Chiral Amplification of Oligopeptides in the Polymerization of α-Amino Acid *N*-Carboxyanhydrides in Water

by **Thomas Hitz** and **Pier Luigi Luisi***

Institut für Polymere, ETH-Zentrum, Universitätstrasse 6, CH-8092 Zürich
(fax: +41-1-6321073; e-mail: luisi@mat.ethz.ch)

Dedicated to Professor *Jack D. Dunitz* on the occasion of his 80th birthday

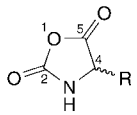
This article is concerned with the chiral amplification of oligopeptides formed in the polymerization of chiral, nonracemic mixtures of the *N*-carboxyanhydride (NCA) of Leu and Glu in aqueous solution. Labeling (deuteration) of one enantiomer and reversed-phase and normal-phase high-performance liquid chromatography mass spectrometry (RP- and NP-HPLC/MS, respectively) were used to determine the product distribution, both with respect to oligopeptide chain length and stereoisomer distribution. Starting the polymerization with an enantiomeric excess (ee) of 20% of the L-enantiomer (L-amino acid/D-amino acid 6:4) leads to an ee of 73% at the level of the homochiral enantiomeric $(Leu)_5$, and of 71% at the level of the homochiral enantiomeric $(Glu)_7$. For the Leu system and in the presence of a solid support (quartz), the ee reached values of up to 100%. We argue that such amplification processes could be relevant for the chemical evolution towards single-handedness.

Introduction. – Theories concerning the abiotic evolution [1] of single-handedness in nature usually deal with two major questions. One concerns the breaking of symmetry (mechanism(s) that produced an initial enantiomeric excess (ee)) [2–4]; the other is concerned with chiral amplification (mechanism(s) that have permitted the increase of this initial ee up to single-handedness) [5–7]. The present paper focuses on the second aspect.

In earlier work [8–10], we investigated the extend of homochiral oligopeptide sequences formed in the polymerization of racemic α-amino acid *N*-carboxyanhydrides[1]) of Leu, Ile, Trp, and Glu (*cf.* general formula **A**) in buffered aqueous solution. The stereoisomer distribution of the oligopeptides with labeled amino acids was analyzed by high-performance liquid chromatography mass spectrometry (HPLC-MS) [8][11]. It was found that the experimentally determined mole fractions of the homochiral oligopeptide sequences were larger than the ones calculated for a theoretical, random polymerization process (binomial distribution). The excess factor varied between 2.5 and 8.3, depending on the oligopeptide chain length and the type of amino acid. Kinetic data for the NCA-Ala [12] and NCA-Val [13] polymerization in aqueous solution are consistent with the preferential formation of oligopeptides with homochiral sequences. This phenomenon was also observed in the polymerization of activated racemic amino acid amphiphiles at the air/water interface [14].

---

[1]) Cyclization products of α-amino acids corresponding to 4-substituted 1,3-oxazolidin-2,5-diones.



A

R = α-amino acid side chain

In the Leu system, we also showed that the presence of a solid support (quartz) strongly increases the mole fraction of homochiral oligopeptides by selectively adsorbing these more-regular oligopeptides out of a mixture of homo- and heterochiral oligomers [10].

Here, we present our results concerning the polymerization of mixtures of *chiral*, nonracemic NCA-Leu and NCA-Glu in aqueous solution with respect to the formation of oligopeptides with homochiral sequence and their chiral amplification. The influence of quartz as a solid support on the stereoisomer distribution was also investigated.

Experimental. – *Materials. N,N*-Carbonyldi[1*H*-imidazole][2]) (CDI), 1*H*-imidazole (> 99.5%), D-Glu (≥ 99%) and aq. trifluoroacetic acid (TFA; ≥ 99%, HPLC grade) were purchased from *Fluka*. D-Leu (> 99%) was purchased from *Bachem*. The deuterated L-amino acids (98%), (D_5)L-Glu and (D_{10})L-Leu, were purchased from *Cambridge Isotope Laboratories* (Andover, MA). MeCN (HPLC grade) was purchased from *Macherey-Nagel*. Both (+)- and (−)-quartz crystals of *Japanese-Industrial-Standard* (*JPS*) quality were a gift of *Toyo Communication Equipment Co*. Deionized H_2O was deionized again before use by a *Milli-Q RG* system from *Millipore*.

Polymerization with Leu. Procedure I: Concentrated Leu solns. of the deuterated L-enantiomer (40 mM) and of the nondeuterated D-enantiomer (40 mM) in 0.4M 1H-imidazole buffer (pH 9) were incubated separately with a 2.5-fold molar excess of CDI at 0° for 2 min. The resulting NCA-(D_{10})L-Leu and NCA-D-Leu solutions were mixed in a molar 6:4 ratio. This *chiral*, nonracemic NCA-Leu soln. was mixed 1:7 (*v*/*v*) with 0.4M 1*H*-imidazole buffer (pH 9) in a 1.5 ml *Eppendorf* tube and incubated for 24 h with shaking at r.t. Then, an equal amount of MeCN was added to bring all oligopeptides into soln. These homogeneous solns. were analyzed by RP-HPLC/MS.

Procedure II: A concentrated Leu soln. of the chiral nonracemic Leu mixture ((D_{10})L-Leu/D-Leu 6:4) (40 mM) in 0.4M 1*H*-imidazole buffer (pH 9) was incubated directly with a 2.5-fold molar excess of CDI at 0° for 2 min. Then, this chiral, nonracemic NCA-Leu soln. was mixed with 0.4M 1*H*-imidazole buffer (pH 9) in a 1:7 ratio (*v*/*v*) in a 1.5 ml *Eppendorf* tube and incubated for 24 h with shaking at r.t. Then, an equal amount of MeCN was added to bring all oligopeptides into soln. These homogeneous solns. were analyzed by RP-HPLC/MS. No difference in the product distribution concerning the oligopeptide chain length and the stereoisomer distribution was observed for *Procedures I* and *II*.

