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Peptide nanotubes self-assembled from leucine-rich alpha helical surfactant-like peptides†

Valeria Castelletto, ‡,Janis Seitsonen, ‡,Janne Ruokolainen, ‡,Cristian Piras, ‡,Rainer Cramer, ‡,Charlotte J. C. Edwards-Gayle, ‡ and Ian W. Hamley ‡,*

The designed arginine-rich surfactant-like peptide R₃L₁₂ (arginine₃–leucine₁₂) is shown to form a remarkable diversity of self-assembled nanostructures in aqueous solution, depending on pH, including nanotubes, mesh-like tubular networks in three-dimensions and square planar arrays in two-dimensions. These structures are built from α-helical antiparallel coiled–coil peptide dimers arranged perpendicular to the nanotube axis, in a ‘cross-α’ nanotube structure. The aggregation behavior is rationalized based on the effects of dimensionality, and the balance of hydrophobic and electrostatic interactions. The nanotube and nanomesh structures display arginine-9 at high density on their surfaces, which may be valuable for future applications.

Peptide nanotubes are biomolecular self-assemblies with remarkable structural and functional properties.1–14 Several classes of peptide nanotube have been reported including those based on helically wrapped β-sheets,15–20 lamimates of cyclic peptide dimers forming antiparallel β-sheet stacks,10,21,22 and stacks of alternating α₁₇-cyclic peptides stabilized by hydrogen bonded antiparallel β-sheets.17,21 In addition, the parallel packing of coiled coil peptide arrays can lead to tubular structures.24–26 Here, we report on a distinct class of peptide nanotube based on a designed surfactant-like peptide (SLP).27 We have recently reported on the self-assembly of a number of SLPs with hydrophobic alanine repeats and various charged ‘headgroups’. These form β-sheet nanofibrils,28,29 and in some cases nanotubes.19,20

‡ Department of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, UK. E-mail: I.W.Hamley@reading.ac.uk
‡ Nanomicroscopy Center, Aalto University, Puumiehenkuja 2, FIN-02150 Espoo, Finland
‡ Diamond Light Source, Harwell Science and Innovation Campus, Didcot, Didcot, Oxfordshire OX11 0DE, UK
† Electronic supplementary information (ESI) available: Experimental procedures including materials, sample preparation, experimental data obtained from several techniques (liquid MALDI-AP MS, CD, SAXS, Cryo-TEM and TEM) and tables listing the parameters extracted from the fitting to the SAXS data and the analysis of cryo-TEM images are included. See DOI: 10.1039/d0cc04299d

Here, we explore the self-assembly of SLPs containing leucine repeats instead of alanine sequences, since leucine has a strong α-helix propensity.31 The peptide studied is R₃L₁₂ (and is capped at both termini, ESI,† Scheme S1). In dilute aqueous solution, nanotube structures comprising α-helical peptide dimers stacked perpendicular to the walls were observed, which have a thickness that corresponds to the length of the dimer comprising antiparallel α-helical peptides. We also unexpectedly observed continuous nanotubular channel network structures that are accessible via pH variation. These structures present arginine residues at the surfaces of the nanotube and tubular network structures, providing a highly cationic surface for future applications. In addition, an ordered square lattice array was observed in thin films under certain conditions of pH. We rationalize the formation of this diverse range of previously unreported peptide nanostructures on the basis of the balance between hydrophobic and electrostatic effects.

We studied pH-dependent self-assembly of R₃L₁₂ at pH 4 (native), or lower, pH values being tuned by addition of 10 mM HCl (pH 2) or 100 mM HCl (pH 1). Circular dichroism (CD) spectra shown in Fig. 1, measured for 0.04 wt% R₃L₁₂, confirm the formation of α-helical coiled coil structures for all solution conditions studied (the CD spectra for 0.07 wt% solutions...
shown in ESI,† Fig. S1 also confirm α-helix structure. 32 For
0.04 wt% R₃L₁₂, the α-helical content fₐ (and [θ]₂₂₂/[θ]₂₀₈ ratio, a
measure of coiled coil aggregation 33,34) are, respectively, 78% (0.85), 42% (0.86) and 17% (1.25) at pH 4, 2 and 1 (inset Fig. 1;
the expressions used to calculate these quantities are provided
in the ESI†). Decreasing the pH reduces fₐ significantly, while
[θ]₂₂₂/[θ]₂₀₈ increases.

