TWO NOVEL SPECTROSCOPIC TECHNIQUES: 2D ROA AND 2D RAMAN/ROA

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Abstract: This contribution describes two new spectroscopic techniques, 2D ROA and 2D Raman/ROA, and their application to studies of conformational transitions in polypeptides. The work focuses on studies with relevance to biological function, such as amyloid formation, and these new techniques are shown to provide new and detailed information on the behaviour of both the polypeptide backbone and sidechains.

Raman optical activity (ROA), is a novel form of chiroptical spectroscopy which measures a tiny difference in the intensity of Raman scattering from chiral molecules in right- and left-circularly polarized incident light or, equivalently, a small circularly polarized component in the scattered light [1-3]. Barron and coworkers have developed the application of ROA to biological systems such as proteins and viruses [4,5]. ROA data are complementary to that from conventional Raman spectroscopy as the two techniques are generated by related, yet subtly different, mechanisms. As a consequence, the corresponding Raman and ROA spectra of a protein or polypeptide constitute a library of structure- and environment-sensitive bands.

However, a widely recognized problem with the analysis of both Raman and ROA spectra of proteins is in obtaining definitive assignments for all component bands, because of band overlaps and a lack of satisfactory models for some structural motifs. A powerful approach to enhancing the visualization and analysis of complex spectra is provided by two-dimensional (2D) correlation spectroscopy. The generalized 2D-spectroscopic correlation technique [6] is based on linear relationships between spectral data obtained under a perturbating influence. Generalized 2D correlation spectroscopy enhances spectral resolution by spreading bands over a second dimension, can provide new band assignments by selectively coupling bands through various interaction mechanisms and can probe the order of spectral changes using synchronous and asynchronous correlations. Heterospectral 2D correlation, which is based on the pairwise comparison of spectra obtained from two different techniques, is a particularly attractive variant as it allows the extraction of complementary information obtained from the two techniques [7]. We present here the first reported application of 2D correlation methods to ROA spectra in the form of two novel methods; 2D ROA and 2D Raman/ROA spectroscopies.

We describe 2D ROA and 2D Raman/ROA studies for the α-helix-to-β-sheet conformational change in polylysine, a model structure for studying fibrilogenesis (8), and the transition from α-helix-to-disordered structure in polyglutamic acid. Several new band assignments for both protein ROA and Raman spectra have been generated through coupling of spectral variations, as shown by examples in Fig. 1 for β-sheet formation in polylysine, which illustrates the enhanced resolution inherent to 2D correlation. The results are shown to be consistent for corresponding 2D ROA, 2D Raman/ROA and 2D Raman correlation spectra of these transitions, while the relative advantages and disadvantages of each method will be discussed. Finally, the incisiveness of these new

techniques is shown by their ability to determine the order of changes in the vibrational modes from the polypeptide backbone and sidechains during these conformational transitions.

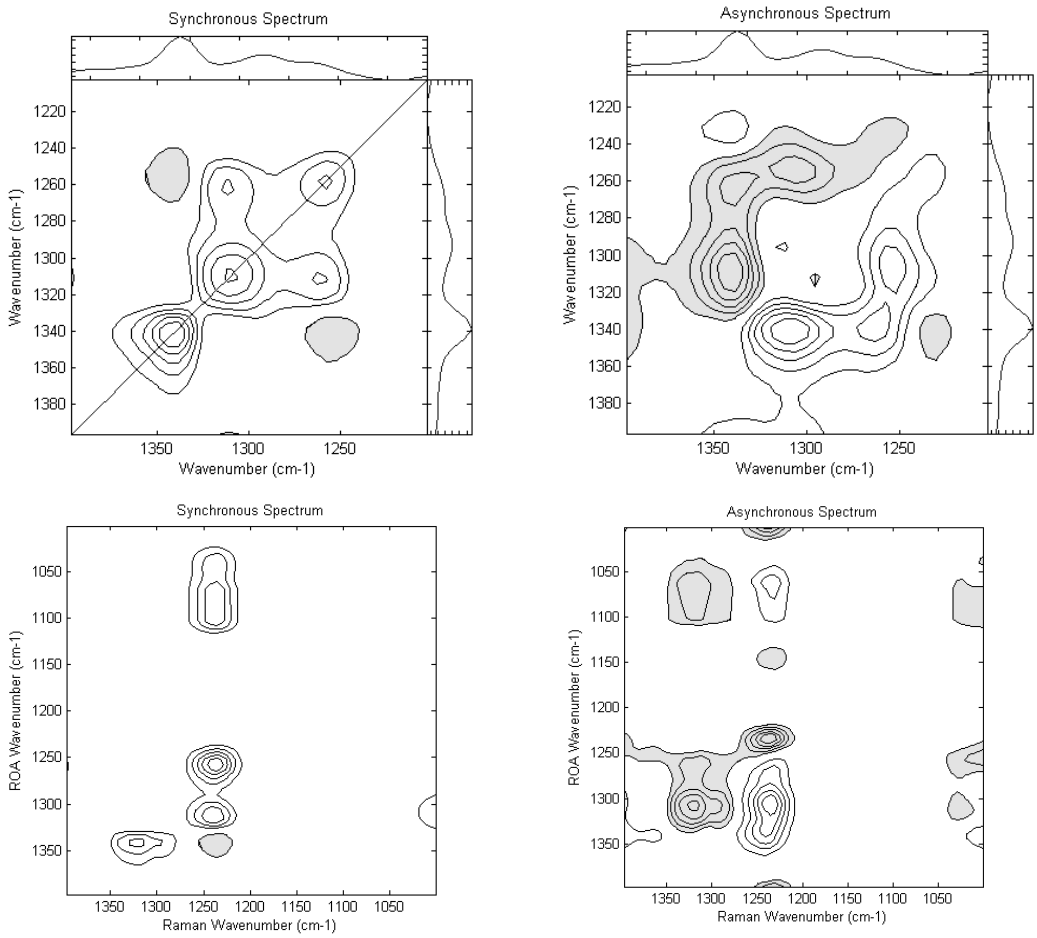

Fig. 1. 2D ROA (top) and 2D Raman/ROA (bottom) synchronous (left) and asynchronous (right) correlation plots around the amide III region for β-sheet formation in polylysine.

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