Supramolecular gel formation and self-correction induced by aggregation-driven conformational changes†

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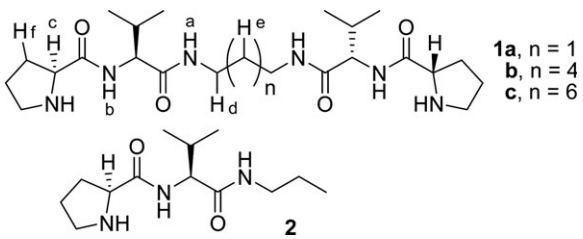
The formation of self-assembled fibrillar networks by low molecular weight peptidomimetics containing a Pro-Val moiety is reported; insight into the aggregation mechanism is provided revealing that it is associated to an unfolding process and that a fibrillar network formed under kinetic control can self-correct into a thermodynamically stable one.

Conformational preferences of the amino acids determine peptide secondary and tertiary structure and play an important role in protein function. For example, it is well reported that misfolding of the peptidic chain may produce unwanted processes that provoke fatal diseases. One of these problematic processes could be peptide assembly or aggregation. For instance, amyloid proteins may suffer conformational changes during folding processes leading to intermediate misfolded structures that can evolve into amyloid assemblies. These assemblies may precipitate as plaques over important parts of the body such as neural tissues and produce the so-called amyloidogenic diseases.[1,2] In general, aggregation of proteins may have a dramatic effect on their function (structural, catalytic or as a carrier). This has lead to an increasing interest in the study of low molecular weight peptidomimetics in order to understand those processes and design active inhibitors for them.[3]

In the last years, our research has been focused in the study of the self-assembly of small peptidomimetic compounds. In this sense, we have studied bolaamphiphilic compounds capable of assemble into fibrillar networks and gels in different solvents.[4] Here we report on the study of peptidomimetics **1a–c** containing L-Pro residues. A bolaamphiphilic L-Val scaffold has been used because we and others had shown before that this is an effective fragment for the construction of fibrillar aggregates.[5] L-Pro residues were introduced for their future application as functional materials. It is well known that L-Pro introduces specific conformational demands due to the presence of the heterocyclic moiety in alpha position to the peptidic bond. For instance, it appears involved in many exotic secondary structure fragments such as polyproline I and II helices or triple helix collagen structures where it is responsible of the formation of turns in the backbone crucial for the final structure.[6] The current study revealed that peptidomimetic compounds **1a–c** are involved in an intriguing folding *vs.* aggregation behavior reminiscent of proteins.

Compounds **1a–c** (Scheme 1) were prepared in good yields by conventional solution peptide synthesis and fully characterized. These compounds showed a high tendency to aggregate in several organic solvents. The aspect of the aggregates was observed either macroscopically (gels or crystalline precipitates) as well as at the microscopic level by SEM (see Fig. 1 and ESI†). All the xerogels revealed the presence of an entangled fibrillar network characteristic of organogels.[7]

The aggregation of compounds **1a–c** in CH_3CN was followed by CD spectroscopy. Samples were prepared by gentle heating of acetonitrile and the solid gelator until complete dissolution. Then spontaneous cooling at 25 °C took place in a few minutes. In the case of compound **1a** upon increasing the concentration of the gelator from 0.6 mM to 30 mM important changes in shape, position and intensity of the bands were found (Fig. 2A). These changes can be associated to the aggregation that results in the formation of a supramolecular gel at concentrations near to 30 mM (minimum concentration for gel formation is 34 mM). Strikingly, very significant differences were observed in the CD spectrum of **1a** if a slow, controlled cooling protocol was used for samples at concentrations close or above 30 mM. In this case, above 40 °C the CD spectrum was basically identical to that obtained by spontaneous cooling. However, at 35 °C a new band appeared at 210 nm that increased in intensity with time (Fig. 2B). Indeed, a strong increase of $\Delta\varepsilon$ for this band was observed after *ca.* 1.5 h together with the formation of a gel. These findings suggested that slow conformational changes were taking place in these samples. It can be reasoned that under *spontaneous* cooling conditions metastable aggregates would be kinetically trapped in the gels whereas *slow*, controlled cooling allowed the evolution into aggregates with increased thermodynamic stability. This difference, depending on the cooling protocol, is also evident in the size and aspect of the materials at the microscopic level (Fig. 1). Xerogels obtained from kinetically trapped acetonitrile gels show larger fibres, whereas those derived from thermocontrolled gels



Scheme 1

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† Electronic supplementary information (ESI) available: Synthesis and characterization of compounds, NMR spectra and additional graphs, SEM pictures and WAXD diffractograms. See DOI: 10.1039/b816234d

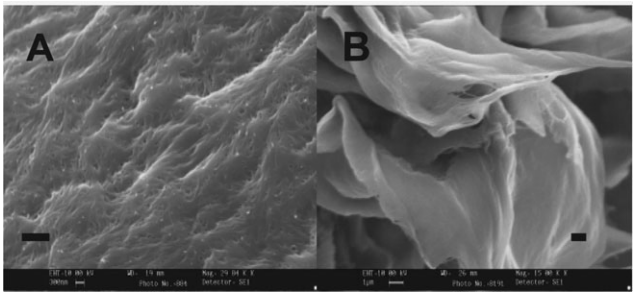


Fig. 1 SEM images of xerogels of compound **1a**: (A) CH_3CN, *spontaneous* cooling, (B) CH_3CN, *slow* cooling; Bars 1 μm.

