Fourier transform IR spectroscopic study of hydration-induced structure changes in the solid state of ω-gliadins

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The hydration of ω-gliadins and partly deamidated and esterified ω-gliadins has been studied by Fourier transform IR spectroscopy. The secondary structure of the fully hydrated proteins was a mixture of β-turns and extended chains, with a small amount of intermolecular β-sheets. The absorption of the glutamine side chain amide groups contributed considerably to the amide I band with two well-defined peaks at 1658 and 1610 cm^{-1}. The amide I band of the dry native sample could not be resolved into single component bands. There the backbone structure seemed to be distorted by extensive hydrogen bonding involving glutamine side chains. With increasing water content, these hydrogen bonds were broken successively by water molecules, resulting in an increase in extended, hydrated structures, which gave rise to the formation of intermolecular β-sheet structures. Above 35 % (w/w) water the β-sheet content fell sharply and was replaced by extensively hydrated extended structures. An amide I band similar to dissolved poly-L-proline proved that parts of the polymer were in a solution-like state. The replacement of many glutamine side chains in the esterified protein produced more resolved secondary structures even in the dry sample. The β-sheet content of the dry sample was higher than in the native ω-gliadins, but hydration generally caused very similar changes. At all hydration levels the spectra indicated a more ordered structure than in the native sample. Overall, the modification caused changes that go beyond the simple presence or absence of glutamine bands.

INTRODUCTION

Alcohol-soluble cereal storage proteins, termed prolamins, consist of a complex heterogeneous mixture of components. They are made up of repetitive and non-repetitive domains, the repetitive domains consisting of short repeated sequences, with high contents of proline and glutamine (up to 30 mol % and 50 mol % respectively) [1]. They are classified into two groups on the basis of their aggregative properties. The gliadins are monomeric proteins and the glutenins form disulphide-linked polymers. The amount and balance of these proteins, in wheat, determine technological use, including breadmaking [1]. Recent experiments with high-molecular-mass subunits of wheat glutenin have shown that disulphide bond cross-linking is important for physicochemical properties, for example viscoelasticity [2]. However, it is likely that the large repetitive domains and their interactions have a significant influence on the behaviour of the polymer(s). In particular, hydrogen bonding by the glutamine side chain amide groups has long been implicated in intermolecular interactions of prolamins [3].

The S-poor prolamins, the ω-gliadins of wheat, C hordeins of barley and ω-secalins of rye, have molecular masses of about 40 kDa from available gene sequences of barley and rye [4]. No gene sequences are available for ω-gliadins of wheat, but N-terminal sequence similarities and comparable amino acid compositions indicate similar sequences to the S-poor prolamins of barley and rye [4]. They consist of short N- and C-terminal domains of approx. 12 and 6 residues respectively, and a central domain consisting of octapeptide repeats of consensus Pro-Gln-Gln-Pro-Phe-Pro-Gln-Gln. They contain few charged residues and no cysteine residues for cross-linking.

CD spectra of C hordeins [5], ω-gliadins [6] and ω-secalins [7] and a synthetic model peptide corresponding to the repeat motif [8] have shown the presence of β-reverse turns and the absence of α-helix and β-sheet structures in solution. Model structures of various repeat sequences incorporating the proposed β-spiral motif [9] agree well with molecular dimensions derived from viscosity measurements, small-angle X-ray scattering and scanning tunnelling micrograph pictures [10]. Similar results were obtained with ^{13}C NMR spectra of C hordein [11].

Fourier transform IR (FTIR) spectroscopy has confirmed the predominance of β-reverse-turn structures in ω-gliadins in acetic acid solution [12], but intermolecular β-sheet structures were observed in the highly hydrated solid [13]. Similar changes were seen with increasing glutenin concentration [14]. This hydrated solid state is more similar to that of the functional state of the proteins. These studies have shown that dilute solution studies do not necessarily reflect the structures in the hydrated solid state [10].

The natural environment in the grain is dominated by intermolecular interactions in a heterogeneous, insoluble polymer and a variable water content. NMR relaxation studies of C hordein [15] and high-molecular-mass subunits [16,17] have shown that the protein becomes very mobile when water is added. An important part in this change is attributed to the side chains of glutamine residues [15]. Such NMR studies did not give direct structural information but they showed that at high water contents there is a large difference in the mobility data compared with globular proteins like lysozyme, which have been used extensively for studies of protein hydration [18].

