Study of Oat Globulin Conformation by Fourier Transform Infrared Spectroscopy

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The conformation of oat globulin dispersions (10% in D_2O) under the influence of pH, chaotropic salts, protein structure perturbants, and heating conditions was studied by Fourier transform infrared (FTIR) spectroscopy. The FTIR spectrum of oat globulin showed major bands from 1670 to 1634 cm^{-1}, corresponding to the four major types of secondary structures, that is, β-turns, β-sheets, α-helices, and random coils. At extreme acidic and alkaline pH conditions, there were changes in intensity in the bands attributed to β-sheet structures (1626, 1634, and 1682 cm^{-1}), and shifts of the bands to higher or lower wavenumbers, indicating changes in conformation. In the presence of some chaotropic salts, the 1626 and 1634 cm^{-1} bands were shifted upward, with a marked decrease in the intensity of the 1634 cm^{-1} peak. The addition of several protein structure perturbants led to a slight shift in the α-helix/random coil bands and a marked reduction in the β-sheet peaks, suggesting protein unfolding. Heating under aggregating conditions led to slight shifts in all of the major bands and progressive changes in the intensity of the α-helix, β-sheet, and β-turn peaks, suggesting protein denaturation. This was accompanied by marked increases in intensity of the two intermolecular β-sheet bands (1682 and 1624−1626 cm^{-1}) associated with the formation of aggregated strands. The IR spectra of soluble and insoluble aggregates showed a redistribution of native and extensively denatured proteins in the two fractions.

Keywords: *Oat globulin; FTIR spectroscopy; protein conformation; denaturation*

INTRODUCTION

Oat globulin, the major protein fraction in oats, is an oligomeric protein with a molecular structure similar to that of other legume 11S globulins (*1*−*3*). The oat globulin hexamer, with an estimated M_r of 320−370 kDa, contains six subunits or monomers, and each subunit is made up of an acidic and a basic polypeptide with M_r values of 32−37 and 20−22 kDa, respectively, linked by disulfide bonds (*3*). Oat protein has been shown to possess good nutritive value (*4*) and functional properties (*5*) and has potential use as a food ingredient. A thorough understanding of the structure−function relationship in oat globulin is important in predicting and controlling the functional performance of oat protein in manufactured foods. Studies of the conformation of proteins under the influence of different environmental conditions can provide crucial information for improving specific functional properties such as gelation and emulsification. The conformation of oat globulin has been studied by differential scanning calorimetry (DSC) (*6*), UV and fluorescence spectrophotometry (*7*), and FT-Raman spectroscopy (*8*).

Fourier transform infrared (FTIR) spectroscopy, similar to Raman spectroscopy, is a vibrational spectroscopic technique that can be used to monitor the secondary structures of proteins (*9*, *10*). FTIR spectroscopy has been used to study the conformation of various food proteins (*11*−*13*) under the influence of various environmental factors (*11*). FTIR spectroscopy has distinct advantages over techniques such as circular dichroism (CD) spectroscopy, which requires transparent samples (diluted protein solutions or transparent gels). FTIR is therefore particularly suitable for studying vegetable proteins with limited solubility. Both Raman and FTIR spectroscopy can be used to investigate protein structural changes during aggregation/gelation because the techniques can be applied to liquid, semisolid, and solid samples (*10*, *14*). FTIR spectroscopy has been shown to be best for monitoring changes in β-sheet, whereas Raman spectroscopy and CD spectroscopy are better at following conformational changes involving the α-helix (*15*, *16*).

When compared with Raman spectroscopy, the FTIR technique has the advantages of higher signal-to-noise ratio, lower cost in instrumentation and maintenance, and ease of setting up a heating system for continuously monitoring conformational changes during thermal treatments. However, Raman spectroscopy can provide additional information on the tertiary structures of proteins (*10*), and because water yields only weak Raman scattering, Raman spectroscopy has advantages over the IR technique for studying proteins in situ (*10*).

