Structure and Dynamics of High Molecular Weight Glutenin Subunits of Durum Wheat (*Triticum durum*) in Water and Alcohol Solutions Studied by Electron Paramagnetic Resonance and Circular Dichroism Spectroscopies

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Complementary information on the structure and dynamics of high molecular weight glutenin subunits (HMW-GS) of durum wheat (*Triticum durum)* was obtained by means of two spectroscopic techniques. Electron paramagnetic resonance was used to investigate the dynamics of the HMW-GS hydrated with two 2-propanol/water mixtures at temperatures between 268 and 308 K by specific spin labeling of their cysteine residues. Spectra were of a composite type, resulting from two populations of spin labels differing in molecular mobility, both undergoing isotropic rotational diffusion. Diffusional coefficients and populations of the fast- and slow-moving spin labels, determined by an accurate spectral line shape analysis, are discussed as a function of temperature and water content in the solvent systems. Far-UV circular dichroism was employed to provide information on the secondary structure of the HMW-GS in three different solvents [aqueous 50% (v/v) 2-propanol, aqueous 0.1% (v/v) trifluoroacetic acid, and trifluoroethanol]. For the first one, the influence of temperature on HMW-GS structure was also investigated.

Keywords: *HMW glutenin subunits; gluten; spin labeling EPR; far-UV CD; protein structure; Triticum durum*

INTRODUCTION

High molecular weight glutenin subunits (HMW-GS) have been found to play a crucial role in determining the quality of bread and pasta wheat (*1*−6). It is now well established that HMW-GS confer elasticity to the dough, even though the mechanism by which this is done is still unclear (*7*).

Over recent years, different techniques have been employed to unravel the structural peculiarities responsible for the properties of these water insoluble proteins (*8*−*13*). Computational analysis has revealed that HMW subunits contain very large amounts of glutamine (35 mol %) and significant amounts of glycine (20 mol %) and proline (10 mol %). They consist of nonrepetitive N- and C-terminal domains flanking a central repetitive domain. The N and C termini are richer in charged residues than the repeat domain and contain most, or all, of the cysteine residues present in the subunits. The repeat domains are characterized by hexa- and nonapeptide repeats in the y-type subunits (PGQGQQ and GYYPTSLQQ) and by hexa-, nona-, and tripeptide repeats in the x-type subunits (PGQGQQ, GYYPTSPQQ, and GQQ) (*14*). Finally, the N- and C-terminal domains contain roughly 15 and 5% of the residues in the proteins, respectively (*10*).

The structure of these domains is the object of active investigation. Computer predictions (*11*) indicated that the repetitive domain has an unusual secondary structure with regularly repeated β-turns, whereas the terminal regions are predominantly α-helical. The evidence obtained from spectroscopic results has partially confirmed these predictions, revealing that the secondary structure of HMW subunits in solution is strongly dependent on the nature of the solvent, in particular, on water content (*10*, *11*, *13*, *15*, *16*). In fact, on the basis of viscometric analysis and circular dichroism (CD) spectroscopy, Field et al. (*10*) proposed that the repetitive central domain of the HMW-GS forms a loose spiral based on repetitive β-turns, whereas the shorter nonrepetitive terminal domains are α-helical in trifluoroethanol (TFE) but random coil in dilute acetic acid and aqueous 50% (v/v) propan-1-ol. The CD spectra of HMW-GS in TFE have been differently interpreted by Hickman (*15*), who suggested that they are associated with a 3_{10} helix. In addition, FT-IR and NMR investigations (*16*) showed that the central repetitive domain consists

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10.1021/jf000800t CCC: $20.00
Published on Web 12/01/2000

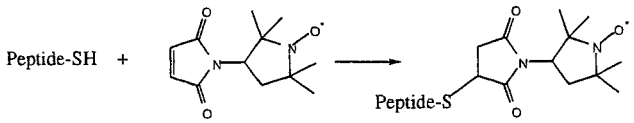

Figure 1. Molecular structure of the spin label 3-MAL.

of β-turns and β-sheets in proportions which vary with water content. All of these studies indicate that HMW-GS are different from the vast majority of investigated proteins; hence, important information may be gained from their further study.