In this study the hydration of ω-gliadins and poly-L-glutamine and derivatized ω-gliadins have been studied by FTIR spectroscopy, to obtain information on structure and interactions in the hydrated solid protein.

MATERIALS AND METHODS

Poly-L-glutamine (26 kDa) was prepared by treating poly-γ-benzyl-L-glutamate (Sigma) with anhydrous liquid ammonia for 10 days at room temperature. The ammonia was allowed to

Abbreviations used: ATR, attenuated total reflectance; FTIR, Fourier transform IR.

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Table 1 Positions, widths and relative areas of the bands fitted to the Fourier-deconvoluted spectra of ω-gliadins and ω-gliadin methyl ester derivatives

For a discussion of secondary structure assignments and error margins see the text.

				Water content (%, w/w)																
	Dry film			3			6			12			15			38			Solution		
Band	Pos. (cm^{-1})	Width (cm^{-1})	Rel. area (%)	Pos. (cm^{-1})	Width (cm^{-1})	Rel. area (%)	Pos. (cm^{-1})	Width (cm^{-1})	Rel. area (%)	Pos. (cm^{-1})	Width (cm^{-1})	Rel. area (%)	Pos. (cm^{-1})	Width (cm^{-1})	Rel. area (%)	Pos. (cm^{-1})	Width (cm^{-1})	Rel. area (%)	Pos. (cm^{-1})	Width (cm^{-1})	Rel. area (%)
Native sample																				
Amide I	1693	13	4	1691	11	4	1691	10	3	1691	9	3	1690	8	2	1690	8	1	1681	13	5
	1683	21	9	1680	18	10	1681	18	10	1680	18	10	1680	17	9	1680	15	5	1669	17	19
	1658	38	60	1658	31	47	1658	32	48	1658	32	47	1659	31	45	1662	33	44	1655	17	16
	1637	13	6	1640	14	6	1641	14	5	1641	13	4	1642	13	4	1644	9	1	1642	16	11
	1629	13	5	1630	16	12	1630	17	17	1630	18	19	1630	19	21	1628	31	38	1632	11	4
																			1619	26	24
	1614	33	16	1616	28	20	1614	22	15	1614	22	16	1613	22	18	1611	15	7	1606	30	21
				1597	14	1	1598	16	3	1597	13	2	1597	12	1	1599	14	4			
Amide II	1582	13	3	1583	12	4	1583	12	5	1583	14	6	1583	14	8	1584	13	7	1584	9	3
	1570	10	2	1567	13	7	1567	15	9	1566	14	10	1566	15	14	1563	22	32	1572	15	10
	1558	19	12	1547	23	33	1547	23	35	1550	19	27	1550	17	25	1550	11	12	1557	20	34
	1541	20	27													1539	12	17	1541	17	25
	1530	11	9	1529	16	22	1529	15	21	1530	21	33	1531	22	35	1527	15	18	1530	10	9
	1515	20	37	1514	14	23	1515	14	22	1514	12	15	1514	11	12	1515	10	10	1518	15	19
	1498	11	10	1499	11	10	1499	11	9	1499	12	9	1499	11	6	1499	10	4	1501	11	3
																			1495	7	2
Ester derivative																					
Ester	1738	15	65	1739	13	47	1738	14	54	1738	13	51	1738	14	52	1738	14	30	1740	13	22
	1722	20	35	1725	24	53	1723	21	46	1723	21	49	1723	21	48	1722	17	51	1722	20	78
																1715	26	19	1706	11	8
Amide I	1695	12	7	1693	11	5	1694	10	3	1694	10	3	1693	11	4				1696	8	0
	1686	12	6	1684	15	9	1686	14	8	1685	14	7	1684	13	9	1684	8	1			
	1672	24	28	1671	14	17	1672	20	18	1672	19	19	1672	25	9	1668	28	43	1669	26	28
	1651	21	30	1655	12	27	1653	23	34	1653	21	30	1653	30	43	1651	16	21	1652	15	13
	1639	11	7	1639	22	18	1638	12	9	1638	13	13	1638	11	5	1639	12	13	1639	12	5
	1628	13	17	1627	17	17	1627	14	21	1627	13	22	1626	13	23	1627	16	3	1628	9	2
	1614	10	3	1614	15	3	1614	10	3	1614	11	3	1613	9	1	1614	10	1	1621	32	52
	1604	12	2	1604	15	3	1605	16	4	1605	16	4	1606	19	5	1612	22	17			
Amide II	1589	22	6	1586	14	4	1586	15	5	1586	13	5	1586	12	4	1585	9	3	1585	10	3
	1570	8	2	1568	12	4	1571	12	2	1570	11	2	1569	16	8	1573	9	2	1570	16	5
	1558	14	8										1554	15	10	1552	30	59	1548	33	67
	1543	18	26	1547	22	35	1544	33	56	1544	34	61	1538	30	55	1539	9	7			
	1531	10	5	1530	17	24	1531	10	4	1531	9	3				1532	10	3	1530	11	6
	1517	27	48	1515	15	24	1516	20	27	1516	19	23	1515	16	16	1520	20	24	1517	15	15
	1499	8	4	1499	11	9	1499	11	7	1498	10	6	1498	12	7	1497	8	3	1498	9	4