In this study, the conformation of oat globulin dispersions was studied by FTIR spectroscopy under the influence of heat treatments and various buffer conditions. The results were compared to our previous data obtained from Raman spectroscopy and other techniques. No attempts were made to quantitatively estimate the proportions of secondary structure types on the basis of least-squares analysis or other mathematical algorithms (*17*, *18*). According to Wilder et al. (*19*), IR alone is not sufficient to unequivocally establish secondary structure without verification by other analytical methods such as CD, X-ray diffraction, and NMR spectroscopy.

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MATERIALS AND METHODS

Oat seeds (variety Hinoat) were grown at the Central Experimental Farm, Agriculture and Agri-Food Canada, Ottawa, Canada. They were dehulled in a pin-mill and defatted by Soxhlet extraction with hexane. Oat globulin was extracted from the defatted oat groats with 1 M NaCl (*20*). The protein content of oat globulin, determined according to the micro-Kjeldahl method (*21*) using a nitrogen to protein conversion factor of 5.80, was 98.9% (*5*). The purity and homogeneity of oat globulin were checked by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (*22*). All chemicals used were of analytical grade. The deuterated reagents (D_2O and DCl) were purchased from Aldrich (Milwaukee, WI).

Sample Preparation. Oat globulin dispersions (10% w/v) were prepared in D_2O instead of H_2O because D_2O has greater transparency in the infrared region of interest (1600−1700 cm^{-1}). To ensure complete H/D exchange, samples were prepared the day before, kept at 4 °C, and equilibrated to room temperature prior to infrared measurements.

To study the effect of pH on FTIR spectral characteristics of oat globulin, protein dispersions (≈10% in D_2O) with the desired pD were prepared by the addition of 1 M NaOD (prepared by dissolving NaOH in D_2O) or 1 N DCl with mixing in a magnetic stirrer, and the protein mixtures were stirred for an hour at room temperature to allow the pD to equilibrate (*6*). Chaotropic salts (sodium chloride, bromide, iodide, and thiocyanate) and protein structure perturbants (sodium dodecyl sulfate, dithiothreitol, and ethylene glycol) were added to oat globulin dispersions prepared in either D_2O or 0.01 M deuterated phosphate buffer, pD 7.0 (pD = pH + 0.4), to give the required concentrations. According to previous studies (*6*, *7*, *23*), chaotropic salts and perturbants at such concentrations will show their effects on the conformation of oat globulin.

For heating experiments, an Omega temperature controller (Omega Engineering, Stanford, CA) was used. The temperature was held at 100 °C, with an accuracy of ±0.5 °C, and the IR spectra were recorded at increasing time periods. Oat globulin dispersions (10%) were heated under buffer conditions (i.e., 0.01 M deuterated phosphate buffer, pD 7, without added NaCl) that prevent gelation (*24*) or extensive aggregation/protein precipitation (*25*). The formation of gels or substantial quantities of aggregates (in buffers containing added salt) will lead to poor quality IR spectra when using a transmission cell.

To prepare buffer-soluble and insoluble aggregates, an aliquot (100 mL) of oat globulin dispersion (1% in 0.01 M phosphate buffer, pH 7.4, containing 1.0 M NaCl) was heated in a 500-mL Erlenmeyer flask covered with aluminum foil in a boiling water bath. Heating conditions were selected such that substantial quantities of both soluble and insoluble proteins could be obtained (*25*). The heated sample was centrifuged at 20000*g* for 30 min, and the residue was washed with distilled water while the supernatant was dialyzed exhaustively against distilled water at 4 °C. Both the buffer-soluble (containing soluble aggregates) and insoluble (containing insoluble aggregates) fractions were freeze-dried. Dispersions (10% w/v) of the freeze-dried samples were prepared in 0.01 M deuterated phosphate buffer, pD 7.0, for infrared measurements. Due to the low solubility of the insoluble aggregates, 1.0 M NaSCN was added to the buffer to improve solubility and spectral quality.

FTIR Spectroscopy. Infrared spectra of oat globulin dispersions were recorded in a Bio-Rad Excalibur FTIR spectrometer (Bio-Rad Laboratories, Cambridge, MA) equipped with a deuterated triglycine sulfate (DTGS) detector. Samples were held in an IR cell with a 25 μm path length CaF_2 window. The scans were performed at 4 cm^{-1} resolution and were averaged. Preliminary data (not shown) showed that increasing the number of scans from 32 to 64, 128, 256, and 512 did not significantly improve the resolution of the IR spectra. For most experiments, 32 scans were used to save time, which was particularly important for monitoring changes during heat treatments.

