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Self-Assembly of a Cyclobutane β-Tetrapeptide To Form Nanosized Structures

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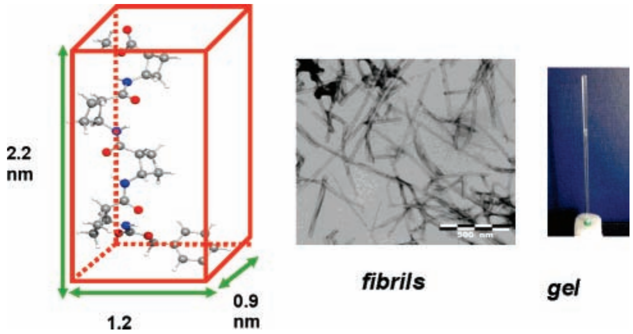
ABSTRACT



A β-tetrapeptide made up of homochiral cyclobutane residues displays conformational bias in solution prompted by the formation of intramolecular hydrogen bonds. Moreover, this compound self-assembles to produce nanosized fibrils and, in some media, it also forms a gel. The combination of NMR, TEM, AFM, and theoretical calculations has proven to be very useful in obtaining insight into the details of these new structures.

Self-assembly of biomolecules in general, and peptides and proteins in particular, underlies both life and disease. To inquire experimentally about the structural details of natural aggregates, amyloid aggregates, constitute one of the ways to increase our knowledge about these processes. An alternative approach is to study the behavior of artificial molecules, as for instance β-peptides, and try to correlate the structures with their observed self-assembling properties. Five- and six-membered carbocyclic rings have been used by authors such as Gellman[2] and Fülöp[3] to induce secondary structures in β-peptides. Nevertheless, data on the formation of tertiary structures by molecular self-assembly are more scarce although recently Fülop described the formation of nanosized fibrils and membranes from small β-peptides containing cyclopentane or cyclohexane rings.[4]

We have shown the ability of the cyclobutane unit to promote the formation of defined foldings in α-[5,6] and β-peptides[6,7] in solution. Herein, we report on the structures

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(1) See, for instance: Carulla, N.; Caddy, G. L.; Hall, D. R.; Zurdo, J.; Gairi, M.; Feliz, M.; Giralt, E.; Robinson, C. V.; Dobson, C. M. *Nature* **2005**, *436*, 554.

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of the cyclobutane β-tetramer **1** made up of homochiral cyclobutane residues. The study of this small peptide in solution revealed a very marked conformational bias prompted by the formation of intramolecular hydrogen bonds. Moreover, this compound self-assembles to produce nanosized fibrils and, in some media, it also forms a gel.

Compound **1** was prepared in 50% yield by coupling, under the usual conditions, of the conveniently protected precursor dimers obtained, in turn, from (1*R*,2*S*)-2-aminocyclobutane-carboxylic acid.[6c] A circular dichroism spectrum for this product, at different concentrations of methanol solutions (ca. 0.2−2.0 mM), presents a negative band around 200 nm, attributable to a nonpolar strand according to the literature data.[8] Changes in time were not observed either for the CD or for the IR spectrum, which showed one single band for the N−H stretching at 3310 cm^{-1} that is typical of associated N*H* protons.

A ^1H NMR study under liquid-state conditions corroborated this feature showing that central N*H* protons in the molecule are involved in the formation of intraresidue N−H−O═C hydrogen bonds, experimentally deduced from temperature coefficient values and with a very good agreement with the H−O distances obtained from theoretical calculations (Table 1). In addition, HH coupling constants and NOE connectivity allowed us to secure a trans stereochemistry for all amide bonds in the major conformation, as previously reported for similar molecules.[7b,c] The rigid and well-defined strand-type conformation of compound **1** is further confirmed from the strong interresidual NOE contacts observed between Hα(*i*) and NH(*i*+1) protons, as depicted in Figure 1.

