Oligopeptide Formation in Crystals

Generation of Oligopeptides with Homochiral Sequences by Topochemical Reactions within Racemic Crystals of Phenylalanine-*N*-carboxyanhydride**

José Geraldo Nery, Gérard Bolbach, Isabelle Weissbuch, and Meir Lahav**

Theories on the origin of life suggest that homochiral biopolymers were already being generated in prebiotic times from racemic mixtures of activated monomeric precursors;[1–3] various scenarios for their formation have been proposed.[4–7]

In our current program we promote a conceivable scheme for the formation of primitive homochiral polymers from mixtures of racemates or nonracemic mixtures of low enantiomeric imbalance, by their self-assembly within two-dimensional (2D) or 3D crystalline architectures of distinct packing motifs, followed by lattice-controlled polymerization reactions.[8–10] The overall process entails two different aspects: a) Generation of racemic mixtures of homochiral polymers from racemates, and b) only one of the two enantiomeric polymers are formed by spontaneous symmetry breaking or by asymmetric induction.

Polymerization reactions within rigid 3D crystals, initiated either thermally or by irradiation, are controlled by the crystal lattice, to the greatest extent, at the onset of the reaction, since the polymeric phase produced is generally much denser than the crystalline monomer reactant. Changes in density and heat release that occur at sites of chain propagation induce local disorder, which brings about the formation of nonstereospecific polymers. Consequently, after years of research, only a limited number of systems including dieth-

[*] Dr. I. Weissbuch, Prof. Dr. M. Lahav, Dr. J. G. Nery
Department of Materials and Interfaces
The Weizmann Institute of Science
76100-Rehovot (Israel)
Fax: (+972) 8-9344-138
E-mail: isabelle.weissbuch@weizmann.ac.il
meir.lahav@weizmann.ac.il

Dr. G. Bolbach
Laboratoire de Chimie Structurale Organique et Biologique
Université Pierre et Marie Curie
75252 Paris Cedex 05 (France)

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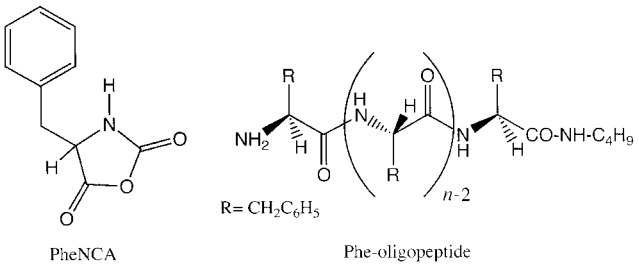
ylenes, diacetylenes, and coordination complexes of nickel were reported that undergo lattice-controlled polymerization beyond dimers and trimers.[11–17] Heterogeneous solid–gas or solid–liquid reactions are even less stereospecific, since the reactant crystal is already damaged at the early stages of the chemical transformation.

Recently, we reported the synthesis of homochiral (isotactic) oligopetides by the polymerization of amphiphilic racemic amino acid derivatives within 2D monolayer crystallites at the air–water interface.[8–10] During these studies, we noticed that when *N*-carboxyanhydride (NCA) derivatives of α-amino acids, bearing long hydrocarbon chains, are arranged within 2D crystallites in a head-to-tail motif at the air–water interface, they undergo a most efficient "zipperlike" stereospecific polymerization reaction to yield syndiotactic (*S*/*R*) oligopeptides. Following these results, we supposed that this mechanism of polymerization should equally operate in the rigid 3D crystals of NCA–α-amino acids with related packing motifs, which would provide another plausible route for the generation of homochiral oligopeptides from racemates. We now report the formation of homochiral oligopeptides by a polymerization reaction in racemic crystals of phenylalanine-*N*-carboxyanhydride (PheNCA) initiated by *n*-butylamine.