Polymerization with Leu in the Presence of (±)-Quartz. The chiral, nonracemic NCA-Leu mixture (NCA-(D_{10})L-Leu/NCA-D-Leu 6:4, 40 mM) in 0.4M 1*H*-imidazole buffer (pH 7) was prepared according to *Procedure II*. The meshed (+)- and (−)-quartz crystals were cleaned and activated with 2M HCl, then washed with H_2O (*Millipore*) to neutral pH. The crystals were washed with acetone and dried for 12 h at 120°. Then, 28.5 µl of the 40 mM chiral nonracemic NCA-Leu soln. in 0.4M 1*H*-imidazole buffer (pH 7) were mixed with 150 µl 0.4M imidazole buffer (pH 7), containing 20 mg of quartz powder (10 mg each (+) and (−)-quartz) in a 1.5 ml *Eppendorf* tube, and incubated with shaking for 24 h at r.t. After centrifugation at 8000 rpm, the supernatant was set aside. Then, 150 µl buffer was added to the remaining quartz powder and, again, 28.5 µl of a soln. of 40 mM chiral, nonracemic NCA-Leu in 0.4M 1*H*-imidazole buffer (pH 7). After 24 h incubation at r.t., the supernatant was set aside after centrifugation at 8000 rpm and analyzed by RP-HPLC/MS. The remaining products were desorbed and solubilized by adding 2 × 500 µl of 100 mM $Na_4P_2O_7$ soln. (pH 10.4, desorption) and 2 × 500 µl of MeCN/H_2O 2:1 (*v*/*v*) (solubilization). The pyrophosphate and MeCN/H_2O solns. were combined and analyzed by RP-HPLC/MS.

---

[2]) Systematic name: 1-[(1*H*-imidazol-1-yl)carbonyl]-1*H*-imidazole.

Polymerization with Glu. Procedure III: Concentrated Glu solns. of the deuterated L-enantiomer (80 mM) and the nondeuterated D-enantiomer (80 mM) in 0.4M 1*H*-imidazole buffer (pH 9) were incubated separately with a 2.5-fold molar excess of CDI at 0° for 2 min. The resulting NCA-(D_5)L-Glu and NCA-D-Glu solns. were mixed in a 6:4 molar ratio in a 1.5 ml *Eppendorf* tube and incubated for 24 h with shaking at r.t. Then, an equal amount of MeCN was added to bring all oligopeptides into soln. These homogeneous solns. were analyzed by NP-HPLC/MS.

Procedure IV: A chiral, nonracemic Glu mixture ((D_5)L-Glu/D-Glu 6:4; 80 mM), in 0.4M 1*H*-imidazole buffer (pH 9) was incubated directly with a 2.5-fold molar excess of CDI at 0° for 2 min in a 1.5 ml *Eppendorf* tube for 24 h with shaking at r.t. Then, an equal amount of MeCN was added to bring all oligopeptides into soln. These homogeneous solns. were analyzed by NP-HPLC/MS. No difference in the product distribution concerning the oligopeptide chain length and the stereoisomer distribution was observed for *Procedures III* and *IV*.

Analysis. Products were analyzed by HPLC (*Thermo Finnigan P4000*) with diode-array (*UV 6000 LP*) and ion-trap MS detection (*Thermo Finnigan LCQ-Deca*). To separate the Leu polymerization products, a C_{18}-reversed-phase column (*EC 250/4*, *Nucleosil 100-5*, *Macherey-Nagel*, Oensingen, Switzerland) was used, with a 1 ml/min flow rate at r.t.; solvent A (0.1% aq. TFA) and B (99.9% MeCN, 0.1% aq. TFA); 2 min isocratic flow at 20% B followed by a gradient to 90% B at a rate of 1.42% B/min. Oligomer separation of the Glu polymerization products was achieved on a normal-phase (NP) column (*TSK Gel Amide-80*, 25 × 0.46 cm, *Tosoh Biosep*, Stuttgart), 0.7 ml/min flow rate at r.t., solvent C (MeCN/H_2O/aq. TFA 97:3:0.1 (*v*/*v*)) and D (MeCN/H_2O/aq. TFA 55:45:0.1 (*v*/*v*); 2 min isocratic flow at 100% C followed by a gradient to 100% D at a rate of 2.63% D/min. The different stereoisomeric subgroups of $(Leu)_n$ and $(Glu)_n$ were monitored by ion-trap MS using selected-ion monitoring (SIM) of the single-charged positive ions. The relative yield (normalized amount) of $(Leu)_n$ and $(Glu)_n$ oligopeptides was deduced from the SIM-peak areas integrated over time by means of calibration curves for the electro-spray ionization (ESI) sensitivity and mass-detection efficiency of oligopeptides with different chain lengths. ESI-Sensitivity and mass-detection efficiency for stereoisomers of the same chain length are in first approximation similar [8–10][14]. For the quantification of the stereoisomer distribution, the SIM peaks of the different stereoisomeric subgroups were integrated over time. The sum of all SIM-peak areas of a particular oligomer with *n* residues corresponds to 1.0. The mole fractions χ were calculated according to the expression exemplified for the (3,0) tripeptide [14]: $\chi\ (3,0) = \chi\ (D_3) =$ SIM-area (3,0)/SIM-areas $[(3,0)+(2,1)+(1,2)+(0,3)]$. The excess of homochiral oligopeptides was calculated according to the expression exemplified for the (3,0) tripeptide [14]: excess $[(3,0)+(0,3)] =$ excess $[(D_3)+(L_3)] = [\chi\ (3,0) + \chi(0,3)]_{exp}/[\chi\ (3,0) + \chi\ (0,3)]_{calc} = [\chi\ (D_3) + \chi\ (L_3)]_{exp}/[\chi\ (D_3) + \chi\ (L_3)]_{calc}$. Typical MS settings are given in [8–10].

Results. – 1. *Chiral Amplification of Oligopeptides in Aqueous Solution*. We will now address the question, whether and to what extent there is chiral amplification in the carbonyldiimidazole (CDI)-induced polymerization [15][16] of chiral, nonracemic mixtures of NCA-Leu and NCA-Glu in buffered aqueous solution. We will compare the experimentally determined stereoisomer distribution of the oligopeptides with a theoretical, random process (binomial distribution of the formed oligopeptides).