In the present work CD spectroscopy is employed to investigate the solution structure of two HMW-GS with very similar molecular masses [97.5 and 100.9 kDa, as determined by sodium dodecyl sulfate (SDS) electrophoretic analysis], extracted as a mixture from gluten of durum wheat cv. Lira. Glutenin subunit 20x is the main component of the mixture (86%), accompained by its linked y-type subunit 20y present in minor amount (14%) (*17*). As reported by Buonocore et al. (*17*), subunit 20x has only two cysteine residues, one in the N-terminal region and the other in the C-terminal domain, whereas subunit 20y contains five cysteines in the N-terminal and two in the C-terminal domain, respectively, as typical of y-type subunits. The CD spectra, recorded in the far-UV region (190−250 nm), arise principally from the peptide bond and are indicative of the backbone structure of the proteins (*18*). The remarkable differences observed in spectra of HMW-GS in different solvents [TFE, 0.1% (v/v) trifluoroacetic acid (TFA), and aqueous 50% (v/v) 2-propanol (IPA50)] confirm the high sensitivity of HMW-GS structure to the nature of the solvent as previously reported for subunits extracted from different wheat cultivars (*10*, *11*).

Even more evident differences were found by applying spin-labeling electron paramagnetic resonance (EPR) spectroscopy to our HMW subunits hydrated by two different 2-propanol/water mixtures, namely aqueous 50% (v/v) 2-propanol plus 0.1 M acetic acid (IPA50A) and aqueous 25% (v/v) 2-propanol plus 0.1 M acetic acid (IPA25A). As is well-known, spin-labeling EPR is a highly sensitive technique for examining protein structure and dynamics (*19*−*21*), even though it has scarcely been used to study gluten HMW-GS (*22*). Here, spin labeling of sulfhydryl groups of the cysteine residues with 3-MAL (see Figure 1) allowed structural dynamics of the HMW-GS terminal regions to be assessed as a function of temperature and water content in the solvent system.

MATERIALS AND METHODS

HMW-GS Extraction and Purification. Flour of durum wheat (*Triticum durum*) cv. Lira was obtained by grinding seeds in a break roller-mill (Labormill 4RB) and stored at 277 K in the dark. The extraction of HMW-GS was performed using a procedure (*23*) based on that of Marchylo et al. (*24*). Routinely, 130 g of flour was extracted twice at 333 K with 0.5 M NaCl (780 mL) for 30 min by continuous agitation of the sample. The suspension was centrifuged for 10 min at 10000*g*. The precipitate was treated at 333 K with 50% (v/v) propan-1-ol (780 mL) for 30 min. The suspension was centrifuged as above, and the precipitate was extracted at 333 K with 50% (v/v) propan-1-ol containing 1% (w/v) dithiothreitol for 30 min and then centrifuged at 10000*g* for 30 min. The supernatant was adjusted to 60% (v/v) propan-1-ol and allowed to rest at 277 K for 1 h. The precipitate was collected by centrifugation, frozen, and lyophilized.

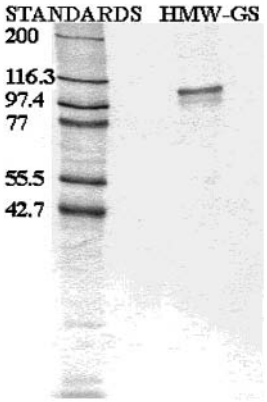

Figure 2. SDS-PAGE of HMW glutenin subunits extracted from gluten of durum wheat cv. Lira.

Samples employed in spectroscopic determinations were further purified by ultrafiltration. Lyophilized HMW-GS were dissolved in aqueous 25% (v/v) 2-propanol (0.2 mg/mL) and subjected at room temperature to repeated ultrafiltrations with a Spectrum C-20K membrane in a Spectrum S-25-10 stirred cell, until no reducing agents were detected in the permeates by HPLC analysis and an absorbance ratio $A_{279}/A_{251} \geq 3$ was found in the UV−vis spectrum of the retentates.

SDS-PAGE Determination of Molecular Masses. The molecular weights of the purified proteins were determined by SDS-PAGE using globular proteins as standards (*25*). Freeze-dried HMW-GS were resuspended in 0.5 mL of 62 mM Tris-HCl buffer (pH 6.8) containing 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, and 0.1% bromophenol blue, boiled for 3 min, and loaded (2 μg) on an SDS slab gel (11% resolving gel and 4% stacking gel). The BDH calibration kit of molecular mass standards was applied to the gel. The gel separation was performed in a mini-gel apparatus (C.B.S. Scientific Co., Solana Beach, CA). Run time was 30 min at 150 V and then 50 min at 200 V. The gel was stained overnight with Coomassie brilliant blue R250, destained with 7.5% (v/v) acetic acid, and then scanned and processed by a digital image analysis program (Sigma Gel, Jandel Corp., San Rafael, CA). The determined molecular masses were 100.9 and 97.5 kDa (see Figure 2).