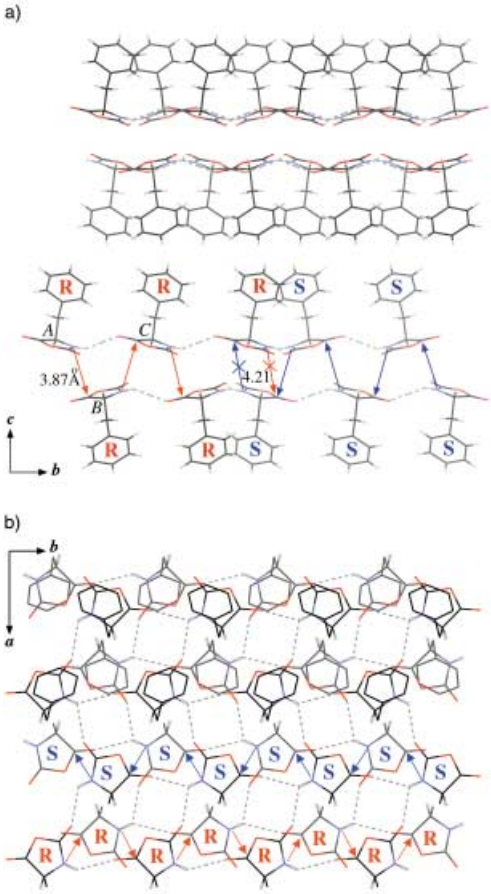


Figure 1. The packing arrangement of (*R*,*S*)-PheNCA crystals viewed along: a) the *a* axis, and b) the *c* axis. For clarity, some molecules in the lower parts of both images are not shown. The reaction pathway is indicated with red and blue arrows for the *R* and *S* molecules, respectively.

Kinetic and crystallographic studies by Kanazawa et al.[18–20] on the polymerization of several NCA monomers in the solid state have demonstrated that the rate of polymerization of these monomers is dependent on the packing arrangements of their crystals. However, since the diastereoisomeric composition of the oligopeptides was not available it was not possible to establish whether such crystals would be appropriate matrixes for the generation of oligopeptides with homochiral sequences. An inspection of the reported packing arrangement of racemic PheNCA[19] suggests that polymerization of this monomer via the zipperlike mechanism should furnish homochiral oligopeptides (Figure 1). The crystal contains two independent, almost identical, molecules per unit cell. The molecules form 2D hydrogen-bonded network layers arranged perpendicular to the *c* axis. For clarity we shall consider two rows of molecules, shown in the bottom bilayer in Figure 1 a. Within each row the molecules are related by translation symmetry, and are arranged in a head-to-tail motif; the two rows are not symmetry related. The separation of the nitrogen atom of molecule A (*R* configuration) residing in one row and the carbonyl carbon atom adjacent to the chiral carbon of molecule B of the same handedness residing in the second row is short (3.87 Å), whereas the same nitrogen atom is separated further from the equivalent carbonyl carbon atom of a nearer *S*-configured molecule (4.21 Å). We anticipated that molecule A (first row) would interact with molecule B (second row), which would serve to bring the latter closer to molecule C, which has the same handedness and resides in the first row (Figure 1 a). The propagation step will proceed preferentially by a zipperlike mechanism, where monomer units are added to the growing chains in an alternating sequential mode from monomers of the two rows. By virtue of symmetry, the enantiomeric monomers in the crystal should react to yield chains of the enantiomeric oligopeptides (Figure 1 b).

To probe the veracity of this mechanism, it was essential to determine the composition of the oligopeptides formed in these crystals. We used racemic mixtures of monomers, in which the phenyl rings of the *S* enantiomer were deuterated.[7,8,21,22] The structure of this "quasi-racemic" crystal of PheNCA is identical to that of its unlabeled counterpart, and different from that of the pure enantiomorph, as determined by X-ray powder diffraction. The polymerization reaction was performed in crystals suspended in hexane and initiated with 0.5 mol % of *n*-butylamine, to yield a complex mixture of diastereoisomeric oligopeptides of various lengths, as determined by MALDI-TOF mass spectrometry. The distribution

