

Molecular Modeling of Unusual Spiral Structure in Elastomeric Wheat Seed Protein

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ABSTRACT

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The structure of the central repetitive domain of the high molecular weight glutenin subunits, a group of elastomeric proteins from the seeds of wheat, were modeled using structure prediction and molecular dynamics. Models were generated with spiral structures, based on repetitive β -reverse

turns, within and spanning the repeat motifs of the central domains. The models were consistent with available data from biophysical studies on the intact proteins and spectroscopic (infra-red and nuclear magnetic resonance) studies of synthetic peptides.

Gluten is the protein-rich mass that remains when dough made from wheat flour is washed to remove starch and other components. It is of particular interest because of its unusual biomechanical properties, a combination of elasticity and viscous flow, which are largely responsible for the end-use properties of wheat in many food systems including breadmaking. The molecular basis for gluten viscoelasticity is therefore of interest in relation to understanding and improving the end-use quality of wheat. Gluten is a mixture of at least 50 proteins that are classified into two groups. The gliadins are monomeric proteins with intrachain or no disulfide bonds while the glutenins form large M_r polymers stabilized by interchain disulfide bonds. The gliadins may be responsible for the viscosity of doughs, while the glutenins are responsible for the elastomeric behavior (Kasarda 1989).

One group of gluten proteins, the high molecular weight glutenin subunits (HMW-GS), has been extensively studied because genetic variation in the amount and composition of its component polypeptides is closely associated with variation in breadmaking quality (Payne 1987). Amino acid sequences of a number of individual HMW-GS were determined by sequencing the corresponding genomic DNA (Shewry et al 1992; Reddy and Appels 1994). The proteins consist of three distinct domains, including a central repetitive domain which varies in length at ≈ 480 –700 residues and consists of tandem and interspersed repeats based on nonapeptide (consensus GYYPTSP/LQQ) and hexapeptide (consensus PGQGQQ) motifs with a tripeptide motif (consensus GQQ) also present in some subunits (Shewry et al 1992). This repetitive domain is flanked by nonrepetitive N- and C-termini of approximately 100 and 40 residues, respectively, that contain most or all of the four to seven cysteine residues that are present in each subunit (Shewry and Tatham 1996).

Structure prediction and spectroscopic studies have indicated that the repetitive domain of the HMW-GS has an unusual secondary structure with regularly repeated β -turns but no β -sheet or α -helical structure (Tatham et al 1984, 1990; Belton et al 1995; van Dijk 1996a). This is supported by hydrodynamic studies that indicate rod-like molecules with diameters of 1.5–1.9 nm and lengths of 50–60 nm, dependent on solvent and conditions (Field et al 1987), and by small angle X-ray scattering (SAXS) diameters of ≈ 6.4 nm and lengths of 70 nm (Thomson et al 1999). Similarly, scanning tunneling microscopy images show a helical secondary structure, with a diameter of ≈ 2 nm and pitch of ≈ 1.5 nm (Miles et al 1991). It has been suggested that this spiral structure consists of β -turns and is

similar to the β -spiral structure demonstrated for peptides related to the pentapeptide repeat motif present in elastin (consensus PGXXX) and it may contribute to the elastomeric behavior of the gluten complex (Tatham et al 1984, 1985). In addition, two computer models of the repetitive domain have been reported. Both give spiral structures but the details differ significantly. Matsushima et al (1990) reported a spiral structure based on β -turns, although this was based on modeling of tandemly arranged nonapeptide motifs (which do not occur in the HMW-GS, nonapeptides always being interspersed with one or more hexapeptide motifs). In contrast, the study of Kasarda et al (1994) gave a spiral based on inverse γ -turns for the tandemly arranged repeats of the hexapeptide sequence.

We have, therefore, reinvestigated the structures of the HMW-GS repetitive domains using structure prediction and molecular dynamics. This has generated three alternative spiral structures, all based largely on β -turns, which are consistent with the available data from spectroscopy and other biophysical studies.

