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ORIGINAL ARTICLE

Catalytic effects of histidine enantiomers and glycine on the formation of dileucine and dimethionine in the salt-induced peptide formation reaction

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Abstract The salt-induced peptide formation (SIPF) reaction takes place readily under mild reaction conditions and proceeds via a copper complex. Its ease of reaction and the universality for prebiotic scenarios add weights to the arguments in favour of the importance of peptide and proteins in the tug of war with the RNA world hypothesis. In addition, the SIPF reaction has a preference for L-form amino acids in dipeptide formation, casting light on the puzzle of biohomochirality, especially for the amino acids with aliphatic side chains. A detailed investigation on the behaviour of aliphatic leucine in the SIPF reaction is presented in this paper, including the catalytic effects of glycine, L- and D-histidine as well as the stereoselectivity under all the reaction conditions above. The results show a relatively low reactivity and stereoselectivity of leucine in the SIPF reaction, while both glycine and histidine enantiomers remarkably increase the yields of dileucine by factors up to 40. Moreover, a comparative study of the effectiveness of L- and D-histidine in catalysing the formation of dimethionine was also carried out and extends the scope of mutual catalysis by amino acid enantiomers in the SIPF reaction.

Keywords Salt-induced peptide formation reaction · Prebiotic chemistry · Leucine dipeptide · Copper complex with amino acids · Homochirality

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Introduction

As a momentous episode in the chemical evolution towards the origin of life, the possibility of abiotic formation of amino acids as well as their polymers, peptides and proteins, has fascinated generations of researchers hitherto. From Miller's initial sparks (Miller 1953) to Plankensteiner's success of discharging in neutral gases (Plankensteiner et al. 2004a, b), the feasibility of synthesis of amino acids and other small biomolecules under primordial earth conditions was realised. For the prebiotic assembly of peptides, several strategies or mechanisms have been proposed, including the 'melting process' (Fox and Harada 1960), numerous condensation reagents (Leman et al. 2004; Ponnamperuma and Peterson 1965; Rabinovitz 1971; Yanagawa et al. 1984), the role of mineral surfaces (Lahav et al. 1978), and the reactions in hydrothermal vents (Huber and Wächtershäuser 1998; Imai et al. 1999), etc.

Compared with all the peptide formation pathways above, the salt-induced peptide formation (SIPF) reaction, discovered in the late 1980s (Schwendinger and Rode 1989a, b) and implemented through drying-and-wetting cycles with the help of divalent copper ions and sodium chloride in aqueous solution under moderate temperature, has repeatedly shown an outstanding facility and universality for the synthesis of small peptides once amino acids appeared on the primitive earth (Rode 1999). It works well with all amino acids investigated so far and prefers α-amino acids with biological relevance (Schwendinger et al. 1995).

Based on theoretical observations of NaCl solutions and Cu(II) complexes with amino acids (Liedl and Rode 1992; Limtrakul and Rode 1985; Limtrakul et al. 1985; Schwendinger and Rode 1989a, b), the key intermediate,

which functions to lower the activation barrier for peptide bond formation, could be described as a distorted structure consisting of one chloride ligand, two amino acids and two water molecules, where the copper ion acts as the coordination centre. In such a complex, one amino acid chelates the Cu(II) ion with its amino nitrogen atom and carboxylate oxygen, while the other amino acid coordinates with the copper centre only through the carboxylate oxygen as the fourth strong binding site is occupied by chloride. The two water molecules in axial positions stay at elongated distances due to the Jahn–Teller effect. The role of NaCl is to act as a dehydrating reagent because at concentrations higher than 3 M the first hydration shell of the Na^+ ions becomes unsaturated.

Previous work on paleogeology (Carver 1981; Hofmann et al. 1999; Levine et al. 1982; Nutman et al. 1996; Ochiai 1978) indicates the abundance of Cu(II) and NaCl and the feasibility of the SIPF reaction on primordial earth, and especially endorses the widespread drying-and-wetting evaporation cycles that occurred in tidal coast regions and lagoons. In addition, the realistic surface temperature of primordial earth was also accordant with the optimum range (60–90°C) for the SIPF reaction.

