Hierarchical Self-Organization from Cyclic Peptide

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Abstract

Our research group is implicated on the design and synthesis of supramolecular structures based on self-assembling cyclic peptides, particularly alpha,gamma-cyclic peptides (CPs). Most of these structures: dimer, nanotubes, fiber, etc, are based on beta-sheet type interactions. The CPs form structures that have allowed preparing a large variety of structures with applications in ion channels, electron and energy transfer process, molecular tweezers and so on. Here, we present the formation of spheres, hollow spheres, fibers, bars, etc. by a hierarchical supramolecular process from alpha,gamma-cyclic peptides with pyridine derivate ligands attached to one of its side chains, that additionally, are able to coordinate metals (Cu, Pd, etc.), entrap molecules, etc. which give to these structures a great potential for catalysis and drug delivery.

Keywords: Self-assembling Cyclic Peptides, Peptide Nanotubes, Hierarchical Self-Organization.

1. Introduction

Bottom-up strategies are powerful methods for the construction of nanometric materials. In this sense, self-assembling processes are one of the most relevant approaches in which small molecules arrange through non-covalent interactions in a specific manner to form well-ordered and thermodynamically most stable structures.[1] Thus, precisely designed small molecules equipped with complementary donor and acceptor groups (functional groups) under appropriate conditions interact one to each other through a variety of equilibria driven by the combination of several non covalent interactions to provide the most stable molecular construction. Furthermore, hierarchical processes, which start from small molecules that form discreet aggregates that further organize into larger materials, are one of the most appealing research topics nowadays.[2] For instance, control of framework morphology at the nanometer to micrometer scale is one of the current challenges in the design of devices for biomedical applications.[3, 4] A range of substances have been used as self-assembling building blocks, in this sense small peptides have emerged as powerful building blocks on the nanoscale because of their biocompability and ease to modify or modulate their shape and properties through the single amino acid exchange.

Here, we present our studies with small six-residues cyclic peptides (CPs) that were designed to form dimers by a self-assembling process,[5] but these specific structures continue into a self-organize process to produce a diversity of architectures, ranging from spheres to fibers. The cyclohexapeptides here reported differ one of each other exclusively on one substituent, a pyridine moiety that is attached to the cyclic peptide (CP) through the serine side chain and this control the structure shape.[6]

2. Materials and methods

2.1. Peptide Synthesis:

The peptide were prepared following a solution strategic previously described.[7]

2.2. Crystallization and images taking for the **CP3** and **CP5**:

A solution of peptide in 0.5 mL of $CHCl_3$ [3.0 mM (1.29 mg, 1.5 μmol) or 0.5 mM (0.22 mg, 0.25 μmol)] was placed in a 10 mL vial that contain a silicon wafer and the resulting solution was allowed to age for 36, 48 or 60 h at room temperature under a saturated atmosphere of hexanes in a closed vial. The silicon wafer was washed with ether and hexanes, air-dried and then used directly without coating for the SEM images.

2.3. Scanning electron microscopy:

The morphologies of the reported materials were investigated by mounting the silicon wafer on a SEM holder. The micrographs were taken either in a Field Emission Scanning Electron Microscopy (FESEM) ULTRA Plus-Zeiss (between 0.5 - 20 kV) or on Variable Pressure Scanning Electron Microscopy [VP-SEM)] EVO LS 15-Zeiss (20 kV at 10 Pa) using SE detector.

3. Results and discussion

We have design CPs decorated with phosphines or alkynes form in the presence of appropriated metals (i.e. palladium or gold) homo- and or heterodimers in which the ligand moieties were oriented as bidentate ligands.[8] Interested by those properties we realized CPs modified with pyridine moieties in the side chains to also act as metal ligands (Fig. 1). In addition, pyridine moieties have been shown to display a variety of supramolecular application guided not only by their coordination properties but also by their implication in hydrogen bonds, halogen bonds, or π-π stacking interactions (donor-acceptor). Thus carboxypyridines could easily be attached to cyclic peptides bearing a serine residue through an ester linkage. And the nitrogen of the

pyridine moiety could be oriented in *ortho*-, *meta*- or *para*- with respect to the anchoring carboxylic group allowing a variety of geometries that could fit the metal coordination sphere or other guesses.

