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Conformation of wheat gluten proteins

Comparison between functional and solution states as determined by infrared spectroscopy

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The conformation of wheat gluten proteins in their functional hydrated solid state (doughy state) has been studied for the first time using attenuated total reflection infrared spectroscopy. The amide I band of functional gluten proteins reveals that, in addition to β-turns and α-helices, these proteins contain a significant amount of intra- and intermolecular extended β-sheet structures. It appears that the solubilization of gluten proteins results in a major decrease of the amount of β-sheet structures accompanied by an increase of the content of the β-turn and α-helical conformations. In addition, the α-helices appears to be more distorded in solution than in the functional state. Furthermore, spectra of ω- and γ-gliadins, which are two types of prolamins of differing amino acid sequence and conformation, confirm the results obtained on the functional protein system. These results suggest that viscoelastic gluten proteins may interact through aligned β-sheets corresponding to their repetitive domains.

Gluten protein; Glutenin; Gliadin; Infrared spectroscopy; Conformation

1. INTRODUCTION

Wheat gluten proteins are composed of monomeric (gliadins) and polymeric or aggregated (glutenins) prolamins [1]. They exhibit the unusual property of being viscoelastic when hydrated and are responsible for rheological properties of wheat flour doughs. Physicochemical origins of their viscoelasticity is not fully understood although roles of glutenin polymers and of intermolecular hydrogen bonds and disulfide bridges have been established [2]. It has been shown that repetitive domains of high molecular weight (HMW) glutenin subunits contain large amount of β-turns [3] and that gluten viscoelasticity may be related to this type of conformation [4]. However, the secondary structure of wheat gluten proteins has been studied mainly by circular dichroism spectoscopy (CD) after complete dissolution of proteins at low concentration, and especially by reduction of inter- and intramolecular disulfide bridges of glutenins [3,5,6]. Structural data on functional (viscoelastic) gluten proteins have not yet been reported so that the relevance of the conformation determined for gluten proteins in solution to those adopted in dough is not known. In this paper, results obtained by transmission and attenuated total reflectance (ATR) infrared spectroscopy on the conformation of gluten proteins after solubilization and in the doughy (viscoelastic) state are compared. Whole gluten and ω;- and γ-gliadins have been studied in order to examine the functional protein system and two types of prolamins of different amino acid sequence and conformation [7].

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2. MATERIALS AND METHODS

Whole gluten was extracted by manual washing with deionised water of a flour dough of wheat Sicco. It was freeze-dried and ground and its total protein content (N × 5.7% dry basis) was 78.3%. Crude gliadin was extracted from gluten (Hardi variety) by solubilization in 70% (v/v) ethanol and fractionated by preparative ion- exchange chromatography on S-Sepharose Fast-Flow as described previously [8]. All fractions were dialysed against 0.01 M acetic acid and freeze-dried. ω-gliadin fraction issued from ion-exchange chromatography was used without additional purification. γ_{45}-gliadin was further purified by hydrophobic interaction chromatography on Phenyl Sepharose CL-4B [9]. ω- and γ- gliadins were identified by acid and SDS-polyacrylamide gel electrophoresis [9]. For spectroscopic measurements of protein solutions, gluten or gliadins were stirred mechanically for 2 h in 0.1 M acetic acid, pH 3.5. Solutions containing about 7% proteins by weight were allowed to stand overnight at 4°C before recording the spectra. Doughy samples were prepared by adding excess water at pH 7.0 to the solids.

Fourier transform infrared spectra (1000 scans) were recorded at a resolution of 2 cm^{-1} on a Bomem DA3-02 spectrophotometer equipped with a narrow band mercury–cadmium–telluride detector. All spectra were corrected for the spectral contribution of water using the method of Dousseau et al. [10]. Gluten proteins contain between 33 and 50% of glutamine side-chains [7] which can contribute to the absorption in the amide I region. Even though Purcell et al. [11] have corrected their infrared spectra of α- and ω-gliadins in solution for the glutamine absorption using molar absorptivities of the free amino acid, progressive deamidation of ω- gliadins, which are prolamins with the highest amount of glutenine residues (40–50%), has demonstrated

that the spectral contribution from this side-chain is fairly weak and does not require correction (unshown results). Fourier deconvolution of the amide I region was done with the method of Griffiths and Pariente [12] using a narrowing parameter, γ, of 7.5 and an apodization filter of 0.14. These parameters were chosen after several trials in order to obtain enough band narrowing to see the major components of the amide I band but without introducing significant sidelobes in the 1690 to 1720 cm^{-1} region where there is no protein band.

Transmission spectra were obtained at 20.0 ± 0.1°C using a cell composed of 2 CaF_2 windows separated by a 6 μm spacer [10]. Spectra of doughy samples were obtained by attenuated total reflection using a single reflection cell (Harrick Scientific, USA) fitted with a zinc selenide prism. Hydrated samples were just pressed against the prism and covered with an excess of water.