1.1. *NCA-Leu System*. RP-HPLC/MS Analysis was used to determine the product distribution with respect to the oligopeptide chain length *n* and with respect to the stereoisomer distribution of the formed oligopeptides. *Perdeuterated* L-Leu (=(D_{10})L-Leu) allows us to distinguish between different stereoisomeric subgroups ($[$D-Leu$]_p[(D_{10})$L-Leu$]_q$) by selected-ion monitoring (SIM) [17]. This means that the ($[$D-Leu$]_p[(D_{10})$L-Leu$)]_q$) subgroups having the same oligopeptide chain length $n = p + q$ will differ by at least 10 and maximally $10 \times n$ mass units [8–10]. The mole fraction χ of the oligopeptide (p,q), where *p* corresponds to the number of unlabeled D-peptide units and *q* to the number of deuterated L-peptide units, was obtained by integrating for a constant *n* the SIM areas of all the stereoisomeric subgroups and by dividing the SIM area of the oligopeptide (p,q) by the sum of all these SIM areas (see *Exper.*).

In the polymerization of NCA-Leu with an ee of 20% of the L-enantiomer, a mixture of oligopeptides with up to five units is formed ($n = 2-5$). The relative yield

(normalized amount) of the oligopeptides relative to the amount of dipeptide (100%) is given in *Fig. 1,a*. It can be seen that the yield decreases weakly from the dipeptides to the tetrapeptides, and strongly from the tetrapeptides to the pentapeptides.

The experimentally determined mole fractions of the stereoisomer groups of the $(Leu)_n$ oligopeptides formed after 24 h are given in *Fig. 1,b* and are compared with the values calculated assuming a theoretical, random distribution. It can be seen that the isolated tetra- and pentapeptides with homochiral sequence have a mole fraction significantly higher than predicted.

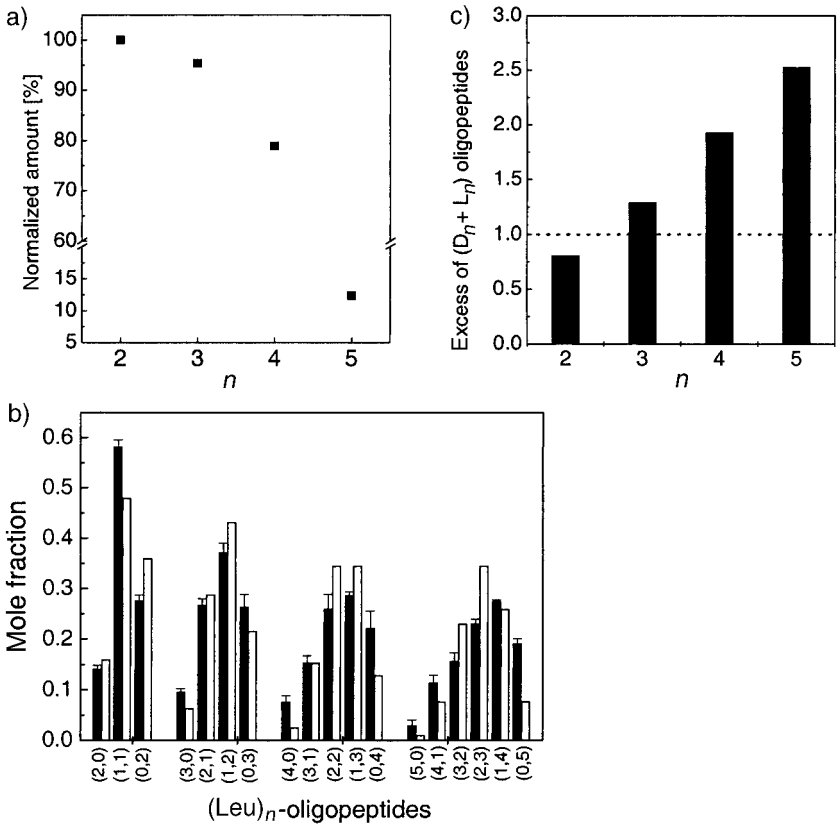


Fig. 1. *RP-HPLC/MS Analysis of the oligopeptides formed in the polymerization of NCA-Leu with an initial ee of 20% of the deuterated* L*-enantiomer* ((D_{10})L-Leu/D-Leu 6:4). *a*) *Relative yield of the different oligopeptides with respect to the amount of dipeptide* (=100%), *as calculated from the SIM areas of the various oligopeptides. b*) *Mole fractions of* (D_p,L_q)*-stereoisomer groups of the* $(Leu)_n$ *oligomers* ($n=2-5$). For each oligomer length, the SIM chromatograms for all the (D_p,L_q)-stereoisomer subgroup masses were integrated over time, and the sum of all SIM-peak areas of a particular *n*-mer corresponds to 1.0. The mole fractions were calculated as described under *Experimental*. The black columns correspond to the experimentally determined mean values of three measurements with standard deviations as error bars. The white columns correspond to the theoretical mole fractions, assuming random polymerization. *c*) *Excess of oligopeptides with homochiral sequence* (D_n and L_n) for $n=2-5$ (*cf. Exper.*). The horizontal dotted line corresponds to a random polymerization process (excess equal to unity).

The excess of oligopeptides with homochiral sequence was calculated by normalizing the experimentally determined mole fraction of the oligopeptides with homochiral sequence to that calculated for a random process [14]. An excess greater than unity indicates a departure of the system from a random polymerization process. This analysis shows a maximum excess-factor of 2.5 for the homochiral pentapeptides. This result is in agreement with previous studies on racemic NCA-Leu polymerizations in buffered aqueous solution [9].