When added to the SIPF reaction systems, especially at 1/8 of the reacting amino acid concentrations, glycine, diglycine and histidine can dramatically enhance the peptide yields from relatively unreactive amino acids in most cases (Plankensteiner et al. 2002; Plankensteiner et al. 2005a, b, c; Reiner et al. 2006; Suwannachot and Rode 1998; Suwannachot and Rode 1999). Such mutual catalysis could be attributed to the ideal reactivity of the catalytic amino acids with the other amino acids, together with the appropriate property for subsequent hydrolysis in order to 'break off' from the freshly formed peptides (Suwannachot and Rode 1998).

Another interesting feature of the SIPF reaction is the stereoselectivity (Plankensteiner et al. 2004a, b) favouring peptide formation from L-amino acids, which together with glycine uniformly compose all the proteins in present organisms. Valine gives the most striking difference in dipeptide formation between L–L and D–D forms in the SIPF reaction (Plankensteiner et al. 2005a, b, c), and so does another aliphatic analogue, alanine (Fitz et al. 2008; Plankensteiner et al. 2004a, b). Slight enantioselectivity was also detected in experiments with tryptophan, serine, lysine (Plankensteiner et al. 2004a, b) and high concentrations of methionine (Li et al. 2008).

One plausible explanation for the stereoselectivity that could, in turn, contribute to biohomochirality (Fitz et al. 2007), is based on the geometry of the active copper complex $[CuCl(aa)(aaH_2)(H_2O)_2]^+$ (aa, short for amino acid). The two axial H_2O ligands are only weakly bound to the copper centre at an elongated distance and, therefore, the equatorial plane consisting of two amino acids and the chloride ligand can be readily distorted towards a tetrahedron-like conformation. Such a distortion provokes a central chirality on the copper centre in addition to the inherent chirality due to the parity violation in weak nuclear interactions, which is relatively high for copper's atomic number ($Z = 29$). According to the parity violation in weak nuclear interactions (Lee and Yang 1956; Rubbia 1985; Weinberg 1980; Wu et al. 1957), all atoms possess an inherent chirality proportional to Z^5 or even Z^6 (Z being the atomic number) and there exists a tiny parity-violating energy difference (PVED) (though this is only 10^{-38} to 10^{-35} J for amino acids) in favour of L-forms between molecular enantiomers (Barron 1994; Berger and Quack 2000; Laerdahl et al. 2000; Mason and Tranter 1983; Mason and Tranter 1984; Tranter 1985). Because of the overlay of the inherent chirality of the copper atom and the central chirality arising from the conformational distortion, a complex containing two L-amino acids can be seen as a diastereomer to its D-counterpart, thereby providing different physical and chemical properties and reactivities.

In the present work, another aliphatic amino acid with a long and hydrophobic side chain, leucine, is investigated in the SIPF reaction. Five independent series of evaporation cycle experiments starting with three different concentrations were performed with L- and D-leucine in SIPF solution (aqueous solution of NaCl and $CuCl_2$). Glycine, L- and D-histidine were respectively added as catalysts to the catalytic series. The reaction solutions in uncapped vials were completely evaporated within 24 h at 85°C and were then refilled with pure water to start the next cycle. Samples were collected after one, four and seven cycles and were prepared for HPLC analysis afterwards. The continuing research on methionine peptide formation catalysed by L- and D-histidine is also discussed in this paper, following the same experimental procedures.

Materials and methods

Materials and reagents

All the amino acids (L- and D-leucine, L- and D-methionine, glycine and L- and D-histidine) and dipeptide standard compounds(L-leu-L-leu and L-met-L-met)utilised were purchased from Bachem AG, Switzerland. NaCl and $CuCl_2{\cdot}2H_2O$ were produced by Merck, D-Darmstadt. Sodium hexanesulfonate monohydrate as an ion-pairing reagent was supplied by Sigma-Aldrich GmbH, Germany, as well as acetonitrile in super-gradient grade for HPLC. KH_2PO_4, concentrated hydrochloric acid and concentrated phosphoric acid were manufactured by Fluka Chemie AG, Switzerland. A water purification system by Barnstead

provided the ultrapure water (18 MΩ cm) for all the aqueous solutions prepared.