MATERIALS AND METHODS

Prediction and Location of β -Turns

The structure prediction methods of Chou and Fasman (1978), Willmot and Thornton (1988), and the Protein Sequence Analysis (PSA) system (Stutz et al 1993; White et al 1994) were used to predict the location and type of β -turns. The consensus hexapeptide (H) motif (PGQGQQ) and nonapeptide (N) motif (GYYPTSPQQ) were considered and a 21-mer comprising two hexapeptides and one nonapeptide (H₂NH).

Energy Calculations

All minimizations were done using 200 steps of steepest descent followed by use of the conjugate gradient minimizer until the maximum derivative was <1 kcal/nm. (All simulations used a time step of 0.001 psec.) The molecular dynamics protocols were as follows.

1. Simulated annealing of isolated models. The temperature was initially raised to 800 K for 3 psec, and then dropped in stages of 50 K followed by 1,000 steps of dynamics. This was repeated to a 150 K, at which time the structure was completely minimized. The whole protocol was repeated 10 times and the lowest energy conformation of the 10 was selected.

2. Molecular dynamics in solution. The calculations of β -turn stability were made in a sphere of water molecules, radius 1.5 nm. A 0.8-nm layer of water molecules was placed around each model. The temperature was raised in stages of 30 K from 0 to 210 K, with 5 psec of equilibration at each stage, with the C_α backbone fixed in position. All constraints were then removed from the system. This was followed by a data collection stage of 20 psec of dynamics at 210 K. Coordinates were saved after each picosecond of the data collection stage. Finally, the solvated structures are completely minimized without constraints. All calculations were carried out using a molecular modeling program (Discover, Biosym Technologies, San Diego, CA).

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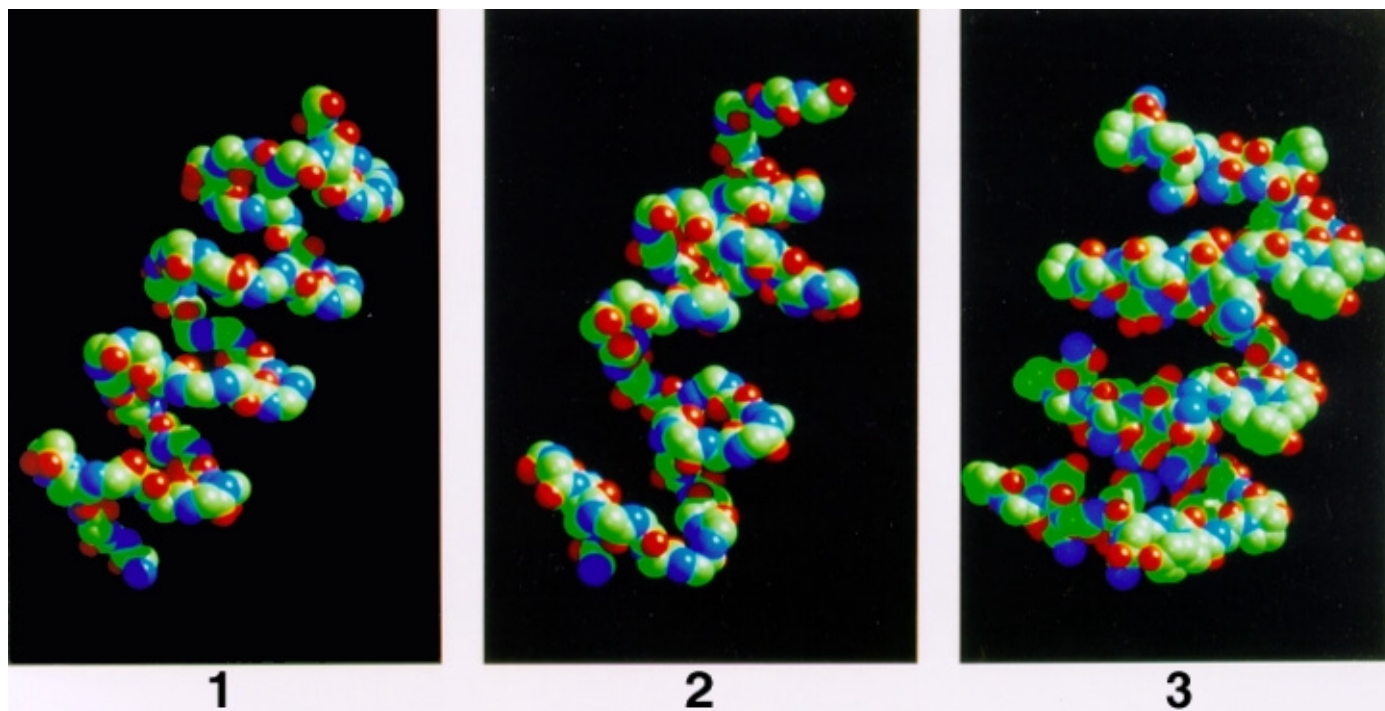


