Templated Peptide Synthesis

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Racemic β Sheets in Biochirogenesis**

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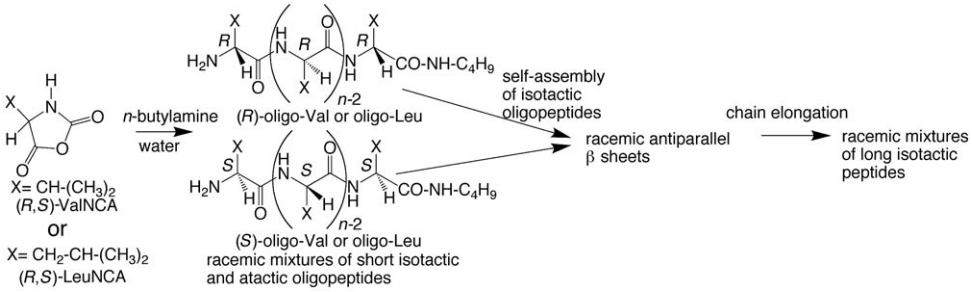
The transition from prebiotic racemic chemistry towards homochiral biology represents one of the unsolved riddles about the origin of life.[1,2] Its elucidation requires the development of possible scenarios for the conversion of racemic monomers into long bio-like homochiral (isotactic) polymers.[3–5] The polymerization of α-amino acids in buffer solutions has been reported to yield small amounts of isotactic oligopeptides, in a process that departs from the Bernoulli random kinetics; however, the mechanism responsible for this deviation was not elucidated.[6]

A way to override the disadvantage of the tendency to form polymeric chains composed from heterochiral repeat units in the polymerization of racemic monomers may require the emergence of supramolecular architectures as intermediates in nonlinear processes.[7–9] A commonly accepted hypothesis for the formation of long primeval peptides of homochiral sequence from racemic precursors suggests the involvement of either α helices, as proposed by Wald,[10] or enantiomorphous pleated β sheets, as suggested by Brack and Spach,[11] as templates. Their formation requires, however, oligopeptides composed from eight (or more) repeat units of the same handedness in the polymerization of racemates, a process that obeys binomial kinetics with a probability of 1 molecule out of 2^8 (256).[12–15] Although it has been demonstrated that both these architectures might exert asymmetric induction in the polymerization reactions of activated α-amino acids,[16] their role in the formation of long homochiral peptides from racemates has not been supported by laboratory experiments.

By contrast, we anticipate on kinetic grounds that racemic β sheets composed of mixtures of isotactic oligopeptides, delineated by chiral rims, might be advantageous architectures, provided that they can operate as templates for the formation of long peptides. The formation of these β sheets should be much faster as it depends upon the concentration of both the *R* and the *S* isotactic chains, whereas that of the enantiopure pleated β sheets depends upon the concentration of only one of the enantiomers. Although racemic antiparallel (*ap*) β sheets had been proposed by Pauling and Corey[17] to be closely similar to the natural pleated *ap* β sheets, they have been overlooked as possible participants in chemobiogenesis.

Here we show that racemic *ap* β sheets play a dominant role in the generation of libraries of isotactic oligopeptides comprising up to 25 repeat units of the same handedness in the polymerization reaction of racemic *N*-carboxyanhydrides of valine (ValNCA) or leucine (LeuNCA) in aqueous solution and in the presence of primary amines (Scheme 1).

(*R*,*S*)-ValNCA and (*R*,*S*)-LeuNCA, enantioselectively tagged with deuterium (98%), were polymerized in water with *n*-butylamine (25 mol%, pH 6–7.5) and analyzed by MALDI-TOF MS. The diastereomeric compositions of oligo-



Scheme 1. The polymerization process leading to the formation of racemic mixtures of long isotactic oligopeptides from racemic monomers dissolved in water.

