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Two-dimensional correlation spectroscopy in protein science, a summary for past 20 years

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ABSTRACT

Two-dimensional correlation spectroscopy (2DCOS) has been widely used to Infrared, Raman, Near IR, Optical Activity (ROA), Vibrational Circular Dichroism (VCD) and Fluorescence spectroscopy. In addition, several new developments, such as 2D hetero-correlation analysis, moving-window two-dimensional (MW2D) correlation, model based correlation ($\beta\nu$ and $k\nu$ correlation analyses) have also well incorporated into protein research. They have been used to investigate secondary structure, denaturation, folding and unfolding changes of protein, and have contributed greatly to the field of protein science. This review provides an overview of the applications of 2DCOS in the field of protein science for the past 20 year, especially to memory our old friend, Dr. Boguslawa Czarnik-Matusewicz, for her great contribution in this research field. The powerful utility of 2DCOS combined with various analytical techniques in protein studies is summarized. The noteworthy developments and perspective of 2DCOS in this field are highlighted finally.

1. Introduction

Two-dimensional correlation spectroscopy (2DCOS) has been widely applied to various research fields to analyze deeply the spectral data obtained under the influence of different kind of external perturbations [1–30]. As a powerful analytical technique, 2DCOS can elucidate subtle structural information in spectral variations for the system of interests [20,22,24,27,31–120]. By the enhanced spectral resolution and improved determination of sequential changes of spectral intensities, particularly 2DCOS has been broadly applied to protein studies [3–30] as it can monitor the behavior of protein both on structural variations and sequential of events. Popular perturbations in protein studies are mainly focus on temperature [27,31–41], pressure [20,22,52–54], pH [24,27,39,42–44], chemical denaturants [45–49], concentration of protein [49–51], as well as H/D exchange [83–85]. Temperature is the most commonly used among the perturbations, since the structural changes in the protein backbone response to thermal treatment are usually accompanied by the nonspecific spectral changes. 2DCOS gives new insights at the molecular level into the exploring of protein unfolding mechanisms, including protein aggregation and fibrillation. Combined with the versatile optical spectroscopies, 2DCOS provides plenty of information about protein structure at different levels and interactions with environment. 2D correlation vibrational spectroscopy has shown its power when investigating the amyloid fibril formation during protein denaturation process, concerning the mis-folding diseases, such as Alzheimer's disease, Parkinson's disease or type II diabetes. Especially, IR spectroscopy is so far the most popular analytical probes used in 2DCOS for protein study [15,17–20,22,24–25,27–28,31,33–35,37,39–41,43,46–52,54–70,101].

For the techniques, being similar to IR, Raman spectroscopy has been widely applied to 2D correlation study for investigation of protein structural changes [16,29,30,44,71–75,103–110]. Raman spectra, which are rich in signatures coming from both side chain and backbone vibrations of the polypeptide, facilitate determination of changes at tertiary level leading to the further unfolding that results in the denaturation. Meanwhile, other spectroscopies including 2D Raman Optical Activity (ROA) [71,72,102,105,106], 2D Vibrational Circular Dichroism (VCD) [73,74] and 2D fluorescence spectroscopy [20,76,111–113], etc. are also quite often to be used in protein study. In addition, several new developments of 2DCOS, such as hetero-correlation analysis [31,80], moving-window two-dimensional (MW2D) correlation [58,92,102,114], model based correlation ($\beta\nu$ and $k\nu$ correlation analyses) [114,116], are also incorporated into the protein research to further deal the sophisticated condition. To be a powerful extension of 2DCOS, hetero 2D correlation analysis is very useful to analyze protein basing on two different types of spectroscopic techniques in probing the same system under the same or different perturbations. Moreover, the combined use of 2DCOS with versatile multivariate chemometric techniques as multivariate

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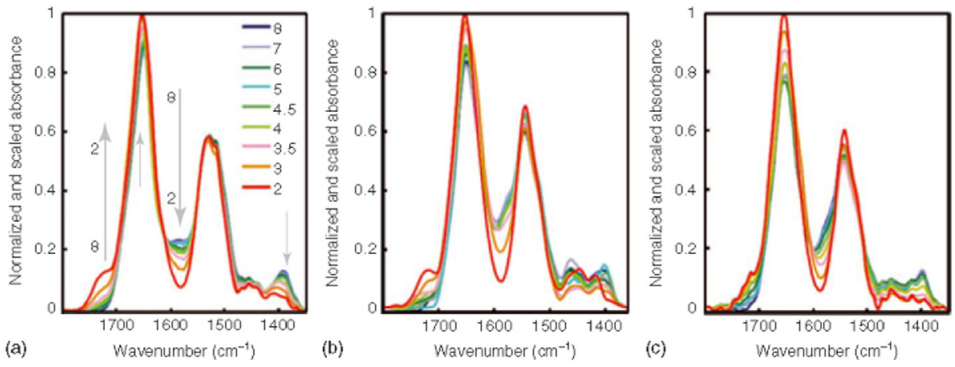

Fig. 1. The normalized spectra for the holoform of bovine α-lactalbumin obtained in the following experiments: FT-IR spectra obtained by (a) ATR mode for dry films, (b) ATR mode for solutions, and (c) transmission model for solutions.
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curve resolution (MCR) and principal component analysis (PCA) also illustrate great potential to reveal the conformation variations of proteins under perturbation [55,60,81–82,116–118].