Evaporation cycle experiments

The drying-and-wetting cycle experiments, simulating primordial coastal tide and terrestrial laguna effects, were implemented in the SIPF solution (500 mM NaCl and 40 mM $CuCl_2$ in ultrapure water) with different starting concentrations (80, 40 and 20 mM) of the leucine and methionine enantiomers dissolved independently. The same solutions were also prepared with glycine or L- or D-histidine as catalysts, at 1/8 (10, 5 and 2.5 mM) of these reactant concentrations. Because of the low solubility of leucine and methionine, concentrated HCl was needed in the proportion of 3–4 drops per 50 ml, only for preparation of the samples containing 80 mM amino acid, from which the 40 and 20 mM solutions could be obtained by dilution with SIPF solution. Such an amount of HCl addition could have little influence on the peptide condensation as the volatile HCl would escape from the reacting solutions when heated.

Subsequently 1 ml of the accomplished solution was transfused into a 2 ml HPLC vial and was evaporated within 24 h at 85°C in an oven, which provided a slow and favourable increase of solute concentration so as to enable peptide formation. After termination of each cycle, 1 ml of ultrapure water was refilled to the residue and the vial was subjected again to the next evaporation cycle. For the leucine experiment the samples were collected after 0, 1, 4 and 7 days and methionine samples were taken daily up to the seventh cycle. All the finished samples were kept dry in a freezer till the HPLC analysis afterwards.

HPLC analysis

The frozen dry residues were dissolved in 1 ml of ultrapure water in an ultrasonic bath, and then filtered through 0.22 μm hydrophilic PVDF syringe filters (Carl Roth GmbH, Germany).

The qualitative and quantitative analysis of the samples was achieved by reversed-phase ion-pairing HPLC on an Agilent 1100 series system with diode array detector, by comparing retention times, UV/Vis spectra and response factors with the standard reference substances in a series of concentrations. For analyses of both leucine and methionine dipeptides the same mobile phases were applied: solvent A consisted of 50 mM KH_2PO_4 and 7.2 mM n-$C_6H_{13}SO_3Na$ as ion-pairing reagent in ultrapure water, adjusted to pH 2.3 with concentrated H_3PO_4 and filtered through 0.2 μm hydrophilic polypropylene membrane filters (GH Plypro, Pall Gelman Laboratory, USA); solvent B was pure acetonitrile at super-gradient grade.

Two micro litre of the samples were injected into an Agilent Hypersil ODS column (5 μm, 2.1 × 200 mm), equipped with a 20 mm precolumn of the same material and a precolumn filter. The following gradient conditions were applied: for leucine system, 0 min 5% B, 0.5 min 7.5% B, 5 min 30% B, 10 min 30% B, 13 min 5% B, flow rate 0.35 ml/min, stop time 17 min, column temperature 40°C; for the methionine system, 0 min 3% B, 10 min 15% B, 13 min 18% B, 15 min 18% B, 17 min 3% B, flow rate 0.35 ml/min, stop time 20 min, column temperature 30°C. For both systems, detection was operated at 200, 4 nm bandwidth with a reference wavelength at 550, 100 nm bandwidth.

Results and discussion

In this study, intensive investigations only determined the formation of L–L and D–D dipeptides that appeared at the same retention time in chromatograms because of the achiral HPLC column employed. Longer peptides or heteropeptides probably produced were not taken into account.

Statistically averaged percentage yields with standard deviations were calculated from five independent parallel repetitions of each experimental item, and are listed in Tables 1, 2, 3, 4 for leucine experiments and Tables 5, 6, 7 for methionine experiments according to different catalytic conditions. The yield comparison factors '*R*' included in these tables are defined by the ratio of dipeptide yields obtained from L-form reactant divided by the data of their D-form analogues, thus an '*R*' value higher than 1 implies that the L-form is more reactive for peptide formation while a value below 1 shows a superiority of the D-form.

Dileucine formation

General

The percentage yields of dileucine keep a steady increase along with reaction cycles as listed in Table 1, and the more dilute the starting concentration is, the higher is the percentage of the leucine that dimerises. However, in the absence of another amino acid as catalyst, dileucine yields are quite low, at only 0.0066% at most for 20 mM L-leucine even after seven-cycle accumulation. This is much lower than 2.10% in case of L–L-dialanine and 0.087% for L–L-divaline (Plankensteiner et al. 2005a, b, c) in the aliphatic side chain category under the same reaction conditions.

