Stereoselectivity Aspects in the Condensation of Racemic NCA−Amino Acids in the Presence and Absence of Liposomes

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ABSTRACT: In previous work dealing with the liposome-assisted polycondensation of NCA (*N*-carboxyanhydride)−amino acids, it has been shown that racemic NCA−Trp condensation affords chains with preferential homochiral sequences: the homochiral blocks become increasingly over-represented with increasing oligomer length if compared with the statistically (Bernoullian) distributions. The present paper deals with three questions related to this stereoselective NCA−Trp condensation. The first question concerns the kinetic mechanism that is responsible for the over-representation of homochiral sequences in the presence as well as in the absence of liposomes. To this aim, we have investigated whether and to what extent the selection of the entering chiral monomer unit is governed by the chirality of the penultimate residue. This analysis allows one to suggest that the polycondensation corresponds to a second- or higher-order Markov process. The second question considered in this paper is whether the preferential formation of homochiral sequences, originally found in NCA−Trp−amino acid condensations, is observed also for amino acids other than Trp as well as for mixtures of different amino acids. To clarify this point, we have analyzed by liquid chromatography mass spectrometry (LC-MS) the condensation products of racemic NCA−D-Leu/perdeuterated NCA−L-Leu and racemic NCA−D-Ile/perdeuterated NCA−L-Ile in an aqueous solution without liposomes. This analysis permits us to show that the preferential homochiral growth occurs also with Leu and Ile. By LC-MS analysis we can show that under the same conditions also mixtures of equal amounts of the L- and D-forms of different amino acids, such as Trp/Leu, Leu/Ile, and Trp/Leu/Ile, exhibit similar behavior. The third question considered in this paper is related to the effect of liposomes on the NCA−Trp condensation, addressing the question of whether the chemical structure of the lipid in the liposomes and consequently their physical state have an influence on the stereoselectivity of the NCA−Trp condensation. To this aim, experiments are carried out at different temperatures in the presence of DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine) liposomes, below as well as above the liquid-analogue/crystalline-analogue transition temperature. The answer to the third question is that basically the same results are obtained independently of the physical state of the lipid.

Introduction

In previous papers[1−3] we have introduced the notion of liposome-assisted polycondensation of amino acids and peptides and described some basic experiments. The main goal of these investigations is to assess whether the membrane of liposomes could operate a selection of the polypeptide chain structure—a question that is also relevant in the field of the origin of life. Operationally, this work is largely based on the use of liquid chromatography mass spectrometry (LC-MS), which permits the identification and quantification of long oligomers, which occur in very low yields. We have shown that the POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine) membrane facilitates the condensation of hydrophobic tryptophan-containing peptides. This gives rise to a relatively long Trp_n sequence with n up to 29.[1] Furthermore, we have observed that the presence of positive charges in the membrane increases the yield of oligopeptides containing negatively charged Glu, and it increases also the yield of oligo-Trp.[2] This shows that electrostatic interactions between the charged liposomes and amino acid side chains may play an important role in the formation of specific peptide structures. In the condensation of racemic[4] mixtures of *N*-carboxyanhydride−D-tryptophan (NCA−D-Trp) and deuterated *N*-carboxyanhydride−L-tryptophan (NCA−L-Trp), product analysis in the presence and absence of POPC liposomes has shown the preferential formation of homochiral sequences.[3] The frequency of the homochiral decades of Trp (L-Trp_{10} and D-Trp_{10}) is for example 40 times larger than the value expected on the basis of a Bernoullian random polymerization. This effect is not dependent on the presence of liposomes.[3] In first approximation the presence of POPC liposomes simply facilitates the synthesis of longer chains that are generally water-insoluble. The data obtained for racemic NCA−Trp condensation are consistent with kinetic and NMR data published for other racemic amino acid condensations.[5−9] However, to the best of our knowledge, a direct stereosequence analysis of the resulting stereoisomeric subgroups up to homochiral decamers has not been carried out before.

There are several questions concerning the preferential homochiral growth of the Trp oligomers.[3] We believe that they may be relevant to the origin of homochiral biopolymers in nature, and the present paper aims at clarifying some of these questions. One first question is relative to the kinetic mechanism of the monomer insertion during the NCA condensation in an aqueous liposome suspension: Is there a stereoselective control of the growing oligopeptide chain by the chain end? A second question is whether the preferential homochiral chain growth is restricted to the case of tryptophan or whether it is also observed with other amino acids. In this context it is also of great interest to see whether the polycondensation with mixtures of equal amounts of the L- and D-forms of different amino acids also leads

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to a preferential formation of homochiral co-oligopeptides. A third question addressed in this paper is whether the chemical structure and the physical state of the liposome membrane affects the formation of oligo-Trp and whether in the case of racemic NCA condensation the physical state has an influence on the stereoisomer distribution.