Labeling of HMW-GS with 3-MAL. The purified proteins (2 mg/mL; pH 6 phosphate buffer) were spin labeled at cysteine residues by incubation with a 20-fold molar excess of 3-MAL at 303 K for 3 h. The spin label excess was removed by ultrafiltration with a Spectrum C-20K membrane in a Spectrum S-25-10 stirred cell. Ultrafiltration was repeated until no spin labels were detected in the permeates by EPR spectroscopy.

Spectrum Measurements and Analysis. EPR measurements were performed with a Varian ESR-E112 spectrometer operating at the X-band frequencies and equipped with a Varian E257 temperature control unity. The spectrometer was interfaced to a 100-MHz personal computer by means of a homemade data acquisition system (*26*, *27*). The spectra were recorded at a field setting of 3290 G, a microwave power of 5 mW, and a modulation amplitude of 1 G. The samples were enclosed in quartz tubes with 1 mm internal diameter. Samples of ∼20 μL with a 15 mg/mL protein concentration were employed.

Calculated spectra were obtained by best fitting the experimental line shapes using the least squares version NLSL of the Slow-Motional EPR Line Shape Calculation Program (*19*, *20*), PC version for Windows NT. A Brownian motional model of isotropic rotational diffusion was adopted. The elements of the hyperfine (**A**) and electron Zeeman (**g**) tensors employed in the calculations were determined by best-fitting spectra in the rigid limit (223 K). They are reported in the legends of Figures 3 and 4.

CD spectra were recorded with a JASCO J40AS spectropolarimeter, using cells with path length from 0.05 cm (0.2−

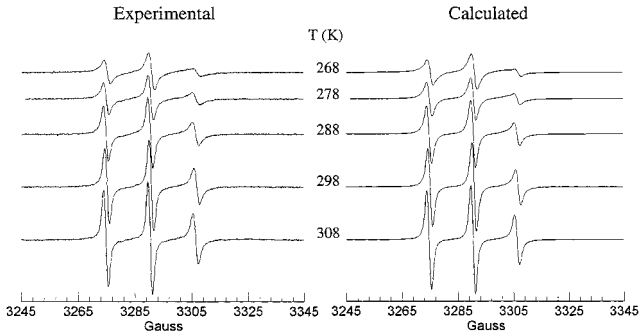

Figure 3. Experimental (left) and calculated (right) EPR spectra of HMW-GS, extracted from gluten of durum wheat cv. Lira and labeled with 3-MAL at cysteine residues, in IPA25A at the indicated temperatures. The following parameters have been used in the calculations: A_{xx}, A_{yy}, and A_{zz} = 7.98, 5.43, and 34.00, respectively gauss; g_{xx}, g_{yy}, and g_{zz} = 2.0086, 2.0079, and 2.0038, respectively; line width = 1.2 G.

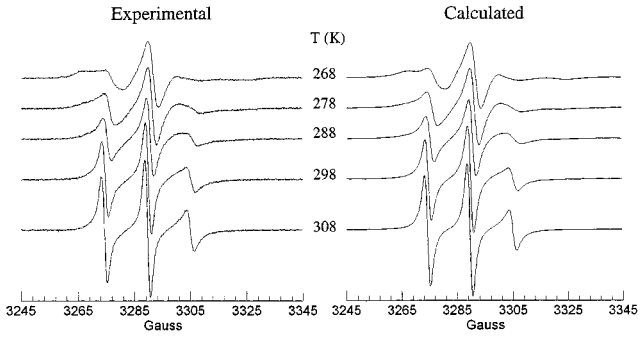

Figure 4. Experimental (left) and calculated (right) EPR spectra of HMW-GS, extracted from gluten of durum wheat cv. Lira and labeled with 3-MAL at cysteine residues, in IPA50A at the indicated temperatures. The following parameters have been used in the calculations: A_{xx}, A_{yy}, and A_{zz} = 6.00, 6.00, and 34.50 gauss, respectively; g_{xx}, g_{yy}, and g_{zz} = 2.0094, 2.0066, and 2.0035, respectively; line width = 1.0 G.

0.8 mg/mL protein concentration) to 1 cm (0.02 mg/mL protein concentration) to control absorbance values (PM voltage up to 500 V). The spectral bandwidth was 2 nm.

Spectral analysis was performed by means of a least-squares fitting procedure using sets of CD basis functions reported in the literature (*28*−*32*).

