Simulation of IR, Raman and VCD amide I band profiles of self-assembled peptides

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Abstract. Vibrational spectroscopy is a suitable and convenient tool to probe the self-assembly of peptides, a biomedically and biotechnologically relevant process. Theoretical efforts to quantitatively analyze vibrational spectra of peptide aggregates have thus far focused on exploring the IR and to a lesser extent the vibrational circular dichroism (VCD) spectra of rather small sized planar or nearly planar β -sheet structure. The current study utilizes an algorithm based on an excitonic coupling model with experimentally or computationally determined parameters to simulate the amide I band profiles of the IR, isotropic Raman, anisotropic Raman and VCD spectra of two- and three-dimensional β -sheet structures. In agreement with earlier calculations we found that the splitting between the two prominent IR bands of an antiparallel β -sheet increases with increasing number of strands until a saturation is reached at approximately eight strands. The dominant isotropic and anisotropic Raman bands of amide I are located between the two IR bands and downshift only slightly with increasing sheet length. The VCD signal is rather weak. We also investigated the influence of sheet stacking on amide I by calculating the respective IR, Raman and VCD profiles for two in-register, facially stacked β -sheets with seven strands per sheet for an antiparallel arrangement of the sheets and six strands per sheet for a parallel arrangement. We found that stacking produces additional bands in the IR and Raman spectrum, in line with the reduced symmetry of the ideal unit cell of the β -sheet. In addition, a more pronounced VCD signal is detected. A comparison with experimental IR, Raman and VCD spectra of gelled (AAKA)₄ reveals a good qualitative agreement between experimental and simulated amide I band profiles.

Keywords: Amide I, VCD, self-assembly, hydrogel, excitonic coupling

1. Introduction

The self-assembly of peptides is an important issue of biomedical, biotechnological and material research. Peptides can form supramolecular structures, such as ribbons, nanotubes and monolayers, which are predominantly composed of some type of underlying β -sheet structures [1,2]. The biomedical relevance of the self-assembly process is due to the fact that several disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease and prion-transmissible spongiform encephalopathies, are believed to result from the self-assembly of peptides/proteins, and their subsequent aggregation into fibrils [3,4]. With respect to biotechnology, the self-assembly process has very positive aspects. It allows for the generation of material with incorporated biofunctionality, such as biocompatibility, and ligand and metal recognition [1]. Hydrogels, i.e. a self-assembled mixture of e.g. peptides and water, are used for tissue engineering and drug delivery [2].

Vibrational spectroscopy is an ideal to probe peptide aggregation in solution [5]. This notion applies particularly to the amide I band, which is the most structural sensitive band among those resulting predominantly from backbone vibrations. The amide I normal mode consists predominantly of a CO stretching vibration with minor admixtures of CN stretch, NH in-plane bending and, if the peptide is dissolved

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in water, HOH bending [6]. The latter is eliminated if the peptide is dissolved in D₂O, where the corresponding mode is termed amide I' [7,8]. The structural sensitivity of amide I results predominantly from two contributions, namely the dependence of its intrinsic frequency on intrapeptide and peptide-solution hydrogen bonding and, to a larger extent, from the excitonic coupling between the excited vibrational states of adjacent peptide groups [9–14]. The latter is particularly pronounced for β -sheets. An ideal antiparallel β -sheet of infinite length can be subdivided into unit cells of C_{2h}-symmetry, which contain four peptide groups. Group theory dictates that one obtains four unit cell modes of A_g, B_g, A_u and B_u symmetry [9]. The splitting between these modes depends on the strength of excitonic coupling. For amide I, the IR active bands assignable to A_u and B_u are substantially split, with the former being downshifted and the latter being upshifted with respect to their position in a β -strand/PPII spectrum (1640–1645 cm⁻¹). The downshifted band (A_u) carries most of the IR intensity, whereas the other band (B_u) is barely detectable [9]. The two *ungerade* modes are both Raman active and are normally positioned between the two IR-bands. Generally, only one Raman band shows up in the spectrum, which is assignable to the A_g-mode [5,9]. The vibrational circular dichroism (VCD) signal of amide I is generally weak owing to the nearly planar structure of an antiparallel β -sheet [15,16]. The parallel β -sheet structure has not been as thoroughly characterized as the antiparallel arrangement. It is clear, however, that the respective spectra are qualitatively similar. Based on results of *ab initio* calculations, Kubelka and Keiderling predicted that the splitting between the two IR components is less pronounced for parallel than for antiparallel β -sheets [15,17]. Since real β -sheets in solution have a finite length and do not adopt the idealized conformations exhibited by crystallized peptides, more than just the above mentioned excitonic modes become activated. As a consequence, the aforementioned bands reflect the strongest contribution in a rather continuous spectrum, which generally covers the region between 1620 and 1690 cm⁻¹ in the IR spectrum [15,17].