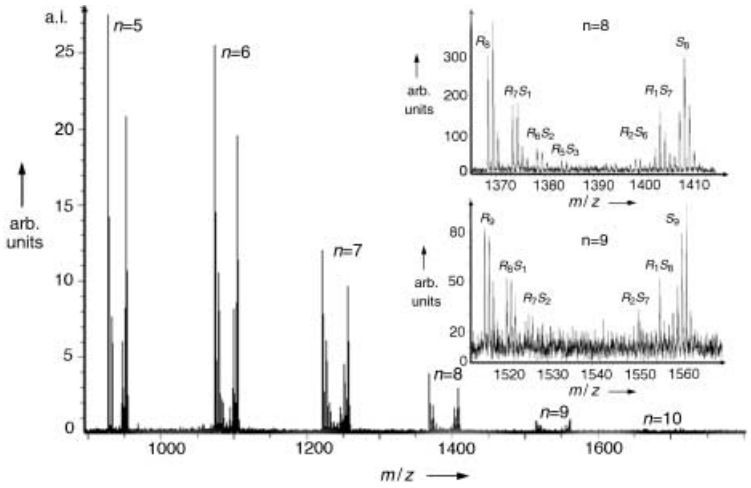


Figure 2. MALDI-TOF mass spectrum of the oligopeptides obtained in the polymerization of (*R*,*S*)-PheNCA at 22 °C showing the *m*/*z* range from penta- to decapeptides. The phenyl ring of the *S* enantiomer was pentadeuterated. The two insets show expanded spectra of the octamer and nonamer ranges. For analysis, samples were reacted with 2:1 (v/v) THF:trifluoroacetic anhydride; the *N*-trifluoroacetyl derivatives are soluble. Dithranol, mixed with NaI dissolved in THF, was used as the matrix.

of the products was analyzed directly after polymerization, or in the form of *N*-trifluoroacetyl derivatives. Figure 2 shows a MALDI-TOF mass spectrum of oligopeptides generated in crystals at 22 °C. Ion species deduced from mass/charge (*m*/*z*) ratios are *N*-trifluoroacetyl derivatives of the oligopeptides, which contain a single C_4H_9NH- group of the initiator, and are detected as Na ions. The intensity of the signals that correspond to different diastereoisomers of the same length are assumed to be proportional to their amount, as a result of identical ionization and detection efficiencies. The MALDI-TOF mass spectra provide a means of determining the relative abundance (r.a.) of the various diastereoisomers, defined as the ratio between the amount of a given diastereoisomer and the total amount of all the diastereoisomers of the same length. No measurable isotope effects were observed and the amount of oligopeptides decreased with an increase in length (Figure 2). The results were quite reproducible from sample to sample and very similar data were obtained using two different spectrometers. Usually, diastereoisomers as large as nonamers were detected but some samples showed also deca- and undecamers. Note that in the polymerization of unlabeled (*R*,*S*)-PheNCA samples, oligopeptides containing up to 17 repeat units were obtained.

Histograms describing the correlation between the r.a. and the composition of the oligopeptides of each length obtained from racemic PheNCA polymerized at 22 °C, 50 °C, and in the melt are shown in Figure 3a, b, and c, respectively. An oligopeptide of length *n*, where $n = h + d$ (*h* and *d* are the number of *R* and *S* repeat units, respectively) is labeled (*h*,*d*). Homochiral oligopeptides of each length *n* are represented at the wings, whereas the heterochiral species are represented in between them (Figure 3). Oligopeptides ($n = 4$–9), primarily exhibiting a homochiral sequence, were obtained in the racemic crystals of PheNCA at 22 °C (Figure 3a), as compared to a random polymerization obeying a binomial law (Figure 3e). Polymerization in the melt (Figure 3c) yielded shorter oligopeptides ($n = 7$), also with a primarily heterochiral sequence, and with an increase in length. Polymerization is still topochemically controlled at 50 °C (Figure 3b), although the degree of enantioselectivity is reduced. Further support for the topochemical nature of this solid-state reaction is gained from the observed different distribution of diastereoisomeric oligopeptides formed upon polymerization of racemic leucine-NCA[23] (Figure 3d), which crystallizes with a different structural motif.