The ee values of the enantiomeric oligopeptides with homochiral sequences are given in *Table 1*, calculated according to $ee = [(L_n - D_n)/(L_n + D_n)]_{exp} \times 100$, n being the oligopeptide chain length. The determined ee values increase from 32 to 73% for the dipeptides to the pentapeptides, respectively. The strong increase of the L_n/D_n ratio is illustrated in *Fig. 2*, which shows the stereoisomer distribution of the pentapeptides (ee = 73%) in the form of a SIM chromatogram. It can be seen that the SIM intensity of the single-charged homochiral $[(D_{10})$L-Leu$]_5$ ion is much higher than the SIM intensity of the single-charged enantiomeric homochiral [D-Leu]$_5$ ion. It is assumed that this strong chiral amplification is *not* due to isotopic effects since the polymerization of racemic NCA-D-Leu/NCA-(D_{10})L-Leu (mass difference per peptide unit: $\Delta m/z = 10$) gave, in a first approximation, a symmetric stereoisomer distribution, *i.e.*, equal mole fractions of the homochiral enantiomeric oligopeptide sequences [9]. Furthermore, studies on the polymerization of activated amino-acid amphiphiles at the air/water interface with strongly deuterated enantiomers ($\Delta m/z = 35$) per peptide unit did not show a substantial isotope effect [14][18]. It is, therefore, concluded that the polymerization of NCA-Leu with an initial ee of 20% is *stereoselective*, showing a strong chiral amplification, for statistical and/or chemical reasons – except for isotope effects (see discussion).

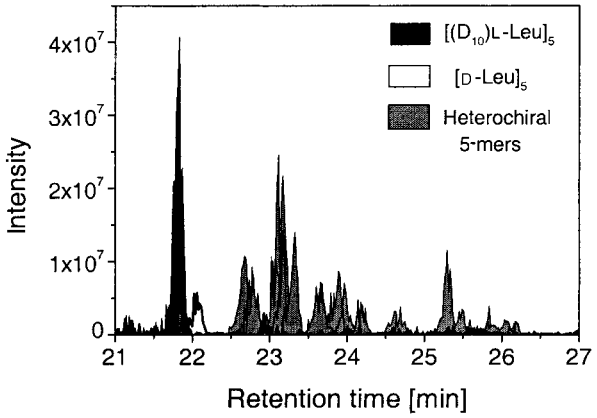


Fig. 2. *SIM-Chromatogram* (intensities of the various peptide ions *vs.* RP-HPLC retention time) *of (Leu)$_5$ after polymerization of NCA-Leu with an initial ee of 20% of the* L*-enantiomer.* The corresponding m/z values (pos. ESI, $z = 1$) of the stereoisomer groups are 634.8 for $[(D_{10})$L-Leu$]_5$, 584.43 for [D-Leu]$_5$, 624.74 for [D-Leu]$[(D_{10})$L-Leu$]_4$, 614.67 for [D-Leu]$_2[$L-(D_{10})Leu$]_3$, 604.59 for [D-Leu]$_3[(D_{10})$L-Leu$]_2$, and 594.51 for [D-Leu]$_4[(D_{10})$L-Leu].

Table 1. *Experimentally Determined ee Values of the Enantiomeric Oligopeptides with Homochiral Sequence.* The polymerization was performed with an ee of 20% of the L-enantiomer (L-amino acid/D-amino acid 6 : 4)[a].

Oligopeptides	Leu, ee [%]	Glu, ee [%]
Dipeptide	32	26
Tripeptide	46	40
Tetrapeptide	48	54
Pentapeptide	73	54
Hexapeptide	[b])	59
Heptapeptide	[b])	71

[a]) Calculated from the experimentally determined mole fractions of the homochiral enantiomeric oligopeptides (see *Fig. 1* and *Fig. 3*) according to the following equation: $ee = [(L_n - D_n)/(L_n + D_n)]_{exp} \times 100$, n being the oligopeptide chain length. [b]) Not detectable.

1.2. *NCA-Glu System*. In the case of Glu, NP-HPLC/MS was used to determine the product distribution with respect to the oligopeptide chain length n and with respect to the stereoisomer distribution of the formed oligopeptides. This technique permits the analysis of hydrophilic oligopeptides that show no retention on conventional RP columns [19]. Pentadeuterated L-Glu (=(D_5)L-Glu) allows one to distinguish between different stereoisomeric subgroups ($[D\text{-Glu}]_p[(D_5)L\text{-Glu}]_q$) by selected-ion monitoring (SIM) [17]. The mole fraction of the oligopeptide (p,q) was calculated as explained for the Leu system (see *Experimental*).

In the polymerization of NCA-Glu with an ee of 20% of the L-enantiomer, a mixture of oligopeptides containing up to eight units is formed ($n = 2-8$) after 24 h. The relative yield of the oligopeptides relative to the amount of dipeptide (100%) is given in *Fig. 3*,*a*. It can be seen that the yield decreases exponentially from the tetrapeptide ($n = 4$) on. Similar trends, as deduced from the UV signal at 220 nm, were observed in the polymerization of NCA-L-Glu at different concentrations[3]).

The mole fractions of the stereoisomer groups with homochiral and heterochiral sequence as a function of the oligopeptide chain length are given in *Fig. 3*,*b* and are compared with the values assuming a theoretical, random distribution. The excess of oligopeptides with homochiral sequence is shown in *Fig. 3*,*c*. From the dipeptides to the hexapeptides, no significant excess of oligopeptides with homochiral sequence is observed: the excess factor varies between 0.75 for $n = 3$ and 1.40 for $n = 6$. Instead, the hepta- and octapeptides show significantly higher mole fractions of oligopeptides with homochiral sequences, the excess factor reaching a value of 5.0 at the octapeptide level. From *Fig. 3*,*b*, it can also be seen that no homochiral (8,0)-oligopeptides and no heterochiral (7,1)-oligopeptides could be detected.