Deconvolution of the infrared spectra was performed using Bio-Rad software (Merlin version 1) and according to the method of Kauppinen et al. (*26*). Band assignment of oat globulin in the amide I region (1600−1700 cm^{-1}) was according to that set forth by Susi and Byler (*15*). All FTIR experiments were performed in duplicate, and reproducible data (with standard deviation <10%) were obtained.

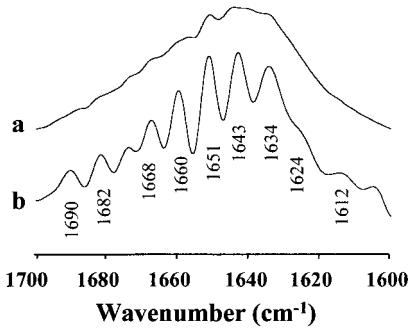

Figure 1. Original (a) and deconvoluted (b) infrared spectra of 10% oat globulin in D_2O.

Table 1. Assignments of Amide I′ Bands in the FTIR Spectrum of Oat Globulin in D_2O

frequency (cm^{-1})	assignment
1612	side chain vibrations
1626	antiparallel β-sheet, aggregated strands
1634	β-sheet
1643	random coil
1652	α-helix
1660	β-turns
1668	β-turns
1682	antiparallel β-sheet, aggregated strands
1690	β-type

RESULTS AND DISCUSSION

Spectral Assignment. Figure 1 shows a typical infrared spectrum (1600−1700 cm^{-1}) of oat globulin dispersion before and after deconvolution. Seven major bands were observed in this amide I region. Table 1 shows the assignments of these bands and others observed under different buffer conditions. From the locations and relative intensities of the infrared bands, it was evident that α-helix (1652 cm^{-1}) and random coils (1643 cm^{-1}) are the major secondary structures in oat globulin, followed by β-sheet (1634 and 1680 cm^{-1}) and β-turns (1660 and 1668 cm^{-1}). According to CD data (*27*), oat globulin, similar to most plant proteins, has a low α-helix content and a large quantity of β-sheet and random coil structures. Discrepancies between CD and FTIR spectroscopy in the quantitative estimation of protein secondary structures have been reported (*15*, *16*).

Effect of pH. Figure 2 shows the infrared spectra of oat globulin dispersions at different pH values. The spectra near neutral pH (pH 5−7) were similar to that of the control (in D_2O without pH adjustment). At highly alkaline (pH 11) pH, the intensities of two bands attributed to β-sheet structures (1626 and 1634 cm^{-1}) were markedly decreased, and the band position was shifted to higher values (1628 and 1636 cm^{-1}). The results indicate a change in hydrogen bonding (*28*) or a change in conformation (*12*). There were also slight decreases in intensity in bands associated with α-helix and unordered structures at pH 9 and 11. At highly acidic condition (pH 3), the 1626 cm^{-1} band was shifted to 1623 cm^{-1}, with increase in intensity. At extreme pH values, the side-chain vibration peak (1612 cm^{-1}) was also shifted, and there was a noticeable decrease in the

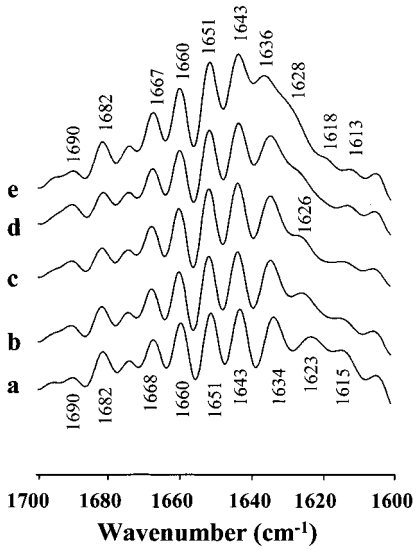

Figure 2. Stacked plot of deconvoluted infrared spectra of 10% oat globulin (in D_2O) at different pH values: (a) pH 3; (b) pH 5; (c) pH 7; (d) pH 9; (e) pH 11.