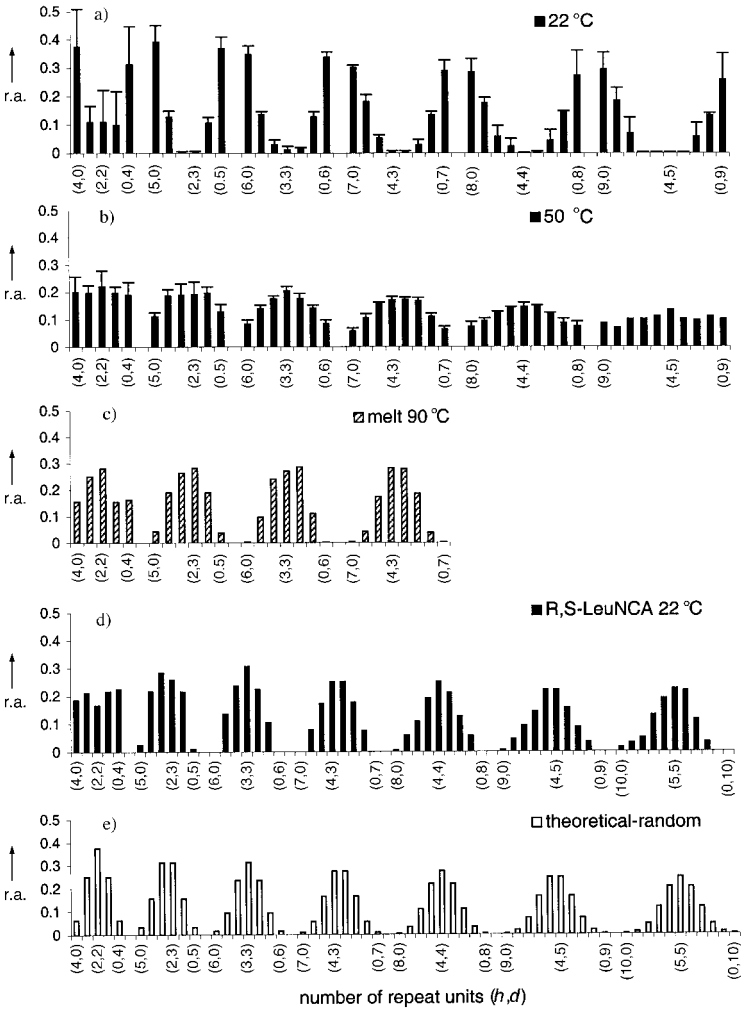


Figure 3. Histograms showing the experimental relative abundance of diastereoisomeric oligopeptides of various lengths obtained from the polymerization in: a,b,c) (*R*,*S*)-PheNCA crystals at 22 °C, 50 °C, and in the melt (90 °C in heptane); d) (*R*,*S*)-LeuNCA at 22 °C. e) The relative abundance calculated for a binomial distribution in a random process. Labels (*h*,*d*) represent the number of *R* and *S* repeat units of each oligopeptide. Histograms a) and b) show the average and standard deviation of three and four experiments, respectively. The nonamer in b) was obtained in one sample only.

It is noteworthy that the r.a. of the homochiral fraction of the longer homochiral Phe-oligopeptides increases along with an increase of the chain length. Figure 4 shows the experimental r.a. (at 22 °C) of homochiral oligopeptides, normalized to that calculated in a theoretical random polymerization, for oligopeptides of various lengths. The results can be rationalized by supposing that a fraction of the short oligomers were formed at defect sites on the surface, which have not propagated towards the interior of the crystals.