Experimental Section

Materials. For all condensation experiments POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine) was purchased from Avanti Polar Lipids (Alabastar, AL). L-DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine), D-DPPC (2,3-dipalmitoyl-*sn*-glycero-1-phosphorylcholin), D-Trp (>99%), *N*,*N*-carbonyldiimidazole (CDI), imidazole (>99.5%), chloroform ($CHCl_3$) (≥99.8%), and trifluoroacetic acid (TFA) (≥99%, HPLC grade) were purchased from Fluka (Buchs, Switzerland). D-Leu (>99%) and D-Ile (>99%) were purchased from Bachem (Dubendorf, Switzerland). The deuterated L-amino acids (98%)—L-Trp(d5), L-Leu(d10), and L-Ile(d10)—were purchased from the Cambridge Isotope Laboratories (Andover, MA). Acetonitrile (ACN, HPLC grade) was purchased from Macherey-Nagel (Oensingen, Switzerland). The deionized water was again deionized by a Milli-Q RG from Millipore (Volketswil, Switzerland).

Liposome Preparation. Liposomes were prepared from POPC or from L- or D-DPPC. First the lipids were dissolved in $CHCl_3$ in a round-bottom flask. After evaporation of the chloroform by rotary evaporation (40 °C, reduced pressure), the lipid film was dried overnight at high vacuum. The dried lipid film was then dispersed in buffer either at room temperature (POPC) or at about 40 °C (DPPC). This led to the formation of mainly multilamellar liposomes of a considerable heterogeneity in size.[10,11] To decrease the number of small liposomes,[12,13] the liposome suspension was frozen 10 times in liquid nitrogen (−195 °C) and thawed in a water bath at 40 °C. To decrease the size and the lamellarity, the liposome suspension was repetitively passed through two Nucleopore polycarbonate membranes from Sterico AG (Dietlikon, Switzerland) with mean pore diameters of 400 nm (10 times), 200 nm (10 times), and 100 nm (10 times) using the extruder (from Lipex Biomembranes Inc., Vancouver, Canada).[12] The POPC liposomes were stored at room temperature and the DPPC liposomes at 37 °C to prevent precipitation. For all experiments only freshly prepared liposomes were used.

CDI-Induced Amino Acid Condensation. In a typical single feeding experiment of racemic amino acids, a concentrated amino acid solution of the deuterated L-isomers (i.e., 40 mM) and of the nondeuterated D-isomers (i.e., 40 mM) in a 0.4 M imidazole/HCl buffer of pH 6.80 was incubated separately with a 2.5-fold molar excess of CDI at 0 °C for 2 min. The resulting activated amino acid solutions were then mixed at different volume ratios. These racemic NCA−amino acid solutions were mixed 1:7 (v/v) with 0.4 M imidazole/HCl buffer, pH 6.80, in a 1.5 mL eppendorf tube containing a magnetic stirrer. The resulting solutions were incubated for 12 h at different temperatures under stirring. In a double feeding experiment the same procedure was repeated. In all the experiments without liposomes, ACN was added to the reaction mixture to bring all oligomers into solution (ACN: oligomer solution 1:2 (v/v)). Dissolution of the condensation products was ascertained by optical density measurements from 400 to 600 nm (Cary, 1E, UV−vis spectrophotometer). If the optical density over this range was zero, it was assumed that all oligomers were dissolved and a homogeneous solution was formed. Afterward 30−70 μL of these oligomer solutions was injected onto the HPLC column. If the amino acid condensation was performed in the presence of liposomes, 30−70 μL of the reaction mixture was injected onto the HPLC column immediately after stirring had been stopped.

Liquid Chromatography Mass Spectrometry. Product analysis was achieved by HPLC (P4000, Finnigan Mat) connected to a diode array detector (UV 6000 LP, Finnigan Mat) and an ion trap mass spectrometry detector (LCQ-Deca, Finnigan Mat). To separate the condensation products, a C_{18} reversed-phase column (ET 250/4 Nucleosil 100-5, Macherey-Nagel, Oensingen, Switzerland) was used. The parameter settings for the HPLC were 1 mL/min flow rate at room temperature, using two buffer solvents A (0.1% TFA) and B (99.9% ACN, 0.1% TFA). Typically one started with a 2 min isocratic flow of 20% B and drove with a gradient of 1.84% B/min up to 90% B. At high percentages of ACN the POPC and the DPPC lipids precipitate on the column, and therefore the column was washed with 100% MeOH after measurements with these two lipids.

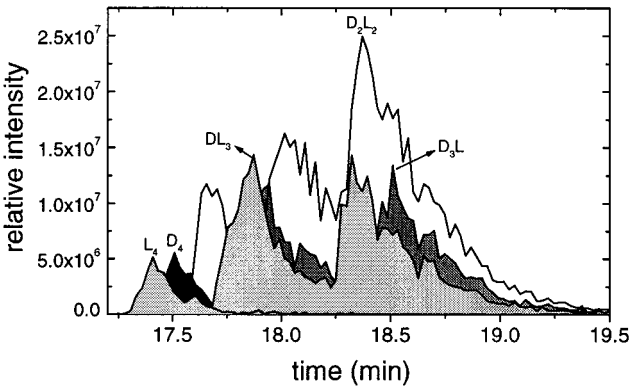

Figure 1. SIM chromatogram peaks for the stereoisomeric subgroups of oligo-D/L-Trp(d5) for $n = 4$.