The reactivity of the formation of dileucine in the SIPF reaction is among the lowest investigated so far, only comparable with the data from tryptophan (0.0016% yield for L–L-ditryptophan starting with 20 mM L-tryptophan

Table 1 L–L/D–D-dileucine yields (%) with standard deviations and corresponding L/D stereoselectivity factors '*R*' with different starting concentrations

Start concentration	Reaction time		
	1 day	4 days	7 days
80 mM			
L–L	0.00041 ± 0.00029	0.0021 ± 0.0009	0.0040 ± 0.0016
R	1.30	1.06	1.18
D–D	0.00031 ± 0.00039	0.0019 ± 0.0009	0.0034 ± 0.0010
40 mM			
L–L	0.00045 ± 0.00068	0.0021 ± 0.0012	0.0045 ± 0.0016
R	0.78	0.78	0.96
D–D	0.00058 ± 0.00056	0.0027 ± 0.0011	0.0047 ± 0.0012
20 mM			
L–L	0.0010 ± 0.0021	0.0034 ± 0.0015	0.0066 ± 0.0007
R	0.92	1.13	1.01
D–D	0.0011 ± 0.0004	0.0030 ± 0.0012	0.0065 ± 0.0008

Table 2 L–L/D–D-dileucine yields (%) with standard deviations and corresponding L/D stereoselectivity factors '*R*' with different starting concentrations with glycine as a catalyst at 1/8 of each starting concentration

Start concentration	Reaction time		
	1 day	4 days	7 days
80 mM			
L–L	0.0055 ± 0.0017	0.039 ± 0.010	0.16 ± 0.02
R	0.86	0.90	1.15
D–D	0.0064 ± 0.0013	0.043 ± 0.009	0.14 ± 0.03
40 mM			
L–L	0.0041 ± 0.0015	0.029 ± 0.007	0.064 ± 0.016
R	1.03	1.07	1.16
D–D	0.0040 ± 0.0015	0.026 ± 0.006	0.056 ± 0.008
20 mM			
L–L	0.0028 ± 0.0026	0.020 ± 0.005	0.036 ± 0.009
R	0.93	1.21	1.22
D–D	0.0030 ± 0.0014	0.016 ± 0.004	0.029 ± 0.008

Table 3 L–L/D–D-dileucine yields (%) with standard deviations and corresponding L/D stereoselectivity factors '*R*' with different starting concentrations with L-histidine as a catalyst at 1/8 of each starting concentration

Start concentration	Reaction time		
	1 day	4 days	7 days
80 mM			
L–L	0.0029 ± 0.0013	0.026 ± 0.006	0.085 ± 0.022
R	1.07	0.94	1.05
D–D	0.0027 ± 0.0014	0.027 ± 0.008	0.080 ± 0.019
40 mM			
L–L	0.0028 ± 0.0008	0.022 ± 0.003	0.054 ± 0.010
R	1.16	1.03	1.04
D–D	0.0024 ± 0.0007	0.021 ± 0.006	0.052 ± 0.026
20 mM			
L–L	0.0024 ± 0.0011	0.025 ± 0.005	0.070 ± 0.016
R	1.09	0.97	1.16
D–D	0.0021 ± 0.0008	0.026 ± 0.005	0.060 ± 0.004

after seven cycles) that is also moderately soluble in water (Plankensteiner et al. 2005a, b, c). These low yields are probably the result of the large and hydrophobic side chain of leucine, which leads to the low solubility in water and high steric hindrance against the condensation of leucine molecules with each other. With such a low solubility, it was not easy to dissolve the leucine in the residue back into solution again when the vials were refilled with water even though leucine could be perfectly dissolved in water when assisted by HCl in the first step of sample preparation. This idea also gives an appropriate explanation for the decreasing reactivity in the SIPF reaction proceeding from alanine, to valine and leucine with more and more voluminous alkyl side chains.

