Poly(L-proline) II Helix Propensities in Poly(L-lysine) Dendrigraft Generations from Vibrational Raman Optical Activity

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Vibrational Raman optical activity (ROA), measured as small circularly polarized components in Raman scattering from chiral molecules, was applied to study the backbone conformations of the first five generations of poly(L-lysine) dendrigrafts (DGLs) in water. Generation 1 was found to support predominantly the poly(L-proline) II (PPII) conformation, the amount of which steadily decreased with increasing generation, with a concomitant increase in other backbone conformations. This behavior may be due to increasing crowding of the lysine side chains, together with suppression of backbone hydration, with increasing branching. In contrast, the ROA spectra of a series of linear poly(L-lysine)s in water show little change with increasing molecular weight. Our results may have implications for the nonimmunogenic properties of DGLs.

Introduction

Dendrimers are highly branched, monodisperse, and symmetrical macromolecules of nanosize dimensions consisting of a central core, branching units, and terminal functional groups.1,2 Hyperbranched polymers also exhibit dendritic architectures, but in contrast to dendrimers, they are nonsymmetric and polydisperse.3,4 They are, however, more easily prepared, which facilitates practical applications. Dendrigraft polymers constitute an important class of hyperbranched polymers, prepared from oligomers or polymers through repeated protect–deprotect grafting steps, each grafting step being called a generation6 (i.e., a graft-on-graft strategy, in contrast to dendrimers, which are prepared from multistep reactions involving monomers). Dendrigraft poly(L-lysine) (DGL) is an example of a water-soluble hyperbranched polymer based on a naturally occurring amino acid monomer making it favorable, inter alia, for biomedical applications.6–8 The physical characterization of dendritic molecules is essential for the rational control of their many applications, which include nanomaterials, diagnostics, drug delivery, biocides, gene transfer, and catalyst supports.9 Here we apply the chiroptical technique of vibrational Raman optical activity (ROA) to monitor changes in the solution conformation with increasing generation of DGL.

ROA measures a small difference in the intensity of vibrational Raman scattering from chiral molecules in right- and left-circularly polarized incident light or, equivalently, the intensity of a small circularly polarized component in the scattered light using incident light of fixed polarization.10–12 The first and second experiments are called incident circular polarization (ICP) and scattered circular polarization (SCP) ROA, respectively. The ability to study aqueous solutions, with no restrictions on molecule size, makes ROA ideal for studying biomolecules, generally,13 and oligo- and polypeptides, in particular, as in the present work.

Experimental Section

Materials. The first five generations of DGLs as trifluoroacetate (TFA) salts were supplied by COLCOM as a gift and used without further purification. Their structures, number average molecular masses ($M_n$), average degrees of polymerization ($N$), and polydispersity indexes ($I$) are listed in Table 1.9 The first three generations are illustrated schematically in Figure 1; more details of the chemical structures are provided elsewhere.8,9 The linear poly(L-lysine) (hydrobromide) samples were purchased from Sigma and used without further purification.

ROA Spectroscopy. The ROA spectra were measured in water using the previously described ChiralRAMAN instrument (BioTools, Inc.),11 which employs the SCP measurement strategy with unpolarized incident light. The ROA spectra are presented as $(I_R - I_L)$ and the parent Raman spectra as $(I_R + I_L)$, where $I_R$ and $I_L$ are the Raman-scattered intensities with right- and left-circular polarization, respectively. All the samples were dissolved in water to a concentration of $\sim 50$ mg/mL and filtered through a 22 µm spin-filter (Millipore) to remove dust particles before transfer into rectangular quartz microfluorescence cells. Experimental conditions: laser wavelength, 532 nm; laser power at the sample, $\sim 300$ mW; spectral resolution, $\sim 10$ cm$^{-1}$; acquisition times, $\sim 32$ h. Solvent water spectra were subtracted from the parent Raman spectra using reference spectra of pure water obtained using the same cell as that used for the particular sample.

Table 1. Number Average Molar Masses ($M_n$), Average Degrees of Polymerization ($N$), and Polydispersity Indexes ($I$) of the First Five Generations of DGLs

<table>
<thead>
<tr>
<th>generation</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_n$ (g/mol)</td>
<td>1450</td>
<td>8600</td>
<td>22000</td>
<td>65300</td>
<td>172300</td>
</tr>
<tr>
<td>$N$</td>
<td>8</td>
<td>48</td>
<td>123</td>
<td>365</td>
<td>963</td>
</tr>
<tr>
<td>$I$</td>
<td>1.2</td>
<td>1.38</td>
<td>1.46</td>
<td>1.36</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Results and Discussion

The ROA spectra of the first five generations of DGLs are displayed in Figure 2, with a set of Raman and ROA spectra of poly(L-lysine)s of roughly similar molecular weight to the DGLs displayed in Figure 3 for comparison. The parent Raman spectrum of each sample was also recorded, but only one parent Raman spectrum is shown at the top of each set of ROA spectra because they are almost identical within each set. The discrepancies observed between the parent Raman spectra of the DGLs
and of the linear poly(L-lysine)s are due to the presence of TFA anions in the DGL samples. As TFA is achiral, this does not influence the comparison of the ROA data.