In the case of co-condensation of nondeuterated D- and deuterated L-amino acids the quantification of the different stereoisomeric subgroups was done by ion trap mass spectrometry. Typical settings were 350 °C capillary temperature, 80 units sheath gas flow rate, 20 units auxiliary gas flow rate, 4.50 kV I-spray voltage, 3−39 V capillary voltage, and −60 to −25 V tube lens offset. Quantification was carried out by integrating the peaks of the SIM (selected ion monitoring) chromatogram peaks over time. Since the reversed-phase column is achiral, the enantiomeric pairs of each oligo-amino acid diastereomer have the same retention time. Therefore, the quantitative analysis is based on the internal standard (deuteration). An example is shown for the stereoisomeric subgroups of oligo-D/L-Trp(d5) for $n = 4$ in Figure 1.

To check whether oligo-amino acid stereoisomers of the same length have similar sensitivities in electrospray-ionization (ES-ionization), nonoverlapping peak areas in the UV chromatograms ($OD_{280\ nm}$: Trp-containing peptides; $OD_{215\ nm}$: peptides without Trp) were compared with the corresponding areas in the MS (mass spectrometry) SIM chromatograms. It was figured out that up of a length of four ($n = 4$) stereoisomers of the same length exhibit to a first approximation similar ionization sensitivities. The comparison was restricted on oligopeptides having the same length, because only stereoisomeric subgroups of the same length were compared directly in this study. The analysis of longer peptides was not possible, because of overlapping UV peaks of the different diastereomers. A detailed analysis in the case of Trp is given elsewhere.[3]

For stereoisomers of the same length it was generally found that the stereoisomeric subgroups consisting of nonhomochiral stereoisomers showed slightly higher ionization sensitivity than the stereoisomeric subgroups consisting of homochiral stereoisomers. But these differences were within the range of the error of several independent measurements. In the case of NCA condensations with mixtures of different amino acids it is assumed that differences in ionization sensitivities of constitution isomers of the same length do statistically not matter (see for example Figure 6C—D_6 and L_6).

Results and Discussion

On the Kinetic Mechanism. To investigate the kinetic properties of the stereoselective process in the presence of liposomes, we have started the polyconden-

sation with a racemic mixture of deuterated NCA−L-Trp (= NCA−L-Trp (d5)) and NCA−D-Trp in the presence of POPC liposomes. The use of a pentadeuterated tryptophan allows to distinguish between different stereoisomeric subgroups ($[\text{D-Trp}]_p[\text{L-Trp(d5)}]_q$) by LC-MS (selected ion monitoring method: SIM).[14,15] This means that the ($[\text{D-Trp}]_p[\text{L-Trp(d5)}]_q$) subgroups having the same length $n = p + q$ will be separated by at least 5 and maximally $5n$ Da. By taking aliquots of the reaction mixture, one can follow the time progress of the different stereoisomeric subgroups. A satisfactory analysis is possible until $n = 4$.

Results are shown in Figure 2. In a Markov process of first order (for a definition of zero-, first-, and second-order Markov process, see ref 16) one would expect no difference in the time progress of the homochiral and nonhomochiral oligomers. This is clearly not observed in the present case. It can be seen that the percentage of the homochiral oligomers ($[\text{D-Trp}]_n$ and $[\text{L-Trp(d5)}]_n$, $n = 2, 3, 4$) decreases with time whereas the percentage of the nonhomochiral oligomers remains stable or increases. This behavior can be explained by a Markov process of second or higher order, in which namely at least the penultimate amino acid residue in the growing oligopeptide chain influences the next elongation step. It can be seen that stereoisomeric subgroups with a higher probability of homochiral blocks (tacticity ≥ 2) on the growing chain end polymerize faster than stereoisomeric subgroups with a lower probability for homochiral blocks. D_2L_2 has for example a probability of homochiral blocks on the growing chain end of 33.3%, D_3L respectively DL_3 of 50%, and D_4 respectively L_4 of 100%. These kinetic properties are probably the reason for the over-representation of long homochiral Trp−oligopeptides observed in racemic NCA-D/L-Trp condensation in this aqueous liposomal system. If one assumes that the POPC bilayer has only the function of facilitating the synthesis of longer chains, then one can conclude that the same kinetic properties are also the reason for the over-representation of homochiral sequences in a liposome free aqueous system.

On the NCA−Amino Acid Condensation of Other Amino Acids Than Trp. We will now address the question of whether the over-representation of homochiral sequences can also be observed with residues other than tryptophan. For this reason, racemic NCA−Leu and racemic NCA−Ile condensations were performed. Since it has been shown previously that POPC liposome membranes have no influence on the oligomerization degree in the case of Leu and Ile, these experiments are performed in the absence of liposomes, whereby again the L-isomer is in both cases 10 times deuterated (d10). Note that Ile possesses an additional chiral center at the C_β atom (*S*-configuration), so that L-Ile and D-Ile are in fact diastereomers and not enantiomers.