Val and oligo-Leu in the product samples are shown in Figure 1a and b. In these spectra, the peaks for the isotactic oligopeptides of each length (labeled R_n and S_n) appear at the wings of each set of signals, whereas the peaks for those oligopeptides containing heterochiral repeat units are located in the middle. For each given length longer than the heptamers, the signal intensities (with consideration of isotopic distributions) of the isotactic oligopeptides are the most pronounced. Previous studies have demonstrated that the molar fraction of diastereomeric peptides of a given length is proportional to the relative intensity of their signals.[9,18,19] (The calculated distribution of the molar fractions of such oligopeptides is given in the Supporting Information.) The molar fractions of the isotactic oligopeptides, normalized to those calculated for a theoretical binomial distribution for each chain length, increase significantly with peptide length (Figure 2a). Such an increase

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

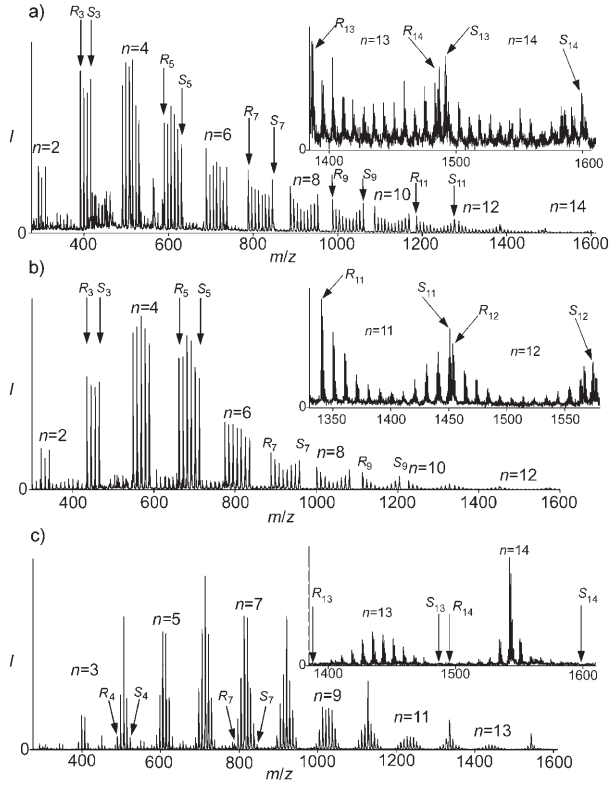

Figure 1. MALDI-TOF mass spectra of the oligopeptides obtained from the polymerization, with *n*-butylamine initiator (25 mol%), of a) and b) (*R*,*S*)-ValNCA and (*R*,*S*)-LeuNCA, respectively, in water and c) (*R*,*S*)-ValNCA in THF. The signals representing the diastereomeric oligopeptides of a given length *n* (where *n* is the total number of repeat units) are labeled. Isotactic oligopeptides are labeled R_n and S_n and arrows point to either the strong signals or their absence when the polymerization was performed in water or THF, respectively. The inserts show an enlargement of the corresponding spectrum.

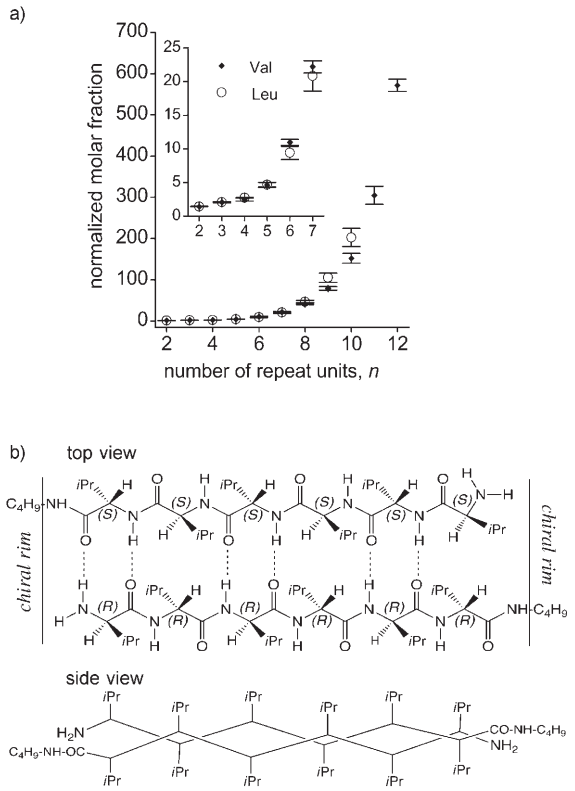

Figure 2. a) Plot of the molar fractions of isotactic oligo-Val (◆) and oligo-Leu (○) of various lengths *n*, normalized to those calculated for a theoretical binomial distribution. Error bars are the average of five samples. The insert shows an enlargement of the plot for $n = 2$–7. For an oligopeptide of a given length, the molar fraction represents the intensity of its signals (with consideration of the isotopic distribution) divided by the sum of the intensity of the signals corresponding to all of the diastereomers of the same length in the MALDI-TOF MS (as shown in Figure 1a and b). b) Top and side view of a racemic *ap* β sheet composed of *R* and *S* strands initiated by *n*-butylamine linked at the C terminus.