The ee values of the enantiomeric oligopeptides with homochiral sequences are given in *Table 1*. The ee values increase from 26 to 71% from the dipeptides to the heptapeptides. The ee value for the octapeptide was not included in *Table 1* because no

[3]) Yields in % of initial L-Glu for the NCA-L-Glu polymerization at different concentrations (0.4M imidazole buffer, pH 7.20, 24 h at r.t.). At 20 mM: 26.3% ($n = 1$), 36.9% ($n = 2$), 19.3% ($n = 3$), 11.1% ($n = 4$), 4.4% ($n = 5$), 1.4% ($n = 6$), 0.6% ($n = 7$); 40 mM: 20.7% ($n = 1$), 23.8% ($n = 2$), 18.9% ($n = 3$), 15.4% ($n = 4$), 11.5% ($n = 5$), 6.0% ($n = 6$), 2.6% ($n = 7$), 1.0% ($n = 8$), 0.31% ($n = 9$), 0.13% ($n = 10$); 80 mM: 17.8% ($n = 1$), 20.5% ($n = 2$), 17.0% ($n = 3$), 12.8% ($n = 4$), 12.3% ($n = 5$), 8.7% ($n = 6$), 5.6% ($n = 7$), 3.1% ($n = 8$), 1.4% ($n = 9$), 0.5% ($n = 10$), 0.22% ($n = 11$).

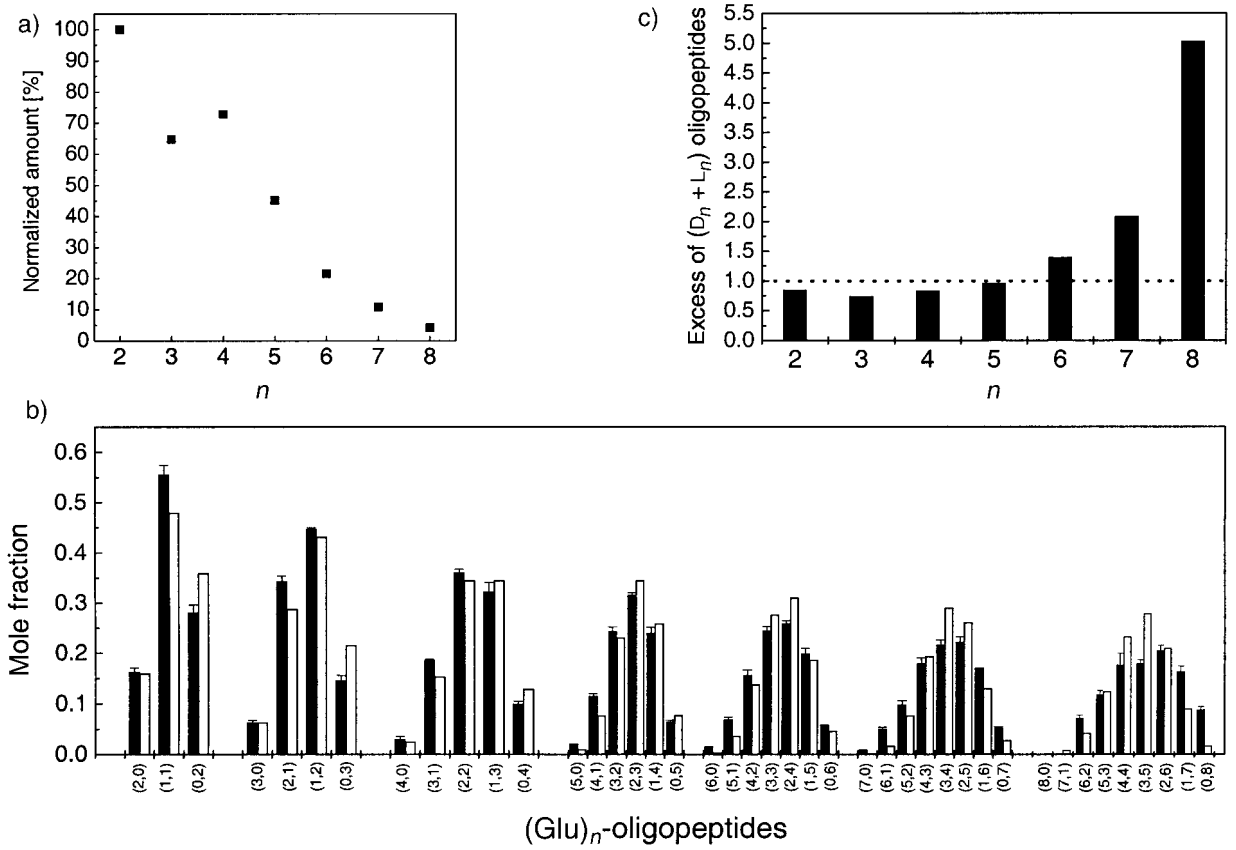


Fig. 3. *NP-HPLC/MS Analysis of the oligopeptides formed in the polymerization of NCA-Glu* ((D$_5$)L-Glu/D-Glu 6 : 4). *a*) *Relative yield of the different oligopeptides with respect to the amount of dipeptide* (=100%), *as calculated from the SIM areas of the various oligopeptides. b*) *Mole fractions of* (D_p,L_q)*-stereoisomer groups of the* $(Glu)_n$ *oligomers* ($n=2-8$). For each oligomer length, the SIM chromatograms for all the (D_p,L_q)-stereoisomer subgroup masses were integrated over time, and the sum of all SIM-peak areas of a particular *n*-mer corresponds to 1.0. The black columns correspond to the experimentally determined mean values of three measurements with standard deviations as error bars. The white columns correspond to the theoretical mole fractions, assuming a random polymerization process. *c*) *Excess of oligopeptides with homochiral sequence* (D_n and L_n) *for* n = *2–8*. The horizontal dotted line corresponds to a random polymerization process (excess equal to unity).

homochiral (8,0)-oligopeptide (D_8-octamer) could be detected. Again, it was observed that the racemic NCA-D-Glu/NCA-(D_5)L-Glu polymerization (mass difference per peptide unit $\Delta m/z = 5$) gave, in a first approximation, a symmetric stereoisomer distribution[4]). Therefore, it is assumed that no significant isotope effect contributed to the observed chiral amplification.