The relative abundances of the D_pL_q stereoisomer groups of the oligo-Leu and oligo-Ile *n*-mers are shown in Figures 3 and 4. It can clearly be seen that also in theses two cases the homochiral oligomers become over-represented and that homochiral sequences become increasingly over-represented with increasing chain length. This indicates that homochiral oligopeptides are generated again preferentially. Note that the distribution of the Ile *n*-mers is not symmetric. In other words the amount of $(\text{L-Ile(d10)})_n$ is different from the amount of $(\text{D-Ile})_n$ ($n = 3, 4, 5$). This asymmetric product distribution probably arises from the asymmetric center at the C_β atom.

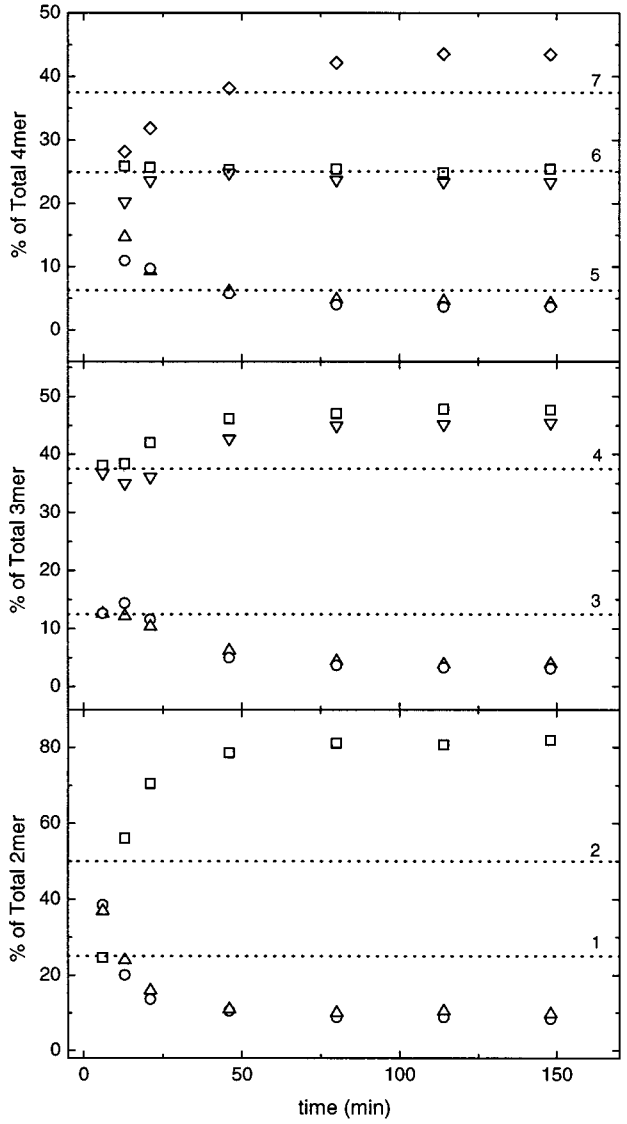

Figure 2. Racemic NCA condensation of NCA-D/L-Trp(d5). Time progress for the formation of homochiral (○: L_2, L_3, and L_4; △: D_2, D_3, and D_4) and non-homochiral (□: DL, D_2L, and D_3L; ▽: DL_2 and DL_3; ◇: D_2L_2) stereoisomers of Trp until $n = 4$ in the presence of a 100 nm extruded POPC liposome suspension. The dotted lines represent the statistical distributions for the corresponding stereoisomeric subgroups (1: L_2 or D_2; 2: DL; 3: L_3 or D_3; 4: DL_2 or D_2L; 5: L_4 or D_4; 6: DL_3 or D_3L; 7: D_2L_2). 417 μL of a freshly prepared 60 mM NCA−D-Trp/NCA−L-Trp(d5), 0.4 M imidazole/HCl, pH 6.80 solution was added to 1250 μL of a 100 nm extruded 100 mM POPC liposome suspension in 0.4 M imidazole/HCl buffer of pH 6.80. After 5 min 45 μL of the reaction mixture was injected for the first time onto the HPLC column and analyzed by an ion trap mass spectrometry detector. By taking 45 μL aliquots of the reaction mixture, one could follow the time progress of the different stereoisomeric subgroups. Points are only shown until 150 min but were collected until 300 min. From 150 min on there was no change in the intensity of the SIM peaks any more.

On the NCA−Amino Acid Condensation with Mixtures of Equal Amounts of the L- and D-Forms of Different Amino Acids. The aim of these investigations, as already mentioned in the Introduction, is to clarify whether the over-representation of homochiral sequences is obtained also in copolymer chains. For this reason we have carried out condensations by using