should arise from a nonlinear reaction pathway comprising a cooperative process of self-assembly of the racemic mixture of isotactic peptide chains into templates and further chain elongation. Chains bearing one or two heterochiral repeat units might be present within the templates provided that these units are located at the termini, as demonstrated by MALDI-TOF-TOF MS analysis (see the Supporting Information).

The X-ray powder diffraction patterns of the oligo-Val and oligo-Leu display, in addition to spacings of 4.7 and 6.9 Å, which are the fingerprint for β sheets, a spacing of 9.7 Å for oligo-Val and one of 12.0 Å for oligo-Leu, values that are indicative of the interlayer spacing. The FTIR spectra of both systems display the C=O amide I stretching vibration band at 1633 cm^{-1}, in keeping with the formation of β-sheet architectures (see the Supporting Information). From these data, however, one cannot differentiate between the various parallel and *ap* β sheets. Antiparallel chains may appear in the following architectures: 1) racemic *ap* β sheets (Figure 2b) with a periodic or nonperiodic arrangement of alternating oligo-*R* and oligo-*S* chains or 2) enantiomorphous pleated *ap* β sheets composed entirely from chains of the same handedness formed though spontaneous segregation of the racemate.

Unambiguous experimental confirmation for the formation of racemic *ap* β sheets can be obtained, provided that the polymerization is initiated with enantiopure α-amino acid methyl esters, for the following reasons. The racemic *ap* β sheets are delineated by chiral enantiotopic growing rims that contain the NH_2 groups belonging to chains of one handedness and repeat units of the initiator attached to the C terminus of chains of opposite handedness (Figure 2b). Consequently, an enantiopure initiator should convert the enantiotopic rims into diastereotopic ones. Such a change in the structures of the growing rims of the peptides (Figure 3a) would desymmetrize the racemic mixtures of the long isotactic oligopeptides formed within the templates. Furthermore, when the reaction is initiated by an S^* amino acid ester, this repeat unit present at the C terminus of the *S* chains should integrate coherently within these strands and thus should not interfere with the regular growth of the neighboring *R* chains. On the other hand, this same S^* repeat unit should induce an imperfection at the C terminus of the *R* chains that will consequently induce steric hindrance (see the Supporting Information) and affect the NH_2 growing sites of the adjacent *S* chains, thereby impeding their growth. As a

