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Vibrational Circular Dichroism Shows Reversible Helical Handedness Switching in Peptidomimetic L-Valine Fibrils

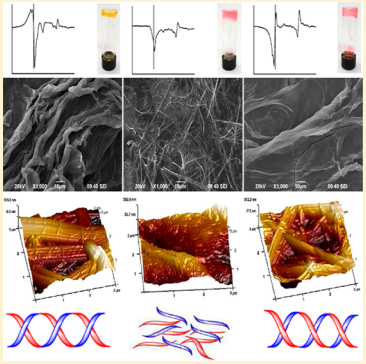
Belén Nieto-Ortega,[†] Vicent J. Nebot,[‡] Juan F. Miravet,[‡] Beatriu Escuder,[‡] Juan T. López Navarrete,[†] Juan Casado,[†] and Francisco J. Ramírez*[,†]

[†]Departamento de Química Física, Facultad de Ciencias, Universidad de Málaga, Málaga 29071, Spain

[‡]Department de Química Inorgànica I Orgànica, Universitat Jaume I, Castellón 12071, Spain

Supporting Information

ABSTRACT: We elucidate the supramolecular organization in the form of microsize fibrils of gels formed by a L-Valine peptidomimetic compound. Analysis was based on circular dichroism spectroscopies, vibrational (VCD) and electronic (CD), supported by microscopy (atomic force and scanning electron). We show how the VCD spectra give account of the micrometric structure of the fibrils formed by the helicoidal arrangement of simpler proto-fibrils, which are organized in a lower hierarchical level. This ability is used to monitorize a fully reversible change in the handedness of the helix by modulating different external stimuli as pH or ionic strength, thus providing the first observation by VCD of such a phenomenon in a short peptide.



SECTION: Glasses, Colloids, Polymers, and Soft Matter

Intermolecular forces drive supramolecular organization of the matter around us, and ultimately originate many of the most relevant physical and chemical phenomena in Nature.[1] This is particularly true in the biological world, where the activity of proteins, carbohydrates, and nucleic acids is the final result of the tridimensional folding and self-assembly of their respective molecular building blocks. Protein fibrillation is a paradigmatic and enigmatic case of supramolecular organization. Almost all peptides and proteins can in fact organize into fibrils; however, their presence and accumulation in living organisms is a sign of disorder and regulatory alterations. A known example is the amyloid fibrils based on cross-β packing of peptides, which have been related with Alzheimer's, Type II diabetes, or spongiform encephalopathy, among others fatal diseases.[2,3] On the positive side, the biotechnological application of protein fibrils is appearing as a promising research field.[4,5]

The first stage in the formation of fibrils consists of peptide folding into protofibrils, which evolve by helical interlinking into bundles or fibrils.[6] The predominant secondary structure of fibrils is β-sheet, although fibrils containing α-helix have also been characterized.[7−9] Evidence has been achieved that fibrilation can occur with short peptide sequences.[10] The chemical composition of the constituting units is also a determinant factor in the process of fibrillation.[6,11,12] For instance, it has been reported that the presence of hydrophobic side-chains stimulates the formation of fibrils.[13,14]

Under certain conditions, the fibrillation process can be mimicked in laboratory experiments by gelation of peptides in water.[7] The properties of fibrilloid hydrogels are highly dependent on the physicochemical conditions, such as pH, ionic strength, concentration, or temperature.[15−17] Hydrogels are therefore suitable biomaterials to insightfully simulate the factors that influence the formation, structure, and stability of fibrils when used as an appropriate peptide-like or peptidemimetic building block.

Gelation processes of small bolaamphiphilic molecules have recently received intense attention.[18−22] Synthetic amphiphilic peptides, built by linking hydrophilic amino acids functionalized with hydrophobic residues, have been designed to form fibrils for biomaterials applications.[23,24] It has been demonstrated that hydrophobic interactions in water solutions, together with hydrogen bonding, play an important role in the robustness of these "artificial" fibrils.[25] A prospective aspect regarding the structural stability of the fibrils is the easiness for the α-helix ↔ β-sheet interconversion as induced by external conditions (pH, temperature, concentration, etc.), a feature mainly described on the basis of CD spectroscopy as the almost exclusive tool of choice.[26] Interestingly, a recent study has demonstrated that α-helix-rich oligomers play a central role in amyloid nucleation, which produces fibrils retaining a very important element of α-helix in their structure.[27] In summary, and in spite of its relevance, there are still many questions about how the

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fibrillation process occurs, about their supramolecular structure, and their stability and/or capacity of transformation.