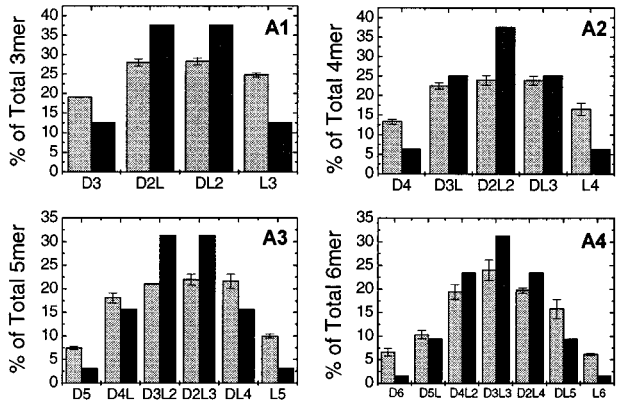

Figure 3. Relative abundances for D_pL_q stereoisomer groups of the oligo-Leu *n*-mers are shown for one racemic NCA−Leu feeding in absence of POPC liposomes (A1−A4). A freshly prepared solution of 40 mM racemic NCA−D-Leu/NCA−L-Leu-(d10) in 0.4 M imidazole/HCl, pH 6.80 was mixed 1:7 (v/v) with a 0.4 M imidazole/HCl buffer, pH 6.80 and incubated for at least 12 h on a shaker. For each oligomer length, the SIM chromatograms for all the D_pL_q stereoisomer subgroup masses were integrated. The total of all SIM-peak areas of a particular *n*-mer corresponds to 100%. The relative abundances of the D_pL_q stereoisomer subgroups are mean values of three measurements. Standard deviations are given as error bars. The black columns correspond to the theoretical distribution, assuming a statistical (Bernoullian) oligomerization.

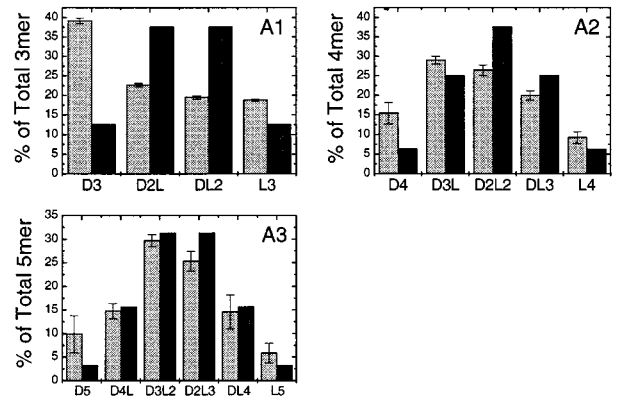

Figure 4. Relative abundances for D_pL_q stereoisomer groups of the oligo-Ile *n*-mers are shown for one racemic NCA−Ile feeding in absence of POPC liposomes (A1−A3). A freshly prepared solution of 40 mM racemic NCA−D-Ile/NCA−L-Ile-(d10) in 0.4 M imidazole/HCl, pH 6.80 was mixed 1:7 (v/v) with a 0.4 M imidazole/HCl buffer, pH 6.80 and incubated for at least 12 h on a shaker. For each oligomer length, the SIM chromatograms for all the D_pL_q stereoisomer subgroup masses were integrated. The total of all SIM-peak areas of a particular *n*-mer corresponds to 100%. The relative abundances of the D_pL_q stereoisomer subgroups are mean values of three measurements. Standard deviations are given as error bars. The black columns correspond to the theoretical distribution, assuming a statistical (Bernoullian) oligomerization.

mixtures of equal amounts of the L- and D-forms of different amino acids, namely with mixtures of Leu/Ile, Trp/Leu, Trp/Ile, and Trp/Leu/Ile. Again, the L-form of the amino acid is deuterated (L-Trp: 5 times; L-Leu and L-Ile: 10 times). All condensations have been performed at different molar ratios of the amino acids participating in the polymerization reaction. Results are shown in Figures 5 and 6 for the highest detectable oligomerization degrees. In all cases an obvious over-representation of homochiral sequences can be observed. It is important to notice that now the homochiral oligomers are formed by all possible amino acid combinations. This can be

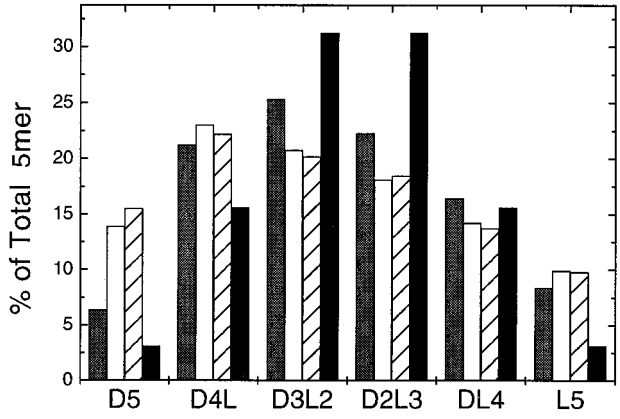

Figure 5. Relative abundances for D_pL_q stereoisomer groups of the oligo-Leu/Ile-5-mer are shown for NCA−Leu/Ile condensations of equal amounts of the L- and D-amino acids at different molar ratios of NCA−Ile:NCA−Leu in absence of POPC liposomes. Freshly prepared solutions of 40 mM NCA mixtures of equal amounts of the L- and D-forms of Leu and Ile in 0.4 M imidazole/HCl, pH 6.80 were mixed 1:7 (v/v) with a 0.4 M imidazole/HCl buffer, pH 6.80 and incubated for 12−15 h on a shaker (again the L-form was deuterated, see text). For each oligomer length, the SIM chromatograms for all the D_pL_q stereoisomer subgroup masses were integrated. The total of all SIM peak areas of the 5-mer corresponds to 100%. The gray columns correspond to a molar ratio of NCA−Ile:NCA−Leu of 1:1, the white ones to a ratio of 1.5:1, and the hatched columns to a ratio of 2:1. The black columns correspond to the theoretical distribution, assuming a statistical (Bernoullian) oligomerization.