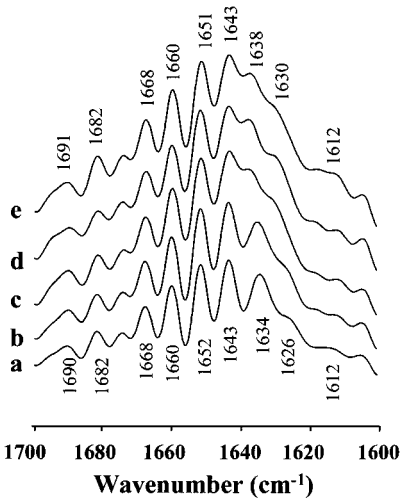

Figure 3. Stacked plot of deconvoluted infrared spectra of 10% oat globulin (in 0.01 M phosphate buffer, pD 7.0) in the presence of 1.0 M chaotropic salts: (a) control (no added salt); (b) NaCl; (c) NaBr; (d) NaI; (e) NaSCN.

intensity of the 1690 cm^{-1} band, which has been associated with the onset of unfolding in β-lactoglobulin (*29*).

Most proteins are stable near their isoelectric pH value, where repulsive forces are low and proteins remain in their native state. At pH values far away from the pI, large net charges are induced, leading to partial unfolding due to intramolecular side-chain charge repulsion and breakup of hydrogen bonds and hydrophobic interactions (*30*). In general, the present data show more marked infrared spectral changes at pH 3 than at pH 9−11. This can be attributed to the fact that the isoelectric pH range of oat globulin, similar to that of most plant proteins, is at the acidic pH of 5−6 (*5*). The present results are consistent with our previous DSC data (*6*), which showed that the peak transition or denaturation temperature (T_d), an index of thermal stability, and enthalpy (ΔH), a measure of the amount of ordered structure, were highest at pH 5−7, suggesting native conformation. The T_d and ΔH values were markedly decreased at pH <4.0 and >9.0, indicating reduced heat stability and partial protein denaturation in oat globulin. The FTIR data also agree with our Raman spectroscopy results (*8*), which showed a transition from β-sheet structure near neutral pH to random coil structure at highly acidic and alkaline pH values. There were also increases in band intensities of the amide I, amide III, and C−H stretching bands at extreme pH conditions, indicating protein denaturation (*8*).

Effect of Chaotropic Salts. In the presence of 1.0 M sodium salts, the β-sheet bands (1626 and 1634 cm^{-1}) were shifted to higher wavenumbers with decreases in band intensity (Figure 3). The progressive changes in the β-sheet band position and intensity, indicative of progressive protein unfolding, followed the order Cl^-, Br^-, I^-, and SCN^- according to the lyotropic or chaotropic series of anions (*31*). Other changes included a progressive decrease in intensity of the random coil band (1643 cm^{-1}), a slight shift in the α-helix band (from 1652 to 1651 cm^{-1}), and slight increases in intensity of the 1682 and 1691 cm^{-1} transitions (Figure 3).

Protein conformation has been demonstrated to be perturbed by salts that affect both electrostatic and hydrophobic interactions via a modification of water structure (*32*, *33*). The extent of water structure modification is dependent on the nature of anions or cations, following the lyotropic series (*31*). Anions (I^- and SCN^-) and cations (Li^{2+} and Ca^{2+}) higher in the series could reduce the free energy required to transfer the nonpolar groups into water and may weaken intramolecular hydrophobic interactions and enhance the unfolding tendency of proteins (*34*). Furthermore, anions lower in the series (Cl^- and Br^-) could promote salting-out and aggregation due to their higher hydration molar surface tension and help to stabilize the protein conformation. I^- and SCN^-, on the other hand, are destabilizing agents due to their high hydration energy and steric hindrance, which promote unfolding, dissociation, and salting-in of proteins (*35*). The FTIR results are consistent with previous DSC (*6*) and Raman (*8*) data in showing the relative effectiveness of these anions in perturbing the conformation of oat globulin. DSC data showed a progressive decrease in enthalpy and T_d (*6*), and Raman spectroscopy showed a progressive shift in the amide I and amide III bands and increases in intensities of amide and C−H bending vibrations (*8*) when the anion was changed from chloride to thiocyanate.

