Homochiral oligopeptides generated *via* an asymmetric induction in racemic 2D crystallites at the air–water interface; the system ethyl/thio-ethyl esters of long-chain amphiphilic α-amino acids†

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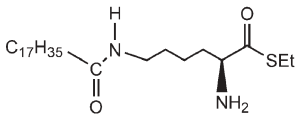
N^{ε}-stearoyl-lysine-ethyl-ester (C_{18}-OE-Lys) operates as an efficient desymmetrizing agent for the generation of homochiral oligopeptides *via* a reaction catalyzed by silver ions in two-dimensional (2D) *quasi*-racemic crystallites of the corresponding thio-ester (C_{18}-TE-Lys) self-assembled on water.

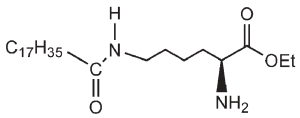
The transition from racemic chemistry towards homochiral biology provides still an unsolved riddle in prebiotic chemistry.[1–3] In particular, it is of interest to design synthetic routes for the preparation of primeval bio-like polymers from racemic monomeric precursors in the presence of chiral initiators. Recently, we proposed a route for the generation of racemic mixtures of homochiral oligopeptides *via* self-assembly of amphiphilic thio-esters[4,5] of α-amino acids into monolayer crystalline domains of appropriate structures at the air–water interface, followed by preferential polymerization between homochiral molecules.[6–9]

Here we report on the role played by esters of amphiphilic α-amino acids as efficient desymmetrization agents in the polycondensation of the corresponding thio-esters within 2D crystallites at the air–water interface leading to the formation of non-racemic libraries of homochiral oligopeptides. Such amphiphilic esters are anticipated to be appropriate chiral auxiliaries for the following reasons: First, they might form *quasi*-racemic 2D crystallites with the corresponding thio-esters, analogous with those well known in three-dimensional crystals.[10,11] Second, the alkoxy group is a poorer leaving group than thiolate group in nucleophilic reaction and previous studies have demonstrated that esters of amphiphilic α-amino acids do not undergo polycondensation.[12] Consequently, the presence of the esters within the enantioselective sites of the thio-ester 2D crystallites might block enantioselectively, in a lattice-controlled process, the chain propagation of the homochiral oligopeptides of a given handedness.

These anticipations were tested by performing the polycondensation of racemic N^{ε}-stearoyl-lysine-thioethyl-ester (C_{18}-TE-Lys) in the presence of (*S*)-N^{ε}-stearoyl-lysine-ethyl-ester (C_{18}-OE-Lys) or with the same enantiomer tagged with 35 deuterium atoms. Solutions in chloroform (0.5 mM) of (*RS*)-C_{18}-TE-Lys, where hydrocarbon chains of the *R*-enantiomer were tagged with 35 deuterium atoms, mixed with 10 mol% of (*S*)-C_{18}-OE-Lys were spread (0.6 mL) on the water surface (927 cm^2) of a Langmuir trough and polymerized by injecting 15 mL of 0.45 mM $AgNO_3$ aqueous solution into the water beneath the monolayer films. The oligopeptide products were collected from the surface after two hours and analysed by matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS).



C_{18}-TE-Lys



C_{18}-OE-Lys

The analysis showed the formation of libraries of oligopeptides containing two to seven repeating units and bearing either an ester (labelled *S′*) or a thio-ester group (labelled *S* or *R*) at the *C*-terminus. The mole fractions of each of the diastereoisomeric oligopeptides were calculated from the intensity of the *m*/*z* signals on the assumption that molecules of the same length display the same ionization efficiency and thus their quantity is proportional to the integrated intensities (see ESI).‡ The distribution of the various oligopeptides and their normalized yields are shown in Fig. 1a, b respectively.§ For comparison, the distribution of the oligopeptides generated from pure racemic C_{18}-TE-Lys is shown in Fig. 1c. The repeating units of the oligopeptides labelled *S* or *R* in Fig. 1a originate from the thio-ester, whereas those originating from the ester are labelled *S′* (*vide infra*). An inspection of Fig. 1b indicates that the amounts of the oligopeptides of any length bearing either an ester or a thio-ester at their *C*-terminus are similar in spite of the fact that the starting concentration of the ester is only 10% that of the thio-ester.¶ Additional support for enhanced reactivity of the ester was provided by its polycondensation reaction in the perdeuterated form mixed with an equimolar amount of the thio-ester of the same absolute configuration. The MALDI-TOF MS analysis of the products (see ESI) shows the formation of oligopeptides containing two to seven repeating units where most of the chains are initiated by the amino group of the ester. A way to explain this result is to assume an entropically-controlled pathway by taking into consideration the formation of dynamic complexes between the Ag^+ ions, amino groups and sulfur atoms of the thio-ester C_{18}-TE-Lys molecules. Since the