Chemicals. 3-Maleimidoproxyl (3-MAL) was purchased from Sigma. Solvents of spectroscopic grade were purchased from Carlo Erba or from Aldrich.

RESULTS AND DISCUSSION

EPR Spectroscopy. EPR measurements were performed on the mixture of the two HMW-GS extracted from gluten of durum wheat cv. Lira, labeled at the sulfhydryl groups of the cysteine residues with 3-MAL (see Figure 1) and hydrated with two different aqueous mixtures, namely, aqueous 25% (v/v) 2-propanol plus 0.1 M acetic acid (IPA25A) and aqueous 50% (v/v) 2-propanol plus 0.1 M acetic acid (IPA50A). The spectra were recorded every 5 degrees on heating in the temperature range between 268 and 308 K; selections are shown in Figures 3 and 4. Their line shape reflects the local environment at the position of the spin labels in the proteins (*19*−*21*), thus allowing a qualitative discussion of changes in local structure and mobility as a function of temperature and solvent composition. In fact, with increasing temperature an evolution of spectral line shapes characteristic of a progressively increasing mobility of the spin labels is observed in both solvent systems (see Figures 3 and 4). Moreover, by comparing EPR spectra recorded at the same temperature on labeled HMW-GS in IPA25A and IPA50A (see Figures 3 and 4), it can be seen that motions in the vicinity of the spin-labeled cysteines decrease with increasing alcohol content in the solvent mixture.

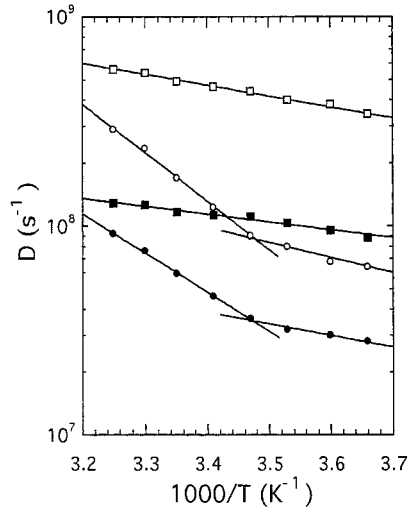

Figure 5. Rotational diffusion constants for the motion of 3-MAL spin labels bound to HMW-GS, extracted from gluten of durum wheat cv. Lira, versus 1000/*T*: (solid squares) slow spin labels in IPA25A; (open squares) fast spin labels in IPA25A; (solid circles) slow spin labels in IPA50A; (open circles) fast spin labels in IPA50A. Rotational diffusion constants are determined with errors up to 0.3%. Solid lines represent fits of the trends of rotational diffusion constants as a function of temperature in terms of the Arrhenius equation as described in the text.

To understand more details of these spectral features, we performed a fitting of the spectral line shapes by means of a computer program based on Freed's theory of slow motional EPR (*19*, *20*). A Brownian isotropic rotational diffusion model was considered for the motion of the spin labels, characterized by a rotational diffusion constant *D*.

The spectral line shapes could be reproduced only by introducing two components associated with rather different rotational diffusion constants, which, in turn, correspond to spin labels with slightly different mobilities, located in two different environments. The two components contribute to the spectra in a different manner at different temperatures; at each temperature, the fitting of the spectra gives *D* values and populations for the slow- and fast-moving spin labels, respectively. As can be observed in Figure 5, in each sample the rotational diffusion constants of slow- and fast-moving spin labels show nearly parallel trends as a function of temperature, indicating that their local environments respond in similar ways to temperature-induced structural changes. Different trends are, however, observed in the two solvent systems.

In IPA25A the rotational diffusion constants of both slow- and fast-moving spin labels regularly increase with increasing temperature. An analysis of these trends in terms of the Arrhenius equation $D = A \exp(-E_a/RT)$ gives the kinetic parameters (i.e., the preexponential factor A and the activation energy E_a) reported in Table 1. Similar activation energies are reported in the literature for the motion of spin labels bound to cysteine residues of hydrated gluten (*33*) and HMW-GS (*22*). The populations of the fast- and slow-