1.3. *NCA-Leu/Quartz System*. In this system, RP-HPLC/MS was used to determine the stereoisomer distribution of oligopeptides formed in the presence of a solid support. (±)-Quartz was selected since it can be considered a prebiotic mineral [20][21]. (±)-Quartz (20 mg) was exposed twice to an aqueous NCA-Leu solution containing the L-enantiomer (20% ee). The supernatant was analyzed by RP-HPLC/MS. To determine the product distribution of the whole system, the quartz surface was exposed to a 100 mM $Na_4P_2O_7$ solution to remove the remaining oligopeptides [22]. This pyrophosphate fraction was also analyzed by RP-HPLC/MS.

In this system, a mixture of oligopeptides with $n = 2-5$ are formed[5]). It was shown that the normalized amount in the supernatant decreases exponentially from the dipeptides to the pentapeptides. The relative yield of the oligopeptides ($n = 3-5$) detected in the pyrophosphate fraction showed a high amount of pentapeptide, indicating a stronger adsorption onto the quartz surface with increasing n [10][20].

The stereoisomer distribution of the oligopeptides detected in the supernatant and in the pyrophosphate fraction is given in *Fig. 4,a*. It can be seen that, for $n = 3-5$, oligopeptides with homochiral sequences have a mole fraction significantly higher than the theoretical values. The excess of oligopeptides with homochiral sequence, presented in *Fig. 4,b*, shows a higher excess factor for the oligopeptides detected in the pyrophosphate fraction than in the supernatant. This indicates that the quartz surface selectively takes up the oligopeptides with homochiral sequence, as it has been shown in racemic NCA-Leu polymerization in the presence of (±)-quartz [10].

It is obvious that the initial ee of 20% of the L-enantiomer is strongly amplified in the oligopeptides. The only detectable tetra- and pentapeptides with homochiral sequence in the supernatant and in the pyrophosphate fraction are [(D_{10})L-Leu]$_4$ and [(D_{10})L-Leu]$_5$. This observation corresponds to an ee of 100% of the whole system (supernatant plus pyrophosphate fraction) for the enantiomeric tetra- and pentapeptides with homochiral sequence, which indicates that the presence of (±)-quartz increases the chiral amplification process observed for the Leu system in buffered aqueous solution (see ee-values for $n = 4, 5$ in *Table 1*).

Discussion. – We have shown that the polymerization of a chiral, nonracemic mixture of NCA-Leu or NCA-Glu solutions shows a strong chiral amplification of the enantiomeric oligopeptides with homochiral sequence, which are formed in excess with

---

[4]) Experimentally determined mole fractions of the Glu-containing oligopeptides with homochiral sequence $[(p,0)+(0,q)] = [D_n + L_n]$ for the dipeptides to the octapeptides after the racemic NCA-D-Glu/NCA-(D_5)-L-Glu polymerization (0.4M imidazole buffer, pH 7.0, 24 h at r.t., mean values of three measurements): $[(0.24 \pm 0.05) + (0.23 \pm 0.02)]$ ($n = 2$); $[(0.082 \pm 0.006) + (0.075 \pm 0.004)]$ ($n = 3$); $[(0.045 \pm 0.009) + (0.045 \pm 0.008)]$ ($n = 4$); $[(0.045 \pm 0.017) + (0.031 \pm 0.009)]$ ($n = 5$); $[(0.0278 \pm 0.0027) + (0.0226 \pm 0.0002)]$ ($n = 6$); $[(0.0185 \pm 0.0021) + (0.0183 \pm 0.0037)]$ ($n = 7$); $[(0.0119 \pm 0.0038) + (0.0154 \pm 0.0011)]$ ($n = 8$).

[5]) Relative yields for the supernatant: 100% ($n = 2$), 50.0% ($n = 3$), 27.3% ($n = 4$), 6.8% ($n = 5$); and for the pyrophosphate fraction: 100% ($n = 3$), 64.3% ($n = 4$), 68.2% ($n = 5$).

respect to a theoretical, random process. The Leu system shows a stronger stereoselectivity than the Glu system, which means that, in the former, the excess of oligopeptides with homochiral sequence is higher at shorter peptide length (*Fig. 1* and *Fig. 3*). In this context, it is of interest that hydrophobic Leu-monomers may interact more strongly with each other in aqueous solution than the negatively charged Glu-monomers [23].