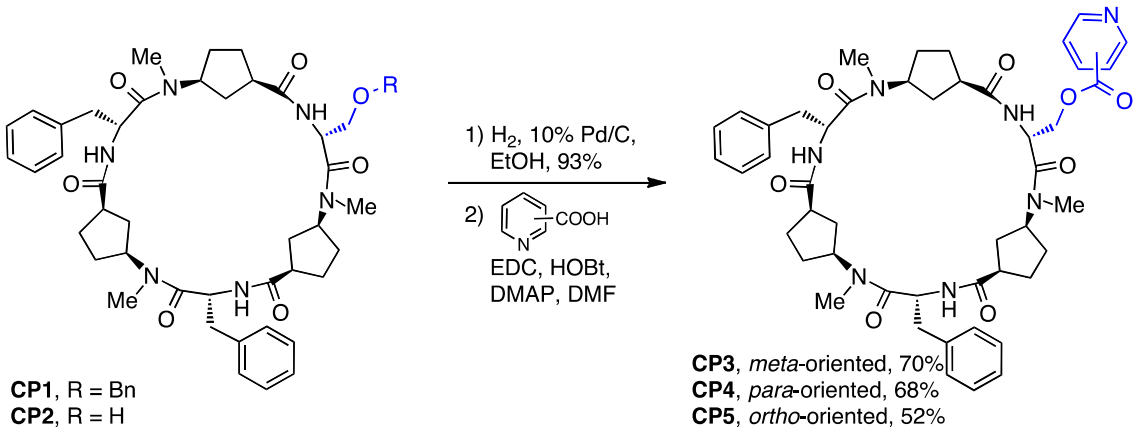

Fig. 1. Synthetic strategy used for the preparation of cyclic peptides **CP3**, **CP4** and **CP5**.

In our preliminary studies,[6] we synthetized the **CP3** (*meta-oriented*) (Fig. 1) and during the characterization process we found the formation of spherical structures though hierarchical process. We carry out several crystallization studies for the optimization formation: solvent, concentration, temperature and time, of these spherical structures. Scanning electron micrograph (SEM) confirmed the formation of solid spherical microstructures with diameters ranging from 10 to 40 μm (Fig. 2, Left). These spheres have a dense solid core of apparently packet needle-shaped crystals. The external surface of the spheres was covered by nanocrystals of 200-300 nm long. These structures has the potential to encapsulate dye (anthracene-9-aldehide and fluorescein) and liberate them, and they can be matallated (metallation) adding salt of Cu or Pd after the sphere formation.

Intrigued for these results, the **CP4** (*ortho-oriented*) and **CP5** (*para-oriented*) (Fig.1) were synthetized. The **CP5** was studied under the better crystallization conditions for **CP3**. **CP5** crystallized in a hierarchical process too giving bars/stick (Fig. 2 Center) that self-organize in

flowers shape structures and hollow spheres that we call “nests” (Fig. 2 Right), unfortunately the “nests” formation is random.

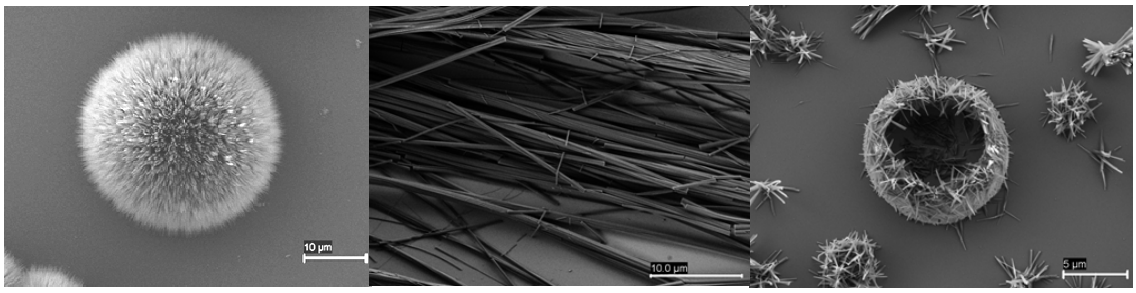

Fig.2. Left: SEM image of a silicon wafer bearing spheres obtained by incubation of a 3.0 mM solution of **CP3** in $CHCl_3$ under a hexane-saturated atmosphere at rt for 48 h. Center: SEM image of microcrystals obtained by equilibration of a 3.0 mM $CHCl_3$ solution of **CP5** in a hexane-saturated atmosphere at rt for 48 h. Right: SEM image from the **CP5** crystalized in $CHCl_3$ with hexane atmosphere (0.5 mM at rt. Age: 48 h).

4. Conclusions

In this work that is firstly structural, we show a set of structures like spheres, bars and nests made in a self-organize crystallization process from self-assembling CPs with a very small change in their structure. The preparation of these novel porous materials with different and well-defined properties and shape could be required for delivery, encapsulation or catalysis, among others.

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