and column temperature 40 °C.

For investigations on the possibility of racemization of Ile and *allo*-Ile during the SIPF reaction, a chiral column Phenomenex Chirex 3126 D-penicillamine (4.6 × 150 mm) was utilized to directly separate and detect the enantiomers of Ile and *allo*-Ile at 235 nm, 4 nm bandwidth and with a reference wavelength at 550 nm, 100 nm bandwidth.

Solvent A was an aqueous solution of 2 mM $CuCl_2$ and must be always present in the tubes to keep the abundance of copper ion for the chiral ligand selector in the column; solvent B was acetonitrile at super-gradient grade. Isocratic elution was applied at flow rate of 0.4 mL/min and at the column temperature of 50 °C, and the gradient parameters could be described as 88 % solvent A, 12 % solvent B, stop time 25 min.

Acknowledgments This work is financially supported by the Austrian Ministry of Education, Science and Culture (BMWF, Grant No. 45.530/0003-11/6a/2007) and the Austrian Science Foundation (Fonds zur Förderung der wissenschaftlichen Forschung, Projekt P19334-N17), which are gratefully appreciated. Feng Li thanks the research grant and maintenance from the Overseas Research Students Awards Scheme (UK) and the Hester Cordelia Parsons Fund (University of Oxford).

References

Bailey J, Chrysostomou A, Hough JH, Gledhill TM, McCall A et al (1998) Circular polarization in star-formation regions: implications for biomolecular homochirality. Science 281:672–674

Breslow R, Levine SM (2006) Amplification of enantiomeric concentrations under credible prebiotic conditions. Proc Natl Acad Sci USA 103:12979–12980

Chyba C, Sagan C (1992) Endogenous production, exogenous delivery and impact-shock synthesis of organic molecules: an inventory for the origins of life. Nature 355:125–132

Cohen BA, Chyba CF (2000) Racemization of meteoritic amino acids. Icarus 145:272–281

Cronin JR, Pizzarello S (1997) Enantiomeric excesses in meteoritic amino acids. Science 275:951–955

Cronin JR, Pizzarello S (1999) Amino acid enantiomer excesses in meteorites: origin and significance. Adv Space Res 23:293–299

Ebbers E, Ariaans GJA, Houbiers JPM, Brugginks A, Zwanenburg B (1997) Controlled racemization of optically active organic compounds: prospects for asymmetric transformation. Tetrahedron 53:9417–9476

Engel MH, Macko SA (1997) Isotopic evidence for extraterrestrial non-racemic amino acids in the Murchison meteorite. Nature 389:265–268

Feinberg G (1978) Handedness of atoms and parity non-conservation. Nature 271:509–512

Hegstrom RA, Kondepudi DK (1990) The handedness of the universe. Sci Am 262(1):98–105

Lahamer AS, Mahurin SM, Compton RN, House D, Laerdahl JK et al (2000) Search for a parity-violating energy difference between enantiomers of a chiral iron complex. Phys Rev Lett 85:4470–4473

Lee TD, Yang CN (1956) The question of parity conservation in weak interactions. Phys Rev 104:254–257

Levine M, Kenesky C, Mazori D, Breslow R (2008) Enantioselective synthesis and enantiomeric amplification of amino acids under prebiotic conditions. Org Lett 10:2433–2436

Li F, Fitz D, Fraser DG, Rode BM (2010) Catalytic effects of histidine enantiomers and glycine on the formation of dileucine and dimethionine in the salt-induced peptide formation reaction. Amino Acids 38:287–294

Liedl KR, Rode BM (1992) Ab initio calculations concerning the reaction mechanism of the copper (II) catalyzed glycine condensation in aqueous sodium chloride solution. Chem Phys Lett 197:181–186

Mason SF, Tranter GE (1984) The parity-violating energy difference between enantiomeric molecules. Mol Phys 53:1091–1111

Mason SF, Tranter GE (1985) The electroweak origin of biomolecular handedness. Proc R Soc Lond A397:45–65

Pizzarello S, Huang Y (2005) The deuterium enrichment of individual amino acids in carbonaceous meteorites: a case for the pre-solar distribution of biomolecule precursors. Geochim Cosmochim Acta 69:599–605

Pizzarello S, Weber AL (2004) Prebiotic amino acids as asymmetric catalysts. Science 303:1151

Pizzarello S, Huang Y, Alexandre MR (2008) Molecular asymmetry in extraterrestrial chemistry: insights from a pristine meteorite. Proc Natl Am Sci USA 105:3700–3704

Robert F, Chaussidon M (2006) A palaeotemperature curve for the Precambrian oceans based on silicon isotopes in cherts. Nature 443:969–972

Rode BM (1999) Peptide and the origin of life. Peptides 20:773–786

Smith GG, Evans RC (1980) Biogeochemistry of amino acids. In: Hare PE, Hoering TC, King, K (eds), Wiley, New York, p 257–282

Smith GG, Evans RC (1989) Effect of the side chain on the racemization of amino acids in aqueous solution. J Org Chem 54:4529–4535

Tranter GE (1985) The parity violating energy differences between enantiomers of α-amino acids. Mol Phys 56:825–838