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† Electronic supplementary information (ESI) available: GIXD patterns of: (*RS*)-C_{18}-TE-Lys, 0.5 : 0.25 : 0.25 (*R*)-C_{18}-TE-Lys : (*S*)-C_{18}-TE-Lys : (*S*)-C_{18}-OE-Lys, 1 : 1 (*R*)-C_{18}-OE-Lys : (*S*)-C_{18}-TE-Lys and reaction products, MALDI-TOF MS analysis from the reaction products of 1 : 1 (*S*)-C_{18}-TE-Lys : (*S*)-deuterated-C_{18}-OE-Lys and reaction products and comments on not measurable isotope effects. See http://dx.doi.org/10.1039/b507457f

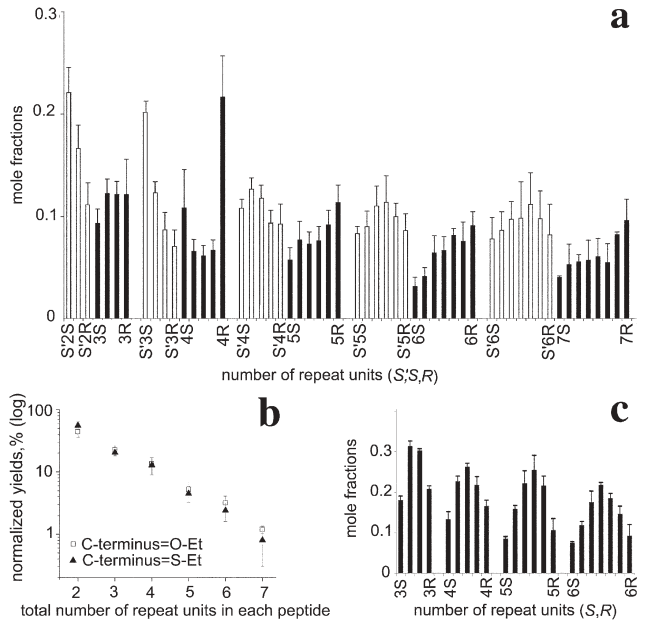
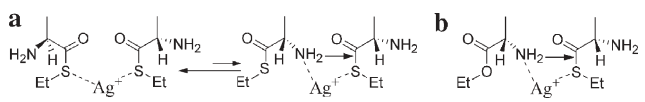


Fig. 1 (a, c) Mole fractions of each of the oligopeptides calculated from MALDI-TOF MS analysis of the products obtained by polycondensation at the air–water interface of (*RS*)-C_{18}-TE-Lys mixed with 10 mol% (*S*)-C_{18}-OE-Lys and pure (*RS*)-C_{18}-TE-Lys, respectively. (b) Normalized yields %, defined as sum of relative intensities of the diastereoisomeric oligopeptides divided by that of the dipeptides, shown for each oligopeptide length with a total number of repeat units n = 2–7. Oligopeptides bearing an OEt or SEt group at the *C*-terminus are shown as open and solid bars, respectively. The error bars represent the average of five experiments. No detectable isotope effects were observed in such a system by interchanging the isotope labeling of the enantiomers (see ESI).‡

affinity of the Ag^+ ions for sulfur is higher than that for nitrogen, the preferred complex will be formed with two sulfur atoms of two neighbouring C_{18}-TE-Lys molecules (Scheme 1a). In view of the product distribution, we proposed that such complexation would place the amino group of one molecule in a non-favourable orientation to the carbon atom of the carbonyl group of the near thio-ester molecule. On the other hand, the Ag^+ ions will bind to the amino group of the ester and the sulfur atom of a nearer thio-ester molecule (Scheme 1b). Such complexation brings the amino group of the ester into a favourable orientation for the reaction. A delayed polymerization initiated by the thio-ester can be rationalised by the formation in a low concentration of a transient Ag^+ ion-bridged complex with an amino group and sulfur atom of two near thio-ester molecules (Scheme 1a) similar to that of the ester shown in Scheme 1b. This reaction scheme is reminiscent of the reaction pathway proposed by Tam *et al.* for the Ag^+ ion-assisted synthesis of cyclic peptides from linear precursors bearing a thio-ester end group.[13,14]

Another result obtained from the distribution of the diastereoisomeric oligopeptides shown in Fig. 1a is that the mole fractions