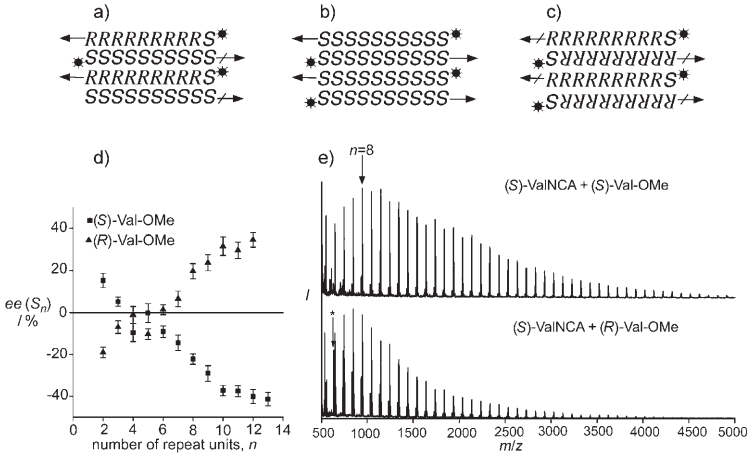

Figure 3. a–c) Different types of *ap* β sheets comprising four strands of homochiral sequences with a S^* repeat unit of the initiator attached at the C terminus: a) racemic β sheet of alternating oligo-*R* and oligo-*S* strands; b and c) enantiomorphous pleated β sheets of either oligo-*S* or oligo-*R* strands. The horizontal arrows represent the direction of chain elongation and the inclined lines represent the sites where the S^* repeat unit of initiator impedes growth of the adjacent strands. d) Plot of the $ee(S_n)$ ($=100([S]-[R_n])/([S]+[R_n])$; [%]) for isotactic oligopeptides of each length *n* obtained in the polymerization of (*R*,*S*)-ValNCA with either (*S*)-Val-OMe (■) or (*R*)-Val-OMe (▲) as the initiator (25 mol%). We have represented the excess of oligo-*S* chains as an *ee* value instead of the diastereomeric excess (*de*) value by not considering the repeat unit of the initiator at the C terminus. Error bars are the average of five samples. e) MALDI-TOF mass spectra of the oligopeptides obtained from the polymerization in water of (*S*)-ValNCA (10 mg mL^{-1}) with (*R*)- or (*S*)-Val-OMe initiator (5 mol%). The signal corresponding to octapeptides is labeled. Note the relatively large amounts of short ($n=5$–8) oligopeptides initiated by hydrolyzed ValNCA, labeled with ⋆, in keeping with a slower polymerization.

result, an S^* initiator is expected to engender libraries of nonracemic mixtures of peptides where the long peptides formed within the racemic templates should be enriched with *R* chains. Likewise, an R^* initiator should produce long peptides enriched with the *S* oligopeptides. By applying the same mechanism to the growth of enantiomorphous pleated sheets, (Figure 3b and c), one anticipates the formation of nonracemic long peptides, albeit with enantiomeric excesses opposite to those formed within the racemic templates. Through use of the same rationale, one may discard the possible formation of parallel β sheets as intermediate templates since, in such architectures, the repeat unit of the enantiopure initiator is located far away from the growing-site exposed NH_2 groups of the two *R* or *S* isotactic chains and therefore it would not exert steric hindrance in their elongation.[20]

An inspection of the MALDI-TOF mass spectra (Figure 4a–c) of samples obtained from the polymerization of (*R*,*S*)-ValNCA (25 mol%) in water, as initiated with either (*R*)- or (*S*)-Val-OMe, and of (*R*,*S*)-LeuNCA with (*S*)-Leu-OMe shows that, up to the tetra- or pentamers, there is an excess of the isotactic peptides in favor of those of the same absolute configuration as the initiator, a result implying the operation of an asymmetric induction in the formation of short oligopeptides. The experimentally determined *ee* values of the isotactic oligopeptides of each length, as calculated from the spectra, are represented in Figure 3d and in the Supporting Information for oligo-Val and oligo-Leu, respectively. There is some discontinuity in the *ee* values in the tetra- to pentamer region where the β-sheet templates are formed. Beyond this length, the *ee* value is dominated by isotactic oligopeptides of opposite handedness to that of the initiator, in keeping with the formation of racemic *ap* β-sheet templates.[21] A similar reversal in the *ee* value is also observed for oligopeptides that bear a few heterochiral repeat units (Figure 4a–c) located at either end of the chains, as proven by MALDI-TOF-TOF MS–MS (see the Supporting Information). The presence of the heterochiral repeat unit of the *R* initiator at the C terminus of the *S* chains induces only an impediment on the rate of growth of the *R* chains without affecting the enantioselectivity of elongation, as shown by the relative amount of these diastereomers appearing at the left wings for each group of signals in Figure 4a–c.

Experimental support for the predicted impediment effect of enantiopure (*S*)-Val-OMe initiator