evaporate and the poly-L-glutamine washed extensively with distilled water [19]. Poly-L-proline (3600 Da) was purchased from Sigma and was used without further purification.

ω-Gliadins from wheat were isolated and purified as described [6]. A methyl ester derivative was prepared by suspending freeze-dried ω-gliadins in anhydrous dioxane/methanol mixture (1:4, v/v). After 48 h of stirring, the protein was rapidly dialysed against ice-cold water and freeze-dried. Approx. 70% of the glutamine side chain amides were converted to methyl ester groups [20].

The water contents of the dry ω-gliadin preparations were determined by Karl-Fischer titration (Orion AF7LC coulometric titrator). Samples of the freeze-dried material were then hydrated over saturated salt solutions, and the water uptake was determined by weighing.

FTIR spectra were recorded on a Bio-Rad FTS-60 spectrometer with a HgCdTe detector. The samples were placed in a MicroCircle attenuated total reflectance (ATR) cell (Spectra-Tech) with a ZnSe crystal.

Typically, 7 mg of freeze-dried protein was stirred into 100 μl of distilled water. The mixture (pH 6) was injected into the ATR cell and a spectrum recorded (256 scans, 2 cm^{-1} resolution, referenced against the empty cell).

The cell was then drained and the protein deposited on the ATR crystal was dried with a stream of dry air. To achieve defined hydration levels, the crystal with the dried protein film was placed for at least 24 h in a jar over a salt solution. At every hydration step a spectrum of the hydrated sample was recorded, then the protein was dried again and the spectrum of the dry sample compared with the previous one to detect irreversible changes in the protein film.

From the spectra of hydrated samples a water spectrum, recorded under identical conditions, was subtracted to remove the contribution of free water. The subtraction criterion was a flat baseline in the region 2500–2000 cm^{-1}, where the protein did not absorb, as well as in the region 1000–800 cm^{-1}, which is dominated by the onset of the very strong water libration modes. The second criterion was used because in samples with low water contents the rather weak 2150 cm^{-1} water combination band might be obstructed by small baseline distortions, which could result in erroneous subtraction factors.

ATR correction and Fourier deconvolution were performed with the spectrometer software. The ω-gliadin spectra were Fourier-deconvoluted [21] with a *K*-factor of 2.0, using a minimum half-width of 20 cm^{-1} and a triangular apodization function.

Band fitting of the Fourier-deconvoluted ω-gliadin spectra was performed with a routine in the Bio-Rad Win-IR software based on the Levenberg–Marquardt algorithm [22]. Starting band parameters were taken from the deconvoluted spectra. Second derivatives of the original spectra were calculated by the Savitsky–Golay method [23] (square polynomial, seven convolution points) as an independent way of verifying the band positions. The initial linewidths were set to 7 cm^{-1} and allowed to vary freely. The spectra were fitted with Gaussian line-shapes until the unconstrained fits reached a stable minimum.

The results of the band-fitting process are summarized in Table 1, grouped in the components of amide I (1700–1595 cm^{-1}) and amide II (1580–1495 cm^{-1}) bands, as well as the $\nu(C{=}O)$ region of the ester derivative. The relative areas of the components were expressed as a percentage of the area of each fitted region, which was equivalent to an area normalization of the amide bands.

Although care has been taken to avoid artifacts in the deconvolution process [24], these figures are still subject to a substantial error from the band-fitting process and the assumption of identical extinction coefficients is questionable [25]. Relative errors of approx. 5% have been reported for β-sheet and turn structure content determinations with this method [26]. These data therefore should rather be used for a qualitative comparison of the samples under different conditions and not be taken as an absolute measure of secondary structure contents.