shown by LC-MS (SIM). The homochiral sequences become also increasingly over-represented with increasing chain length (data not shown). Because of analytical reasons, the LC-MS-analysis shown in Figure 6 has to be restricted to the homochiral and nonhomochiral oligomers. A resolution into all different stereoisomeric subgroups is not possible. Nevertheless, these experiments clearly show on the level of a direct product analysis that a preferential homochiral growth occurs also when the growing chain consists of more than one type of amino acid.

If one compares Figure 6A,B with Figure 6C, a slight increase of the over-representation of homochiral sequences can be observed. This means that homochiral sequences with three different amino acids (Trp, Leu, and Ile) are over-represented with respect to the homochiral sequences that consist of two different amino acids (Trp,Leu and Trp,Ile). This opens the interesting question whether the increasing number of amino acids directly affects the increasing homochirality.

On the Influence of the Chemical Structure of the Lipid. One may conceive that the liposome-assisted polycondensation is going to be influenced by the physical state of the lipid bilayer, as this may affect the interaction with the growing tryptophyl chain. Above T_m (the main phase transition temperature), the bilayer is in a disordered liquid-analogue state, whereas below T_m it assumes an ordered, crystalline-analogue state.[17,18] POPC has a T_m of about − 3 °C,[19] and all the experiments carried out so far[1−3] in the presence of liposomes have been under conditions where the liposome bilayer is fluid (above T_m). In the case of POPC measurements below T_m are not possible, due to the freezing of water. As an alternative lipid, chiral L-(1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine) and D-DPPC (2,3-dipalmitoyl-*sn*-glycero-1-phosphorylcholine) have been chosen,

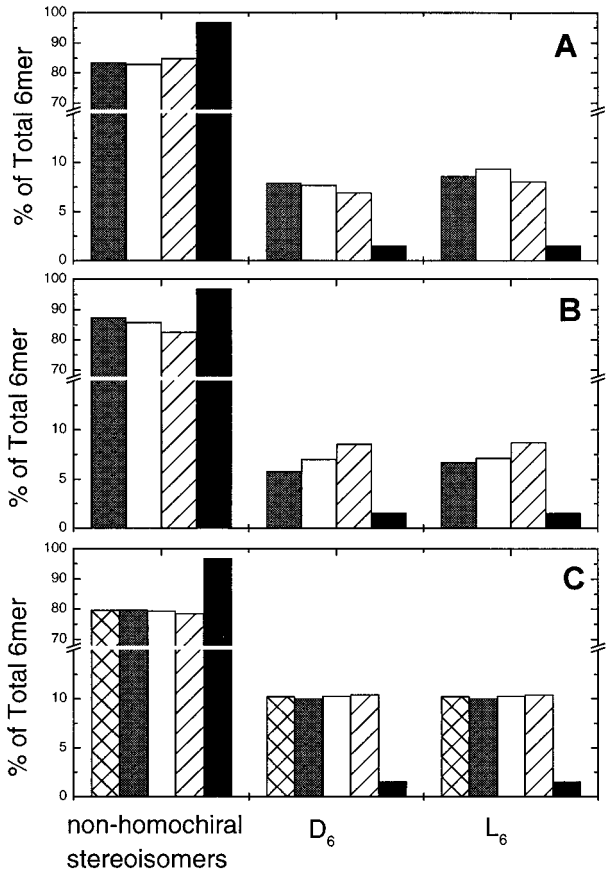

Figure 6. Relative abundances for the homochiral (D_6 and L_6) and the non-homochiral stereoisomer groups of the oligo-Trp/Leu (A), oligo-Trp/Ile (B), and oligo-Trp/Leu/Ile (C) 6-mers are shown for different molar ratios of the involved amino acids in the absence of POPC liposomes. The molar ratios varied for A (Trp:Leu) from 1:2 (white); 1:1 (gray) to 2:1 (hatched), for B (Trp:Ile) from 1:2 (hatched); 1:1 (white) to 2:1 (gray) and for C (Trp:Leu:Ile) from 1:1:1 (crosshatched); 2:1:2 (gray); 2:2:1 (white) to 1:2:2 (hatched). Freshly prepared solutions of 40 mM NCA mixtures of equal amounts of the L- and D-forms of the different amino acids in 0.4 M imidazole/HCl, pH 6.80, were mixed 1:7 (v/v) with a 0.4 M imidazole/ HCl buffer, pH 6.80 and incubated for 12 h at room temperature on a shaker. The same volumes of the mixtures were added again in a second feeding experiment and incubated for another 12 h. The quantification of the different stereoisomeric subgroups was performed as described above. The black columns correspond to the theoretical distribution, assuming a statistical (Bernoullian) oligomerization.