Fig. 1. Space filling models of model 1 with type II β -turns at QPGQ, YPTS, SPQQ, and QGQQ; model 2 with type II β -turns at QGY Y and QPGQ and type I/III β -turns at SPQQ and YPTS; model 3 with distance-based β -turns at QPGQ, YPTS, and SPQQ, the largest continuous stretch of residues without β -turns, GQGQQGY, has values consistent with β -sheet.

Model Building

Models were based on combining standard β -turn types at locations indicated by theory and experiment with a variety of torsional angles for those regions connecting the β -turns using MOLEDT (Biosym Technologies). Initially, the ϕ value for these torsion angles was restricted to accommodate proline residues at any position (i.e., $\phi = -60 \pm 20^\circ$). The ψ values were not restricted. Models were also created using a more general definition of a β -turn as suggested by Wilmott and Thornton (1988). This definition requires that a β -turn be described in terms of the $C_\alpha(i) - C_\alpha(i+3)$ distance, which is <0.7 nm, in a β -turn. Models were compared to the physical dimensions provided by experiment and accepted or rejected for further study based on fit to this data.

RESULTS

The repetitive domains of the HMW-GS consist largely of hexapeptide and nonapeptide motifs, with the hexapeptides occurring as tandem repeats and interspersed with nonapeptides. The nonapeptides only occur interspersed with hexapeptides and not in tandem.

The distribution and types of β -turns within the consensus hexapeptide (H) and nonapeptide (N) motifs were initially predicted (Table I). β -Turns of type II are predicted in the hexapeptide motif over residues QPGQ. Proline shows a preference for position $i+1$ of a β -turn and glycine for position $i+2$, and turns with proline and glycine in these positions are usually type II. In the nonapeptide motif, overlapping turns are predicted of mixed turn types.

Predictions based on both the type I and type II discrete state-space server (DSM) from the PSA server give similar results. However, no turns are predicted to lie within the nonapeptide motif, but a low probability of β -strand was predicted. The results of the energy calculations on the turn structures, together with the calculated probabilities and the predicted turn types, are shown in Table II. The energy calculations are consistent with the predicted results. In all cases, except for the turn at SPQQ, solvation in a sphere of water serves to increase the stability of the theoretically

preferred conformation with respect to the isolated model.

When the effects of overlapping turns were considered, the results were less easily interpreted. Four heptapeptides were considered, based on the consensus hexapeptide and nonapeptide motifs and spanning the junctions of four motifs: A, QGY YPTS; B, SPQQPGQ; C, YPTSPQQ; and D, QPGQQQ. The peptides were subject to energy minimization (Table III). These results differ from those calculated for the single turn peptides. The differential stabilization energy between the two turn types in the isolated and solvated peptides is reduced rather than increased as in the single turn peptides. Therefore, single turns are predicted to be the more stable structure.

A large number of initial models were constructed based on combining standard β -turn types at locations suggested by theory and experiment with a variety of torsional angles for those regions connecting the β -turns. All models were compared to the physical dimensions and secondary structures provided by experiment and accepted or rejected for further study, based on fit to this data. The three final models selected for further development were constructed using a HNH sequence motif repeated three times plus an additional N-terminal H motif and a two residue C-terminal partial H motif (total motif HHNHHNHHNHH). A final set of three models were constructed based on turns defined only for the H motif and combinations of the H and N motifs.