Table 1. Arrhenius Parameters for the Motion of 3-MAL Spin Labels Bound to HMW-GS Extracted from Durum Wheat Cv. Lira in IPA25A and IPA50A

		fast spin labels		slow spin labels	
solvent	temp range (K)	A (s^{-1})	E_a (kcal/mol)	A (s^{-1})	E_a (kcal/mol)
IPA25A	268−308	$(2.88 \pm 0.41) \times 10^9$	2.40 ± 0.08	$(2.15 \pm 0.34) \times 10^9$	1.71 ± 0.09
IPA50A	268−288	$(9.84 \pm 7.75) \times 10^{15}$	10.59 ± 0.48	$(1.16 \pm 0.42) \times 10^{14}$	8.58 ± 0.22
IPA50A	288−308	$(2.88 \pm 1.95) \times 10^{10}$	3.31 ± 0.38	$(3.04 \pm 1.15) \times 10^9$	2.55 ± 0.21

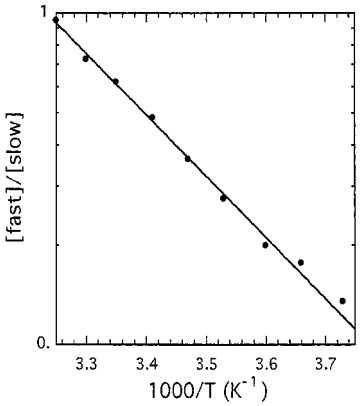

Figure 6. Population ratios [fast-moving spin labels]/[slow-moving spin labels] versus 1000/T for labeled HMW-GS, extracted from gluten of durum wheat cv. Lira, in IPA25A. Populations were determined with errors up to 5%. The line represents the fit of experimental points in terms of the equilibrium equation [fast]/[slow] = exp($\Delta S/R$) exp($-\Delta H/RT$).

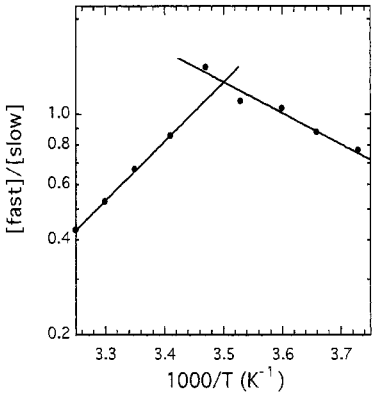

Figure 7. Population ratios [fast-moving spin labels]/[slow-moving spin labels] versus 1000/T for labeled HMW-GS, extracted from gluten of durum wheat cv. Lira, in IPA50A. Populations were determined with errors up to 8%. Lines represent fits of experimental points in terms of two equilibrium equations of the type [fast]/[slow] = exp($\Delta S/R$) exp($-\Delta H/RT$), as described in the text.

Table 2. Thermodynamic Parameters for 3-MAL Spin Labels Bound to HMW-GS Extracted from Durum Wheat Cv. Lira in IPA25A and IPA50A

solvent	temp range (K)	ΔS (eu)	ΔH (kcal/mol)
IPA25A	268−308	27.29 ± 0.75	8.44 ± 0.23
IPA50A	268−288	16.23 ± 2.09	4.50 ± 0.59
IPA50A	288−308	-29.53 ± 0.59	-8.57 ± 0.18

moving spin labels change regularly with temperature, so that the [fast]/[slow] ratio depends on the temperature as shown in Figure 6. These results can be associated with the presence of an increasing number of spin labels in less hindered sites with increasing temperature. An analysis of these data in terms of the equilibrium equation [fast]/[slow] = exp($\Delta S/R$) exp($-\Delta H/RT$) yields the entropy and enthalpy differences as summarized in Table 2. It may be noted that ΔS is positive, indicating that higher entropy is associated with the environment of fast-moving spin labels with respect to that of slow-moving spin labels. On the other hand, the sites of slow spin labels have lower enthalpy than those of the fast ones. The combined effects of ΔS and ΔH result in a significant change of the population ratio: whereas at low temperature [slow] $\gg$ [fast], at high temperature [slow] $\cong$ [fast]. Analogous trends of populations were reported for the dynamics of gluten labeled with 3-MAL (*33*) and other spin labels (*22*). However, in those cases a 4−5 order of magnitude difference between the diffusion constants was found instead of the <1 order difference here determined (see Figure 5).