Effect of Protein Perturbants. The effects of some protein structure perturbants on the IR spectral characteristics of oat globulin are shown in Figure 4. In the presence of 10 mM dithiothreitol (DTT) (Figure 4b), 40% ethylene glycol (EG) (Figure 4c), and 10 mM sodium dodecyl sulfate (SDS) (Figure 4d), marked decreases in the intensity of the β-sheet bands (1626 and 1634 cm^{-1}) and shifts in band positions for several transitions (from 1616 to 1617 cm^{-1}, from 1626 to 1628 cm^{-1}, from 1644 to 1643 cm^{-1}, and from 1652 to 1651 cm^{-1}) were observed. The results suggest extensive protein denaturation.

SDS is an anionic detergent that binds to proteins by noncovalent forces and increases the net charge, leading to ionic repulsion and unfolding of polypeptides (*36*). DTT is a reducing agent and can break up disulfide bonds in the oat globulin oligomers to create a destabilized conformation. EG can destabilize protein conformation by lowering the dielectric constant of water and weaken the nonpolar interactions between protein

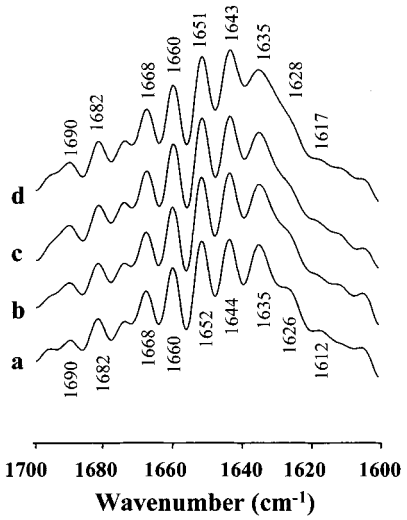

Figure 4. Stacked plot of deconvoluted infrared spectra of 10% oat globulin (in 0.01 M phosphate buffer, pD 7.0) in the presence of protein perturbants: (a) control; (b) 10 mM dithiothreitol; (c) 40% ethylene glycol; (d) 10 mM sodium dodecyl sulfate.

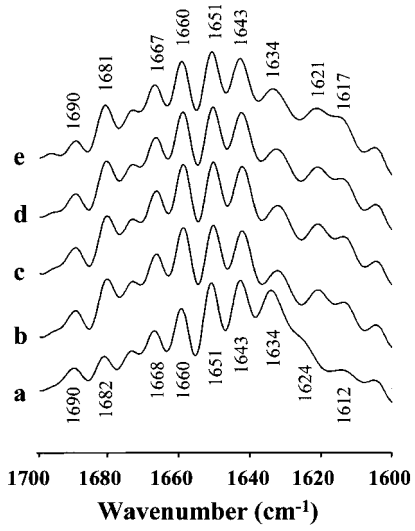

Figure 5. Stacked plot of deconvoluted infrared spectra of 10% oat globulin (in 0.01 M phosphate buffer, pD 7.0) heated at 100 °C for (a) 0 min, (b) 15 min, (c) 30 min, (d) 60 min, and (e) 120 min.

molecules (*37*). The present data are again in agreement with our previous DSC and Raman results (*6*, *8*). In the presence of these perturbants, thermal stability and enthalpy of oat globulin were decreased (*6*), and pronounced a shift in the Raman amide III band to higher frequency and increases in the intensity of the amide I and C−H stretching transitions were observed (*8*), indicating extensive perturbation of the protein conformation.