The strong conformational sensitivity of amide I' makes it a rather ideal tool for structure analysis. Generally, amide I band profiles are used to determine the fraction of different secondary structures in a (folded) protein or peptide [5]. Over the past ten years, the excitonic coupling between amide I modes have been used for a more refined analysis of mostly unfolded short peptides [14,18,19]. With respect to β -sheets, theoretical and computational studies have focused on amide I in the respective IR and, to a more limited extent, in the VCD spectra [15,17,20]. These studies utilized force fields transferred from DFT calculations on small and short β -sheet units and semi-empirical calculations. All these studies revealed that the splitting between the A_u and B_u band in the IR-spectrum increases with the number of strands in a β -sheet up to a number of 8 strands, while this splitting was found to be practically independent on the strand length.

In this short paper, we will, for the first time use the excitonic coupling model to calculate the amide I bands, not only for the IR and VCD spectra, but also for the isotropic and anisotropic Raman bands of β -sheet structures. To make contact with the results from recent experiments in our laboratory, we consider different two- and three-dimensional arrangements of sheets composed of 16-mers and octamers. This study shows that a combination of the above listed spectroscopies can be used for a rather detailed structure analysis of β -sheet structures.

2. Theory

The excitonic coupling theory for the amide I bands of polypeptides has been described in detail in several papers [14,21]. Briefly, we calculated the IR and Raman intensities of amide I by writing the

respective transition dipole moments and Raman tensors of excitonic states as:

$$\begin{aligned}\vec{\mu}'_i &= \sum_{j=1}^N a_{ij} \vec{\mu}'_j, \\ \hat{\alpha}'_i &= \sum_{j=1}^N a_{ij} \hat{\alpha}'_j,\end{aligned}\tag{1}$$

where i labels the excitonic state, $\vec{\mu}_j = \partial \vec{\mu}_j / \partial q_j \cdot q_j$ and $\hat{\alpha}'_j = \partial \hat{\alpha}'_j / \partial q_j \cdot q_j$ denote the transition dipole moment and the Raman tensor of the i th of N peptide units and q_j is the normal coordinate of the amide I mode associated with the j th unit. The IR and Raman band intensities are directly proportional to the square of the transition dipole moments and the square moduli of Raman tensor elements of the excitonic states, respectively. The coefficients a_{ij} are obtained by diagonalizing the Hamiltonian:

$$\hat{H} = \sum_{j=1}^N \hat{H}_j^0 + \sum_{j=1}^{N-1} \hat{H}_{j,j+1} + \sum_{j=2}^N \hat{H}_{j,j-1} + \sum_{j=1}^{N-2} \sum_{k=j+2}^N \hat{H}_{j,k} + \sum_{k=3}^N \sum_{j=1}^{k-2} \hat{H}_{j,k},\tag{2}$$

where $\hat{H}_j^0$ is the Hamilton of the unperturbed, j th local oscillator. The second and third term describe nearest neighbor coupling, whereas non-nearest neighbor coupling is fully accounted for by the fourth and fifth term. Intra-strand nearest neighbor coupling was calculated as described by Schweitzer-Stenner [19]. All other coupling between nearest neighbors, and second, third, etc. nearest neighbors, were calculated by using the transition dipole formalism:

$$\hat{H}_{j,k} = \xi \left[\frac{\vec{\mu}_j \cdot \vec{\mu}_k}{R_{jk}^3} - \frac{3(\vec{\mu}_j \cdot \vec{R}_{jk})(\vec{\mu}_k \cdot \vec{R}_{jk})}{R_{jk}^5} \right],\tag{3}$$

where $\xi = 9.047 \times 10^{15} \text{ esu}^{-2}$ and $\vec{R}_{jk}$ is the distance vector between the j th and k th oscillator.

The structural dependences of the amide I band profiles have different sources. First of all, excitonic coupling is *per se* orientational dependent, which affects IR absorption, and isotropic and anisotropic Raman scattering. The IR-intensity distribution additionally reflects the angles between the transition dipole moments in a peptide assembly, whereas the anisotropic Raman intensity distribution reflects the angles between the normals of the peptide planes [21].