RESULTS

The structure assignment of the amide bands of ω-gliadins had to take into account the unusual amino acid composition of the proteins (40–45 mol% glutamine, 26–32 mol% proline and 7–9 mol% phenylalanine [1]). The intensity of the ring vibration of the phenylalanine side chains at approx. 1602 cm^{-1} is relatively small [27], but, owing to their high contents, proline and glutamine are expected to be the major contributors to the intensity of the amide bands. Therefore, for comparison purposes, spectra of poly-L-glutamine and poly-L-proline in the dry and hydrated states have been recorded as an aid to band assignment.

Figure 1 shows the IR spectra of dry and hydrated poly-L-glutamine. The 1627 cm^{-1} band is due to the β-sheet peptide backbone, but the band at 1658 cm^{-1} has been previously assigned to the C=O stretching vibration of the side chain amide groups [19]. The band at 1605 cm^{-1} is due to the NH_2 scissoring vibration [28]. Hydration of the polypeptide had only limited effect on the positions of the 1627 and 1605 cm^{-1} bands. The most obvious difference was the intensity of the 1658 cm^{-1} band, which was visibly smaller in the hydrated sample. We suppose that this was likely to be due to a change in the extinction coefficient, because no shifts occurred.

The amide bands of poly-L-proline were much more affected by hydration. The spectra of the dry sample and of an aqueous solution are compared in Figure 2. In solution the amide I band was shifted from 1643 to 1617 cm^{-1} and the C–H deformation band from 1427 to 1456 cm^{-1}. This is consistent with a previous study [29] on poly-L-proline II in ^2H_2O, which showed a similar shift in the opposite sense, caused by aggregation of polyproline II helices, but did not reflect a change in the secondary structure of the polypeptide. The observed difference between dry and

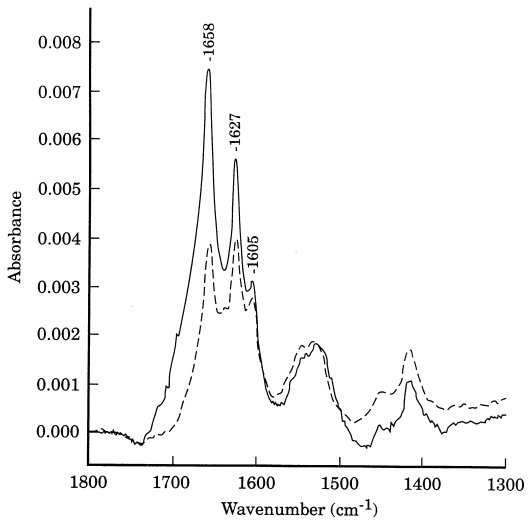

Figure 1 FTIR spectra of poly-L-glutamine

Solid line, dry sample; broken line, sample suspended in water, ^1H_2O spectrum subtracted.

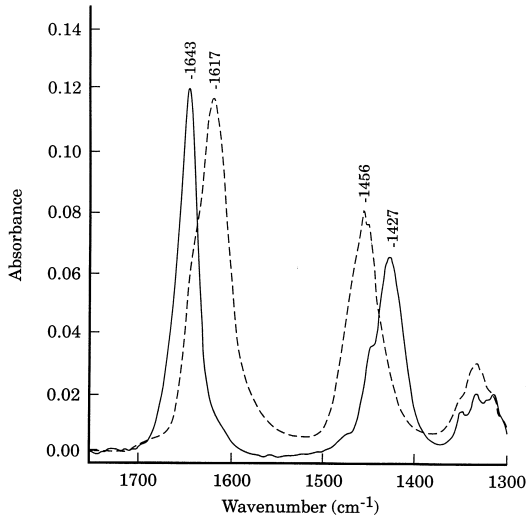

Figure 2 FTIR spectra of poly-L-proline

Solid line, dry sample; broken line, sample in aqueous solution, ^1H_2O spectrum subtracted.