Having established the conformation of SLP R₃L₁₂, we then
used the powerful combination of cryo-TEM, TEM (TEM: trans-
mission electron microscopy) and SAXS (small-angle X-ray
scattering) to probe self-assembly behavior. Fig. 2 displays
representative cryo-TEM images obtained for 0.04 wt% solu-
tions of R₃L₁₂ at pH 4, 2 and 1. These images (and others shown
in ESI,† Fig. S2–S4) show the presence of short nanotubes at
pH 4 (Fig. 2a and d). Decreasing the pH from 4 to 2 the nano-
tubes become better defined, and their population and length
both increase (Fig. 2b, e and Fig. S3, ESI†). Decreasing the
pH even further to pH 1 leads to the formation of a continuous
tubular network aggregates (Fig. 2c, f and Fig. S4, ESI†).

The shape of the nanotubes at pH 2 is very well defined,
several cryo-TEM images clearly show the nanotube cross
section (Fig. S3, ESI†). The continuous tubular network assem-
bles at pH 1 seem to mainly comprise three-fold connecting
nodes, such as those highlighted in Fig. S4 (ESI†). To determine
the nanotube diameter and wall thickness, histograms were
created (Fig. 2g and h) based on a series of cryo-TEM images for
samples at each pH value. These results will be discussed below
together with the parameters extracted from the fitting of the
SAXS data.

Fig. 3 shows synchrotron SAXS data for 0.04 wt% solutions
of R₃L₁₂. Samples at pH 4 and pH 2 present strong oscillations
arising from nanotube wall interference features in the form
factor, with a period set by the nanotube diameter. This features
is absent from the data for the pH 1 sample, which as shown by
cryo-TEM (Fig. 2) forms a tubular network structure instead of
narrow dispersity diameter nanotubes. As described in detail in
the ESI,† high quality form factor fits (shown as lines in Fig. 3) of
the SAXS data were performed which provide the nanotube
diameter, as well as wall thickness. The SAXS fitting parameters
are listed in Table S1 (ESI†). Table S2 (ESI†) compares the
parameters measured from cryo-TEM images with those calcu-
lated from the fitting of the SAXS curves. The results are in good
agreement and these results indicate that, from TEM, the thick-
ness of the nanotube wall, tₛ is 3.4 ± 0.5 nm, 3.7 ± 0.6 nm and
3.4 ± 0.5 nm pH 4, 2 and 1 respectively. From SAXS, the wall
thickness is 3.3 ± 0.1 nm, 3.0 ± 0.1 nm and 3.0 ± 0.1 nm at pH 4,
The nanotube wall thickness corresponds closely to the length of continuous tubular network structure (Fig. 2c, f and Fig. S4, ESI†). The nanotube thickness is approximately 3 nm within the experimental error. The cryo-TEM and SAXS results also show that the nanotube diameter dramatically decreases at pH 1, i.e., in the continuous tubular network structure (Fig. 2c, f and Fig. S4, ESI†). The nanotube wall thickness corresponds closely to the length of R3L12 estimated using average residue spacings,\textsuperscript{35} \( l = (1.5n) + (3.4p) = 28.2 \text{ Å} (\approx 3 \text{ nm}) \) where \( n = 12 \) (number of \( \ell \)-residues in the \( \alpha \)-helix) and \( p = 3 \) (number of R-residues).

We thus propose that the nanotube walls comprise opposed dimers of \( \alpha \)-helices oriented perpendicular to the main axis of the nanotube, as shown in Fig. 4. The antiparallel configuration is also reasonable as it will minimize electrostatic repulsion between arginine residues (peptide charge +3 under all pH conditions studied). The nanotubes and nanotubular network structures formed are coated with arginine on inner and outer surfaces. Nanotube formation results from the balance of hydrophobic and hydrogen bonding of the leucine residues that form antiparallel coiled coil dimers and electrostatic repulsion of arginine residues. The formation of the tubular network structure at very low pH 1 is ascribed to the unfavorable electrostatic penalty associated with having uncharged tubes exposed to large H\textsuperscript{+} ion concentrations, leading to closure into tubular networks.