The VCD signal of the amide I band is another very sensitive probe of peptide and protein structure, and is directly proportional to the rotational strength. In the framework of the excitonic coupling model, the rotational strength is written as [14]:

$$R_i = \text{Im} \left[\sum_{j=1}^N a_{ij} \vec{\mu}'_j \cdot \left(\sum_{k=1}^N a_{ik} \vec{m}'_k - \frac{i\pi}{2} \left(\sum_{l=1}^{N-1} \sum_{m=l+1}^N \tilde{\nu}_{lm} \vec{R}_{lm} \times (a_{il} \vec{u}'_l - a_{im} \vec{u}'_m) \right) \right) \right],\tag{4}$$

where $\vec{m}'_k$ is the intrinsic magnetic transition dipole moment of the k th oscillator.

3. Material and methods

Materials. Ac-(AAKA)₄-NH₂ was synthesized by Celtek Peptides (Nashville, TN, USA) and used without further purification. The peptide was dissolved in D₂O at a concentration of 5 mg/ml (~3.5 mM). It has been shown previously that this peptide forms a hydrogel in the presence of Cl⁻ anions [22]. To form the hydrogel, a sample of the prepared peptide stock solution was added to a pre-weighed amount of NaCl, to yield a final salt concentration of 2 M.

Spectroscopy. The FTIR and VCD spectra were recorded with a ChiralIR™ instrument from BioTools (Jupiter, FL, USA), with a spectral resolution of 8 cm⁻¹. The Ac-(AAKA)₄-NH₂ hydrogel was placed into 100 μm CaF₂ BioCell™ (BioTools). Three separate spectra were recorded, with total experiment times of 10, 12 and 15 hours. The resulting three spectra were averaged. For the purpose of illustration, the IR band resulting from residual trifluoroacetic acid (TFA), which was present in the Ac-(AAKA)-NH₂ sample (at 1674 cm⁻¹), was manually subtracted with the spectral analysis software MULTIFIT [23].

The 442 nm excitation from a HeCd laser (Model IK 4601R-E; Kimmon Electric US) was used to obtain the Raman spectra. The Raman set-up in our lab has been described previously [24]. Approximately 100 μl of the Ac-(AAKA)₄-NH₂ hydrogel was deposited onto a conventional microscope slide, and the Raman spectra were acquired. A total of 5 *x*-polarization spectra and 10 *y*-polarization spectra were acquired for a total of 10 s each.

4. Results and discussion

In a first step, we simulated the IR, isotropic Raman, anisotropic Raman and VCD band profiles of amide I for antiparallel β-sheets as a function of the number of incorporated strands. In order to allow for a comparison with experimental data, we ensured that the single strand of our model reflects the properties of the 16-mer Ac-(AAKA)₄-NH₂. We recently showed that Ac-(AAKA)₄-NH₂ aggregates in solution and eventually forms a hydrogel in the presence of Cl⁻ ions [22]. The respective amide I profiles of the peptide's gel phase are shown in Fig. 1. The spectra in Fig. 1 are indicative of an antiparallel β-sheet with a large splitting between the intense low wavenumber and the weak high wavenumber component of the IR spectrum of the amide I (~78 cm⁻¹) and a rather continuous distribution of intensities between both, which decreases with increasing wavenumber, as predicted [20]. The Raman bands are unusually broad, most likely a result of inhomogeneity of the hydrogel sample. In contrast to what is predicted for an ideal antiparallel β-sheet, isotropic and anisotropic Raman scattering was observed at the position of the strong IR band at ca. 1620 cm⁻¹. It should be noted that the intensity of anisotropic scattering is unusually large owing to intrinsic scattering. The VCD signal of amide I' is unusually strong and depicts a positively biased couplet close to the low wavenumber band in the IR spectrum. It should be noted that these amide I' band profiles are somewhat different from the respective spectra of a different hydrogel of (AAKA)₄ [10] for which we obtained rather regular amide I' profiles indicative of a more ideal β-sheet structure.