Effect of Heat Treatments. Figure 5 shows the FTIR spectra of oat globulin dispersions heated at 100 °C for different time periods, and Figure 6 shows the changes in integrated intensity of several IR bands with heat treatment. Results show that the intensity of the bands associated with the formation of intermolecular antiparallel β-sheet structures and aggregated strands (1621−1624 and 1681−1682 cm^{-1}) were progressively increased with increase in heating time (Figures 5 and 6), leveling off at ∼30 min (Figure 6). The band positions were also shifted toward lower wavenumbers (Figure

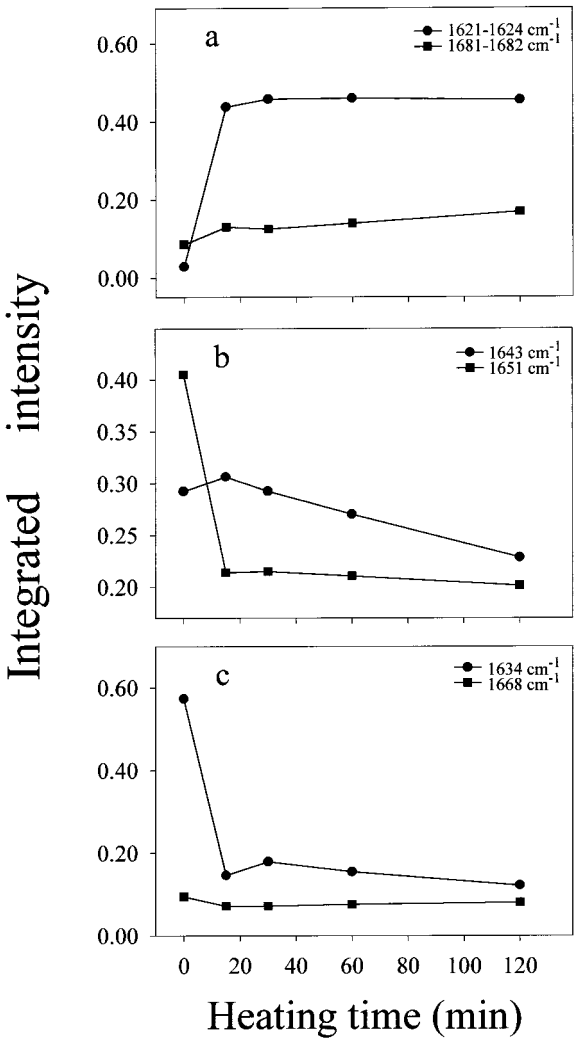

Figure 6. Plot of integrated intensity of (a) 1621−1624 and 1681−1682 cm^{-1} bands, (b) 1643 and 1651 cm^{-1} bands, and (c) 1634 and 1668 cm^{-1} bands in the infrared spectra of 10% oat globulin (in 0.01 M phosphate buffer, pD 7.0) heated at 100 °C for different time periods.

5). Marked intensity decreases in the β-sheet (1634 cm^{-1}) and random coil (1643 cm^{-1}) bands were observed, again leveling off at 15−30 min after heating (Figure 6). Slight decreases in intensity were also observed in the α-helix (1651 cm^{-1}) and β-turn (1668 cm^{-1}) transitions (Figure 6). Other spectral changes included a decrease in intensity in the β-type band (1990 cm^{-1}) and a shift of the side-chain vibration band (1612 cm^{-1}) to a higher wavenumber (Figure 5).

The infrared data indicate marked changes in the conformation of oat globulin when heated at a temperature well below its T_d, 110 °C (*6*). Our previous DSC data did show that heating at 100 °C for various time periods caused a progressive decrease in enthalpy, indicating protein denaturation, although the extent of changes was much lower than that observed at 110 °C (*23*). Significant changes in the conformation of oat globulin were observed only at 110 °C when monitored by ultraviolet and fluorescence spectrophotometry (*7*) and Raman spectroscopy (*8*). This suggests that FTIR is more sensitive in detecting the thermal denaturation of oat globulin than the other techniques we attempted. In the study of the thermal denaturation of β-lactoglobulin by FTIR and DSC (*29*), the DSC onset temperature rather than the peak temperature was found to

correlate with the denaturation temperature based on the amide I bands of FTIR spectra (loss of 1648, 1636, and 1624 cm^{-1} bands). This again indicates the higher sensitivity of FTIR compared with DSC in detecting the thermal denaturation of a protein.

In view of the exceptionally high thermal stability of oat globulin, the present results also indicate that the protein can aggregate under relatively mild heat treatments (at 10 °C below the T_d). The data are consistent with our previous study (*25*), which indicated that oat globulin can undergo thermal aggregation at 100 °C, although at a much lower rate than at 110 °C.

