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Influence of the Architecture on the Molecular Mobility of Synthetic Fragments Inspired from Human Tropoelastin

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ABSTRACT
This work deals with the vibrational, thermal and dielectric characterization of a synthetic peptide (S4) released during the proteolysis of human tropoelastin. This peptide was shown to form amyloid-like fibers implied in neurodegenerative pathologies. The comparison between the linear peptides and the associated amyloid-like fibers evidences the strong influence of the secondary structures on the physical structure and chain dynamics of these polypeptides.

Index Terms - thermally stimulated currents, permittivity measurements, infrared spectroscopy, calorimetry, biological materials.

1 INTRODUCTION
ELASTIN, the protein responsible for elasticity of tissues such as lung, skin and arterial wall consists of a three-dimensional network whom turn-over is almost absent under physiological conditions. Under certain pathological conditions elastin is attacked by the metalloproteinase MMP12 releasing short polypeptides able to give rise to amyloid-like fibers [1]. Amyloid fibrils are highly ordered parallel cross β-sheet structures that self-assemble from small proteins or peptides, and they are usually associated with many diseases, such as Alzheimer’s disease, Parkinson’s disease, type II diabetes and more. As depicted in Figure 1, conformational and microscopy studies showed that the polypeptide (S4), corresponding to a sequence of 22 amino-acids released during the proteolysis of human tropoelastin could form amyloid-like fibers in vitro [2]. The aim of this work is to get an insight into the vibrational, thermal and dielectric characteristics of the soluble, linear peptides S4 (labeled S4 peptides) and their aggregated forms (labeled S4 fibers) mainly using FTIR, Differential Scanning Calorimetry (DSC), Dynamic Dielectric Spectroscopy (DDS) and Thermally Stimulated Currents (TSC). These techniques are well-suited for the analysis of biological materials both in solution [3] and in the condensed state [4–6], since the insolubility of the fibers is not an obstacle to the analysis. DSC can reveal ordered and amorphous structures at the mesoscopic level, connecting the molecular level (accessible through vibrational techniques) and the microscopic/macroscopic level (accessible through microscopy and turbidimetry). Dielectric techniques are useful to scan the dynamics of proteins over a wide range of frequency/lengths. In previous studies[7] we investigated the chain dynamics of native elastin in the nanometer range, showing that dry elastin possessed the peculiar features of a fragile liquid, associated with the strength and nature of interactions.

Figure 1. Schematic representation of S4 peptides and associated S4 fibers.
2 MATERIALS AND METHODS

2.1 SYNTHESIS OF S4 PEPTIDES AND AGGREGATION OF S4 FIBERS

The S4 peptides (LVGAAGLGGLGVGGLGVPGVGG, molecular weight: 1734 g.mol\(^{-1}\)) were synthesized by solid-phase methodology. These peptides were freeze-dried and purified by reversed-phase high performance liquid chromatography and their purity was assessed by electrospray mass spectrometry.

According to previous turbidimetry experiments showing the self-aggregation of S4 peptides into amyloid-like structures [2], S4 peptides were dissolved in TBS buffer (Tris (50 mM), NaCl (1.5 M), and CaCl\(_2\) (1.0 mM), pH 7) with a concentration of 30 mg/mL (17mM); the solution was stirred at 80°C for 3 h in order to precipitate S4 peptides into S4 fibers. Once formed, S4 fibers were separated from the solution by 3 cycles of centrifugation (15000 RPM, 5 min) and washed in pure distilled water prior the freeze-drying process.

2.2 PHYSICAL TECHNIQUES OF CHARACTERIZATION

2.2.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier transform infrared spectroscopy/attenuated total reflectance (FTIR/ATR) spectra were collected using a Nicolet 5700 FTIR (THERMO FISHER SCIENTIFIC, Waltham, MA) equipped in ATR device (diamond crystal) with a KBr beam splitter and a MCT/B detector. Spectra (64 accumulations) were recorded over the region of 4000–450 cm\(^{-1}\).