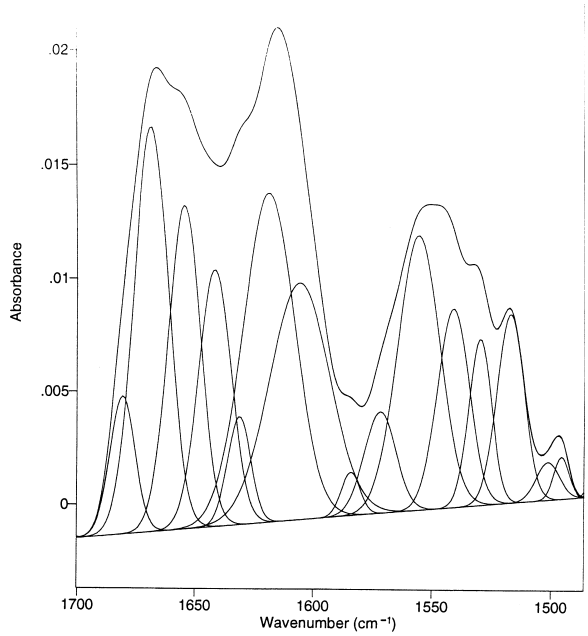

Figure 3 Fourier-deconvoluted amide I and amide II bands of fully hydrated ω-gliadins, fitted with a sum of Gaussian bands

The reduced χ^2 of this fit was 5.40, with a correlation coefficient of 0.99979 and a standard error of 0.00002365.

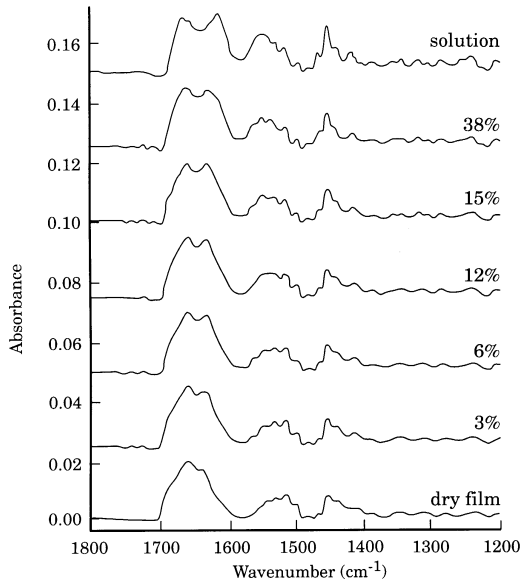

Figure 4 Fourier-deconvoluted spectra of ω-gliadin

Spectra stacked vertically with increasing hydration level, given in percentage (w/w). ^1H_2O spectrum subtracted from hydrated samples.

dissolved poly-L-proline is therefore caused by different environments, i.e. protein–protein interactions in the dry sample and protein–water interactions in solution.

Figure 3 shows the Fourier-deconvoluted amide I and amide II bands of fully hydrated ω-gliadin samples, fitted with Gaussian band-shapes. In accordance with the previously discussed glutamine spectra the sharp component band at 1655 cm^{-1} was assigned to the carbonyl stretching of the glutamine side chain, whose NH_2 scissoring also contributed to the intensity at 1606 cm^{-1}. The largest band component at 1619 cm^{-1} seems very similar to the band of hydrated polyproline and was presumably caused by hydrated chains containing this amino acid. The bands at 1669 and 1642 cm^{-1} were assigned to β-turns (non-bonded and internally hydrogen-bonded carbonyls respectively [30]). This is in good agreement with IR studies of a peptide model, which according to CD experiments had a β-turn structure and showed bands at 1666 and 1634 cm^{-1} in trifluoroethanol [8]. Our results therefore proved that even at very high hydration there was a substantial amount of β-turn structure as well as a small quantity of β-sheets in the protein. In contrast, the peptide model showed only one broad band at 1635 cm^{-1} in ^2H_2O, which was interpreted as an extended (β-sheet) structure in solution.

The Fourier-deconvoluted spectra of ω-gliadins at different water contents are shown in Figure 4. Figure 5 shows the corresponding spectra of the methyl ester derivative. Fitting of the resolution-enhanced spectra of the dry ω-gliadin sample yielded one major component at 1658 cm^{-1} that coincided with the maximum of the original amide I band complex and accounted for approx. 60% of its total absorption. Another 16% of the amide I band area was contributed by a broad band centred at 1614 cm^{-1}. The remainder was made up of smaller bands at 1693, 1683, 1637 and 1629 cm^{-1} (Table 1).

The bands at 1658 and 1614 cm^{-1} were assigned to the glutamine side chain absorptions. However, although the spectra of poly-L-glutamine showed an increased extinction of the side chain C=O group in the dry state, the intensity and width of the 1658 cm^{-1} band were larger than expected, and no distinct bands from the protein backbone were observed. Obviously the broad band at 1658 cm^{-1} contained contributions from more than one source, which could not be separated. Purcell et al. [12] have described the failure to find a distinct band of the glutamine side chains in deconvoluted IR spectra of gliadins. They concluded that a large number of closely spaced components restricted the spectral resolution.