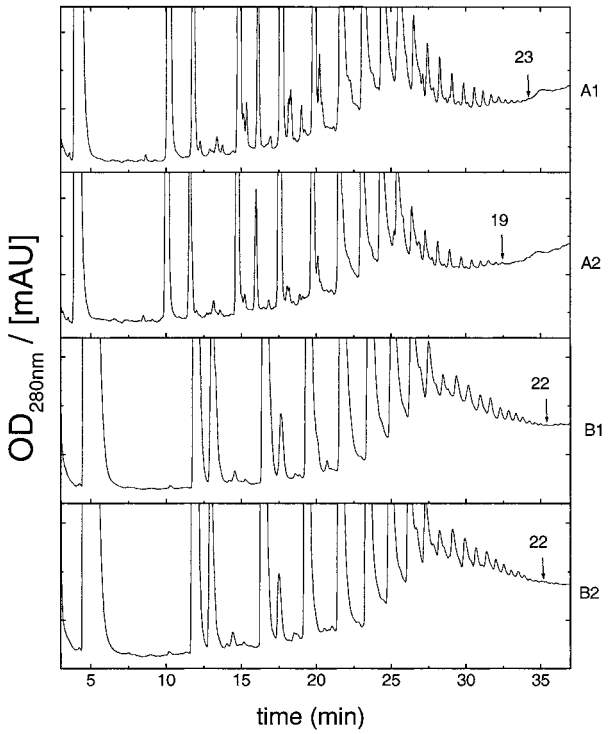

Figure 7. Effect of DPPC liposomes on the oligomerization of NCA−Trp at two different temperatures. HPLC absorption chromatograms are shown at 280 nm (C18 column, Macherey-Nagel, ET 250/4 Nucleosil 100-5). The numbers indicate the degree of oligomerization. Absorption chromatograms are shown for the NCA−Trp condensation at 37 °C (A) and 25 °C (B) of L-Trp (A1, B1) and of D-Trp (A2, B2) in the presence of L-DPPC liposomes. 60 mM NCA−Trp (L-Trp or D-Trp) in 0.4 M imidazole/HCl, pH 6.80 was mixed 1:3 (v/v) with a 100 nm extruded 50 mM L-DPPC liposome suspension in 0.4 M imidazole/HCl, pH 6.80 and incubated for 12−15 h stirring at 25 or 37 °C.

and NCA−Trp condensations at 5, 25, 37, and 50 °C have been performed. Because DPPC has a T_m of 41 °C,[20] it is now possible to perform the NCA−amino acid condensations below the phase transition temperature.

In the absence of liposomes the NCA−L-Trp condensations at temperatures between 5 and 50 °C yield an oligomer distribution which is in a first approximation independent from temperature. The quantification of the different oligomers has been possible until $n = 6$ (n = oligomerization degree).[21] An obvious difference at higher temperatures is the increasing rate of hydrolysis of preformed NCA−Trp and the higher yield of the longest detectable oligomer, the hexamer, at 50 °C.

When the NCA−Trp condensation is performed in the presence of L-DPPC liposomes, the longest detectable oligomer is in both cases for 25 and 37 °C around the 20-mer (Figure 7). Even at 5 °C (data not shown) oligomers >15 could be obtained. At 50 °C was no analysis possible because of precipitation. The maximal detectable chain length in the case of 50 mM DPPC at 25 °C (below T_m) is at least the 20-mer (Figure 7), and in the case of 100 mM POPC at 25 °C (above T_m) it is the 29-mer.[1] Please note that in the case of POPC the liposomes have been fed twice with activated amino acids while single feeding has been used in the experiments with DPPC. The oligomerization degrees in the case of POPC and DPPC are obviously of the same range. This indicates that the phase transition temperature does not have a significant influence on the degree of oligomerization of NCA−L-Trp and NCA−D-Trp. Therefore, it seems to be likely that in the case of NCA−Trp and $(Trp)_n$ the binding to phosphatidylcholine membranes is largely based on electrostatic and/or π-cationic[22] interactions and not on hydrophobic ones. Further experiments are, however, necessary to confirm this.

In the case of racemic NCA−L-Trp(d5)/NCA−D-Trp condensations (Table 1) in the presence of L- and D-DPPC-liposomes at 25 and 37 °C it can clearly be seen again that homochiral sequences become over-represented in the longer oligopeptides. But again, like in the case of POPC liposomes,[1] the bilayer has mostly the effect of increasing the length of the longest oligopeptide and does not seem to affect significantly the symmetry of the stereoisomer distribution (Table 1). Only at 25 °C in the presence of L-DPPC liposomes the homochiral oligo-Trp−L-mer (9-mer) seems significantly over-

Table 1. Relative Abundances for D_pL_q Stereoisomer Groups of the Oligo-Trp *n*-mers (n = 8, 9) Are Shown for One Racemic NCA−Trp Feeding in Presence of L- or D-DPPC Liposomes at 25 or 37 °C[a]