The decomposition of the amide I/II bands on the renormalized spectra was then performed with a Gaussian curve fitting procedure using the second derivative spectra to extract positions of the different overlapping bands.

2.2.2 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Analyses were performed using a DSC Pyris calorimeter (PERKIN ELMER, Waltham, MA). The calorimeter was calibrated using cyclohexane and indium as standards, resulting in a temperature accuracy of ±0.1°C and an enthalpy accuracy of ± 0.2 J/g. Samples, 5 mg in weight, were sealed into non hermetic aluminum pans. Experiments done in triplicate were performed between 5 and 225°C with a heating rate of 20°C.min\(^{-1}\) under N\(_2\) atmosphere.

2.2.3 DYNAMIC DIELECTRIC SPECTROMETRY (DDS)

The dielectric measurements were performed using a broadband dielectric spectrometer BDS 4000 system (NOVOCONTROL TECHNOLOGIES GmbH & Co. KG, Hundsgangen, Germany). Samples were kept in a special cell usually devoted to biological samples consisting of two stainless steel electrodes surrounded by a Teflon ring. The diameter of the electrodes was 15 mm, and the thickness of the samples was 0.1 mm (compressed powdered sample).

Isothermal measurements of the complex dielectric function \(\varepsilon^* = \varepsilon' - i\varepsilon''\) were performed at every 5°C degree, with an isothermal stability of ± 0.1°C, from -150 to 200°C in the frequency range 10\(^{-2}\) to 3.10\(^6\) Hz after a first heating at 120°C to dehydrate the sample. Amplitude of measuring voltage was 1 V. The experimental limit for the loss factor (\(\tan\delta = \varepsilon''/\varepsilon'\)) was about 10\(^{-5}\).

2.2.4 THERMALLY STIMULATED CURRENTS (TSC)

Complex Thermally Stimulated Currents thermograms were carried out using a TSC/RMA Analyzer (SETARAM Instrumentation, Caluire, France). Samples were placed between two stainless steel electrodes surrounded by a Teflon ring. Before experiments the cryostat was flushed and filled with dry He to insure good thermal exchanges. For complex experiments, the sample was polarized by an electrostatic field E=800 V mm\(^{-1}\) over a temperature range from the poling temperature (\(T_p=30°C\) and \(T_p=140°C\)) down to the freezing temperature \(T_0=-160°C\). Then, the field was turned off and the depolarization current was recorded with a controlled heating rate (\(q=+7 °C.min^{-1}\)); the equivalent frequency of the TSC thermograms was about 10\(^{-3}\) Hz.

2.2.5 FRACTIONAL POLARIZATIONS (TSC/FP)

Elementary TSC thermograms were obtained with a polarization window of 5°C. The field was removed and the sample cooled to a temperature \(T_{cc}=T_p-40°C\). The depolarization current was recorded with a constant heating rate \(q=+7 °C min\) min. The series of elementary thermograms was generated by shifting the polarization window by 5 °C toward higher temperature.

3 RESULTS AND DISCUSSION

3.1 SECONDARY STRUCTURES

The FTIR spectra of S4 peptides and S4 fibers in the 1750-1500 cm\(^{-1}\) range are presented in Figure 2.

![Figure 2. FT–IR spectra of S4 peptides (A) and S4 fibers (B) in the amide I–II zone (bold line: experimental curve; fine lines: decomposition into Gaussian peaks).](image-url)
This zone corresponds to the amide I (C=O stretching) and amide II (C-N stretching and N-H bending) bands. Some distinct features can be observed between S4 peptides and S4 fibers on these global spectra; in peculiar the amide I band located at 1645 cm\(^{-1}\) in S4 peptides is narrower and shifted at 1627 cm\(^{-1}\) in S4 fibers. Since the amide I-II region consists of several bands strongly dependent on secondary conformations, it was subjected to curve fitting in order to resolve the various underlying and overlapping spectral features that contribute to this complex region\[8–11\]. The decomposed FTIR spectra of the amide I-II region are superimposed on global spectra in Figure 2 and the assignments of the vibrational bands are reported in Table 1.