The supramolecular arrangements in biological systems structures are often chiral. The control of supramolecular or second-order chirality (to differentiate from intrinsic chirality) is therefore central to establish reliable structure−activity interconnections. The chiral properties of macromolecules can be reached by using complementary techniques such as X-ray crystallography, electron and atomic force microscopies, or CD spectroscopy. However, in most of the cases, the result is still insufficient. In this scenario, VCD spectroscopy combines the high sensivity of the dichroic techniques to monitor the self-assembly of chiral structures with the molecular level information provided by infrared spectroscopy. It has been recently proved how protein fibrillation in solution produces enhancements of the VCD signal by 1−2 orders of magnitude, which are ascribed to the extremely high sensitivity of collective molecular vibrations to the high regime of organization promoted by long-range supramolecular organization in chiral systems.[30,31]

The present work focuses in the case of a peptidomimetic compound, **Pval3** in Figure 1. This molecule is formed by the

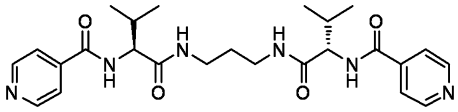

Figure 1. Molecular structure of the bolaamphiphilic **Pval3**.

union, through a trimethylene bridge, of two L-Valine (L-Val) amino acids attached to pyridyl moieties. The bolaamphiphilic L-Val scaffold has been shown to be effective to form fibrillar aggregates in several solvents.[21,22,28,29] In this work, the structural organization into fibrils of **Pval3** gels has been followed by chiro-optical spectroscopies (CD and VCD) and microscopy (SEM, AFM). We will attempt to modify the structure of the fibrils by external factors and evaluate the impact and extent on the macromolecular modification. We will show how VCD spectroscopy delineates a fully reversible structural chirality switch in peptide-mimetic fibrils formed in hydrogels of **Pval3**.

Figure 2 shows the chiro-optical spectra of the **Pval3** hydrogels, in D_2O, formed at three different pD values and at a fixed concentration 18 mM. All the spectra were obtained using the same sample, in which VCD and CD were consecutively recorded without removing the samples from the spectroscopic cell (a demountable cell for liquids provided with CaF_2 windows; see the Supporting Information for detailed experimental settings). The initial pD at which the hydrogel was formed (pD = 6 in deuterium oxide) was lowered to pD = 2 by direct addition of deuterated hydrochloride acid. The pD cycle was finished by restoring the initial, close to neutral pD, by addition of deuterated sodium hydroxide. The structural reversibility of the process was confirmed by exactly recovering the VCD spectrum of the initial hydrogel. FTIR spectra were also simultaneously recorded, which displayed identical patterns before and after pD changes (Supporting Information).

The VCD spectra at pD = 6.0 and pD = 2.0 show band sign inversion, almost mirror images, in the region 1800−1500 cm^{-1}. The strong bisignate band around 1640 cm^{-1} is assigned to the Amide I mode, which is largely a combination of the C═O stretching vibrations. The band at 1554 cm^{-1} is the Amide II

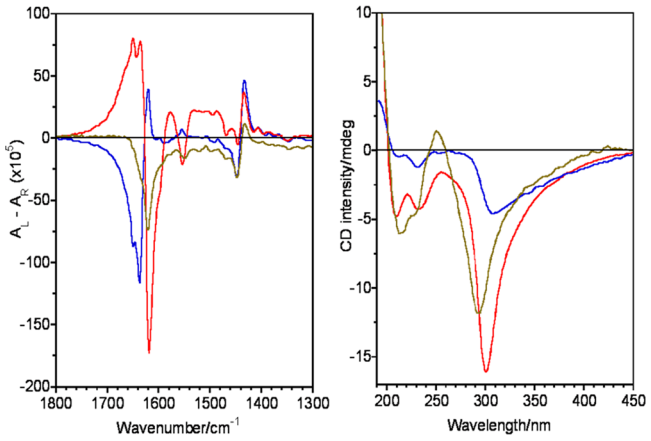

Figure 2. VCD (left panel) and CD (right panel) spectra of **Pval3**, as fibrillar hydrogels, at pD 6.0 (red lines), 2.3 (brown lines), and 2.0 (blue lines). All the spectra were obtained from one hydrogel formed in pure deuterium oxide at a concentration 18 mM. The pD of the hydrogel was changed by direct additions of deuterated hydrochloride acid. Spectra were recorded after 10−20 min after stabilization.