This article reviews mainly the application of 2DCOS in the protein science. In addition, conventional 2DCOS probes with various analytical techniques, and the noteworthy developments of 2DCOS are also discussed to highlight the useful applications of this unique technique in protein investigations. Finally, the conclusion and perspective remarks of 2DCOS in protein research are summarized.

2. Two-dimensional Correlation Spectroscopy in Protein Study

2.1. Two-dimensional Infrared Correlation Spectroscopy

IR spectroscopy combined with 2DCOS is the most commonly used in the study of protein researches. It is very well suited to processes of protein folding and/or unfolding, including the aggregation and the fibril formation from β-type proteins. A primary limitation for proteins in IR experiments is the uncertainties caused by serious peak overlapping. Besides, it is also usually impossible to distinguish bands from the same secondary structure components located at quite different regions in the protein. Many physical parameters such as temperature and pressure, even the common protein denaturants, including organic solvents, detergents, and extreme pHs etc., are used as perturbations for protein studies either.

By using 2D IR correlation spectroscopy, Litwińczuk et al. investigated the pH-induced transition of holo-form of bovine α-lactalbumin (bLA) from the native (N) to the acidic (A) state. The pH-induced IR structural evolution, detected by both the attenuated total reflection (ATR) and the transmission (TR) mode, were analyzed [55].

Fig. 1 shows the FT-IR spectra of the holoform of bovine α-lactalbumin in solid film and in solutions collected either by ATR or reflection mode, which looks similar to each other. All the spectra were treated by Pareto scaling before the 2D correlation analysis to improve the contribution from weak spectral features. Thus the important spectral changes related to the secondary structure of protein are clearly disclosed both in the synchronous and asynchronous 2D contour maps (Fig. 2). As shown, the synchronous peaks are very similar both for signs and magnitudes, indicating the spectral changes induced by the pH decreases from 8 to 2 are quite similar.

In synchronous 2D correlation spectra of three systems, positive cross peaks at (1660, 1721/1717) and (1660, 1539) cm^{-1} and negative cross peaks at (1660, 1400), (1660, 1580), (1660, 1490) and (1660, 1460) cm^{-1} were observed. Bands at 1721/1717, 1660, and 1539 cm^{-1} are assigned to the $\nu_{(C=O)}$, amide I band, and amide II, respectively. Intensity of these three bands increase together with pH decreases. Especially the positive cross peak at (1660, 1721/1717) cm^{-1} suggests that the conformational variations primarily relates to the structural fragments close to the acidic amino acids. Because a large amount of the pH-sensitive residues are located in the helical domain, those changes corresponding to the more significant adjustments of the helical domain to the new pH conditions could be prominent for the amide vibration. Negative cross peaks explain that the intensity of band at 1660 cm^{-1} increases while those at 1580, 1490, 1460 and 1400 cm^{-1} decrease during pH changes. Bands near 1400 and 1580 cm^{-1} are assigned to the symmetric and asymmetric stretching vibrations of the carboxyl groups of the Asp side chains, respectively. Two separated bands at 1490 and 1460 cm^{-1} assigned to the Trp and Phe aromatic ring-stretching vibrations, respectively, and the higher frequency peak is additionally intensified by the vibrational characteristics of Tyr residues. Although all signs of cross peaks in synchronous 2D correlation spectra are same, there are clear differences in intensity variations of three systems. The deficiency in the interactions with water

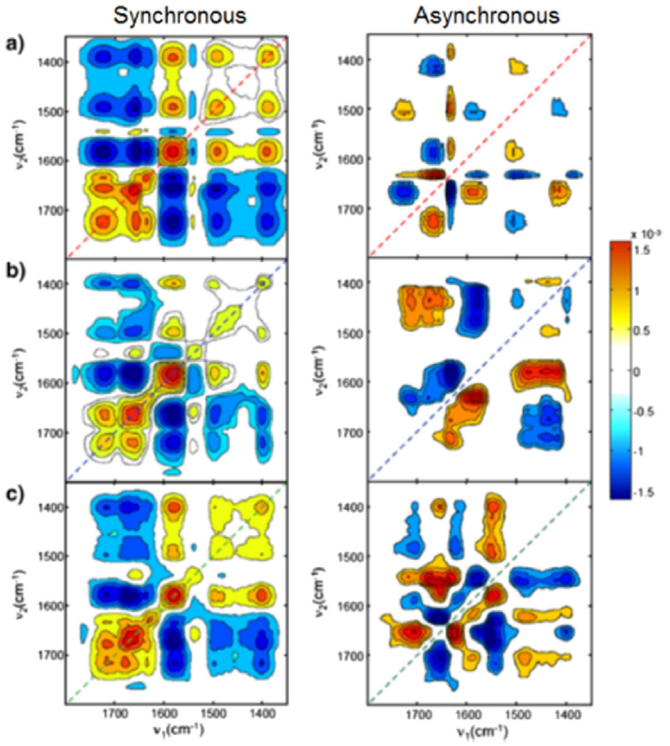

Fig. 2. The synchronous and asynchronous 2D correlation spectra calculated from the infrared spectra measured under different conditions: (a) film in ATR mode, (b) solution in ATR mode, and (c) solution in transmission mode. The color bar, which is in common for the three maps, is displayed to the right of the plot.
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