The resolution enhancement process should have been able to

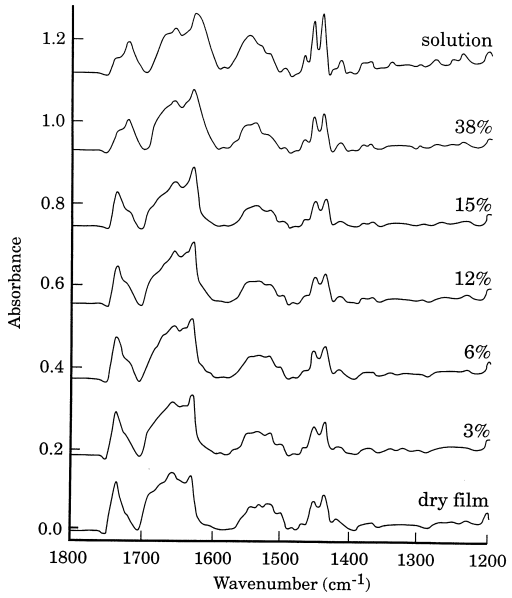

Figure 5 Fourier-deconvoluted spectra of ω-gliadin methyl ester derivative

Spectra stacked vertically with increasing hydration level, given in percentage (w/w). ^1H_2O spectrum subtracted from hydrated samples.

separate sharp bands from well-defined secondary structure elements superimposed on a broad band from glutamine side chains, but the merging of the whole band complex around 1658 cm^{-1} into one broad band indicated a comparatively wide range of conformations in the dry protein. It could be that intra- and inter-molecular hydrogen bonds between glutamine side chain and backbone peptide groups distorted the secondary structure. The role of these hydrogen bonds was highlighted by the disappearance of this band broadening in solution (see above), where most of the glutamine side chains were hydrated. This interpretation is supported by the fact that two distinct amide I band components at 1672 and 1651 cm^{-1} were observed in dry samples where the glutamine side chain amide groups had been largely deamidated and esterified (Figure 5 and Table 1). Here the absence of interfering glutamine amide groups revealed more information about the secondary structure of the dry protein, although it has to be considered that the secondary structure of this sample might have been changed during the modification process.

The most likely assignment of the band at 1672 cm^{-1} would be to β-turns. Assignment of the 1651 cm^{-1} band to α-helix could be considered, because polyglutamine esters adopt this conformation [19], but the abundance of proline residues in the ω-gliadins would make such a structure unfavourable. The observed intensity of the band (30%; Table 1) is of the same order of magnitude as the proline content of the protein, and it was therefore assigned to this amino acid. The shift of 8 cm^{-1} compared with the poly-L-proline II model would indicate a somewhat different geometry.

There was some evidence for β-sheet structures in the dry ω-gliadins from a characteristic band at 1629 cm^{-1} [27]. However, the amide bands of both β-sheet and turns are usually split and have small high-frequency components in the region 1680–1690 cm^{-1}. The difficulty of assigning the two bands at 1683 and 1693 cm^{-1} conclusively to either β-sheet or turns made it impracticable to obtain the total area of the β-sheet bands and thus the β-sheet content of the proteins had to be approximated by the area of its major band component. Although this approach did not yield the absolute β-sheet contents, it was nevertheless useful in comparing different structures. Judging by this, the amount of β-sheet structure was apparently higher in the modified sample. Presumably it was also more regular, as shown by the comparatively narrow shape of the very distinct band at 1628 cm^{-1}.

The bands below 1620 cm^{-1} were much weaker in the spectrum of the dry esterified sample (approx. 4%, compared with 16% in the native ω-gliadins). This feature was therefore assigned to the NH_2 scissoring vibration of the glutamine side chains. Their position at 1614 cm^{-1} was approx. 10 cm^{-1} higher than in the poly-L-glutamine model. This suggests a different, probably less regular, hydrogen-bonding environment in the dry prolamins.

The amide II bands of native and modified ω-gliadins were resolved into six distinctive bands at 1570, 1558, 1541 (1543), 1530 (1531), 1515 (1517) and 1498 (1499) cm^{-1} (Table 1). There seemed to be a striking similarity between both samples and this might indicate similar backbone configurations in the dry proteins. However, the amide II band is less reliable for secondary structure determination than the amide I band [25], and, as will be seen later, is much more sensitive to hydration than to secondary structure change. Therefore small differences in the structures of the dry samples might go undetected here.