mode, which is mainly composed of N−H bending modes coupled with C−N stretching vibrations. The structural information contained in this spectral VCD behavior is twofold: (i) The rather low frequency position of the Amide I mode indicates the presence of strong intermolecular C═O···H−N hydrogen bonds; and (ii) there is a supramolecular organization in the network of these intermolecular bonds that makes their VCD spectra (i.e., the C═O and N−H motions) highly sensitive to it, a chiral superstructure that produces the mirror-like VCD response with lowering pD. This point is a fundamental structural−spectroscopic relationship that unequivocally links the VCD effect with the existence of long-range well-defined supramolecular organizations. The bisignate band centered at 1442 cm^{-1} could be assigned to either pyridine C−H bending vibrations or methylene scissoring modes.[32,33] Significantly, inversion for this feature is not observed over the whole pD range, although the bands do receive VCD enhancement. This result suggests that these moieties are not involved in the supramolecular organization, which is consistent with their location far from the groups responsible of the long-range ordering.

The VCD spectrum at pD = 2.3 seems to correspond with an intermediate state in which the hierarchical organization responsible for the VCD mirror-like behavior is seemingly absent. At this stage, the gel becomes a little more fluid, although its rheological consistency is recovered at pD = 2.0. At pD below 2.0, the sample turns out to be a highly fluidic solution, and its VCD spectrum is flat (Supporting Information), reinforcing further the supramolecular origin of the VCD signal in the hydrogels. All these experiments succeeded with starting 18 mM solutions, while experimentation in more dilute solution gave insufficient VCD signal. This reveals further the supramolecular origin of the VCD spectra and its modulation with the pD.

Every CD spectra of Figure 2 was recorded just after its corresponding VCD spectrum. The CD spectrum at pD = 6.0 shows the typical two-band pattern of the α-helix secondary structure of the peptide-mimetic hydrogelator with bands at 210 and 232 nm. A strong negative feature at 300 nm was assigned to an n→π^* transition within the pyridine rings. They were measured at 212, 231, and 307 nm at pD 2.0, although the

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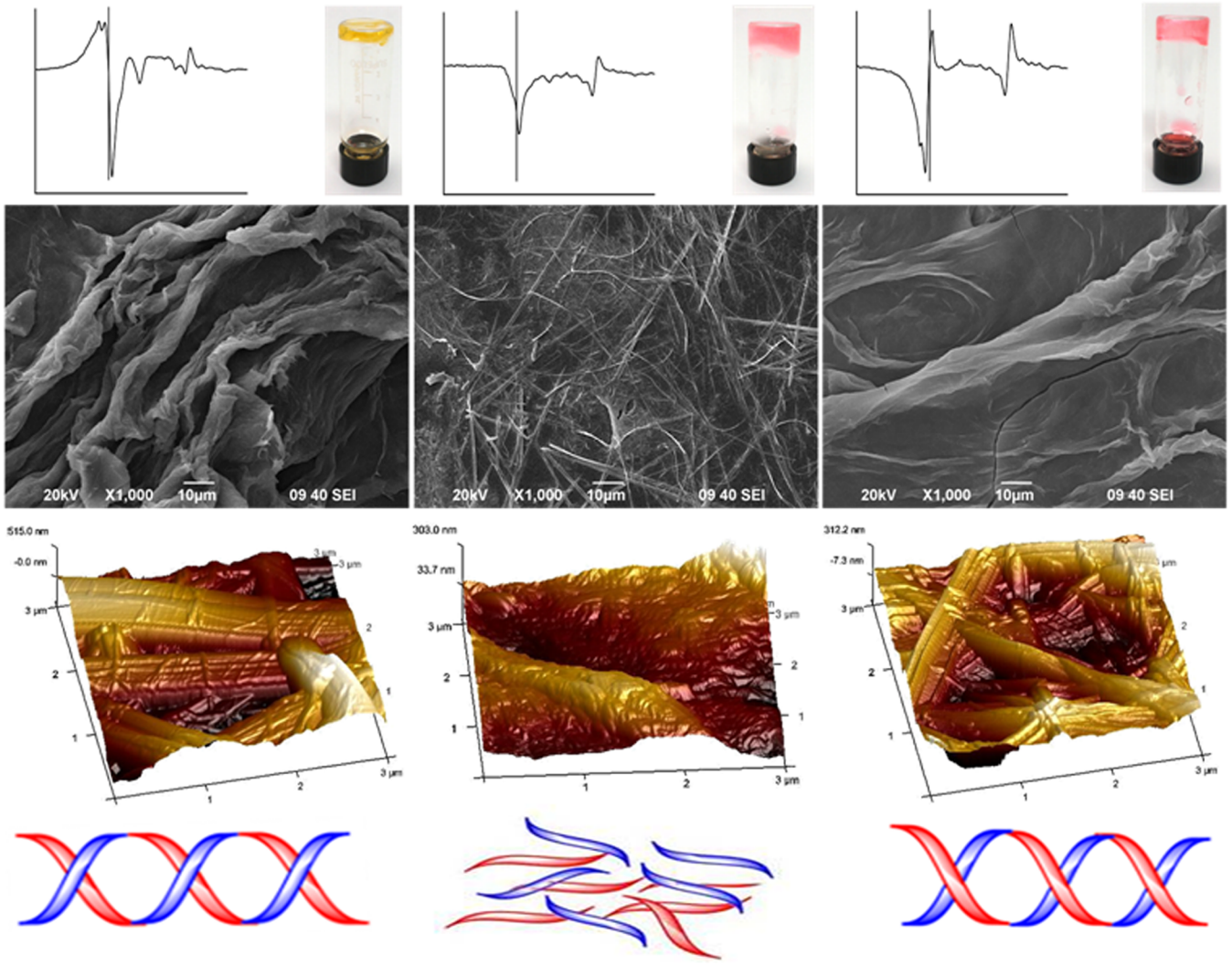