	L-DPPC		D-DPPC		
	% distribution at 25 °C	% distribution at 37 °C	% distribution at 25 °C	% distribution at 37 °C	% statistical (Bernoullian) distribution
D_8	1.83 ± 0.43	2.24 ± 1.27	1.61 ± 0.10	1.38 ± 0.10	0.39
D_7L	6.75 ± 0.40	6.68 ± 0.48	6.70 ± 0.22	6.30 ± 0.70	3.13
D_6L_2	16.23 ± 0.66	14.76 ± 1.10	13.99 ± 0.11	13.93 ± 0.49	10.94
D_5L_3	18.30 ± 0.36	18.57 ± 1.13	17.64 ± 0.36	18.00 ± 0.41	21.88
D_4L_4	18.69 ± 0.24	18.70 ± 0.23	18.13 ± 0.23	18.37 ± 0.55	27.34
D_3L_5	16.74 ± 1.15	17.31 ± 0.51	17.67 ± 0.42	18.40 ± 1.36	21.88
D_2L_6	12.32 ± 1.88	13.20 ± 1.13	14.95 ± 0.43	15.06 ± 0.24	10.94
DL_7	6.30 ± 0.10	6.21 ± 0.27	7.70 ± 0.40	7.21 ± 0.87	3.13
L_8	2.85 ± 0.52	2.35 ± 0.78	1.62 ± 0.13	1.38 ± 0.15	0.39
D_9	1.32 ± 0.27	not detectable[b]	1.27 ± 0.23	not detectable[b]	0.20
D_8L	5.20 ± 1.12		4.23 ± 0.17		1.76
D_7L_2	11.95 ± 0.36		10.66 ± 0.38		7.03
D_6L_3	15.62 ± 0.70		15.52 ± 0.26		16.41
D_5L_4	16.22 ± 0.86		16.28 ± 0.52		24.61
D_4L_5	15.80 ± 0.59		15.89 ± 0.34		24.61
D_3L_6	14.34 ± 1.08		16.04 ± 0.61		16.41
D_2L_7	11.08 ± 0.60		11.57 ± 0.70		7.03
DL_8	5.40 ± 0.48		6.15 ± 0.40		1.76
L_9	3.07 ± 0.35		2.40 ± 0.57		0.20

[a] Freshly prepared solutions of racemic 60 mM NCA−D/L-Trp(d5) in 0.4 M imidazole/HCl, pH 6.80 was mixed 1:3 (v/v) with a 100 nm extruded 50 mM L- or D-DPPC Suspension in 0.4 M Imidazole/HCl, pH 6.80, and incubated for 12−15 h stirring. [b] The S/N ratio (S: signal, N: noise) was too low, so that the SIM chromatogram areas could not be integrated.

represented when compared with the corresponding oligo-Trp−D-mer (9-mer). But an opposite behavior in the presence of D-DPPC liposomes is not observed. So one can conclude that the chirality of the DPPC lipid has no measurable influence on the chirality of the growing Trp chain. Trp oligomers may bind on the surface of the liposomes, close to the tertiary amine, which is at considerable distance to the chiral center of the phospholipid.

Concluding Remarks and Outlook

Our kinetic studies of racemic NCA−D/L-Trp condensation in the presence of liposomes indicate that the control of the stereoselective process is not only due to the last (ultimate) residue but also at least to the penultimate one. These kinetic properties may also be the reason for the over-representation of homochiral sequences in an aqueous system without liposomes.

In the case of liposome-assisted racemic NCA-D/L-Trp condensation the chiral structure of the lipid and the corresponding liposome phase transition temperature do not seem to influence significantly the distribution of the stereoisomeric subgroups of the resulting Trp oligomers. This may at first sight be surprising. On the basis of a simple entropic consideration and assuming that hydrophobic forces play a major role, it is expected that a temperature increase leads to an increased binding. On the other hand, molecular dynamic studies have shown that in some cases hydrophobic and amphiphilic polypeptides may not be spontaneously inserted into the hydrophobic interior of a membrane.[23,24] This indicates that interactions on the surface of liposomes (i.e., π-cation) may play a more important role than the hydrophobic effect. If this is applicable to our system, it would mean that the growing peptide chains are probably mostly bound on the surface of the liposomes and are not inserted into the hydrophobic core of the bilayer. Therefore, the contact to the chiral center of the lipid may be weak or even absent.

We believe that the most interesting observation of our study is the fact that the preferential homochiral chain growth, observed in the case of Trp^3 and Leu (Figure 3) or Ile (Figure 4) can be observed also with mixtures of equal amounts of the L- and D-forms of two or even three different amino acids (Figures 5 and 6). By inference, one is tempted to say that this may also be the case if the NCA−amino acid condensation is performed with a much larger number of different amino acids in an aqueous solution or in the presence of liposomes. If it is so, homochirality would assess itself. This observation, once proven experimentally, would be particularly interesting if long enantiomeric polypeptides could be physically separated. This in fact might lead to some useful hint for the origin of homochirality of biopolymers in nature.

Work in this direction is in progress in our group.

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