Remarkably, distinct structures were observed in dried films of the pH 1 sample. TEM images (representative examples shown in Fig. S5, ESI†) consistently showed a population of sheet structures containing prominent lattices with square arrays 3 nm × 3 nm in size. This size corresponds approximately to the estimated length of an antiparallel \( \alpha \)-helical dimer,\textsuperscript{35} considering the closely packed leucine residues, but allowing for splaying of the longer terminal arginine residues.

We propose that this structure is a rotator-type phase, in which electrostatic repulsion of arginine residues is minimized by 90° rotations of helical dimers (Fig. S5c, ESI†), analogous, for example, to certain 2D spin lattice nanomagnet systems.\textsuperscript{36} We propose that this structure is favored in dried films due to confinement which leads to \( \alpha \)-helices parallel to the interface, whereas in bulk the helical dimers are able to orient perpendicular to the nanotube surfaces.

Additional studies were performed on solutions containing a higher concentration, 0.07 wt%, of R3L12. Fig. S6–S8 (ESI†) display cryo-TEM and fitted SAXS form factor data. Nanotube parameters obtained from the fitting to the SAXS curves and measures of the cryo-TEM images are listed in Tables S1 and S2 (ESI†). Cryo-TEM images at pH 4 show unwrapped nanotubes (Fig. S6, ESI†), coexisting with a population of short folded nanotubes (inset Fig. S6a, ESI†). Increasing the pH to 2 gives well defined long nanotubes (Fig. S7a–c, ESI†), and a tubular structure network is formed at pH 1 (Fig. S8, ESI†). These results are similar to those at the lower concentration 0.04 wt%, with the exception of the unwrapped nanotubes observed at pH 4. This points to the subtle interplay of electrostatic and hydrophobic interactions, modulated by peptide concentration.

Nanotube dimensions were obtained from cryo-TEM and SAXS (Fig. S7d, e, S8c, d and Tables S1 and S2, ESI†) and are generally very similar to those measured for 0.04 wt% R3L12 samples, as discussed above. Analysis of CD spectra (Fig. 1 and ESI† Fig. S1) indicates that there is a slight increase in \( \alpha \)-helical content and a stronger tendency to form coiled coil helices at 0.07 wt% compared to 0.04 wt% peptide. The stability of R3L12 in very acidic solution (pH 1) was investigated using state-of-the-art liquid AP-MALDI MS. The spectra shown in ESI† Fig. S9 show that no peptide degradation was detectable for the solutions at pH 4 or 1.

In summary, designed SLP R3L12 exhibits remarkable pH-dependent self-assembly of \( \alpha \)-helical peptide structures, including nanotubes and tubular network structures with molecular thickness arginine-coated walls based on opposed dimeric coiled coils. Our results contrast with observations for the related peptide K3L12 which forms bilayer discs, fibrils or vesicles depending on pH, resulting from self-assembly of \( \alpha \)-helical peptides.\textsuperscript{37} It is also distinct from the behavior of longer “block” polypeptides such as R60L20 which self-assembles to form vesicles.\textsuperscript{38} The self-assembly of SLP R3L12 can also be contrasted with conventional coiled coil peptides with heptad repeats which can be engineered to form extended coiled-coil assemblies, such as fibrils and nanotubes,\textsuperscript{34,39–42} since these generally contain arrays of helices.
parallel to the long axis, although radial arrangements have been reported, in particular in the recently discovered cross-α fibril structure. Peptide R_{L12} forms distinctive cross-α nanotube structures. As well as remarkable self-assembly properties, R_{L12} may have interesting bioactivities and functionalities (for example potential cell-penetrating, antimicrobial or biocatalytic activities) arising from the high-density arginine-coating of the nanotubes and tube networks.

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Conflicts of interest

There are no conflicts to declare.

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