Figure 3. SEM and AFM images of **Pval3**, as fibrillar hydrogels, at pD 6.0 (left), 2.3 (middle), and 2.0 (right). The VCD spectrum and a picture of the hydrogel are shown over each image. The gel color is due to methyl orange, which turns in the pD range 3.1−4.4. The effect of pD on the fibril folding is represented below.

whole spectrum exhibits intensity lowering together with an intensity reversal of the 200 and 250 nm bands. The CD spectrum at pD = 2.3 shows slight differences. The secondary structure seems to be preserved, since neither β-sheet nor random-coil marker bands are detected. However, we would like to point out that a reported marker band is the optical response of a secondary structure formed by a great number of amino acids, which is not our case. In this sense, although the CD signal is consistent with an α-helix, β-sheet or random coil chains could be also present. In any case, a slight change in the secondary structure is required to accommodate to the supramolecular transition supported by the VCD spectra. It is worth mentioning that the sign of the intense pyridine absorption does not change with pD, contrarily to that reported for self-assembled nanofibers of pyridine containing L-glutamic lipids.[34] Nevertheless, a slight blue-shift is observed at pD = 2.3 as a consequence of the mandatory unfolding prior to the fibril winding reversal.

SEM and AFM images were obtained for the same hydrogels after suitable preparation (see details in Supporting Information). The images are displayed in Figure 3 as a function of the pD. These pictures monitor, at a microscopic level, the supramolecular transition inferred by VCD. It is worth taking into account that the stability of the hydrogels up to pD = 2.3 is in agreement with the pK_a of 2.9 calculated for the two pyridine units of **PVal3**, which implies that at this pD 25% of the pyridine rings remains still unprotonated. Two different morphologies are clearly distinguished. The central SEM image at pD = 2.3 shows an agglomeration of scattered thin fibrils. In the left and right images, at pD = 6.0 and pD = 2.0 respectively, these fibrils (hereafter protofibrils) are organized at a higher hierarchical level, likely, in mature fibrils of micrometer size showing signs of helicity generated by the interlinking of the protofibrils unorganized at pD = 2.3. Interestingly at the microscopic level, this supramolecular organization is only exhibited in the hydrogels with enhanced mirror-like VCD spectra. This establishes a connection between the origin of the VCD signal and the micrometric structure of the peptide-mimetic hydrogelator. Assuming a helicoidal arrangement in the fibrils, the mirror-like behavior of the peptidic infrared VCD bands with variable pD must be interpreted as a reversal of the helix winding in the fibrils from pD = 6 to pD = 2 going through a intermediate stage with a critical concentration of D^+ that completely destroys the