Scheme 1 Schematic representation of the proposed complexes formed between the Ag^+ ions and adjacent thio-ester or thio-ester/ester molecules.

of the ester-initiated tri- and tetra-peptides of homochiral sequence *S*′2*S* and *S*′3*S* are twice and three times as large as the corresponding *S*′2*R* and *S*′3*R* (Fig. 1a shown as open bars). By contrast, the mole fractions of all the thio-ester initiated homochiral oligopeptides (Fig. 1a shown as solid bars) composed of only *R*-repeating units (*nR*) are larger than those of the corresponding enantiomers of *S*-configuration (*nS*).‖ These distributions are very different from those obtained from pure racemate shown in Fig. 1c. A way to rationalize these results is to assume that the (*S*)-esters replace (*S*)-thio-ester molecules in the 2D crystallites of (*RS*)-C_{18}-TE-Lys to form *quasi*-racemates and the remaining (*S*)-C_{18}-TE-Lys and (*S*)-C_{18}-OE-Lys molecules can also make separate 2D enantiomorphous crystallites. Indeed reactivity within such domains takes place as described above (see ESI).

The formation of *quasi*-racemic 1 : 1 (*R*)-C_{18}-TE-Lys : (*S*)-C_{18}-OE-Lys 2D crystallites with a packing arrangement ** very similar to that of the pure (*RS*)-C_{18}-TE-Lys and different from those of the two enantiomers was directly demonstrated by performing grazing incidence X-ray diffraction (GIXD) measurements[15] using synchrotron radiation.[6] The GIXD patterns are shown in Fig. 2a–c. In the *quasi*-racemic crystallites the thio-ester molecules are related by a *pseudo*-glide symmetry to the ester molecules (Fig. 2d).

On the basis of the reactivity of the pure (*RS*)-C18-TE-Lys where the reaction occurs preferentially between homochiral molecules in comparison to the binomial distribution, and according to Scheme 1, the (*S*)-ester molecules occluded into quasi-racemic crystallites are anticipated to react faster than and mostly with thio-ester molecules of the same handedness. The (*R*)-C_{18}-TE-Lys molecules that remain in excess in the crystallites will start to react in a later stage to yield an enhanced concentration of non-racemic oligopeptides of homochiral sequence.

The preferred enantioselective reactivity of the C_{18}-OE-Lys was demonstrated in a model system, by performing the polycondensation reaction in three-component mixtures of composition

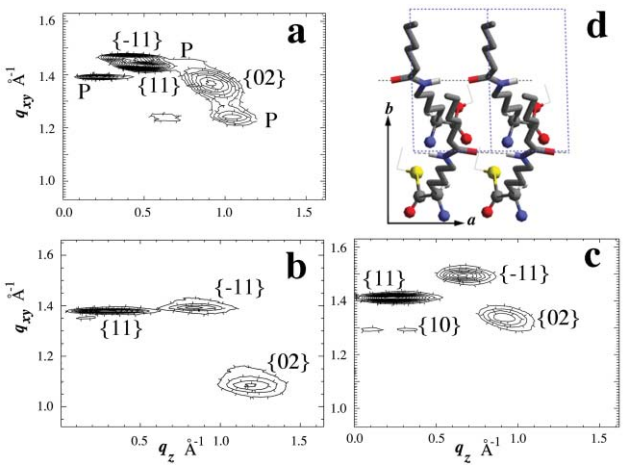


Fig. 2 GIXD patterns as 2D contour plots of scattered intensity as a function of the horizontal q_{xy} and vertical q_z components of the scattering vector, $I(q_{xy},q_z)$, of the self-assembled 2D crystallites of: (a) 1 : 1 (*R*)-C_{18}-TE-Lys : (*S*)-C_{18}-OE-Lys; (b) (*R*)-C_{18}-OE-Lys; (c) (*S*)-C_{18}-TE-Lys. (d) Packing arrangement of the crystallites in (a) viewed along *c*-axis. For clarity, only parts of the chains are shown. Note that in (a), the weak peaks labelled P belong to a small amount of product phase (see ESI).