For our simulations, we utilized the amide I wavenumber shifts caused by a A → K substitution, and the amide I transition dipole moments of AA, AK and KA segments reported by Measey et al. [25]. For the intrinsic wavenumber of non-terminal alanine residues, we used the value of 1656 cm⁻¹ obtained for the central residue of tetraalanine [26]. The wavenumber positions of the terminal amide I modes are generally different from those of the remaining peptide groups. For an acetylated peptide,

Fig. 1. IR, isotropic Raman, anisotropic Raman and VCD spectra of an Ac-(AAKA)₄-NH₂ hydrogel of the amide I' region. The hydrogel was prepared by adding NaCl to a 5 mg/ml (3.5 mM) solution of Ac-(AAKA)₄-NH₂ in D₂O, to yield a final salt concentration of 2 M.

the N-terminal amide I band lies at 1628 cm^{-1} , which is nearly 30 cm^{-1} lower than the wavenumbers of non-terminal residues [27]. The carbonyl group at the C-terminal generally shifts the corresponding amide I' mode to the $1645\text{--}1648\text{ cm}^{-1}$ region [26,27]. All these wavenumber values reflect the influence of hydrogen bonding of the peptide to water, and of water to the respective amide proton [28,29]. Generally, one can assume a similar influence of interstrand CO–NH hydrogen bonding on the intrinsic wavenumbers (Choi, private communication). Therefore, we performed the simulations with the above introduced wavenumber values. The antiparallel β -sheet was constructed so that the shortest CO–NH distance between peptide groups in adjacent strands was $2.0\text{ \AA}$. This yields interpeptide transition dipole coupling values which are very close to those reported by Lee and Cho [20]. The chosen dihedral angles corresponded to the canonical values for an ideal antiparallel β -sheet structure, namely $\phi = -137^\circ$ and $\psi = 133^\circ$. Each band associated with an excitonic transition was described by a Gaussian band profile of 20 cm^{-1} halfwidth.

Figure 2 depicts the IR, isotropic Raman, anisotropic Raman and VCD profiles of amide I for β -sheets containing the indicated number of (AAKA)₄ peptide strands. Aggregation causes the IR-profile to split, with the low wavenumber component carrying most of the intensity. The split substantially increases with an increasing number of strands until saturation is reached at approximately 8 strands. Concomitantly, the low wavenumber IR band narrows and the peak intensity increases, which reflects a decreasing number of excitonic transitions contributing to the overall band profile. This issue will be discussed in more detail below. The maximal shift obtained by our simulation moves the band to 1622 cm^{-1} , which is not too far away from the 1616 cm^{-1} at which this band is positioned in the experimental IR spectrum of (AAKA)₄. The corresponding weak band peaks at 1683 cm^{-1} , which is approximately 10 cm^{-1} lower than the corresponding experimental value ($\sim 1694\text{ cm}^{-1}$). The isotropic Raman profile of the 8-strand aggregate is dominated by a band at 1652 cm^{-1} . This wavenumber is only slightly lower than the experimental value of $\sim 1657\text{ cm}^{-1}$. The anisotropic Raman profile exhibits an asymmetric band whose maximum is slightly non-coincident with the 1652 cm^{-1} band in the isotropic spectrum, which is again congruent with our experimental observation. Additionally, the anisotropic band profile displays a shoulder at ca. 1674 cm^{-1} , which is not so pronounced in the experimental spectrum. The VCD spectrum is weak, and exhibits a small couplet in the 1670 cm^{-1} region. Experimentally, we observed something different, namely a negative couplet, with the positive maximum occurring near the position of the intense IR band at $\sim 1614\text{ cm}^{-1}$. Generally, however, the simulated amide I profiles are in good qualitative agreement with the respective experimental spectra of the gel phase of (AAKA)₄.

One might be tempted to assign the two IR and the two Raman bands to the A_u, B_u, A_g and B_g representation of the unit cell of an ideal antiparallel β -sheet, respectively. A simulation carried out with a higher spectral resolution (the bandwidths were reduced to 1 cm^{-1}) reveal that the intense IR and Raman bands are indeed dominated by a single excitonic transition (Fig. 3), so that the assignment to unit cell modes appears justified.

In a second step, we calculated the influence of stacking on the amide I band profiles. To this end, we considered two different cases, i.e. a parallel and an antiparallel facial in-register arrangement of two sheets with six and seven strands per sheet, respectively. The sheet–sheet distance was adjusted to $7.5\text{ \AA}$, in accordance with Aggelli et al. [30]. The result of the simulation is shown in Fig. 4.