Table 1. Position and assignments of the amides I and II bands in S4 peptides and S4 fibers.

<table>
<thead>
<tr>
<th>Position (cm(^{-1}))</th>
<th>Assignment</th>
<th>Position (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1694</td>
<td>Anti-parallel (\beta) sheets</td>
<td>1697</td>
<td>Anti-parallel (\beta) sheets</td>
</tr>
<tr>
<td>1680</td>
<td>Anti-parallel (\beta) turns</td>
<td>1669</td>
<td>Anti-parallel (\beta) turns</td>
</tr>
<tr>
<td>1667</td>
<td>Turns, loops</td>
<td>1651</td>
<td>(\alpha) helices, unordered</td>
</tr>
<tr>
<td>1653</td>
<td>(\beta) sheets</td>
<td>1657</td>
<td>(\beta) sheets</td>
</tr>
<tr>
<td>1552</td>
<td>(\beta) sheets</td>
<td>1525</td>
<td>(\beta) sheets</td>
</tr>
<tr>
<td>1537</td>
<td>unordered</td>
<td>1504</td>
<td>Side chains</td>
</tr>
<tr>
<td>1518</td>
<td>(\beta) sheets</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In S4 peptides different structures are present, such as anti-parallel \(\beta\) sheets and turns, unordered conformations and helices. The amid I region of S4 fibers is very distinct from the amid I region of S4 peptides, with a prominent component at 1627 cm\(^{-1}\) ascribed to intermolecular \(\beta\) sheets in cross \(\beta\) structures [12], together with a band of increasing intensity at 1697 cm\(^{-1}\), which is typical for the antiparallel \(\beta\) sheet conformation. The predominance of the cross \(\beta\) structure resulting from aggregation in S4 fibers is evidenced by the presence of two main components in the amide II region at 1552 and 1525 cm\(^{-1}\), that are representative of the \(\beta\) sheet conformation [2,12].

### 3.2 PHYSICAL STRUCTURE

Thermograms of freeze-dried S4 peptides and freeze-dried S4 fibers are reported in Figure 3. The specific thermal answer of wholly dehydrated S4 peptides is characterized by a well-defined glass transition at 147°C (\(\Delta C_p=0.45 \text{ J.g}^{-1}.\text{K}^{-1}\)), reversible on successive scans. Freeze-dried S4 peptides possess an amorphous region, with a lack of long-range order, like elastin [13,14]. The main event detected on the second thermogram of S4 fibers is a sharp and weak endothermic peak at 145.6°C associated with a first order transition, which can be considered as the thermal signature of amylloids fibers. This transition, not observed on the third scan, is irreversible. In contrast with S4 peptides, no glass transition is detected in the DSC thermograms of S4 fibers, revealing the high degree of order of these fibers.

Figure 3. DSC thermograms of freeze-dried S4 peptides and freeze-dried S4 fibers (second and third heating runs after a first run to 150°C to dehydrate the sample).

This thermal behavior is consistent with the periodic sub-nanoscale crystalline ordering of amyloid fibers, consisting of stranded-crystalline regions, which are perpendicular to the axis of the fibril and which are linked by hydrogen bonds. It must be pointed out that such an irreversible transition in the 140-150°C range has already been reported in \(\beta\)-amyloid self-assembled model peptides and associated with the irreversible crystalline phase transformation from a hexagonal structure to a orthorhombic one [15].

### 3.3 CHAIN DYNAMICS

Dynamic dielectric spectroscopy and thermally stimulated currents have been used to characterize the molecular mobility of S4 peptides and fibers over a wide frequency range. The three-dimensional representation of the dielectric loss of S4 peptides, i.e., the imaginary part of the dielectric function \(\varepsilon''\) versus frequency and temperature is presented in Figure 4.