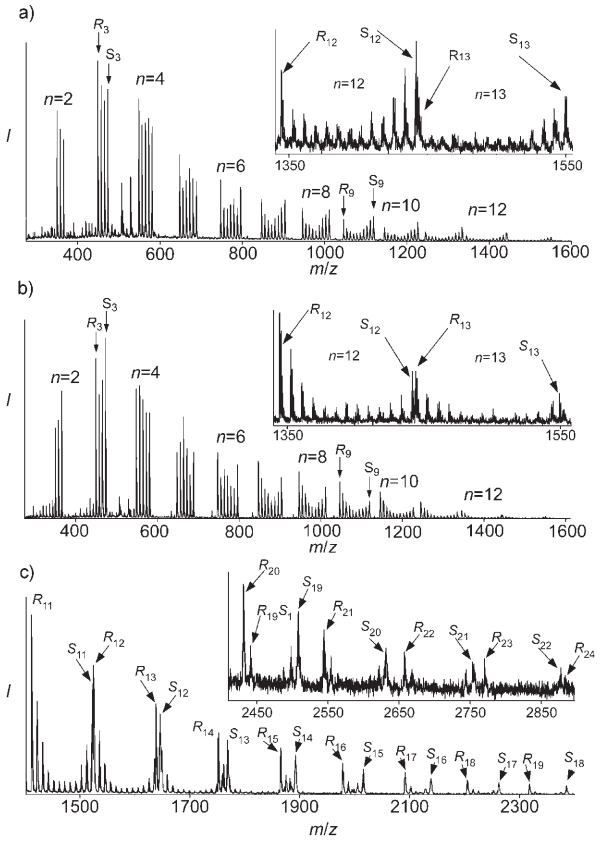

Figure 4. MALDI-TOF mass spectra of the oligopeptides obtained from the polymerization in water, with initiator (25 mol%), of: a) (*R*,*S*)-ValNCA with (*R*)-Val-OMe, b) (*R*,*S*)-ValNCA with (*S*)-Val-OMe, and c) (*R*,*S*)-LeuNCA with (*S*)-Leu-OMe. The signals representing diastereomeric oligopeptides of a given length *n* are labeled. In (c), only oligopeptides longer than $n=11$ are shown, for clarity. The R_n and S_n labels and arrows point to isotactic oligopeptides. The inserts show an enlargement of the corresponding spectra in the range of the long oligopeptides.

on the pleated β sheets, if enantiomorphous β sheets were to operate as templates, was provided by the comparative polymerization of (*S*)-ValNCA with initiators (5 mol%) of either handedness. The MALDI-TOF mass spectra (Figure 3e) clearly show that beyond octapeptides, after self-assembly into pleated β sheets, the oligopeptides obtained in the presence of (*S*)-Val-OMe are more numerous than those obtained with (*R*)-Val-OMe.

On the basis of all of the above results, we propose the following reaction route. Polymerization starts in the aqueous solution, with the formation of a library of short oligopeptides. The isotactic tetramers and pentamers are almost water-insoluble as compared to the heterochiral diasteroisomers, as determined by the MALDI-TOF MS analysis of the water-soluble fraction of oligo-Val and oligo-Leu (see the Supporting Information). For this reason, these amphiphilic peptides self-assemble preferentially and precipitate as racemic *ap* β sheets that serve as templates for further chain elongation (Scheme 1). The reaction ensues enantioselectively at the interface between the solid-like template, at the hydrated structured pockets where the active NH_2 groups are located, and the aqueous solution. The role played by the water solvent in various steps of the reaction is essential, as illustrated if the same reactions are performed in THF or dioxane. Under such conditions, the diasteroisomeric distribution of the oligopeptides is driven towards randomness and the isotactic peptides are not formed beyond octamers, as shown in Figure 1c and in the Supporting Information.[22]

In conclusion, we have demonstrated the role played by racemic *ap* β sheets as templates for the generation of long homochiral oligopeptides of amphiphilic α-amino acids in aqueous solutions, by starting from racemic NCA precursors. These results might have some relevance to prebiotic chemistry since recent studies have suggested that α-amino acid NCAs might have operated as intermediates for the formation of the early peptides.[23–25] These results support a scenario in which racemic *ap* β-sheet architectures may have played an important role in biochirogenesis by preceding the previously commonly proposed enantiomorphous templates such as α helices or pleated β sheets. Such a conjecture also provides additional backing to previous claims that nonchiral crystals, such as glycine and glycylglycine,[2] or minerals, such as calcite,[26,27] delineated by chiral faces, could have played a topical role in "mirror-symmetry breaking" in the prebiotic world.

Finally, homochiral peptides of the length reported here might have served as primers for further amplification of chirality by self-replicating mechanisms[28] or asymmetric synthesis.[29] The present synthetic approach is currently being extended for the generation of homochiral copeptides that also include hydrophilic amino acids.

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