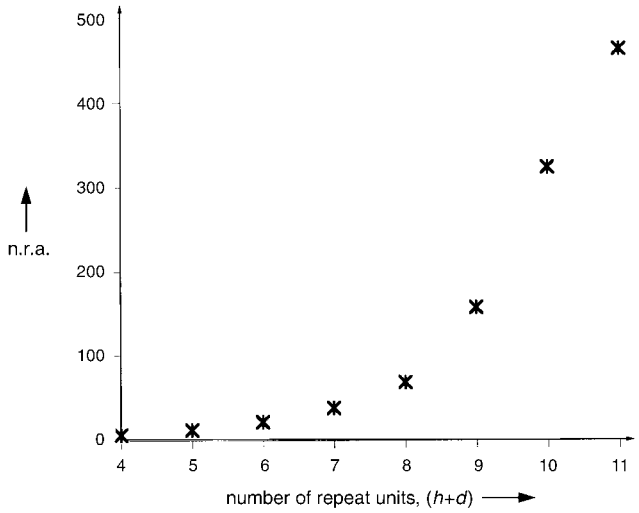


Figure 4. Enhancement of the experimental relative abundance of the homochiral [(*h*,0) + (0,*d*)] oligopeptides normalized to that calculated for a theoretical random process for molecules of any length $n = h + d$ (n.r.a. = normalized relative abundance). Note that deca- and undecapeptides were detected only in some of the polymerization experiments.

In conclusion, a process that comprises the self-assembly of activated α-amino acids into crystalline architectures, followed by lattice-controlled reactions, provides a plausible route for the formation of homochiral oligopeptides of 10–17 units in length. The generation of homochiral oligopeptides by this route is not confined for reactivity in 3D crystals, but should be also applicable within less organized architectures such as membrane bilayers and vesicles.

In the present study we have focused on the formation of racemic mixtures of homochiral oligopeptides. However, reactivity in crystals can be further exploited for the preparation of enantiopure oligopeptides, generated either by the polymerization of appropriate racemic monomers in the presence of small amounts of enantioselective initiators and inhibitors,[24] or by the amplification of chirality originating from nonracemic mixtures[8,25] of monomers of low enantiomeric imbalance that undergo phase separation.

Experimental Section

NCA monomers were prepared following a reported procedure.[26] *R*-Phe (1 mmol) and *S*-Phe (ring D5 98 % Cambridge Isotope Laboratories; 1 mmol) were suspended in dry THF and heated to 40 °C under argon. Solid bis(trichloromethyl) carbonate (3.5 mmol) was added gradually for about 1 h until a clear solution was obtained; the reaction then proceeded for additional 3 h. Hexane was then added and the NCA crystals were formed overnight at −5 °C. The thin platelike (*R*,*S*)-PheNCA crystals obtained after filtration were analyzed by IR spectroscopy and X-ray powder diffraction. (*R*,*S*)-LeuNCA was prepared in a similar way using deuterated (*S*)-[D_7]leucine.

Solid-state polymerization was performed (under argon atmosphere) in crystals suspended in hexane, using *n*-butylamine (0.5 mol %) as an initiator, for 12 and 72 h at 50 and 22 °C, respectively. The gel-like product was washed with dry THF and ethyl acetate until all the monomer was removed, as confirmed by infrared spectroscopy. The product in its final form was centrifuged and dried. Melt polymerization was performed in heptane, heated to 90 °C.

Samples for MALDI-TOF mass spectral analysis were prepared by either dissolving the dry material in a 2:1 (v/v) mixture of THF and trifluoroacetic anhydride (trifluroacetylation of the N-terminal of the oligopeptides) or THF:trifluoroacetic acid until a clear solution is obtained. 0.5 μL of this solution was deposited on to a matrix deposit (1:1 (v/v) mixture of dithranol solution in chloroform and a NaI saturated solution in THF) on the instrument holder. The MALDI-TOF positive-ion mass spectra were obtained in reflector mode from two different instruments at the Weizmann Institute (Bruker Biflex 3) and at the University of Paris VI (Perceptive Biosystems, Voyager Elite), both equipped with a N_2 laser.

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