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Heat-induced gel formation of plasma proteins: New insights by FTIR 2D correlation spectroscopy

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ABSTRACT

Generalized 2D correlation spectroscopy (COS) has been applied to FTIR spectra of porcine plasma proteins to elucidate the sequence of events leading to pH- and/or thermal-induced protein unfolding and aggregation. Changes in the amide I′ region of the infrared spectra (in the pH range between 7.5 and 4.5, at 0.5 pH intervals) at 30 °C were especially evident as the pH approached the p*I* of serum albumin (4.8), with the globulin fraction in the plasma proteins undergoing denaturation prior to serum albumin. The effect of increasing temperature (from 30 to 90 °C, in increments of 5 °C) on the secondary structure of the plasma proteins at pHs in the range of 7.5–6.0 revealed that a decrease in alpha-helical structures is taken place previously to diminish native beta-sheets. So, the overall results of this study demonstrate that serum albumin and the globulin fraction differ in their sensitivity to pH and temperature.

1. Introduction

Heat-induced gelation of proteins is considered to occur via complex multi-step events. Both the shape and the size of the protein are only mildly altered during heating (Baier & McClements, 2005; Clark, Kavanagh, & Ross-Murphy, 2001; Matsumoto & Inoue, 1991; Tobitani & Ross-Murphy, 1997), but the secondary structure is often disrupted (Belloque & Smith, 1998; Bouraoui, Nakai, & Li-chan, 1997; Clark et al., 2001; Clark, Saunderson, & Suggett, 1981; Qi et al., 1997). The way in which each protein unfolds during heating is unique and is affected by environmental factors such as pH, temperature and time, among others. The extent of protein aggregation during subsequent network formation affects the rheological properties of the gel (Tobitani & Ross-Murphy, 1997). A means of studying these events in order to gain an improved understanding of heat-induced gelation processes is provided by FTIR spectroscopy owing to the sensitivity of the infrared spectra of proteins to changes in secondary structure and protein aggregation (Byler & Susi, 1986; Ismail, Mantsch, & Wong, 1992; van Stokkum et al., 1995). An even better understanding may be achieved through the application of generalized two-dimensional correlation spectroscopy (2D COS) (Noda, 1993) in conjunction with FTIR spectroscopy. Because 2D COS analysis spreads the spectral data over a second dimension (e.g., increasing temperature or heating time), spectral features not readily observable by conventional one-dimensional (1D) FTIR spectroscopy may be clearly discerned. Furthermore, the synchronous and asynchronous 2D correlation plots generated by 2D COS analysis of FTIR spectra recorded as a function of temperature or heating time provide a means of investigating the sequence of events that result in heat-induced protein unfolding and aggregate formation (Filosa, Wang, & Ismail, 2001; Ismoyo, Wang, & Ismail, 2000).

In the present study, generalized 2D COS was used to characterize the sequence of events leading to heat-induced unfolding and intermolecular association of porcine plasma proteins. In a previous FTIR study in which we examined the thermal behavior of porcine plasma proteins, the spectra recorded under conditions leading to heat-induced gelation indicated that while the proteins retained a substantial amount of native secondary structure after heating, new intermolecular hydrogen-bonded β-sheet structures were formed (Saguer, Fort, Alvarez, Sedman, & Ismail, 2008). Several authors maintain that the formation of such intermolecular hydrogen-bonded β-sheet structures is essential for the formation of the gel network (Allain, Paquin, & Subirade, 1999). Taken together with the limited extent of protein unfolding observed in our work, this suggests that the particular secondary structure domains that unfold as well as the rate and extent of the subsequent formation of intermolecular hydrogen-bonded β-sheet structures could be important in governing the network integrity and, consequently, the rheological properties of the gel. Further information is provided by the important role of pH, which is evidenced by the finding that at pH values far above the p*I* of serum albumin (~4.8), the most abundant plasma protein, porcine plasma proteins form gels that have excellent texture and water retention capacity (Parés,

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Saguer, Saurina, Suñol, & Carretero, 1998; Saguer et al., 2008). Thus, the present 2D COS study was undertaken to elucidate the sequence of events leading to heat-induced gelation of porcine plasma proteins under different pH conditions.

2. Materials and methods

Spray-dried porcine plasma (AP920) was generously provided by American Protein Corporation (Ames, Iowa) and used as received. Protein and water contents were $92.5 \pm 0.5\%$ (w/w) and $4.9 \pm 0.1\%$ (w/w), respectively.

2.1. Solution preparation

To determine the effect of pH/temperature treatments on secondary structure, 7% (w/v) protein solutions were prepared in D_2O (99.9% D, Sigma–Aldrich, St. Louis, MO, USA) from spray-dried plasma and adjusted to different pH values (from 7.5 to 4.5, in increments of 0.5 pH units) using DCl (99% D, Sigma–Aldrich St. Louis, MO, USA). The reported pH values are direct readings from the pH meter and have not been transformed to pD values ($pD = pH + 0.4$). Samples were stored at 4 °C overnight to allow for full H–D exchange of solvent-exposed amide groups.