3. RESULTS AND DISCUSSION

The infrared spectrum in the amide I region of the wheat gluten in the functional state (Fig. 1A) shows that ATR technique is a valuable tool to study the secondary structure of gluten proteins in their viscoelastic state. In order to emphasize the main spectral features, spectra were Fourier deconvolved, a computational technique that decreases the width of the infrared bands. The Fourier deconvolved spectrum of functional gluten (Fig. 1B) unravels at least four bands at 1616, 1632, 1652 and 1671 cm^{-1}. The 1632 cm^{-1} feature is highly characteristic of amide groups involved in the extended β-sheet structure [13,14] while the low-frequency amide I band at about 1616 cm^{-1} has been associated with the presence of intermolecular β-sheet networks since it occurs readily on aggregation of proteins [15] and polypeptides [16]. The prominent band at 1652 cm^{-1} is assigned to the α-helical conformation, although the spectral contribution from the unordered conformation cannot be completely ruled out at this frequency [13]. Finally, the 1672 cm^{-1} band is assigned to the β-turn conformation [13,14]. This type of structure is predicted in repetitive domains of sequences of gluten polypeptides and was observed by CD in highly repetitive prolamins [17].

Fig. 1 further reveals that major conformational changes occur on the solubilization of gluten proteins. In the spectrum of the proteins in solution, the intensity of the β-sheet bands at 1615 and 1632 cm^{-1} is markedly lower whereas the band due the α-helical conformation is stronger and now appears at 1657 cm^{-1}, suggesting that the intramolecular hydrogen bonds in the α-helices are weaker for the proteins in solution. In addition, there is a significant increase of the intensity of the β-turn band at 1674 cm^{-1} for the solubilized gluten proteins.

The methods of Dousseau and Pézolet [18] and Lee et al. [19] have been used to quantify the secondary structure content of gluten proteins in solution. The results obtained with the method of Dousseau and Pézolet [18] were, however, subjected to large prediction errors. This might be due to the spectral contribution of the amidated side-chains in the amide II region since

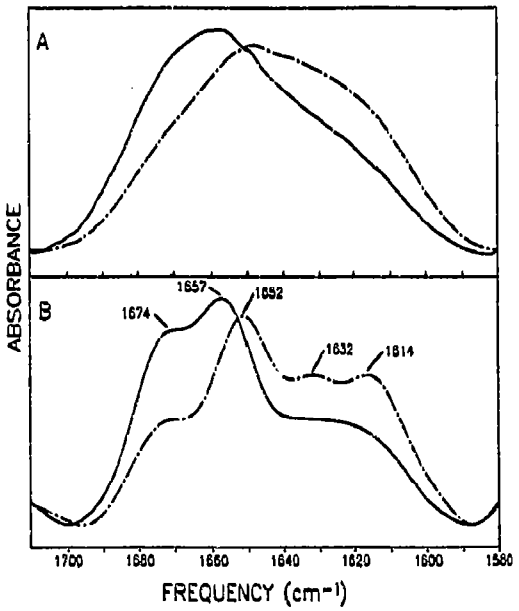

Fig. 1 (A) Original and (B) Fourier deconvolved infrared spectra in the amide I region of wheat gluten in the doughy state (-.-.-) and in solution in 0.1 M acetic acid (----).

both the amide I and amide II bands are analysed with this method while only the amide I band is considered with the method of Lee et al. [19]. The results obtained with the latter method (Table I) show that gluten proteins in solution contain approximately equal amounts of α-helices, β-sheets and β-turns. The α-helix content determined by infrared spectroscopy is in fairly good agreement with that obtained by CD on gliadins and low-molecular weight (LMW) glutenins in solution, these proteins accounting for about 75% of the gluten proteins [6,20]. The amount of β-turns and undefined structure is also in good agreement with that predicted from the amino acid sequences of gluten proteins [17]. The relatively high content of the β-sheet conformation determined by infrared spectroscopy is, however, more surprising since the CD results have shown that HMW-glutenins are almost devoided of β-sheets [3] whereas this type of conformation accounts for only 10 to 20% of the secondary structure of gliadins [20] and LMW-

Table I

Secondary structures of wheat gluten proteins in 0.1 M acetic acid solution

Proteins	Secondary structure content (%)*			
	α-helices	β-sheets	β-turns	Undefined
Whole gluten proteins	31	28	27	15
γ_{40}-gliadin	36	25	28	11
ω-gliadin	22	31	31	16

*From the method of Lee et al. [19].

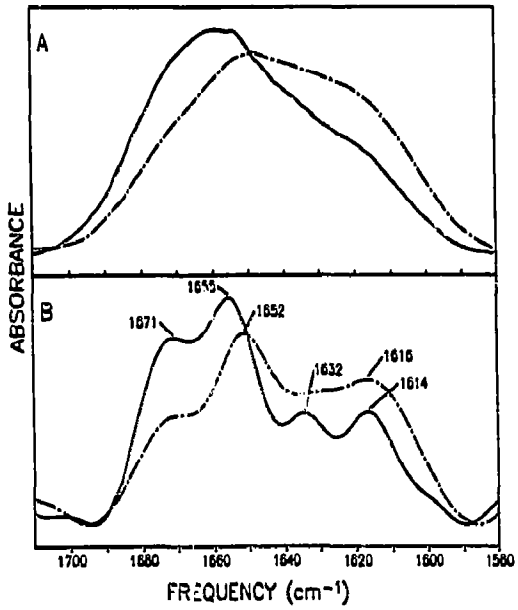

Fig. 2 (A) Original and (B) Fourier deconvolved infrared spectra in the amide I region of wheat γ_{46}-gliadin in the doughy state (-.-.-) and in solution in 0.1 M acetic acid (----).