Table 1. Chemical Shifts, Coupling Constants, Interresidual NOEs, Temperature Coefficients, and Hydrogen Bond Lengths for the N*H* Protons of the Predominant Conformer of **1**

NH^a	δ^b [ppm]	J_{HH} [Hz]	NOE (length^c)	$\Delta\delta/\Delta T^d$ [ppb/K]	hydrogen bond (length^c)
H_{10}	5.96	9.9		−1.8	O_{15} (2.11)
H_{16}	6.70	11.3	H_{14} (2.25)	−0.7	O_{21} (1.92)
H_{22}	6.70	11.3	H_{20}	−1.5	O_{27} (2.07)
H_{28}	6.51	8.5	H_{26} (2.23)	−2.2	O_{34} (2.32)

^{a} See Figure 1 for atom numeration. ^{b} In $CDCl_3$ at 298 K. ^{c} Internuclear distance by theoretical calculations, in Å. ^{d} In $CDCl_3$

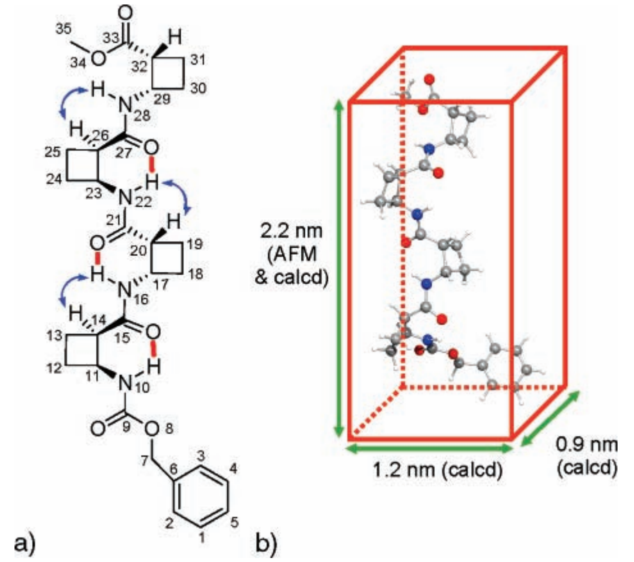

Figure 1. (a) Structure of tetrapeptide **1** where atom numeration, significant NOEs (arrows in blue), and hydrogen bonds (in red) are defined from NMR data. (b) Major conformation for **1** obtained by DFT theoretical calculations in solution showing calculated and AFM (height) molecular dimensions.

A conformational search afforded five representative structures, which were optimized at the B3LYP/6-31(d,p) level of calculation in chloroform solution. The structure of the most stable conformer is shown in Figure 1b and presents intramolecular hydrogen bonds involving NH and O═C groups bonded to the same cyclobutane ring, with the only exception of NH_{28}, which is closer to O_{34} than to the carbonyl oxygen. This conformer is 5.8 kcal mol^{-1} lower in Gibbs energy than the second most stable one. Therefore, it must be by far the most populated one at room temperature.

These results show that β-tetrapeptide **1** tends to adopt a strand-mimicking secondary structure in solution following trends similar to those observed in smaller cyclobutane β-peptides previously synthesized in our laboratory, in excellent agreement with the experimental findings.

Otherwise, transmission electron microscopy (TEM) images show ribbon-like fibrils after incubation of a 1 mM

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solution of **1** in MeOH at room temperature for 1 day (Figure 2a). The length of the fibrils is in the micrometer range and their width is uniformly in the range 23−25 nm.

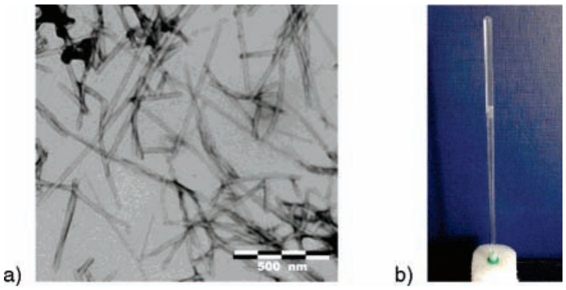

Figure 2. (a) TEM image of the fibrils formed by **1** from a 1 mM solution in MeOH (1 day incubation) placed onto a carbon-film coated copper grid and stained with 2% uranyl acetate. (b) Gel formed from a 3:1 acetone−hexane 1 mM solution.