The hydration of the ω-gliadins to 3% (w/w) water content resulted in a sharp decrease in width and intensity of the 1658 cm^{-1} band (Table 1). The band at 1630 cm^{-1} increased and the maximum of the amide II band was shifted from 1514 to 1546 cm^{-1}. The deconvoluted amide bands of the samples at 6, 8, 12 and 15% (w/w) water content showed a continuous decrease of bands at 1691 and 1514 cm^{-1} and an increase of the 1630 and 1566 cm^{-1} bands. The integrated intensities of the amide II band around 1566 cm^{-1} (shown in Table 1) increased linearly with the water content. The band from the NH_2 scissoring vibration of the glutamine side chain amide groups was shifted to slightly lower wavelengths, but its intensity remained unchanged.

Comparable variations were observed in the esterified sample. The band at 1627 cm^{-1} increased at the expense of the 1653 cm^{-1} band. In the amide II region there was a continuous shift of intensity from the 1517 cm^{-1} band to 1548 cm^{-1}. The increased hydration was also documented by the relative intensities of the free and hydrogen-bonded ester carbonyl bands at 1738 and 1722 cm^{-1} respectively.

In both the native and esterified samples the initial hydration up to 15% (w/w) water content resulted in small, localized increases or decreases. However, the overall pattern of the amide bands appeared unchanged at these low hydration levels. Thus it has to be concluded that these spectral features were brought about by the limited binding of water molecules, which did not cause a profound structural change in the protein.

The band pattern began to change at higher water contents. At 38% (w/w) in the ω-gliadin the 1628 cm^{-1} band was almost as big as the 1658 cm^{-1} band. However, in the esterified samples no comparable increase in β-sheet content was observed. Instead the appearance of a strong band at 1612 cm^{-1} suggested the formation of hydrated, extended chains. Band positions around 1620 cm^{-1} have been reported for extensively hydrated extended chains containing proline residues [30]. This agreed with the observed amide I band frequency of 1617 cm^{-1} in dissolved poly-L-proline. Thus the additional intensity in this region could be explained by a partial solubilization of the protein.

In solutions of the ω-gliadin samples the broad band complex at 1658 cm^{-1} could be further separated and the band at 1655 cm^{-1}, which we assigned to the glutamine side chain

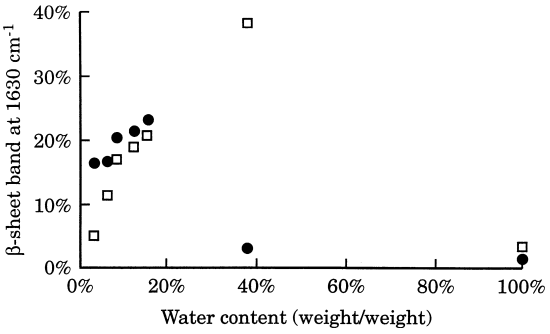

Figure 6 Change in the β-sheet structure content as a function of hydration, indicated by the 1630 cm^{-1} amide I band component

Symbols: □, native ω-gliadins; ●, methyl ester derivative.

carbonyl, was clearly visible. Now also in the native ω-gliadin sample only a small remainder was left of the β-sheet band at 1630 cm^{-1}. The band maximum was shifted down to 1616 cm^{-1}, whereas the original 1614 cm^{-1} band (NH_2 scissoring) was found at 1610 cm^{-1}. The 1619 cm^{-1} band, owing to fully hydrated proline residues, indicated the conversion of the β-sheet into fully hydrated, extended chain structures. It is conspicuous that such a change coincides with a remarkable increase in relaxation times in the NMR experiments [15], which indicates a large increase in mobility.

In the esterified sample a similar change took place. The ester carbonyl bands showed a marked increase in the hydrogen-bonded (hydrated) form, and in the fully hydrated sample the main band at 1621 cm^{-1} showed the presence of dissolved protein strands.

The biggest difference in the hydration behaviour of native and esterified sample was the extent of the change in the band around 1630 cm^{-1}, which is an indicator of the β-sheet content as discussed above. Figure 6 compares the relative areas of this band for the two samples as a function of hydration level. During the initial hydration steps up to 15% (w/w) this increased from 5% to 21% in the native sample, but from 17% to 23% in the esterified ω-gliadins. In the native sample it peaked at 38% (w/w) water content, but in the modified protein it was already drastically reduced at this hydration level. In both of the fully hydrated samples it decreased to less than 5%. The different values at 38% (w/w) water content might indicate a slight difference in the solution behaviour of the modified protein compared with the native ω-gliadins.