For HMW-GS in IPA50A the trends of diffusion constants of both slow- and fast-moving spin labels were not regular in the whole range of temperature explored, but a marked change in the slope was observed at ~288 K (see Figure 5). We analyzed these trends in terms of two different Arrhenius equations, one valid between 268 and 288 K and the other between 288 and 308 K, obtaining the kinetic parameters reported in Table 1. Thus, whereas below 288 K the motion of spin labels in IPA50A shows activation energies similar to those obtained for spin labels bound to the HMW-GS in IPA25A, at higher temperatures the motion of the spin labels is more hindered in the former solvent. It must be emphasized, however, that fast spin labels in IPA50A have lower (2−5 times) rotational diffusion constants than fast spin labels in IPA25A and even lower than slow spin labels in IPA25A between 268 and 288 K (see Figure 5). These results confirm the observation that lower water content in the solvent system induces a decrease of spin label mobility, that is, favors the presence of more hindered cysteine environments. Following Affleck et al. (*34*) this behavior can be associated with a reduction of the dielectric constant of the solvent, indicating that motions in proteins are governed in part by electrostatic interactions.

Moreover, we found that in IPA50A the populations of slow- and fast-moving spin labels show peculiar changes with temperature: the [fast]/[slow] ratio, which shows values up 1.4, increases with increasing temperature between 268 and 288 K, whereas it decreases between 288 and 308 K (see Figure 7). These results can be related to a conformational change, involving the local environments of both slow- and fast-moving spin labels, occurring in the proteins in IPA50A at ~288 K. An analysis of the population ratios in terms of the equilibrium equation [fast]/[slow] = exp($\Delta S/R$) exp($-\Delta H/RT$) gave the thermodynamic parameters reported in Table 2. At low temperatures (268−288 K) positive ΔS and ΔH values were found, whereas negative values were determined at high temperatures (288−308 K). As a consequence, an increase of the population of spin labels with both higher enthalpy and entropy is observed as the temperature is increased in the whole range of explored temperatures, that is, of fast-moving spin labels between 268 and 288 K and of slow-moving ones between 288 and 308 K.

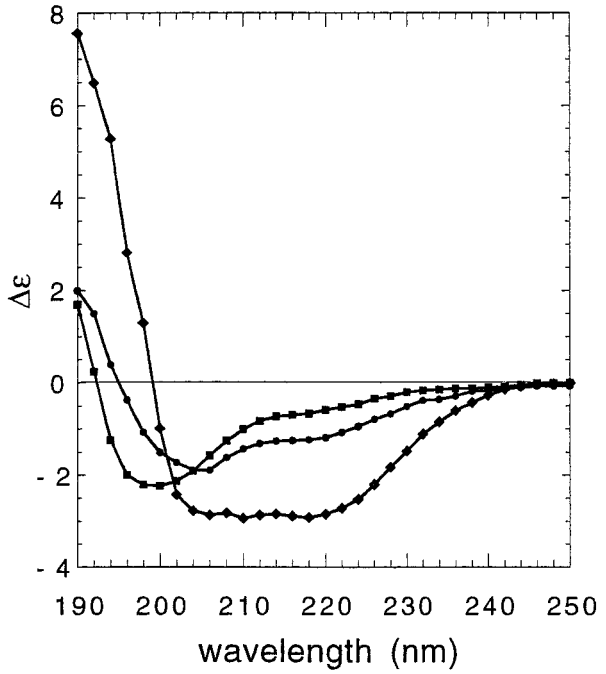

Figure 8. Far-UV CD spectra recorded at room temperature on HMW-GS, extracted from gluten of durum wheat cv. Lira and labeled with 3-MAL in IPA50 (circles), aqueous 0.1% (v/v) TFA (squares), and TFE (rhomboids).

Far-UV CD Spectroscopy. The influence of the solvent and temperature on the structure of the HMW-GS extracted from durum wheat cv. Lira was investigated also by means of far-UV CD spectroscopy. In fact, the CD spectrum in the far-UV region (below 250 nm) arises principally from the peptide bond and, thus, is indicative of the backbone structure of proteins (*18*). The room temperature spectrum of our HMW-GS labeled with 3-MAL in IPA50 shows a minimum at 205 nm and a shoulder at ~220 nm (see Figure 8). Analogous spectra are reported in the literature for different HMW-GS in mixtures of aqueous 50% (v/v) propan-1-ol and TFE (*10*) and interpreted as indicative of a regular structure rich in β-turns, with minor contribution from α-helix conformations (*35*). A similar spectrum, with almost no contribution from α-helix, is observed here for our HMW-GS labeled with 3-MAL dissolved in aqueous 0.1% (v/v) TFA (see Figure 8) and was observed by other authors for HMW-GS extracted from a different wheat cultivar in 0.05 M acetic acid. On the contrary, the spectrum of our HMW-GS labeled with 3-MAL in TFE is typical of a protein relatively rich in α-helix, with minima at around 208 and 221 nm and a crossover point at 200 nm (see Figure 8). These results are in agreement with those reported by Field et al. (*10*) and can be attributed to the fact that in TFE the nonrepetitive N- and C-terminal domains of the HMW-GS adopt an α-helical-rich conformation, whereas in IPA50 and aqueous 0.1% (v/v) TFA these regions would not appear to be predominantly in the α-helix conformation, which, on the contrary, has been indicated as the predominant one by structure predictions (*11*, *12*). Some authors (*36*, *37*) reported that certain organic solvents increase the α-helical content of proteins when added in high concentrations, the decreased dielectric constant of the solvent increasing protein structure contact (*34*, *38*). It is unlikely that the repetitive central domain of HMW-GS could be induced by the solvent to form an α-helix, owing to the high content of proline in the repeat motifs (*39*). Structure predictions (*11*, *12*) indeed indicate that the central repeat domains of HMW-GS are rich in β-turns. In our case, because the intensity of the α-helix spectrum is greater than the intensities of the various β-turn types, the CD spectrum of the central repeat domain could not be observed in TFE, whereas a decreased α-helix content in IPA50 would allow the β-turn spectra to be observed.

