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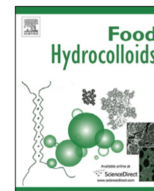

Critical desiccation state Raman spectroscopy for simple, rapid and sensitive detection of native and glycosylated protein

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ABSTRACT

A simple, rapid, and sensitive method named critical desiccation state Raman spectroscopy (CDSRS) has been developed for detecting native and glycosylated protein. Raman spectroscopy was performed within 1 min and without background subtraction. Results showed that Raman intensity of 1 μL of low concentration dispersions of biomacromolecule analytes remarkably increased from weak to high signal-to-noise ratio during the progressive desiccation process, and high-quality CDSRS spectra of biomacromolecule analytes could easily be obtained under the critical desiccation state, especially those such as zein and whey protein isolate-dextran conjugate which were difficult and even impossible to detect from their solid powders or low concentration dispersions by conventional Raman spectroscopy. Therefore, CDSRS would provide an opportunity to obtain high-quality Raman spectra from biomacromolecule analytes, which would be beneficial to elucidate the relationship between secondary structures and functional properties of protein and to control the formation of food hydrocolloids with desirable physicochemical properties.

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1. Introduction

Infrared and Raman spectroscopies, usually considered as two complementary vibrational spectroscopy techniques, have been used to investigate information on protein secondary structure and on intra-and inter-molecular interactions (Krimm & Bandekar, 1986). The water signal at 1640 cm^{-1} in infrared spectroscopy strongly overlaps with the protein amide I band, whereas water is a relatively weak Raman scatterer (Keating & Byrne, 2013). Infrared spectrum is largely dominated by the protein backbone amides, whereas Raman spectrum is not only sensitive to the protein backbone amides but also the protein side chains (particularly aromatic residues). Therefore, Raman spectroscopy has more advantages than infrared spectroscopy to characterize biological materials in solution. However, due to the inherently weaker Raman scattering, conventional Raman spectroscopy could not acquire the desirable Raman signals from low protein concentration dispersions. To overcome this problem of sensitivity and capacity of conventional Raman spectroscopy, several Raman techniques have been developed in the past few decades. The most emerging techniques include resonant Raman spectroscopy, coherent anti-Stokes Raman spectroscopy, surface-enhanced Raman spectroscopy, tip-enhanced Raman spectroscopy, and drop coating deposition Raman spectroscopy (Keating et al., 2013; Ortiz, Zhang, Xie, Ribbe, & Ben-Amotz, 2006). Although the sensitivity of the Raman spectroscopic techniques has significantly been enhanced, there are many shortcomings to be overcome. For example, the major disadvantages of resonant Raman spectroscopy are the interference of fluorescence and possible omittance of some important structural information (Morris & Wallan, 1979). The primary obstacles of coherent anti-Stokes Raman spectroscopy are the presence of a strong nonresonant solvent signal and a strong solvent water resonant signal with broad spectral width (Volkmer, Cheng, & Sunney Xie, 2001). The important challenges of surface-enhanced Raman spectroscopy are poor reproducibility, inherent instability, sensitivity against environmental conditions, and possibly different surface-enhanced Raman signals compared with conventional Raman spectroscopy (Cialla et al., 2012; Zheng & He,

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2014). The main drawbacks of tip-enhanced Raman spectroscopy are the reproducibility of fabrication of active tips suitable for tip-enhanced Raman spectroscopy, tip contamination, and comparability of tip-enhanced Raman signals compared with conventional Raman spectroscopy (Yeo, Stadler, Schmid, Zenobi, & Zhang, 2009). Understanding the structure-function relationships of proteins before and after glycosylation will facilitate the preparation of stable glycosylated protein-based nanocarriers, such as micelles, emulsions, and nanoparticles for nutrient and nutraceutical delivery. However, to the best of our knowledge, the drop coating deposition Raman spectra of glycosylated protein through Maillard reaction by dry heating have not been reported in the literature. Glycosylated proteins show better solubility, thermal stability, and emulsification properties compared with the original proteins (Liu & Zhong, 2012; Xia et al., 2015). Stable nanoparticle dispersions against pH and salt could be prepared by glycosylated protein (Kasran, Cui, & Goff, 2013a, Kasran, Cui, & Goff, 2013b; Li, Yu, Yao, & Jiang, 2008; Qi, Yao, He, Yu, & Huang, 2010).

The objective of the present study was to develop a simple, rapid, and sensitive method for detecting native and glycosylated protein, especially those which were difficult and even impossible to determine from their solid powders or low concentration dispersions by conventional Raman spectroscopy. This new technique was named critical desiccation state Raman spectroscopy (CDSRS). High signal-to-noise ratio Raman spectrum can easily be obtained by CDSRS from the molecules of close contacts in small clusters. The small clusters in a coffee ring resulted from a microscale volume of low analyte concentration dispersions under the critical desiccation state, which was the transition stage from aqueous dispersion to solid state. The critical desiccation state could easily be differentiated during the progressive desiccation process by a laser confocal microscopy. CDSRS not only can overcome the limitations of the above-mentioned Raman techniques and keep their high sensitivity, but it also has other important advantages: good reproducibility, low background, excellent comparability, and simple measurement. Therefore, CDSRS would be beneficial to elucidate the relationship between structure and function of protein and to control the formation of food hydrocolloids with desirable physicochemical properties.