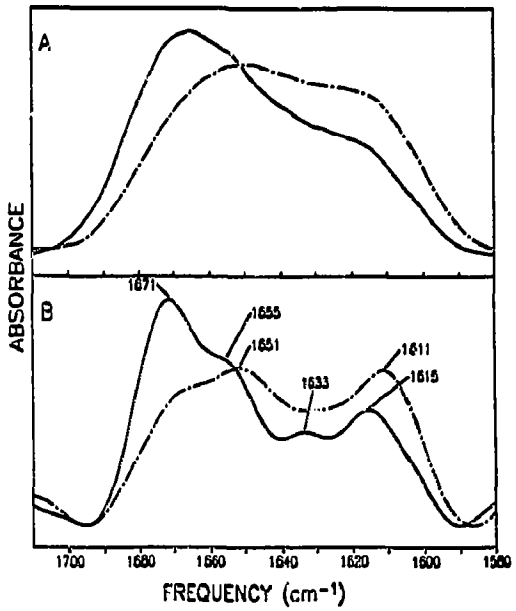

Fig. 3 (A) Original and (B) Fourier deconvolved infrared spectra in the amide I region of wheat ω-gliadin in the doughy state (-.-.-) and in solution in 0.1 M acetic acid (----).

glutenins [6]. This discrepancy might be due either to the fact that infrared spectroscopy is more sensitive than CD for the determination of the β-sheet conformation since the characteristic bands due to this type of structure are well resolved, or to the higher protein concentration used for infrared measurements since gluten proteins have a high tendency for intermolecular association.

It has not been possible to obtain quantitative results with low prediction errors on the conformation of functional gluten proteins since the methods of Dousseau and Pézolet [18] and Lee et al. [19] have been developed for proteins in solution. Nevertheless, it is clear from Fig. 1 that functional gluten proteins display a higher content of intra- and intermolecular β-sheets and a lower amount of β-turns and α-helices compared to the proteins in solution. In addition, the frequency of the amide I band associated with the α-helical conformation suggests that the α-helices are more stable in functional gluten proteins than for those of the proteins in solution due to the presence of stronger intramolecular hydrogen bonds. These results highlight for the first time the presence β-sheet conformation in domains where intermolecular interactions between the protein polypeptide chains in functional gluten exist.

In order to generalize the results obtained on whole gluten proteins, infrared spectra of purified γ_{46}- (Fig. 2) and ω-gliadins (Fig. 3) were also recorded. These proteins are particularly interesting since the structure of ω-gliadins is non globular [9] and rich in β-turns [5,17] while the structure of γ_{46}-gliadin is more compact because of the presence of intramolecular disulfide bonds and has an α-helical content of about 30% corresponding to the C-terminal nonrepetitive domain of the protein [5,21]. Comparison of Figs. 1 and 2 shows that the spectra of gluten and γ_{46}-gliadin are quite similar for both the doughy and solution states. In the solution spectra, the components of the amide I band are more well resolved for γ_{46}-gliadin than for the gluten proteins since gluten is composed of several proteins with different conformation while γ_{46}-gliadin is a pure protein. Nevertheless, the quantitative results on the secondary structure content of γ_{46}-gliadin and gluten proteins in solution are quite close (Table I). The values obtained for γ_{46}-gliadin are in fairly good agreement with those determined by CD on dilute solutions [5,21], except for the higher content of β-sheet structure, as also observed for gluten. On the other hand, in the spectrum of ω-gliadin in solution (Fig. 3), the 1671 cm^{-1} band due to β-turns is the prominent feature, while in the spectra of gluten (Fig. 1) and γ_{46}-gliadin (Fig. 2) in solution, the α-helix band at 1657 cm^{-1} is the strongest one. Qualitatively, the undeconvolved spectrum of Fig. 3 is in agreement with the corrected spectrum of ω-gliadins obtained by Purcell et al. [11] in D_2O. Table I shows that the main difference between the secondary structure of ω-gliadins and that of γ_{46}-gliadin is in the content of α-helices which is markedly higher for the latter protein. As for gluten proteins and γ_{46}-gliadin, the secondary structure of ω-gliadin is richer in both intermolecular β-sheet (1611 cm^{-1}) and α-helical (1651 cm^{-1}) structures in the doughy state than in solution.

In conclusion, ATR infrared spectroscopy shows for the first time that the solubilization of either whole gluten proteins or purified prolamins results in the decrease of the amount β-sheets, especially intermolecular β-sheets, and an increase of the α-helix and β-turn contents. This suggests that viscoelastic gluten proteins may interact through aligned β-sheets corresponding to their repetitive domains.

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