ROA is proving valuable in the study of unfolded proteins and disordered polypeptides, especially for the identification of the poly(L-proline) II (PPII)-helical conformation in such states. Although originally defined for the conformation adopted by polymers of L-proline, the PPII helix can be supported by amino acid sequences other than those based on L-proline. It consists of a left-handed helix with 3-fold rotational symmetry in which the $\phi, \psi$ angles of the constituent residues are restricted to values around $-75^\circ, +145^\circ$, corresponding to a region of the Ramachandran surface adjacent to the $\beta$-region. The extended nature of the PPII helix precludes intrachain hydrogen bonds, with protein X-ray crystal structures indicating that the structure is stabilized instead by main chain hydrogen bonding with water molecules, with side chains modulating PPII helix-forming propensities in various ways. This produces a rather flexible, adaptable structure that is becoming increasingly recognized as a major conformational element of disordered polypeptides and intrinsically disordered (natively unfolded) proteins in aqueous solution and which may be important in the functional role of intrinsically disordered sequences.

As discussed in earlier work, the ROA spectra of the disordered poly(L-lysine) samples contain bands characteristic of PPII structure, especially the strong positive $\sim 1318$ cm$^{-1}$ band and the weaker positive $\sim 1675$ cm$^{-1}$ band. The assignments of these bands, which are often prominent in the ROA spectra of disordered peptides and intrinsically unfolded/disordered proteins generally, have been confirmed by ab initio quantum chemical simulations. The ROA spectrum of DGL generation 1, which is simply an eight-residue linear L-lysine peptide, is most similar to that expected for the model PPII helix conformaion. It is reassuring that a seven-residue L-lysine peptide has previously been shown, using circular dichroism (CD), to take up a predominantly PPII conformation in neutral aqueous solution.

Although the ROA spectra of all five generations of DGLs in Figure 2 show positive bands at $\sim 1318$ cm$^{-1}$, the intensities vary considerably, starting with a strong sharp band in generation 1 that gradually degrades through the series to become very weak in generation 5, with concomitant changes elsewhere in the spectra. This indicates that PPII structure is steadily replaced by other backbone conformational elements with increasing branching. A simple explanation could be that increased branching leads to increased crowding of the lysine side chains, which forces the peptide backbone into other conformations. A further important factor could be that branching also tends to inhibit water solvation of the backbone. Studies on short lysine peptides have shown that water molecules stabilize the PPII conformation of the backbone even under conditions in which side-chain charges are heavily screened or even neutralized, in contrast with long homopolymers of lysine for which neutral side chains encourage $\alpha$-helix formation. Both factors could have the effect of increasing the $\alpha$-helical propensities at the expense of the PPII propensities in the core backbone sequences, evidenced by the steady increase of the characteristic positive $\alpha$-helix ROA bands at $\sim 1300$ and $1340$ cm$^{-1}$ and concomitant decrease in the positive PPII band at $\sim 1318$ cm$^{-1}$ through the series. In contrast, the ROA spectra of the series of linear poly-(L-lysine)s in Figure 3 show little change with increasing...
molecular weight, and certainly no decrease in the positive $\sim 1318 \text{ cm}^{-1}$ PPII band.

Suppression of the PPII content of DGLs with increasing branching could be associated with their nonimmunogenic properties (http://www.colcom.eu). Intrinsic disorder is known to be crucial in the immune response.\textsuperscript{23,24} Short intrinsically disordered peptide sequences are good antigens, whereas long disordered regions and intrinsically disordered proteins initiate only weak immune responses or are even completely nonimmunogenic.\textsuperscript{23,24} The flexible adaptable character of PPII structure, previously described as a “careful disorderliness”,\textsuperscript{13,20} has been suggested to be important in certain aspects of protein function. The careful disorderliness of a relatively short sequence supporting PPII structure may be associated with the ability to readily adapt, perhaps in a cooperative manner, to other conformations compatible with the structure of the antigen binding site. The conformational heterogeneity of the more random conformations required by the structure of the antigen binding site. The conformational heterogeneity of the more random structure found in the short separate lysine sequences within DGLs, which would also have reduced mobility due to steric crowding, may prevent them from adapting so readily.

Conclusions

The PPII content of DGLs has been shown by ROA spectroscopy to steadily decrease with increasing branching, with a concomitant increase in other backbone conformations. The fact that the ROA spectra of a series of linear poly(L-lysine)s with comparable molecular weights show little change with increasing molecular weight, all being dominated by similar amounts of PPII structure, supports the suggestion that loss of PPII is due to increasing crowding of the lysine side chains, together with suppression of backbone hydration, with increasing branching. Our results may have implications for the nonimmunogenic properties of DGLs, with immunogenicity itself being associated with the ability of short sequences of PPII structure to readily adapt to other conformations compatible with antigen binding sites.

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References and Notes

(8) Tsogias, I.; Theodossiou, T.; Sideratou, Z.; Paleos, C. M.; Collet, H.; Rossi, J. C.; Romestand, B.; Commeyras, A. Biomacromolecules 2007, 8, 3263–3270.
(9) Cottet, H.; Martin, M.; Papillaud, A.; Souaïd. E.; Collet, H.; Commeyras, A. Biomacromolecules 2007, 8, 3235–3243.