Deep UV resonance Raman spectroscopy for studying protein folding and aggregation

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Recent advancements in laser technology and the development of high efficiency array detectors for the entire UV-visible region have made Raman spectroscopy a user-friendly technique that has many practical applications. UV excitation allows for a substantial increase in the efficiency of Raman shattering due to both the resonance enhancement and stronger scattering of more energetic photons. Shifting the excitation below ~250 nm results in a dramatic increase in the signal-to-noise ratio of Raman spectroscopic measurements because of the absence of fluorescence interference. All these factors open exciting opportunities for numerous applications of UV Raman spectroscopy in various areas of chemistry and biology. Deep UV excitation ($\lambda_{ex} \leq 210$ nm) has been proven to be especially useful for biological studies due to resonance enhancement of Raman scattering from the polypeptide backbone and, as a result, provides information about the secondary structure of proteins [1]. A high sensitivity of deep ultraviolet resonance Raman (DUVRR) spectra to the protein secondary structure is based on extremely complexed nature of amide modes involving coupling of various vibrations. Amide I mode consists of carbonyl C=O stretching, with smaller contribution from C-N stretching and N-H bending. Both Amide I and Amide II bands involve significant C-N stretching, N-H bending, and C-C stretching. The C_α-H bending vibrational mode involves C_α-H symmetric bending and C-C_α stretching [2]. Amide III and (C)C_αH bending vibrational modes are most sensitive Raman bands to the amide backbone conformation. Asher et al.[3] have demonstrated that this sensitivity results from the coupling of amide N-H motion to (C)C_αH motion that depends on a Ramachandran dihedral Ψ angle. As a result, the Ramachandran Ψ-angular distributions could be obtained from an Amide III deep UV Raman band shape [1]. Unlike tryptophan and tyrosine, phenylalanine has a low fluorescence quantum yield that limits its application as a natural fluorescence probe. The Raman scattering of phenylalanine is resonantly enhanced on excitation with wavelengths below 200 nm [2]. For example, the 1000-cm^{-1} (ν12) phenylalanine Raman band was evident in all 197-nm excitation spectra of lysozyme and a dramatic decrease in its Raman cross-section with temperature and incubation time was assigned to the increased exposure of phenylalanine residues to water resulting from lysozyme unfolding.

The development and application of digital cameras for acquiring Raman spectra composed of hundreds of points open new opportunity for extracting additional information via statistical analysis of spectral datasets [4]. For example, the early stages of lysozyme fibrillation were quantitatively characterized by two-dimensional correlation deep UV resonance Raman spectroscopy in terms of the sequential order of events and their characteristic times [1]. The evolution of secondary and tertiary structure was established, including the formation of the fibrillation nucleolus. Combining hydrogen-deuterium exchange (HDX) with DUVRR spectroscopy and Bayesian statistics allowed for characterizing the fibril core structure [5]. Raman bandwidth of the highly ordered cross-β sheet of lysozyme fibrils was found comparable to that of peptide microcrystals, indicating no inhomogeneous broadening due to various amino acid residues involved into the cross-β core. This is in contrast to the Raman spectra of native globular protein β-sheets which exhibit broader Raman peaks than those of homopolypeptides. The conformation of the parallel β-sheet in the $A\beta_{1-40}$ fibril core is atypical for globular proteins, while the antiparallel β-sheet in $A\beta_{32-42}$ fibrils is a common structure in globular proteins. In contrast to globular proteins, the conformations of parallel and antiparallel β-sheets in Aβ fibril cores are substantially different, and these two types of β-sheet can be differentiated by DUVRR spectroscopy. We were also able to modeling the Raman signature of the $A\beta_{1-40}$ fibril core based on the known structure. HDX-DUVRR spectroscopy allowed for probing the change in the cross-β core structure due to the spontaneous refolding of apo-*a*-lactalbumin fibrils from one polymorph to another [1].

References

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