Catalytic effect of glycine and L- and D-histidine

With glycine, L- or D-histidine as a catalyst, the formation of dileucine is significantly boosted by 5- to 40-fold for both leucine enantiomers at different starting concentrations as shown in Fig. 1 and Tables 2, 3, 4. In the 80 mM system, glycine is more efficient than L- and D-histidine in catalysing the formation of dileucine since the yields with glycine are twice as high as the ones with histidine after seven cycles. On the contrary, in the 20 mM case this trend is totally reversed as L- and D-histidine contribute to the doubled dileucine yields compared to the reaction with glycine after seven cycles. Interestingly, for the in-between 40 mM series, the catalytic effects of glycine, L- and D-histidine are quite comparative despite of a little preponderance of glycine.

With regard to the possibility of different efficiencies of L- and D-histidine as catalysts (see Tables 3, 4), the D-form seems to perform better in catalyzing the reactions starting with 80 mM of leucine and also in heightening the dileucine yields after one evaporation cycle for every initial concentration, while both enantiomers have no clear catalytic difference in other cases. Furthermore, the two histidine enantiomers are appreciably less powerful than glycine in higher starting leucine concentrations (80 and 40 mM) whereas in 20 mM experiments this advantage of glycine is reversed.

In addition, the higher is the starting concentration of leucine, the higher are the dileucine yields enabled by these three amino acid catalysts, which is just the opposite of the circumstances without catalyst. Such a phenomenon could probably be ascribed to the poor de facto yield due to the low solubility of leucine as well as other negative factors stated above. Without mutual catalysis, the low solubility and reactivity of leucine leads to a low dependence on starting concentrations, which means that the de facto yields do not change proportionately with starting concentrations of leucine.

Table 4 L–L/D–D-dileucine yields (%) with standard deviations and corresponding L/D stereoselectivity factors '*R*' with different starting concentrations with D-histidine as a catalyst at 1/8 of each starting concentration

Start concentration	Reaction time		
	1 day	4 days	7 days
80 mM			
L–L	0.0040 ± 0.0013	0.0359 ± 0.0021	0.105 ± 0.003
R	1.32	0.996	1.02
D–D	0.0031 ± 0.0017	0.0361 ± 0.0053	0.104 ± 0.011
40 mM			
L–L	0.0038 ± 0.0014	0.024 ± 0.004	0.051 ± 0.004
R	1.08	0.97	1.01
D–D	0.0036 ± 0.0020	0.025 ± 0.006	0.050 ± 0.007
20 mM			
L–L	0.0044 ± 0.0019	0.0301 ± 0.0025	0.062 ± 0.003
R	1.25	1.01	0.97
D–D	0.0035 ± 0.0011	0.0298 ± 0.0061	0.064 ± 0.011

Enantioselectivity

Although not so obvious as in the results from alanine or valine (Fitz et al. 2008; Plankensteiner et al. 2005a, b, c), the preference for the formation of L–L-dileucine in the absence of a mutual catalyst is still quite apparent for most reaction systems except in the case of 40 mM leucine. However, such 'defects' in the 40 mM case without catalyst may be remedied by glycine and L-histidine added as catalysts, while in other samples catalysed by glycine and L- and D-histidine as indicated in Fig. 1, no general preference for one chiral form of leucine can be found, although the L–L-dileucine yields are slightly higher in the majority of the experiments.

Nevertheless, the exciting preference for L–L-dileucine formation can be detected in almost all the results after seven reaction cycles and is catalysed by all three amino acids as well, which implies the possibility of more significant stereoselectivity of leucine after enough evaporation cycles in the SIPF reaction.

Dimethionine formation

The detailed behaviour of methionine in the SIPF reaction and the catalytic effects of glycine and L-histidine have

Table 5 L–L/D–D-dimethionine yields (%) with standard deviations and corresponding L/D stereoselectivity factors '*R*' with different starting concentrations