For the antiparallel arrangement, the low frequency IR band broadens and its intensity decreases. The corresponding high-frequency component shifts up to ca. 1690 cm^{-1} and a band at 1640 cm^{-1} gains some IR intensity. The dominant bands in the isotropic and anisotropic Raman spectra shift down to ca. 1638 cm^{-1} , which is close to the new IR band. This separates them from the weaker bands at 1670 cm^{-1} ,

Fig. 2. Simulated amide I band profiles of the IR, isotropic Raman, anisotropic Raman and VCD spectra of different oligomer of Ac-(AAKA)₄-NH₂. The number of strands in the considered sheet monomer is indicated. The parameters used for the simulation are described in the text.

Fig. 3. Excitonic structure of the amide I band profiles of the IR, isotropic Raman, anisotropic Raman and VCD spectra of an Ac-(AAKA)₄-NH₂ octamer. The spectra were simulated with a reduced halfwidth of cm^{-1} to enhance resolution.

Fig. 4. Simulated amide I band profiles of the IR, isotropic Raman, anisotropic Raman and VCD spectra of an Ac-(AAKA)₄-NH₂ octamer (solid black) and of two octamers with in-register facial stacking (antiparallel orientation: dashed, parallel orientation: dashed-dot-dot).

which are now clearly resolved. Additionally, a weak band appears at 1610 cm^{-1} . The corresponding VCD signal is still weak with two positive bands at the positions of the two canonical IR bands.

A rather different behavior is observed for the parallel arrangement. The strong IR band is less broadened, but the splitting between the two IR bands is significantly reduced to ca. 37 cm^{-1} . The dominant Raman band is only slightly up-shifted from the respective position in the spectrum of a two-dimensional sheet with 8 strands. The weaker, high-energy band, however, is significantly enhanced, particularly in the anisotropic spectrum.

The VCD spectra of both stacking arrangements exhibit a more pronounced amide I signal than that of the two-dimensional single sheet. For the parallel arrangement, the amide I region displays two pronounced negative Cotton bands on the low and high wavenumber sides of the low- and high-wavenumber IR bands, respectively. The VCD spectrum of the antiparallel arrangement shows a relatively pronounced couplet close to the low wavenumber band of the IR profile.

A comparison of the simulations of the amide I for two facially stacked β -sheets with the experimental (AAKA)₄ spectra in Fig. 1 yields mixed results. The splitting between the low and high wavenumber bands in the IR spectrum is only slightly underestimated by the antiparallel arrangement, and more so by the parallel arrangement. For the former, the dominant Raman band appears at substantially lower wavenumbers than the corresponding experimental band. On the contrary, however, the position of the experimentally observed Raman band is actually close to the corresponding value obtained for the parallel arrangement. The broadening of the anisotropic profile in the simulation is consistent with the rather broad experimental spectrum. The simulated VCD spectrum of the antiparallel arrangement is very close to the experimental one, in that both display a positive band at the position of the intense low wavenumber IR band.

It is not unreasonable to assume that the experimental amide I' profiles displayed in Fig. 1 reflect the coexistence of both two-dimensional tapes of β -sheets and higher order aggregates with parallel stacked β -sheets. This would particularly explain the fact that the observed low wavenumber amide I' band is heavily asymmetric towards higher wavenumbers. One might argue that this might also reflect twisting and other deformations of the β -sheet structure, but our simulations (results not shown) show that this would reduce the band splitting and thus not be consistent with our experimental data. A more serious concern arises from the fact that the positive charges on the lysine residues should prevent stacking. Recent MD simulations have provided evidence for an antiparallel β -sheet structure of (AAKA)₄ in which the lysine residues of neighboring strands point into opposite directions to avoid Coulomb repulsion [31]. We would have to assume substantial shielding of these charges by Cl⁻ anions to make any stacking arrangement plausible. However, atomic force microscopy data indicate that fibril formation precedes gel formation, which would require some kind of stacking. A plausible architecture of the (AAKA)₄ aggregates is shown in Fig. 5, where antiparallel strands form sheets which stack in a parallel fashion.

5. Summary

This paper demonstrates that the classical excitonic coupling approach is suitable to calculate not only the IR, but also the isotropic Raman, anisotropic Raman and VCD profile of rather large β -sheets, which would be out of range for any *ab initio* calculations. Rather than transferring tensors and force constants from DFT calculations on smaller units, we used empirical values for intrinsic wavenumbers and oscillator strengths to obtain remarkable agreement with experimental data.

Fig. 5. Cross section of a possible architecture of the parallel stacking of (AAKA)₄ β -sheets.

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