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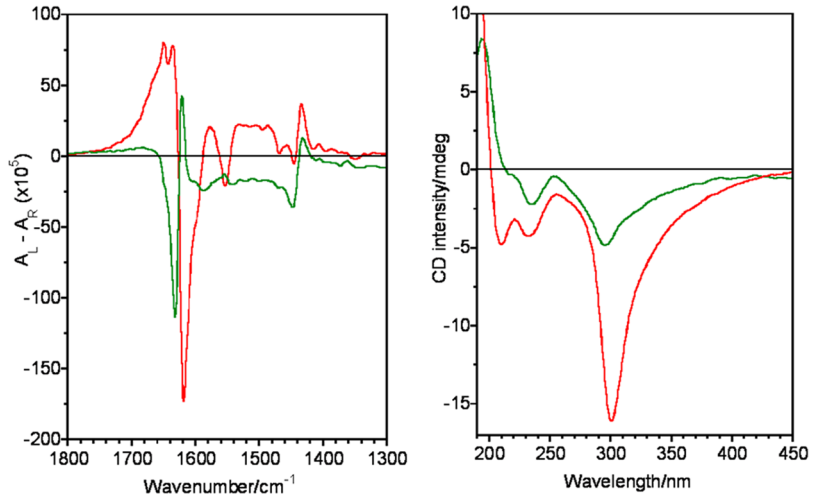

Figure 4. VCD (left panel) and CD (right panel) spectra of **Pval3**, as fibrillar hydrogels, in pure deuterium oxide (red lines) and after addition of sodium chloride until reaching an ionic strength of 0.002 M (green lines).

fibrils. Interestingly, pD reversal allows the recovering of the gel properties highlighting the reversibility of the structural modification with the pD.

Inversion of helical structures is a known phenomenon in the biological world. In 1972, Pohl and Jovin described an inversion of the CD spectra of a synthetic poly(dG-dC) under a high saline concentration,[35] ascribed to a double helix right-to-left handed structural modification. This leads us to further test the modulation of the supramolecular structure of our peptide-mimetic hydrogelator with the ionic strength by VCD spectroscopy. Figure 4 displays VCD and CD spectra of hydrogels of **Pval3** in deuterium oxide before and after addition of sodium chloride up to an ionic strength of I = 0.002 M. This spectrum mimics that with pD = 2.0 in Figure 2. The most outstanding fact, again, is the inversion of the three-peptide vibrational VCD bands in the region 1800−1500 cm^{-1} or mirror-like behavior. Similarly to the experiments changing the pD, the CD spectra do not show noticeable changes. As a consequence, a change in the sense of the fibril winding with the increase of the ionic strength can be invoked again. As in the case of pD, the FTIR spectra did not exhibit appreciable changes when the ionic strength is increased (Supporting Information) further emphasizing the sensitivity of the VCD signal to the long-range organization and its collective character. A subtle difference between the pD and ionic strength fibril winding reversal concerns its kinetics, which is now under investigation.

The complete self-assembly process of a low molecular mass hydrogelator, **Pval3**, has been scrutinized by vibrational circular dichroism. **Pval3** is a peptide-mimetic compound that enables one to simulate in vitro the self-organization properties of artificial proteins and its dependence with the physicochemical conditions. In particular, we have studied how **Pval3** self-assembles by interlinking of many single chain α-helix into protofibrils and mature fibrils of micrometric size. Fibrils likely result in the highest levels of hierarchical organization giving rise to a long-range ordering consisting of a fine helical winding of substructures. We have found that these supramolecular structures modulate their helical disposition in response to external pD and ionic strength stimuli, in a reversible way, which supposes a relevant connection between biomimetic material structure (morphology) and easy controllable chemical inputs. Thus, we achieve the first observation of enhancement of the VCD signal produced by a long-range helicoidal pattern of a short peptide.

Protein fibrillation is of considerable interest, and many of the details of its process are still unknown. This is in part due to the absence of structural techniques able to give accurate account of it in different conditions. In this paper, we show a nice example of the versatility of the VCD technique to provide precise information of the organization of a peptide-mimetic hydrogelator in a variety of physicochemical situations. Due to the consistency of the VCD data obtained, it is possible to recognize how the fibril structure modulates its form, sometime offering the same structural response, to different external stimuli, such as the pD and the ionic strength. The VCD technique is able to insightfully account for the micrometric organization of biomaterials due to its extremely high sensitivity to coherent collective long-range molecular motions in helicoidal dispositions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

FTIR and VCD spectra, enlarged SEM and AFM images, and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ramirez@uma.es.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

VCD, vibrational circular dichroism; CD, electronic circular dichroism; FTIR, Fourier transform infrared; SEM, scanning electron microscopy; AFM, atomic force microscopy

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