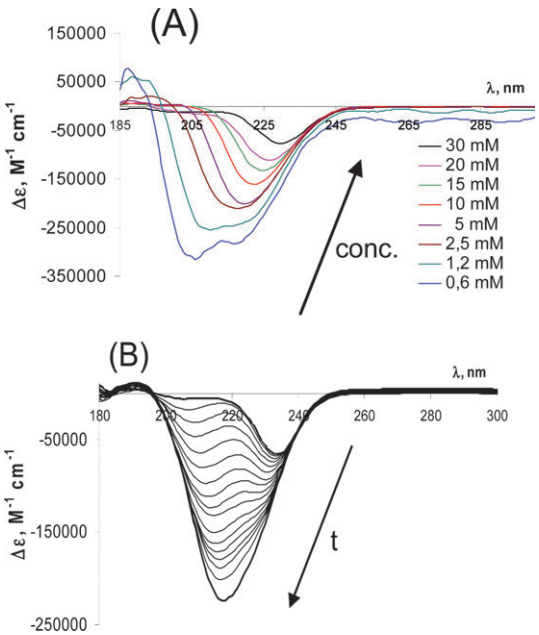


Fig. 2 (A) CD spectra of compound **1a** at different concentrations in CH_3CN under *spontaneous* cooling. (B) Evolution of CD spectrum of **1a** (30 mM) in CH_3CN at 35 °C after *slow* cooling (step 5 min).

reveal a network of thinner fibrils that form a cloth-like high surface material.

^{1}H NMR was used to investigate the structure of these compounds in solution and in the gels. It was envisaged that, as previously reported for other analogues, the presence of a flexible alkyl spacer and several H-bonding groups may allow intramolecular folding in solution.[5a] Analogue **2** was prepared for comparison and amide NH signals (*a*, *b* in Scheme 1) were taken as probes. A relevant finding was that amide signal *b*, appearing at 8.1 ppm was quite insensitive to concentration, temperature and solvent polarity for all the compounds suggesting that even at low concentrations this proton is involved in a strong intramolecular H-bond with Pro N^{α} lone pair.[8] On the other hand, amide signal *a* that appeared at 6.6 ppm for compound **2** was shifted downfield in diluted samples of bola analogues (**1a**, 7 ppm, **1b**, 6.7 ppm and **1c**, 6.7 ppm). These results indicate that amide protons *a* are most likely involved in intramolecular H-bonding and that this effect particularly strong for compound **1a** which presents the shortest alkyl spacer. NOE experiments revealed the spatial proximity of proton *a* to *c* and *f*, and proton *b* to

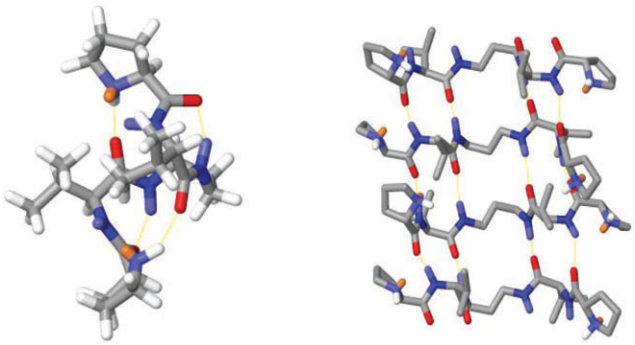
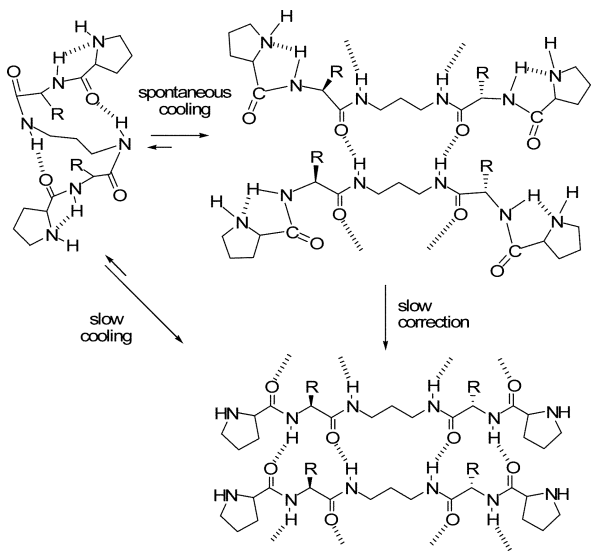