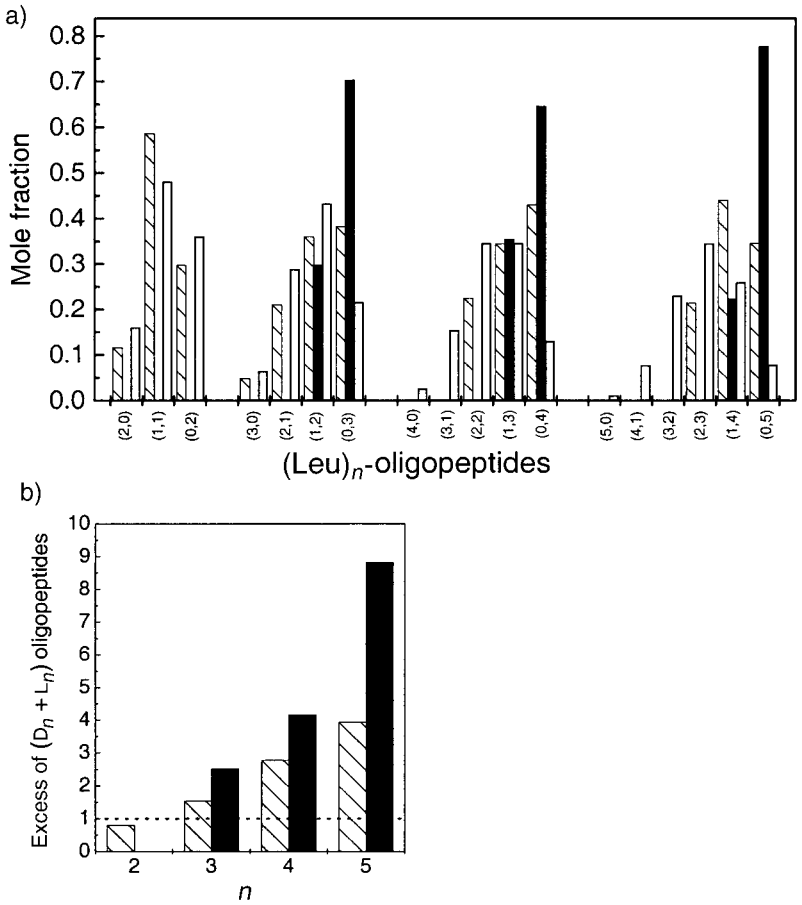


Fig. 4. *RP-HPLC/MS Analysis of the oligopeptides obtained in the polymerization of NCA-Leu* ((D$_{10}$)L-Leu/D-Leu 6:4) *in the presence of (±)-quartz*. a) *Mole fractions of* (D$_p$,L$_q$)*-stereoisomer groups of the (Leu)*$_n$ *oligomers* ($n=2-5$) *determined for the oligopeptides present in the supernatant* (hatched columns) *and in the* $Na_4P_2O_7$ *fraction* (black columns). The white columns correspond to a theoretical, random polymerization process. *b*) *The corresponding excess of oligopeptides with homochiral sequence* (D$_n$ and L$_n$) for $n=2-5$. The horizontal dotted line corresponds to random polymerization (excess equal to unity).

The time dependence of the mole fractions of the oligopeptides with homo- and heterochiral sequence suggests higher order in the polymerization process (data not shown). It has been demonstrated that hydrophobic natural α-amino acids can form structured clusters (enantiomorphous domains) at the air/solution interface [24][25].

Recent ESI-MS investigations on Ser in aqueous solution suggest that natural α-amino acids may form structured clusters even in bulk aqueous solution [26][27] (for a critical review, see [28]). Furthermore, it is known that higher order occurs also later in the polymerization process by means of penultimate amino acid residue effects in the growing peptide chain [29], chain aggregation [29], monomer-chain association equilibria [29], and, of course, secondary-structure formation [30–33]. Especially in the Glu system, which showed no significant excess of homochiral sequence for $n = 2$–6, secondary structures may be the most-important factor for the observed sharp increase of the excess factor from the heptapeptide to the octapeptide (*Fig. 3*, *c*). It is reasonable to assume that several of the effects mentioned above contribute to the observed overrepresentation of oligopeptides with homochiral sequence in this study, and it is of interest to quantitatively analyze the influence of each of these effects on the whole stereoselective process.

In the following discussion, we will analyze the strong chiral amplification observed for the Leu and the Glu systems. Let us first consider a theoretical, random system and start with a molar ratio of 6:4 (L-amino acid/D-amino acid). In this random polymerization process, the ratio of the mole fractions of oligopeptides with homochiral sequence (L_n/D_n) increases according to $(L_n/D_n)^n$, with n being the oligopeptide chain length. The theoretical ee values are given in *Table 2*. It can be seen that this trivial binomial propagation brings about a strong increase of the initial ee. At the octapeptide level, it reaches already 92%. At the same time, however, the mole fraction of the oligopeptides with homochiral sequence becomes smaller and smaller due to the high number of heterochiral diastereoisomers simultaneously formed. Therefore, the probability of obtaining oligopeptides with homochiral sequence becomes negligible with increasing chain length n [34][35]. This is illustrated in *Table 3*. The mole fraction of the L-amino acid oligomer drops from 0.36 in the dimer to 0.0168 in the octamer. Thus, the chiral amplification in a theoretical, random process is irrelevant because the mole fractions of the oligopeptides with homochiral sequence become infinitely small in longer oligomers.

Table 2. *Theoretical* ee *Values of the Enantiomeric Oligopeptides with Homochiral Sequence for Random Polymerization* (L-amino acid/D-amino acid 6:4)

Oligopeptides	Calc. ee [%][a])
Dipeptide	38
Tripeptide	54
Tetrapeptide	67
Pentapeptide	77
Hexapeptide	84
Heptapeptide	89
Octapeptide	92

[a]) $ee = [(L_n - D_n)/(L_n + D_n)]_{calc} \times 100$,

In the present study, we observed an increase of the ee values with increasing oligopeptide chain length, as statistically expected (*Table 1*). In both cases (Leu and Glu), the ee values remain below the theoretical values (*Table 1* and *Table 2*), which shows that both polymerization processes do not proceed randomly. Nevertheless, we

think that the chiral amplification is mostly due to the binomial propagation mentioned above. Since the mole fraction of oligopeptides with homochiral sequence does not become negligibly small in the longer oligopeptide chains (*e.g.*, Leu system), 'statistical chiral amplification' seems to be a true phenomenon.

Table 3. *Theoretical Mole Fractions* χ *of* (D_p,L_q)-*Stereoisomer Groups of the Oligopeptides Formed in a Random Polymerization* (L-amino acid/D-amino acid 6 : 4)

(D_p,L_q)	χ	(D_p,L_q)	χ
(2,0)	0.16	(6,0)	0.00410
(1,1)	0.48	(5,1)	0.03690
(0,2)	0.36	(4,2)	0.13824
		(3,3)	0.27648
(3,0)	0.064	(2,4)	0.31104
(2,1)	0.288	(1,5)	0.18666
(1,2)	0.432	(0,6)	0.04666
(0,3)	0.216		
		(7,0)	0.00163
(4,0)	0.0256	(6,1)	0.01720
(3,1)	0.1536	(5,2)	0.07740
(2,2)	0.3456	(4,3)	0.19350
(1,3)	0.3456	(3,4)	0.29030
(0,4)	0.1296	(2,5)	0.26127
		(1,6)	0.13060
(5,0)	0.01024	(0,7)	0.02799
(4,1)	0.0768		
(3,2)	0.2304	(8,0)	0.00066
(2,3)	0.3456	(7,1)	0.00786
(1,4)	0.2592	(6,2)	0.04129
(0,5)	0.07776	(5,3)	0.12386
		(4,4)	0.23222
		(3,5)	0.27869
		(2,6)	0.20902
		(1,7)	0.08958
		(0,8)	0.01680

Finally, let us have a closer look at the NCA-Leu quartz system. The most-interesting finding is probably the strong tendency of the oligopeptides, detected in the pyrophosphate fraction, towards single-handedness (*Fig. 4*,*a*, black columns). The (±)-quartz most likely increases the mole fraction of the oligopeptides with homochiral sequence by selectively adsorbing these more-regular oligopeptides formed in aqueous solution [10]. Thus, such a simple heterogeneous system may be very useful in accumulating homochiral oligopeptides of single-handedness formed in the polymerization of chiral, nonracemic mixtures of activated amino acids according to the following three observations:

i) Preferential formation of oligopeptides with homochiral sequence [8][9][14][18]
ii) Strong, probably statistical amplification of the initial ee in the oligopeptides
iii) Selective adsorption of the longer oligopeptides with homochiral sequence on different minerals [10].