Start concentration	Reaction time						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
80 mM							
L–L	1.30 ± 0.18	2.49 ± 0.25	2.79 ± 0.13	2.75 ± 0.13	2.09 ± 0.07	1.63 ± 0.08	1.09 ± 0.09
R	0.92	1.19	1.13	1.17	1.09	1.08	1.06
D–D	1.41 ± 0.05	2.07 ± 0.42	2.47 ± 0.09	2.35 ± 0.18	1.91 ± 0.06	1.50 ± 0.05	1.02 ± 0.04
40 mM							
L–L	0.29 ± 0.01	0.71 ± 0.03	0.80 ± 0.04	0.68 ± 0.04	0.46 ± 0.04	0.26 ± 0.01	0.10 ± 0.01
R	0.90	0.99	1.07	1.03	1.13	1.29	1.48
D–D	0.32 ± 0.03	0.72 ± 0.07	0.75 ± 0.03	0.66 ± 0.09	0.40 ± 0.03	0.21 ± 0.03	0.067 ± 0.012
20 mM							
L–L	0.014 ± 0.006	0.031 ± 0.011	0.042 ± 0.006	0.035 ± 0.008	0.025 ± 0.007	0.027 ± 0.005	0.0072 ± 0.0026
R	0.54	0.96	0.74	0.76	0.92	3.16	1.54
D–D	0.026 ± 0.009	0.032 ± 0.019	0.057 ± 0.019	0.047 ± 0.021	0.027 ± 0.001	0.0083 ± 0.0011	0.0047 ± 0.0034

Table 6 L–L/D–D-dimethionine yields (%) with standard deviations and corresponding L/D stereoselectivity factors '*R*' with different starting concentrations with L-histidine as a catalyst at 1/8 of each starting concentration

Start concentration	Reaction time						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
80 mM							
L–L	1.09 ± 0.03	1.68 ± 0.09	2.09 ± 0.07	2.56 ± 0.08	2.52 ± 0.04	1.92 ± 0.08	1.47 ± 0.03
R	0.85	0.87	0.93	1.02	1.07	1.07	1.02
D–D	1.29 ± 0.13	1.93 ± 0.16	2.24 ± 0.08	2.51 ± 0.12	2.34 ± 0.06	1.81 ± 0.16	1.44 ± 0.096
40 mM							
L–L	0.48 ± 0.05	1.01 ± 0.10	0.92 ± 0.05	0.62 ± 0.14	0.35 ± 0.12	0.15 ± 0.04	0.055 ± 0.021
R	0.65	0.84	0.81	0.87	0.86	0.80	0.69
D–D	0.75 ± 0.04	1.21 ± 0.04	1.14 ± 0.09	0.71 ± 0.08	0.41 ± 0.07	0.19 ± 0.06	0.079 ± 0.037
20 mM							
L–L	0.038 ± 0.013	0.13 ± 0.02	0.17 ± 0.02	0.10 ± 0.01	0.044 ± 0.004	0.022 ± 0.006	0.0076 ± 0.0055
R	0.42	0.68	0.83	0.93	1.02	2.27	1.35
D–D	0.091 ± 0.012	0.19 ± 0.03	0.20 ± 0.02	0.11 ± 0.02	0.043 ± 0.020	0.097 ± 0.011	0.0056 ± 0.0054

Table 7 L–L/D–D-dileucine yields (%) with standard deviations and corresponding L/D stereoselectivity factors '*R*' with different starting concentrations with D-histidine as a catalyst at 1/8 of each starting concentration

Start concentration	Reaction time						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
80 mM							
L–L	1.06 ± 0.06	1.70 ± 0.12	2.10 ± 0.09	2.28 ± 0.19	2.27 ± 0.34	2.19 ± 0.65	1.53 ± 0.46
R	0.86	0.91	0.92	0.93	0.94	1.01	1.01
D–D	1.23 ± 0.15	1.87 ± 0.14	2.29 ± 0.09	2.45 ± 0.16	2.43 ± 0.17	2.18 ± 0.49	1.51 ± 0.41
40 mM							
L–L	0.45 ± 0.11	0.92 ± 0.20	0.96 ± 0.17	0.67 ± 0.14	0.36 ± 0.09	0.15 ± 0.06	0.052 ± 0.029
R	0.69	0.82	0.92	0.91	0.96	0.89	0.91
D–D	0.65 ± 0.15	1.12 ± 0.20	1.05 ± 0.13	0.74 ± 0.09	0.38 ± 0.09	0.17 ± 0.06	0.057 ± 0.024
20 mM							
L–L	0.050 ± 0.023	0.11 ± 0.08	0.16 ± 0.08	0.10 ± 0.05	0.057 ± 0.016	0.018 ± 0.009	0.011 ± 0.005
R	0.51	0.56	0.73	0.96	1.13	1.19	1.18
D–D	0.099 ± 0.036	0.19 ± 0.06	0.22 ± 0.03	0.11 ± 0.01	0.050 ± 0.019	0.015 ± 0.014	0.009 ± 0.008

been investigated previously (Li et al. 2008), and hence the present study on dimethionine formation focuses on the roles of L- and D-histidine, respectively.