The FTIR results show that aggregation of oat globulin started to occur (as indicated by marked intensity increases in the aggregation bands at 1624 and 1682 cm^{-1}) within relatively a short heating time (15−30 min) and leveled off after the initial sharp increases (Figure 6). This is consistent with our previous data, which showed that heating of oat globulin solutions (1%) at 100 or 110 °C led to rapid precipitation of protein (formation of insoluble aggregates) followed by leveling off after 15−30 min (*25*). It should be noted that oat globulin was heated under different conditions in the two studies. In the previous investigation, oat globulin was prepared in 1% (w/v) solution in buffer containing 1.0 M NaCl, whereas in the present study, 10% dispersions in buffer with no added salts were used to prevent extensive protein precipitation. It has been shown that both high protein concentration and the presence of added NaCl enhanced thermal aggregation of oat globulin (*25*). Heating of oat globulin at concentrations >5% in the presence of added salt will lead to thermal gelation of the protein (*24*). The study of the thermal gelation of oat globulin would require attenuated total reflectance (ATR) FTIR spectroscopy, which has been shown to be particularly suited to the determination of secondary structure of globular proteins in functional states such as a gel (*12*).

For monomeric proteins, such as ovalbumin and bovine serum albumin, thermal aggregation is normally preceded by denaturation, following the scheme N ⇌ D → A, where N denotes native protein, D denatured molecule, and A the aggregate (*38*). For oligomeric proteins with complex quaternary structures such as soy glycinin and oat globulin, heat may cause association/dissociation of the oligomer, and disruption of the quaternary structure itself may result in aggregation (*39*).

When thermal denaturation and aggregation of β-lactoglobulin were studied by FTIR and DSC (*29*), it was suggested that the DSC onset temperature corresponded to the denaturation temperature based on the FTIR amide I bands. The DSC peak temperature, on the other hand, was similar to the temperature at which distinct aggregation bands (1618 and 1684 cm^{-1}) could be observed in the FTIR spectra. These results suggested that unfolding of β-lactoglobulin actually occurred at a lower temperature than the DSC peak temperature and that the latter appeared to be more indicative of aggregation than denaturation. In oat globulin, DSC (*24*) and FTIR data did suggest that the onset temperature (100.8 °C) in the DSC thermogram was related to the temperature (100 °C) at which denaturation was observed by FTIR. However, because aggregation was also observed by FTIR near the onset temperature, it is not possible to suggest that the DSC peak temperature (110 °C) corresponds to the aggregation temperature of oat globulin.

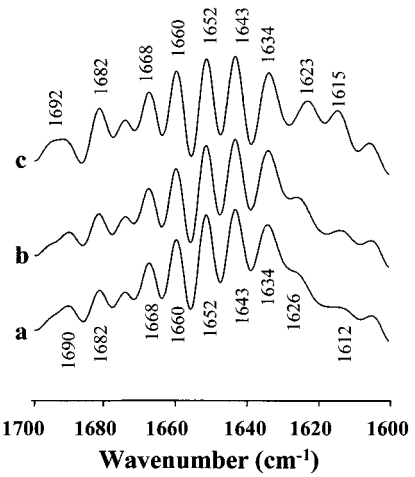

Figure 7. Stacked plot of deconvoluted infrared spectra of 10% dispersions of (a) oat globulin, (b) buffer-soluble aggregates, and (c) buffer-insoluble aggregates in 0.01 M phosphate buffer, pD 7.0.

IR Spectral Characteristics of Aggregates. Figure 7 shows the IR spectra of buffer-soluble and insoluble aggregates separated by centrifugation of oat globulin dispersions heated at 100 °C for 20 min. Due to the low solubility of the insoluble aggregates leading to poor IR spectral resolution, and therefore difficulty in comparing with the unheated control, 1.0 M NaSCN was added to the buffer to increase the solubility of the aggregates. In a previous study (*7*), 1 M NaSCN was also used to obtain >70% solubilization of the buffer-insoluble aggregates for UV and fluorescence spectroscopic analysis. Thiocyanate is higher than chloride in the lyotropic series of salts with greater protein perturbing and solubilizing effects (*33*). Preliminary results (not shown) showed that the IR spectra of the NaCl-insoluble aggregates in buffers with or without NaSCN were similar in terms of band positions and relative intensities, indicating that the use of a salt higher in the lyotropic series did not cause additional spectral changes in the heat-aggregated protein.