Figure 4. Imaginary part (\(\varepsilon''\)) of the dielectric spectrum of S4 peptides as a function of frequency and temperature from DDS experiments.
The different dielectric losses detected in this map show the complexity of the molecular mobility of S4 peptides. In addition to the increase of $\varepsilon''$ measured at low frequency and high temperature and attributed to conductivity, four modes are pointed out and indexed in Figure 4 for S4. The same DDS protocol applied to S4 fibers also evidences four dielectric modes. For more convenience and better comparison we have reported in Figure 5 selected isochronal plots of $\varepsilon''$ and $\varepsilon$ versus temperature for S4 peptides and S4 fibers. The associated temperature dependence of the relaxation time $\tau(T)$ of each mode was plotted in Figure 6 and resumed in Table 2 for S4 peptides and S4 fibers. S4 peptides and S4 fibers are characterized by a similar $\beta$ mode occurring in the same frequency-temperature range obeying the Arrhenius law of equation (1):

$$\tau(T) = \tau_0 e^{-E_a/RT}$$

(1)

Where $R$ is the universal gas constant, $E_a$ is the activation energy and $\tau_0$ the pre exponential factor. As shown in Table 2, these activation parameters are very close for S4 peptides and fibers. Such an Arrhenius-like behavior is found in literature data for the $\beta$ mode of a wide class of proteins and polypeptides and attributed to the localized orientation of carbonyl groups[16-17]. With an estimation of $\sim 23$ kJ/mol for the enthalpy of hydrogen bonds, the values of $E_a$ roughly correspond to the energy needed for the breaking of two hydrogen bonds[16-17], allowing the further orientation of polar groups. Moreover, the value of $\tau_0$, related to the activation entropy $\Delta S$ in the Eyring's theory by:

$$\tau_0 = \left(\frac{h}{kT}\right)e^{-\Delta S/R}$$

(2)

(where $h$ is the Planck constant and $k$ the Boltzmann constant) is indicative of slightly cooperative modes ($\Delta S=0$ for a value of $\tau_0$ close to $10^{15}$ s) according to Stark weather’s criterion [18]. So we can assume that the distinct architecture of S4 peptides and fibers does not induce difference at this scale of mobility.

This fact is consistent with the peculiar nanoscale crystalline ordering of S4 peptides and fibers. Amyloidogenic peptides consist of numerous homogenous crystalline regions of a few nanometers dimension divided by the regions of no covalent bonding [20]. It was recently shown that some amyloidogenic sequences had intrinsic high electrical dipole moment, their self-assembly leading to macroscopic dipole moment oriented along the tubes axis. That would explain why the dielectric experiments are peculiarly sensitive to conformational changes in these structures in contrast with DSC. At higher temperature, the $\alpha$ mode of S4 peptides is a composite mode characterized by isoenthalpic processes at low and high frequency and a temperature-frequency dependent behavior obeying the Arrhenius law between 1 and 1500 Hz. This peculiar feature must be ascribed to overlapping transitions including structural first orders transitions of the nanocrystalline regions as well as the segmental process associated with the dielectric manifestation of the dynamic glass transition, which was detected in this temperature range by DSC. It is noteworthy that the $\alpha$ relaxation obeys an Arrhenius’ law (and not a Vogel-Tamman-Fulcher’s law) allowing us to ascribe this polypeptide to a strong glass in the Angell’s classification [21] as already observed in ordered proteins with an high density of hydrogen bonds[7]. As for

![Figure 5](image)

**Figure 5.** Real ($\varepsilon'$) and imaginary ($\varepsilon''$) parts of S4 peptides and S4 fibers as a function of temperature at selected frequencies from DDS experiments.

In contrast with the $\beta$ mode, the MT1 and MT2 modes are quasi-isothermal processes occurring at distinct temperatures for S4 peptides and fibers. Characterized by maxima on the $\varepsilon'$ curves at the same temperatures as observed on the $\varepsilon''$ curves (as shown in Figure 5), MT1 and MT2 modes can be attributed to structural phase transitions [19], not detectable by DSC in this temperature zone.