All in all, the L- and D-form of histidine perform similarly as catalysts, especially for the data within the first four cycles in the 80 mM case and the results after four or more cycles with 40 and 20 mM starting concentrations, where dimethionine yields catalysed by histidine are also the nearest to the ones without catalyst in Tables 5, 6, 7. That means, under such reaction conditions, histidine seems to have the weakest effect on dimethionine formation in the SIPF reaction; consequently, the difference between the two histidine enantiomers as catalysts being the smallest goes without saying.

No overwhelming difference was discovered, concerning their effects on the stereoselectivity. Nevertheless, in the 20 mM samples where histidine has the strongest effect on elevating the yields of dimethionine, L-histidine contributes more to the preference for L-met-L-met after six and seven cycles according to the '*R*' values of 2.27 and 1.35 in Table 6 compared with 1.19 and 1.18 in Table 7. A similar preference for L–L-dimethionine also occurs in the 80 mM samples after four cycles or more (see the last four '*R*' values in the 80 mM rows of Tables 6, 7), but in most of the cases when either L- or D-histidine is present as a catalyst, D-methionine provides higher dipeptide yields than its L-form.

One point to be mentioned is that the yields of dimethionine start to decline after the summits after three or

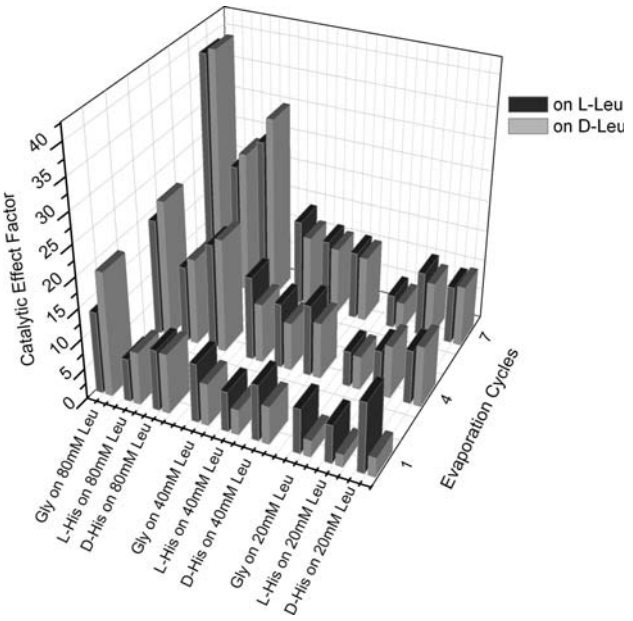

Fig. 1 Catalytic effect factors of glycine, L- and D-histidine on the formation of L–L- and D–D-dileucine starting with 80, 40 and 20 mM of L- and D-leucine

four cycles. Such a phenomenon could probably be pinned on the hydrolysis without the protection of mineral surfaces (Lambert 2008), and the possibility of further or side reactions leading to longer or other peptides and byproducts should not be neglected either.

Conclusion

Unlike other aliphatic analogues such as alanine and valine in the SIPF reaction, leucine has a relatively low reactivity among the amino acids investigated thus far, and shows the slight preference for its L-enantiomer during the formation of dileucine only in quite high or low starting concentrations. As catalysts, glycine, L- and D-histidine can remarkably enhance the dileucine yields but only function positively for dilute concentrations of methionine. Both L- and D-form of histidine have similar catalytic effects on peptide formation of leucine and methionine, respectively, in spite of the fact that they can differently affect the stereoselectivity in certain of reaction conditions. The experimental results presented in this paper add to existing research on aliphatic amino acids in the SIPF reaction, which have quite clear implications for the origin of biohomochirality, and also offer more information on the catalytic behaviour of L- and D-histidine.

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