We thank *Peter Walde* and *Matthias Voser* for help with literature search and for stimulating discussions.

REFERENCES

[1] W. A. Bonner, *Orig. Life Evol. Biosph*. **1992**, *21*, 407.
[2] B. L. Feringa, R. A. van Delden, *Angew. Chem.*, *Int. Ed*. **1999**, *38*, 3419.
[3] M. Avalos, R. Babiano, P. Cintas, J. L. Jiménez, J. C. Palacios, *Chem. Commun.* **2000**, *11*, 887.
[4] M. Bolli, R. Micura, A. Eschenmoser, *Chem. Biol*. **1997**, *4*, 309.
[5] F. C. Frank, *Biochim. Biophys. Acta* **1953**, *11*, 459.
[6] J. Podlech, *Cell. Mol. Life Sci*. **2001**, *58*, 44.
[7] I. Sato, H. Urabe, S. Ishiguro, T. Shibata, K. Soai, *Angew. Chem.*, *Int. Ed*. **2003**, *42*, 315.
[8] M. Blocher, T. Hitz, P. L. Luisi, *Helv. Chim. Acta* **2001**, *84*, 842.
[9] T. Hitz, M. Blocher, P. Walde, P. L. Luisi, *Macromolecules* **2001**, *34*, 2443.
[10] T. Hitz, P. L. Luisi, *Helv. Chim. Acta* **2002**, *85*, 3975.
[11] L. Addadi, E. Gati, M. Lahav, L. Leiserowitz, *Isr. J. Chem*. **1977**, *15*, 116.
[12] P. D. Bartlett, R. H. Jones, *J. Am. Chem. Soc*. **1957**, *79*, 2153.
[13] A. Commeyras, H. Collet, L. Boiteau, J. Taillades, O. Vandenabeele-Trambouze, H. Cottet, J. P. Biron, R. Plasson, L. Mion, O. Lagrille, H. Martin, F. Selsis, M. Dobrijevic, *Polym. Int*. **2002**, *51*, 661.
[14] I. Weissbuch, G. Bolbach, H. Zepik, E. Shavit, M. Tang, J. Frey, T. R. Jensen, K. Kjaer, L. Leiserowitz, M. Lahav, *J. Am. Chem. Soc*. **2002**, *124*, 9093.
[15] K. W. Ehler, L. E. Orgel, *Biochim. Biophys. Acta* **1976**, *434*, 233.
[16] A. R. Hill Jr., L. E. Orgel, *Orig. Life Evol. Biosph*. **1996**, *26*, 539.
[17] M. S. Lee, E. H. Kerns, *Mass Spectrom. Rev*. **1999**, *18*, 187.
[18] H. Zepik, E. Shavit, M. Tang, T. R. Jensen, K. Kjaer, G. Bolbach, L. Leiserowitz, I. Weissbuch, M. Lahav, *Science* **2002**, *295*, 1266.
[19] T. Yoshida, *Anal. Chem*. **1997**, *69*, 3038.
[20] V. A. Basiuk, T. Y. Gromovoy, E. G. Khil'chevskaya, *Orig. Life Evol. Biosph*. **1995**, *25*, 375.
[21] A. G. Cairns-Smith, 'Seven Clues to the Origin of Life', Cambridge Univ. Press, Cambridge, UK, 1985.
[22] R. K. Iler, 'The Chemistry of Silica: Solubility, Polymerization, Colloid and Surface Properties and Biochemistry of Silica', John Wiley & Sons, New York, 1979.
[23] B. Palecz, *J. Am. Chem. Soc*. **2002**, *124*, 6003.
[24] I. Weissbuch, L. Addadi, Z. Berkovitch-Yellin, E. Gati, M. Lahav, L. Leiserowitz, *Nature* **1984**, *310*, 161.
[25] I. Weissbuch, L. Leiserowitz, M. Lahav, *J. Am. Chem. Soc.* **1991**, *113*, 8941.
[26] R. G. Cooks, D. Zhang, K. J. Koch, F. C. Gozzo, M. N. Eberlin, *Anal. Chem*. **2001**, *73*, 3646.
[27] K. J. Koch, F. C. Gozzo, S. C. Nanita, Z. Takats, M. N. Eberlin, R. G. Cooks, *Angew. Chem.*, *Int. Ed*. **2002**, *41*, 1721.
[28] C. A. Schalley, P. Weis, *Int. J. Mass Spectrom*. **2002**, *221*, 9.
[29] H. R. Kricheldorf, in 'Models of Biopolymers by Ring-Opening Polymerization', Ed. S. Penczek, CRC Press, Boca Raton, FL, 1990.
[30] R. D. Lundberg, P. Doty, *J. Am. Chem. Soc*. **1957**, *79*, 3961.
[31] H. Weingarten, *J. Am. Chem. Soc*. **1957**, *80*, 352.
[32] G. Wald, *Ann. N. Y. Acad. Sci*. **1957**, *69*, 352.
[33] E. R. Blout, P. Doty, J. T. Yang, *J. Am. Chem. Soc*. **1957**, *79*, 749.
[34] W. A. Bonner, *Orig. Life Evol. Biosph*. **1999**, *29*, 615.
[35] J. S. Siegel, *Chirality* **1998**, *10*, 24.

Received February 10, 2003