2.2. FTIR measurements

FTIR spectra were recorded on a Nicolet 8210 FTIR spectrometer (Thermo Electron Corporation, Madison, WI, USA) equipped with a deuterated triglycine sulphate (DTGS) detector. The spectrometer was continuously purged with dry air from a Balston dryer (Balston, Haverhill, MA, USA). The samples (8 μL) were placed in a thermostated IR transmission cell between two CaF_2 windows separated by a 50-μm Teflon™ spacer. To study the effect of temperature on secondary structure, the protein solutions were heated in the IR cell from 30 to 90 °C in 5 °C increments. At each temperature, the sample was equilibrated for 5 min prior to recording of its spectrum. The temperature of the cell was regulated by an Omega temperature controller (Omega Engineering, Laval, QC, Canada). The reported temperatures are accurate to within ±0.5 °C. Each spectrum was obtained by co-addition of 512 scans at 4-cm^{-1} resolution and ratioed against an open-beam background spectrum recorded at the beginning of the temperature run. Fourier self-deconvolution (FSD) was performed between 1800 and 1600 cm^{-1} (encompassing the amide I′ region) using Omnic 6.0 software (Thermo Electron Corporation, Madison, WI, USA), with a bandwidth of 27.2 cm^{-1} and a resolution enhancement parameter of 2.6. The deconvoluted spectra were normalized to unit area of the amide I′ band to compensate for minor pathlength variations. The FSD spectra were devoid of water vapor absorptions, confirming the efficacy of the dry-air purge of the spectrometer, and accordingly no corrections for residual water vapor were required.

2.2.1. Generalized 2D correlation spectroscopic analysis

Two-dimensional correlation (2D COS) analysis was performed on the FSD spectra. Synchronous and asynchronous correlation intensities were computed from the spectra recorded as a function of decreasing pH or increasing temperature. 2D COS analysis was carried out using the KG2D program (written by Y. Wang and described by Wang et al., 1998), which was implemented within Grams/32 software (Version 4; Thermo-Galactic, Salem, New Hampshire, USA); to facilitate examination of weak features, the 2D correlation maps were also displayed as 3D contour maps. The synchronous map consists of two types of peaks: auto-peaks, on the diagonal, which are always positive and are indicative of changes in band intensity induced by the pH or temperature changes and cross-peaks, at the off-diagonal positions, which indicate that the changes in band intensity at the corresponding wavenumbers on the x and y axes are correlated; a positive cross-peak indicates that the intensities of the two bands change in the same direction (e.g., both bands increase or decrease with increasing temperature), while a negative one indicates that the changes are in opposite directions. On the asynchronous map, auto-peaks are not observed, and the presence of cross-peaks indicates that changes in the correlated bands are taking place out-of-phase, i.e., accelerated or delayed with respect to each other. A positive sign of the synchronous and asynchronous cross-peaks at wavenumbers $x = \nu_1$ and $y = \nu_2$ indicates that the intensity change at ν_1 occurs prior to that at ν_2; if the change at ν_1 takes place after that at ν_2, the sign of the cross-peak on the asynchronous map is negative. If the cross-peak on the synchronous map is negative, the sign convention is reversed. Thus, the sequence of events is established by comparing the signs of cross-peaks in the synchronous and asynchronous maps (Noda, 1993).

3. Results and discussion

3.1. Changes in 1D FTIR spectra of plasma proteins as a function of pH

The FSD spectra of plasma proteins in the amide I′ absorption region as a function of increasing pH at 30 °C are shown in Fig. 1. As discussed in a previous publication (Saguer et al., 2008), the relative proportions of the serum albumin fraction, comprising ~60% of the plasma proteins, and the globulin fraction, composed primarily of immunoglobulins and representing ~36% of the plasma proteins, can be ascertained from the FSD–FTIR spectra. More specifically, the two strong bands at 1653 and 1638 cm^{-1} observed in the spectrum recorded at pH 7.5 are, respectively, assigned to α-helical structures, which are predominant in the serum albumin fraction (Carter & Ho, 1994; Peters, 1996), and intramolecular antiparallel β-sheet structures, mainly present in the globulin fraction of plasma (Saguer et al., 2008). Accordingly, the differences in the relative intensities of these bands in Fig. 1 as compared to the corresponding spectra in the previous publication (Saguer et al., 2008) may be attributed to a slightly higher proportion of the globulin fraction in the porcine

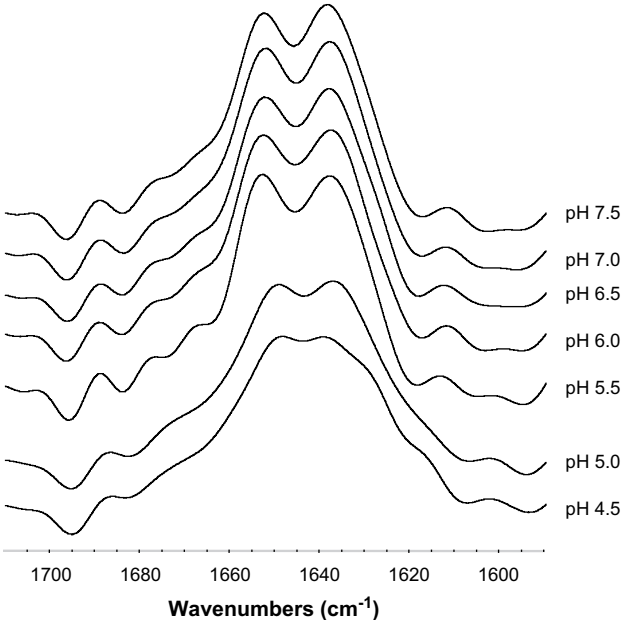

Fig. 1. Fourier self-deconvolved infrared spectra obtained at 30 °C from plasma protein solutions adjusted to different pHs.