![Figure 6](image)

**Figure 6.** Temperature dependence of the relaxation times of the four dielectric modes ($\beta$, MT1, MT2 and $\alpha$) recorded by DDS experiments for S4 peptides and S4 fibers.

<table>
<thead>
<tr>
<th>Mod</th>
<th>S4 Peptides</th>
<th>S4 Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>$E_a=47$ kJ/mol</td>
<td>$E_a=48.5$ kJ/mol</td>
</tr>
<tr>
<td>T=17°C</td>
<td>$\tau_0=3.5\times10^{-15}$ s</td>
<td>$\tau_0=4.9\times10^{-15}$ s</td>
</tr>
<tr>
<td>MT1</td>
<td>Isothermal</td>
<td>Isothermal</td>
</tr>
<tr>
<td>MT2</td>
<td>Isothermal</td>
<td>Isothermal</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>$E_a=240.5$ kJ/mol</td>
<td>$E_a=189$ kJ/mol</td>
</tr>
<tr>
<td>T=105°C</td>
<td>$\tau_0=1.6\times10^{-24}$ s</td>
<td>$\tau_0=4.8\times10^{-24}$ s</td>
</tr>
<tr>
<td>T=140°C</td>
<td>Isothermal</td>
<td>Isothermal</td>
</tr>
</tbody>
</table>

Table 2. Temperature-frequency dependence of the four modes of S4 peptides and S4 fibers and associated parameters.
the high temperature mode of S4 fibers, it is shifted toward high temperature, reflecting structure stiffening in the fibers; in this temperature-frequency zone the α mode seems to be mainly governed by the segmental relaxation associated with the dielectric manifestation of the glass transition of a strong glass forming biomaterial (not detectable in DSC). An enlargement of the graph (not shown) indicates a discontinuity at 145°C, corresponding to the irreversible phase transition between two crystalline phases and associated with hydrogen bonds breaking. In contrast with the β mode, the activation parameters of the α mode are very distinct in S4 peptides and fibers; the high values of the apparent activation energies together with the very low values of the pre-exponential factor are indicative of cooperative processes in both cases.

In order to complete this dielectric analysis, Thermally Stimulated Currents have been used. The various peaks appearing in TSC the thermograms characterize the operative molecular mechanism through which an electret stores its charge. In this non isothermal relaxation process, mainly the dipoles and free charges of the polymer are expected to get relaxed.

The low temperature TSC thermograms of S4 peptides and S4 fibers are reported in Figure 7.

![Figure 7. TSC thermograms of S4 peptides and S4 fibers obtained after a poling at 30°C.](image)

For S4 peptides and S4 fibers the low temperature β mode is recorded at around -100°C. It corresponds to the β mode already detected in DDS experiments (there are similar features between TSC thermograms and the low temperature zone of the Figure 5). Once again the similarity of the β mode in both materials indicates that this localized relaxation is not ultra-structure dependent. Nevertheless, in contrast with DDS experiments, the low equivalent frequency of TSC technique allows us to detect an additional mode (β') at -64°C for S4 peptides, reversible on successive scans. The multiplicity of the localized motions can be associated with the distinct environments of the C=O in S4 peptides as revealed by FTIR. In the case of S4 fibers, a reverse peak (R), well reproducible on successive scans, is noted at -67°C. This peak was shown to arise only with poling; according to literature data[22], such a reverse current peak could be due to the presence of interfaces in S4 fibers acting as traps and giving rise to homocharges accumulation during electrode charge injection. As a matter of fact, it is noteworthy that amyloid fibers, organized in cross β spines structures, can be considered as multi interfacial materials[23].

Nevertheless the low temperature value of this reverse peak is quite unusual for such a homocharge current peak, generally recorded at higher temperature[24].

TSDC peaks of opposite polarity are also the signature of poly(zwitterions), in which a thermo reversible reaction between low dipole moment species and their conformational isomers of high polarity occurs with relaxation times smaller than the orientational relaxation time[25].