Far-UV CD spectra were recorded at different temperatures in the 278−298 K range on solutions of our HMW-GS in IPA50 (spectra not shown). A significant lowering of the intensity was observed in the spectral range between 220 and 250 nm with decreasing temperature (~30% at 230 nm going from 298 to 278 K), which could be attributed to a reduction of the α-helix content in the protein, that is, in the terminal regions where this structure is possible and where the spin labels are located.

To quantitatively interpret the room temperature CD spectra of HMW-GS labeled with 3-MAL in TFE and IPA50 (see Figure 8), attempts of deconvolutions were made by means of four different methods (*28*−*32*); the obtained results are reported in Table 3. In all cases a higher contribution of α-helix to the spectrum is found in TFE with respect to IPA50, as expected. Unfortunately, all methods present severe limitations, as demonstrated by the fact that negative contributions of some structures to the spectra are obtained from the calculations and that the contributions do not sum to 1. These limitations are related to the fact that the proteins of the basis sets used in the calculations are different from HMW-GS under many aspects (i.e., amino acid composition, globular structure, solubility, etc.). In particular, two methods (*28*−*30*) consider only two structures (α-helix and random coil) among those predicted for HMW-GS, giving quite high errors in the calculated structure contributions to the spectra. The method of Chang et al. (*31*) seems to be more reliable because it includes a β-turn structure. The highest number of β structures is considered in the method of Hennessey and Johnson (*32*), which can thus be considered as the more appropriate for the analysis of our CD spectra. The deconvolution results obtained using this method indicate 25% of α-helix for the HMW-GS in TFE and 4% in IPA50 (see Table 3). However, the secondary structures used in the Hennessey and Johnson method are unable to fully represent HMW-GS conformations, notwithstanding their demonstrated ability to reproduce the CD spectra of enzymatic proteins (*32*). Nevertheless, because HMW-GS terminal domains can assume α-helix structures contrarily to the central repetitive domain, we believe that the α-helix percentages above-reported substantially indicate that the terminal domains are almost completely in the α-helix conformation in TFE, whereas an α-helix content of ~15% can be estimated for our HMW-GS in IPA50.

Very similar CD spectra were observed in the same solvent systems for labeled and unlabeled HMW-GS (spectra not shown), indicating that labeling results in no significant alteration of the protein secondary structure, that is, that disulfide bonds do not play an essential role in the formation of structure. These results also confirm that the spin labels can be seen as extraneous side-chain residues of the proteins, and responses of the neighboring protein to a change in environment (i.e., an increase in temperature or a

Table 3. Results of Deconvolutions of Room Temperature CD Spectra of HMW-GS Extracted from Durum Wheat Cv. Lira in IPA50 (Roman Typeface) and TFE (Italic Typeface)