2. Materials and methods

2.1. Materials and reagents

Whey protein isolate was purchased from Hilmar Ingredients (Hilmar, California). Dextran (40 kDa), zein (25–29 kDa), hydrochloric acid and sodium hydroxide were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All materials were used without any further purification. All aqueous dispersions were prepared with deionized water.

2.2. Preparation of native or glycosylated protein and/or polysaccharide dispersions

Whey protein isolate-dextran conjugate was prepared according to the method previously described (Sun et al., 2011). Briefly, whey protein isolate and dextran were dissolved in deionized water at a weight ratio of 1:1. After storing overnight at 4 °C to ensure complete hydration, the mixed dispersion was adjusted to pH 7.0 and then lyophilized. The resultant powder spread on Petri dish was incubated in a desiccator containing saturated KBr solution with a 79% relative humidity at 60 °C for 7 days. Whey protein isolate, dextran, whey protein isolate/dextran mixture, and whey protein isolate-dextran conjugate stock dispersions were prepared by dissolving their corresponding powders in deionized water. Zein stock dispersion was prepared by dispersing its powder in 85% (v/v) ethanol. Whey protein isolate, dextran, and zein concentrations in different stock dispersions were adjusted to 10 mg/mL. Aqueous zein dispersion was produced by injecting zein stock dispersion into deionized water as antisolvent and removing ethanol through a rotary evaporator under reduced pressure at 40 °C. Whey protein isolate/dextran/zein mixture or whey protein isolate-dextran conjugate/zein mixture dispersions were prepared by injecting zein stock dispersion into whey protein isolate/dextran mixture or whey protein isolate-dextran conjugate stock dispersions and removing ethanol as described above. Whey protein isolate, dextran, and zein concentrations in different dispersions were diluted with deionized water to 1.0, 1.0, and 0.5 mg/mL, respectively. All dispersions were adjusted to pH 7.0.

2.3. Raman spectroscopic measurement

Raman spectrum of each sample was determined at room temperature (25 °C) using a laser confocal microscopy Raman spectrometer (LabRAM HR Evolution, Horiba Jobin Yvon SAS, Longjumeau Cedex, France). Samples (1 mg solid powder or 1 μL dispersion) were placed on glass slides wrapped in aluminum foil. White light microimages of samples were acquired at magnifications of 5 or 50 ×. A 532 nm laser with power of 20 mW was used to acquire Raman spectra of samples after white light focused on samples at a magnification of 50 ×. Raman spectra were collected in the range 400–2000 cm^{-1} after an acquisition time of 20 s. During the progressive desiccation process, white light microimages of the edge of the drop on the aluminum foil gradually changed from opaque thick drop to transparent thin film. The critical desiccation state was the transition stage from aqueous dispersion to solid state. From a morphological point of view, the white light microimage of the critical desiccation state was the initial stage of transparent thin film formation. Therefore, the critical desiccation state was easily differentiated during the progressive desiccation process by a laser confocal microscopy. The LabSpec 6 software (Horiba Scientific) was used for spectral acquisition and analysis. All spectra represented the average of three replicate analyses without background subtraction and baseline correction.

3. Results and discussion

3.1. CDSRS spectrum of zein

A simple, rapid, and sensitive method named CDSRS was developed to detect Raman spectra of biomacromolecules. As shown in Fig. 1, Raman intensity of 1 μL of low zein concentration dispersion remarkably increased from weak to high signal-to-noise ratio during the progressive desiccation process. Because no Raman signals of aluminum foil substrate were observed (data not shown) and the spectral interference from water was insignificant under the critical desiccation state, high-quality Raman spectra of zein could be obtained without background subtraction (White light microimage in Fig. 1B6 and Raman spectrum in Fig. 1A6). However, it was quite difficult to obtain the Raman signals of zein powder. Previous studies reported that Raman intensity of zein powder was extremely weak (Fernandez, Torres-Giner, & Lagaron, 2009; Hu et al., 2015; Navdeep, Banipal, Kaur, & Bakshi, 2016). This might be due to the fact that protein powders are often composed of very thin fibers or flakes, in which the physical state might not be suitable for producing strong Raman scattering (Zhang, Mrozek, Xie, & Ben-Amotz, 2004). Additionally, proteins (such as light-harvesting proteins from higher plants) in crystalline powder displayed a very limited number of intermolecular contacts, due to the

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