A contribution from increasingly hydrated proline residues to the increased 1630 cm^{-1} band was considered, but was rejected on the grounds that this caused the formation of a separate band at 1619 cm^{-1} in the hydrated samples and was also inconsistent with the decrease in intensity seen in the fully hydrated samples.

DISCUSSION

Two different approaches to the problem of glutamine side chain contributions to the amide I band have been reported in the literature [12,13]. In ^1H_2O, deamidation reduced the overall intensity of the broad-band complex, but did not significantly alter the amide I band-shape compared with the original spectra. Pézolet et al. [13] reported spectra for ω-gliadin without correction for the glutamine side chains. The spectra of Purcell et al. [12] were recorded in ^2H_2O at p^{2}H 3. There the glutamine absorption is shifted to 1635 cm^{-1} [31]. This distorted the band shape and only after subtraction did the band resemble qualitatively that obtained in acetic acid solution [13]. Under the conditions of our study, the comparison of the deconvoluted spectra of ω-gliadin showed changes in the spectral band patterns that went beyond the simple presence or absence of glutamine bands. This was seen especially in the band broadening in the dry ω-gliadin samples, which indicated a certain amount of intramolecular hydrogen bonding involving the glutamine side chains. Spectral subtraction of the glutamine contribution would therefore distort the amide I band considerably.

Many proteins have a higher content of β-sheet in the solid state than in solution, owing to intermolecular interactions [32]. The ester-modified ω-gliadins fit into this picture. The slight increase in the β-sheet content with hydration might be due to an increased extinction coefficient of this band in the hydrated sample. Its disappearance in solution suggests that this structure arises from intermolecular interactions. In contrast, in the native ω-gliadin sample there was little β-sheet (even taking into account different extinction coefficients) in the dry state, where intermolecular interactions would be expected to be strongest. Furthermore an increase in β-sheet content should restrict the protein mobility, yet NMR relaxation measurements showed an increase.

To answer this question we postulate that in the dry protein there is a rigid, compact structure, stabilized by many inter- and intra-molecular hydrogen bonds between glutamine side chains and backbone peptide groups. Intermolecular β-sheet structures are formed in solid proteins because of the close contact with neighbouring molecules. In the entangled solid of prolamins, neighbouring molecules and different parts of the same molecule appear essentially similar because of the highly repetitive structure. There is potentially no difference between intramolecular and intermolecular β-sheets, but the observed stiffness of the molecules would favour the latter. The high frequency of proline residues in the sequences of ω-gliadins and other prolamins would make long stretches of β-sheet structures unfavourable. Instead it is likely that many short stretches, a few residues long, are formed, flanked by more or less loose extended chain structures. This imperfect structure might be more susceptible to distortion by hydrogen bonds with the glutamine side chains than regular β-sheet, which would explain why in the native ω-gliadins the content of extended β-sheet structures is apparently much lower and the content of unordered structures higher than in the dry esterified sample.

The binding of water molecules will occur preferentially at the polar amide/peptide groups, and will break internal hydrogen bonds, especially those involving polar side chains, removing their distorting effect on the secondary structure and giving the protein more freedom to move. The increased mobility of the hydrated parts would allow structure rearrangements like the formation of β-sheets. The competition between protein–protein and protein–water interactions is determined by the availability of binding partners. With increasing water content, more and more intermolecular contacts are broken, and at very high water levels extensively hydrated, extended chains appear instead of the β-sheets.

The general plasticizing effect of water binding has been observed with all proteins so far studied. However, prolamins have no 'core' structure, and hydration could occur at many positions in the molecule. The structure data from fully hydrated ω-gliadin samples indicated that stretches of hydrated extended chains coexisted with structured regions containing β-sheets and turns. There might even be an equilibrium between folded and unfolded parts of the proteins. The formation of transient stretches of dissolved protein chains agrees with NMR relaxation

data indicating that at hydration levels above 60–70 % (w/w) the molecules are as mobile as a fully dissolved sample (even if it might be still out of solution), and therefore the solvation of individual residues has to be considered as complete. This is in striking contrast with the behaviour of globular proteins, where the molecule as a whole dissolves and the interior residues are fixed in place in a durable secondary structure. It is tempting to suggest that the hydration behaviour, in which the hydrogen-bonding capacity of the glutamine side chains plays an important role, might have profound implications for the biological accessibility of the prolamins as well as their technical properties.

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