After incubation for longer periods of time, fibrils became longer but their width was kept constant. NMR spectra of freshly prepared CD_3OD or $CDCl_3$ solutions, recorded under liquid-state conditions, were super imposable to those resulting from solutions incubated for 24 h showing that fibrils coexist with molecular species.[9]

The self-assembly of molecules of **1** was also observed by atomic force microscopy (AFM) (Figure 3). This technique allowed us to determine the molecule height for **1**. The surface aggregates height for the tetrapeptide deposited onto a mica substrate from a fresh 50 μM diluted solution in MeOH, in the range 2.1−2.3 nm, is in very good accordance with the calculated parameter in the conformation displayed in Figure 1b. The absence of preferred aggregation directions suggests that the surface aggregates could be formed during the drying stage.[10]

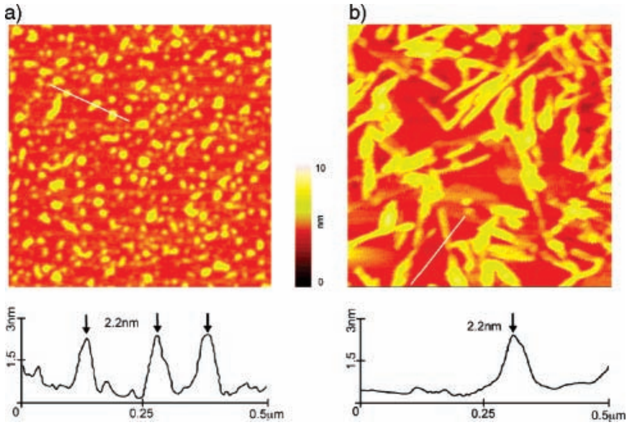

Figure 3. (a) A (1.5 × 1.5) μm^2 tapping mode AFM image of the tetrapeptide molecule deposited onto a freshly cleaved mica substrate from a fresh 50 μM diluted solution in MeOH. A topographic profile on the same image shows a constant surface aggregates height of 2.2(±0.1) nm. (b) A (1.5 × 1.5) μm^2 tapping mode AFM image of the tetrapeptide molecule deposited onto a freshly cleaved mica substrate from a 24 h old solution (1 mM in MeOH). Focusing at the lowest aggregates topographic profile shows a height of 2.1(±0.1) nm.

At higher concentrations and longer incubation times, fibrils with a similar shape to those observed by TEM are formed. Different heights were observed, probably due to piling of individual fibrils at the high concentration. Nevertheless, the measured height for the lowest aggregates (2.1 nm, Figure 3b) is in good agreement with the value observed from diluted solutions (Figure 3a). The observation of equivalent structures by both AFM and TEM techniques in spite of the use of two different substrates (graphite and mica) strongly supports that fibrillar aggregates were already present in the solution coexisting with dissolved molecular species.[11,12]

Moreover, an organogel, which was stable for several days, was formed when **1** was dissolved in 3:2 ethyl acetate−hexane (1 mM) by heating to boiling, then air cooled and left to stand. A similar result was obtained from 3:1 acetone−hexane 1 mM solution but the gel was less stable in this medium and precipitated by standing for 1 day both at room temperature and at 5 °C (Figure 2b). Evidence for the formation of this gel was provided by the ^{1}H NMR spectrum where broad signals were observed due to the increase of viscosity in the gel state (see Figure S7 in the Supporting Information).

In conclusion, cyclobutane β-peptides have been shown to combine rigidity and amphipaticity in order to provide different ways of self-assembling. The combination of solution NMR, TEM, AFM, and computational methods has proven to be very useful in obtaining insight into the details of these new organic nanosized structures. Further investigations addressed to establish the molecular interactions implied in the self-assembly process are in progress in our laboratories.

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Supporting Information Available: Synthesis and fully characterization (NMR spectra and theoretical studies) of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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