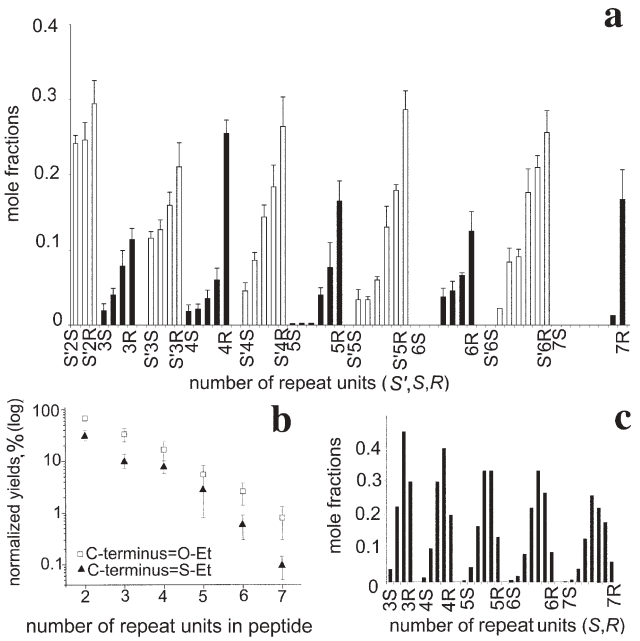


Fig. 3 (a) Mole fractions of each of the oligopeptides calculated from MALDI-TOF MS analysis of the products obtained by polycondensation at the air–water interface of 0.5 : 0.25 : 0.25 (*R*)-C_{18}-TE-Lys : (*S*)-C_{18}-TE-Lys : (*S*)-C_{18}-OE-Lys mixtures. (b) Normalized yields %, shown for each oligopeptide length with a total number of repeat units n = 2–7. (c) Mole fractions calculated for a binomial distribution in a theoretically random process for mole ratio 1 : 2, *S* : *R*. Oligopeptides bearing an OEt or SEt group at the *C*-terminus are shown as open and solid bars, respectively. The error bars represent the average of five experiments.

0.50 : 0.25 : 0.25 of (*R*)-C_{18}-TE-Lys : (*S*)-C_{18}-TE-Lys : (*S*)-C_{18}-OE-Lys, respectively. The GIXD patterns of these crystallites are very similar to those of the pure racemate. Fig. 3a shows the experimental distribution of the diastereoisomeric oligopeptides in comparison with a binomial distribution of 2 : 1 mixtures of *R* : *S* thio-esters (Fig. 3c) calculated on the assumption that the ester would have been inert. The normalized yields of the oligopeptides of each length are shown in Fig. 3b. The ratio between the mole fractions of the tri-peptides *S*′2*S* and *S*′2*R* is 0.82, whereas the ratio of the starting thio-ester monomers *S* : *R* is 0.50, reconfirming that in the early stages the reaction proceeded preferentially between (*S*)-esters and (*S*)-thio-esters depleting the *quasi*-racemic crystallites from (*S*)-thio-ester monomer. As a result, the oligopeptides initiated by thio-ester molecules commence to react in a later stage in a milieu already enriched with the (*R*)-thio-esters leading to an enhanced formation of homochiral oligopeptides (*nR*) primarily of a single handedness.††

In conclusion, the ability of the esters of α-amino acids to form, on the one hand, *quasi*-racemic 2D crystallites with the corresponding thio-esters, and on the other hand, to operate as an efficient initiator but not participate in the chain propagation reaction in the Ag^+ ion-mediated lattice-controlled polycondensation of the thio-esters within crystalline assemblies, makes such molecules suitable auxiliary desymmetrization reagents for the generation of non-racemic mixtures of homochiral oligopeptides in reactions. Differences in reactivity between the thio- and ester-initiated oligopeptides are currently being considered for the preparation of longer enantiopure oligopeptides of prebiotic relevance.

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Notes and references

‡ Detailed information on the absence of measurable isotope effects is available in the supporting information.

§ The dipeptides are formed at non-ordered parts of the monolayer film even in the absence of catalyst. Their quantities vary from one experiment to another and are not shown for clarity.

¶ Similar trends were also observed for the distribution of the oligopeptides obtained from the polycondensation of the mixture 0.50 : 0.45 : 0.05 (*R*)-C_{18}-TE-Lys : (*S*)-C_{18}-TE-Lys : (*S*)-C_{18}-OE-Lys.

‖ Note that the formation of larger amounts of the homochiral tetrapeptides implies a preferred reaction between dipeptides of the same handedness.

** The molecules pack in a *pseudo*-rectangular unit cell, with a = 4.94 Å, b = 9.04 Å, γ = 86.1° and their hydrocarbon chains are tilted by 33° from the surface normal in a direction almost parallel to the b axis.

†† Due to the enantiomeric enrichment of the 2D crystallites, the longer ester-initiated oligopeptides (*S*′*nR*) are formed in larger concentrations than the *S*′*nS* in all the experiments starting with different monomer compositions.

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