	Greenfield and Fasman method	Chen et al. method	Chang et al. method	Hennessey and Johnson method
α-helix	0.244 ± 0.019	0.192 ± 0.017	−0.049 ± 0.033	0.036 ± 0.001
	0.537 ± 0.022	*0.501 ± 0.034*	*0.264 ± 0.049*	*0.250 ± 0.030*
antiparallel β-sheet	−0.314 ± 0.065	−0.074 ± 0.111	−0.180 ± 0.036	0.166 ± 0.050
	−0.522 ± 0.074	*−0.167 ± 0.149*	*−0.259 ± 0.055*	*0.038 ± 0.008*
parallel β-sheet	no	no	no	0.035 ± 0.001
	No	*no*	*no*	*0.026 ± 0.004*
random coil	0.069 ± 0.038	0.328 ± 0.006	0.350 ± 0.118	0.140 ± 0.020
	−0.270 ± 0.043	*0.189 ± 0.076*	*0.254 ± 0.180*	*−0.185 ± 0.100*
β-turn I	no	no	0.176 ± 0.022	0.037 ± 0.006
	no	*no*	*0.019 ± 0.034*	*0.0057 ± 0.0002*
β-turn II	no	no	no	0.016 ± 0.004
	no	*no*	*no*	*−0.018 ± 0.005*
β-turn III	no	no	no	0.006 ± 0.001
	no	*no*	*no*	*0.005 ± 0.001*

change of solvent) will be manifested by the behavior of the spin labels.

CONCLUSIONS

In this study some important information on the structure and dynamics of HMW-GS in water and/or alcohol solutions has been achieved.

The spin label EPR study has shown that HMW-GS extracted from gluten of durum wheat cv. Lira contain terminal regions with different structures and mobilities. In fact, the observed spectral line shapes result from the superposition of two components ascribable to spin labels differing in mobility and population, located in two different environments, as also reported for gluten and gluten subfractions with different compositions (*22*, *33*, *40*).

The dynamics of the spin labels is influenced by the temperature and by the nature of the solvent interacting with the HMW-GS, the mobility increasing with increasing temperature and water content in the solvent mixture (i.e., the dielectric constant of the solvent). In particular, in the water-rich solvent IPA25A no transition in the segmental flexibility of HMW-GS was observed in the temperature range explored (268−308 K) and the activation energies determined for the motion of both fast- and slow-moving spin labels could be related to low-energy bonds in the immediate surrounding of the labels, due to both solvent−protein and protein−protein interactions. On the contrary, in IPA50A a discontinuous trend of diffusion constants as a function of temperature was found for both fast- and slow-moving spin labels, indicating that at temperatures >288 K a protein structure more sensitive to temperature is present.

Moreover, we found a marked influence of temperature and water content in the solvent on the populations of fast- and slow-moving spin labels. In particular, a temperature increase favors spin labels with higher enthalpy and more degrees of freedom, that is, fast-moving spin labels in IPA25A between 268 and 308 K and in IPA50A between 268 and 288 K and slow-moving spin labels in IPA50A between 288 and 308 K. The higher enthalpy and entropy associated with these spin labels most probably reflect less tight solvent−protein and protein−protein interactions in the environments of the corresponding cysteine residues. The peculiar trend of spin label populations observed for HMW-GS in IPA50A could be associated with conformational changes occurring at ∼288 K involving the cysteine residues, that is, the structure of the protein terminal regions.

The far-UV CD study has shown that some solvents, such as TFE, help the HMW-GS to assume regular secondary structures, that is, α-helix structure in the terminal regions and/or β-turns in the repetitive central regions, whereas in aqueous solvents more disordered structures are preferred. These findings are in good agreement with results reported by other authors for HMW subunits extracted from different wheat cultivars (*10*−*12*, *15*). Unfortunately, a complete physically significant description of the HMW-GS secondary structure could not be obtained due to the lack of basis sets suitable for the analysis of CD spectra of plant proteins.

Moreover, a lowering of the α-helix structure content was found in IPA50 by decreasing the temperature from 298 to 278 K. Because cysteine residues and, in turn, spin labels are located in the terminal domains of HMW-GS, we could infer that the structural changes observed by EPR are promoted by the modest conformational modifications of these sole domains. In other words, the terminal regions seem to be the solvent- and temperature-sensitive parts of HMW-GS. Finally, it must be emphasized that concentration effects cannot be excluded because the spectroscopic techniques here employed require different protein concentration ranges.

ABBREVIATIONS USED

IPA, propan-2-ol or isopropanol (2-propanol); IPA50, aqueous 50% (v/v) 2-propanol; IPA50A, aqueous 50% (v/v) 2-propanol/plus 0.1 M acetic acid; IPA25A, aqueous 25% (v/v) 2-propanol plus 0.1 M acetic acid; TFE, trifluoroethanol; TFA, trifluoroacetic acid; 3-MAL, 3-maleimidoproxyl; HMW-GS, high molecular weight glutenin subunits; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography.

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Received for review June 29, 2000. Revised manuscript received October 16, 2000. Accepted October 16, 2000. This work was supported by MIPAF, MURST, and CNR (Rome, Italy).

JF000800T