The data show that the IR spectrum of the buffer-soluble aggregates (Figure 7b) was similar to that of the unheated control (Figure 7a), suggesting that oat globulin in the soluble aggregate fraction did not undergo extensive conformational changes. The IR spectrum of the insoluble aggregates (Figure 7c) showed pronounced increases in intensity of the aggregation peaks at 1682 and 1626 cm^{-1} (shifted to 1623 cm^{-1}). There were no marked changes in the intensity of the other major bands, but increases in band intensity and shift in band position were observed in two minor peaks, namely, the side-chain vibration band at 1612 cm^{-1} and β-type band at 1691 cm^{-1} (Figure 7c).

The results again showed that relatively mild heat treatment (100 °C for 20 min) can lead to marked conformational changes in oat globulin. However, there seems to be a redistribution of the native and denatured proteins in the soluble and insoluble fractions, respectively. The data are consistent with our previous studies, which showed that soluble aggregates, prepared even by heating at a higher temperature (110 °C), displayed DSC (*23*) and UV and fluorescence spectral characteristics (*7*) similar to those of the unheated control. The insoluble aggregates, on the other hand, did not show

any endothermic response (*23*) and displayed significant red shift in the UV absorption spectrum and pronounced blue shift in the fluorescence emission spectrum (*7*), indicating that the fraction contained extensively denatured proteins. These results are consistent with the generally accepted view that aggregation is preceded by denaturation (*38*). The data also show that soluble aggregates of oat globulin can be formed from undenatured proteins, resulting probably from the disruption of the quaternary structure (*39*).

Because the buffer-soluble aggregates showed only a slight increase in the 1623 cm^{-1} aggregation band (Figure 7b), it can be deduced that the pronounced increases in the two FTIR aggregation bands during heating (Figures 5 and 6) were mainly due to the formation of buffer-insoluble aggregates. The other observed FTIR spectral changes indicating protein denaturation may also be attributed to the insoluble aggregates formed during heat treatment.

Conclusion. The present study demonstrated that the conformation of oat globulin was influenced by pH, chaotropic salts, some protein structure perturbants, and heat treatments. Thermal aggregation was observed in the heat-treated samples as indicated by prominent bands associated with aggregated strand structures. In general, the β-sheet structures seem to be more sensitive to environmental changes and heat treatments than the β-turn and α-helix structures. This may be attributed to the predominance of β-sheet conformation in oat globulin, as demonstrated by CD data (*27*), but is not clearly indicated in this study. The results are consistent with our previous data obtained by other techniques including DSC, UV, and fluorescence spectrophotometry and FT-Raman spectroscopy. However, FTIR can detect protein unfolding and aggregation at temperatures lower than other techniques, indicating its higher sensitivity. The study indicates that FTIR spectroscopy, similar to DSC and Raman spectroscopy, is an appropriate technique for monitoring the conformation of plant proteins such as oat globulin with limited solubility. These techniques can also be used to monitor heat-induced protein aggregation/precipitation in situ. Although FTIR spectroscopy, in the transmission mode, seems to be less suitable than Raman spectroscopy and DSC to study extensive thermal aggregation or gelation of proteins in situ, the problem could be overcome by operating under total attenuated reflectance mode. Because both FTIR and Raman are vibrational spectroscopic techniques, they can provide complementary data on the secondary structures of oat globulin.

As a potential food ingredient, oat globulin will be subjected to various processing conditions during food manufacturing, leading to conformational and structural changes in the protein. It is essential to monitor these changes because they could be either beneficial or detrimental in terms of the nutritional or functional properties of the processed food systems (*10*). As demonstrated by the advantages of FTIR spectroscopy listed in this study, it should be a valuable analytical tool for monitoring the structural changes in oat globulin during processing.

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