Fig. 3 Models of compound **1a** in solution (left) and in the gel (right) obtained with MACROMODEL 8.0, AMBER* force field.

d and *e* supporting the folded structure of compounds **1a–c** in diluted solution. Additionally, CD spectra of a 0.6 mM solution of **1a** showed the typical shape of an helix with two negative bands at 217 and 205 nm and a positive lobe at *ca.* 187 nm. A possible model obtained by molecular mechanics that agrees with these evidences is shown in Fig. 3 (see ESI†).

Concentration dependent ^{1}H NMR of compounds **1a–c** before gel formation under *spontaneous* conditions showed a downfield shift of amide NH a evidencing its participation in aggregation by intermolecular H-bonding. On the other hand the chemical shift of the amide signal *b* did not change in that range of concentrations. These results indicate that the initial stages of *spontaneous* aggregation are associated to the rupture of some the intramolecular H-bonds of the folded conformations (Scheme 2). This unfolding mechanism was confirmed by transfer-NOE experiments with the formed gels. This experiment, widely used in protein science and only recently applied by us in the field of supramolecular gels, gave information about the structure of **1a–c** in the aggregated state.[9] The comparison of the NOE correlations obtained for gel and diluted samples confirmed the partial unfolding of the molecule upon the aggregation that takes place upon spontaneous cooling of concentrated samples of gelator (see ESI†). Indeed, the unfolding process was also evidenced by CD where the



Scheme 2

helical band found in diluted solution suffered a red shift until 235 nm associated with the conformational change and aggregation.

The observed evolution of the CD of concentrated mixtures upon *slow* cooling suggested that an additional slow conformational reorganization was taking place yielding a thermostable gel (Scheme 2).‡ The higher degree of organization in this gel is clearly supported by several facts. First, the WAXD pattern of the xerogels obtained by slow cooling showed sharp reflections denoting a high degree of crystallinity in the fibers whereas that of the xerogel obtained after spontaneous cooling gelation only showed a broad signal at 4.5 Å and a background of an amorphous material, in accordance with a loosely defined aggregation as a result of the reduced number on intermolecular interactions. This peak is typical for intermolecular distance in the H-bonding direction (see Fig. 3 and ESI†) as described for analogues.[4a] Secondly, a kinetic study of the aggregation under controlled cooling conditions at 35 °C revealed an activation free energy for the reorganization process of 28 kJ mol^{-1} in agreement with the rupture of several hydrogen bonds. Finally, the solubility of the gel formed by **1a** under kinetic conditions determined by ^{1}H NMR was 12 mM whereas for the thermodynamic gel was 8 mM reflecting stronger intermolecular interactions and increased gelation ability in the latter case.[10] These properties are related to the higher thermal stability measured for the gels obtained by slow cooling (T_{gel} = 75 °C) as compared to the kinetic gels (T_{gel} = 55 °C).

Very interestingly, a kinetically trapped gel was able to self-correct when it was kept for 48 h at 30 °C being converted into a gel with properties similar to those of the material obtained by *slow* cooling (solubility and WAXD pattern of the xerogel).

Some differences were observed in the aggregation of compounds **1b** and **1c** as compared to **1a**. Firstly, *spontaneous* cooling had to be employed in order to obtain gels whereas *slow* cooling produced crystalline precipitates. On the other hand, CD investigation of the aggregation process did not reveal a strong influence of the cooling methodology as described for compound **1a**. Finally, WAXD of xerogels and precipitates were identical (see ESI). Altogether these results suggested that in these two compounds the energetic barrier for conformational changes is much lower than for compound **1a** probably related to the higher flexibility of the alkyl spacer that, as already mentioned, led to weaker intramolecular H-bonds and in consequence to lower stability of their folded conformations. Nevertheless, transfer-NOE experiments on those gels supported the partial unfolding of the molecule as a general mechanism.

In summary, we have presented an example of small peptidomimetics that evidence the paramount importance of folding and aggregation even for *a priori* simple molecules. We have shown that very subtle structural and environmental changes may have a dramatic effect on supramolecular aggregation. We have also proved that, upon overcoming the required energy barrier these reversible supramolecular systems are capable of self-correction to produce a more stable and organised material. Current work is being done with the aim of controlling the switching between soluble and aggregated states.

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Notes and references

‡ CD experiments of gels formed by fast cooling at different temperatures (10, 0, −20 °C) confirmed the strong influence of cooling rate and allowed the observation of kinetically trapped intermediate partially unfolded conformations.

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