The high temperature TSC thermograms of S4 peptides and S4 fibers are reported in Figure 8.

![Figure 8. TSC thermograms of S4 peptides and S4 fibers obtained after a poling at 140°C.](image)

In this temperature zone are evidenced shoulders in the [10;40°C] zone both for S4 peptides and S4 fibers (which can be connected to MT1 and MT2 modes previously observed by DDS) and a main peak labelled HT peak. Because of conductivity at high temperature, the eventual additional relaxation peaks cannot be detected. Once again significant differences are evidenced between S4 peptides and fibers, with an enhancement of the modes in the [0,40°C] for S4 fibers and a shift towards high temperature of the HT peak, certainly associated with the densification of hydrogen bonds network. The fine structure analysis of the TSC peaks was achieved through the TSC/FP method in [-150, 80°C] for both materials. Nevertheless, because of the reverse peak occurring at -67°C in S4 fibers, in this case the analysis of the elementary peaks was limited to the high temperature zone. In this procedure, each isolated spectrum is well approximated by a single relaxation time, allowing Bucci-Fieschi's analysis[26]. The FP method allowed us to access the distribution of elementary relaxation times \( \tau \) involved in the different TSC modes in the scanned temperature range. By plotting the variation of \( \tau_e(T) \) versus temperature, we noted that all the extracted relaxation times were well fitted by an Arrhenius’ law (equation (1)). We reported on Figure 9 the variation of \( E_a \) versus temperature for all the isolated relaxation times as well as the Starkweather’s function corresponding to non cooperative processes[18].

It can be observed from Figure 9 that the lowest temperature processes β and β' of S4 peptides are slightly cooperative (as already observed by DDS) in contrast with the complex MT
modes, more detectable with the FP analysis and presenting a significant gap with the Starkweather’s function, indicative of cooperative processes. The structural transition associated with these modes become more cooperative in S4 fibers probably due to the long range order present in S4 fibers in contrast with S4 peptides.

![Figure 9. Variation of activation energies of the elementary relaxation times extracted from TSC/FP procedure versus temperature.](image)

### 7 CONCLUSION

A close correlation is evidenced between structural analysis (FTIR) and physical characterization (DSC, DDS and TSC) for the studied polypeptides. Peculiar signatures of the amyloid fibers can be detected both at the localized and delocalized levels in the condensed state. Amyloid-like S4 fibers constitute an insoluble and less plastic/elastic material, and it is liable to have negative biological consequences. Dielectric experiments are peculiarly sensitive to conformational changes in these amyloidogenic peptides. These nanocrystalline materials possess several phase transitions between crystalline structures of different polarities. As shown for some model peptides, their self-assembly into amyloid fibers probably lead to macroscopic dipole moments explaining the enhancement of their self-assembly into amyloid fibers probably leading to macroscopic dipole moments explaining the enhancement of their self-assembly into amyloid fibers probably leading to macroscopic dipole moments explaining the enhancement of their self-assembly into amyloid fibers probably leading to macroscopic dipole moments explaining the enhancement of.

### REFERENCES


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Briglia Bochicchio received the Ph.D. degree in chemistry in 2003 both at the University of Basilicata (Italy) and at University of Reims Champagne-Ardenne (France). Since 2007, she is an assistant professor at the Department of Science of the University of Basilicata where she teaches bioorganic chemistry at the School of Chemistry. Brigida’s research interests are in the field of Protein-Inspired materials.

Antonietta Pepe is an assistant professor in organic chemistry, at the Department of Science, University of Basilicata, Italy. She get her Ph.D. degree in natural product sciences at the University of Naples in 1998, under the supervision of Prof. L. Mayol. Following her Ph.D. degree she was recruited as a post-doctoral research fellow at the Department of Chemistry of the University of Basilicata (Prof. A. M. Tamburro, supervisor. Her research interests are based on molecular and supramolecular studies of model peptides of elastomeric proteins, such as elastin, abductin and lamprin, and resilin, is now oriented on the synthesis and development of protein-inspired biomaterials.