Model 1. The simplest model put a type II β -turn at the highest probability positioning the H motif at QPGQ. Residues in regions linking the turns were assigned ϕ and ψ values of -54° and 120° , respectively. The spiral was created by generating a repeating H-motif-only structure with each H motif with the above conformation (any repeating conformation of residues creates some form or helix or spiral). The resulting spiral had dimensions consistent with the experimental diameter of 1.7 nm and pitch of 1.3 nm. The correct HNH motif sequence was then fitted to this structure, forcing turns at YPTS, SPQQ, and QGQQ (in addition to QPGQ) (Fig. 1A). These sequences are found to some extent in turns in proteins but their best turn type is not type II. However, any attempt to replace the type II turns at these positions with the most favored turn type

for the sequence (type I/III) resulted in loss of the spiral structure. The length of the molecule, assuming a repetitive domain length of 650 residues, was 53.5 nm.

Model 2. Five turns were placed solely within the HNH motifs (Fig. 1B). Type II turns were located at QGYI and QPGQ and type I/III turns were located at SPQQ and YPTS. This is in agreement with the predicted and calculated turn-type preferences for these residues. Linking residues have ϕ and ψ values of -60° and 130° respectively. The calculated pitch of the spiral is 1.6 nm and the diameter is 2.0 nm. However, the calculated length was only 37.7 nm.

Model 3. The final HNH motif model selected used none of the standard β -turn types (Fig. 1C). Instead, β -turns were based on the distance criteria of Wilmott and Thornton (1988). The β -turns were placed at QPGQ, YPTS, and SPQQ. The largest continuous stretch of residues without β -turns, GQGQGY, with ϕ and ψ values consistent with β -strands. The calculated pitch of the spiral was 1.5 nm and diameter was 2.1 nm. The model is roughly elliptical in plan view, with a cross-section of $\approx 1.7 \times 2.5$ nm. The length was calculated as 54.5 nm.

The three models were subject to energy minimization followed by a simulated annealing protocol. The structures proved to be intrinsically unstable during the simulated annealing, resulting in com-

plete loss of all initial structural assignments. Further investigations of these loose spiral structures were made with the C_α in each model fixed. This allowed investigation of side chain to side chain and side chain to backbone hydrogen bonding. The resulting structures were analyzed for hydrogen bonding networks and other stabilizing factors (Table III). Model 3 is clearly the most stable, but this is not just due to an enhanced hydrogen bonding network, as all models display a similar number of hydrogen bonds, $\approx 0.5/\text{residue}$. This number of hydrogen bonds is significantly smaller than that obtained from an α -helix or β -sheet, where there is approximately one hydrogen bond per residue. The models display little interturn hydrogen bonding, reducing the possibility of stabilizing the large pitch structures.

Analysis of the type of hydrogen bond in vacuo (Table IV) shows that in models 1 and 2, the majority of the hydrogen bonds are *i, i+3*, whereas in model 3, the majority are *i, i+2* hydrogen bonds. Model 1 displays considerably more long-range hydrogen bonds than models 2 or 3. In fact, the hydrogen bonding in the energy-minimized models changed little from that in the initial input models.

The intrinsic instability of these large pitch structures in vacuo led to the analysis with a hydration shell around each model. The supporting effect of the solvent molecules allowed removal of all constraints on the backbone. Simulations were conducted on the solvated models.

The calculated energetics of the solvated models are shown in Table V. The models are energetically similar. In the isolated models, model 3 is 200 kcal more stable than either model 1 or model 2 (data not shown), but this difference is reduced to 50 kcal in the solvated models. Comparison of the intramolecular hydrogen bond types predicted in vacuo and solvated (Table IV) shows a large decrease in the numbers of backbone to backbone hydrogen bonds. The total numbers of hydrogen bonds were reduced $\approx 50\%$, with the number of backbone to backbone hydrogen bonds being reduced $\approx 80\%$. The overall effect of solvation was to disrupt the intramolecular hydrogen bonding in each of the models in favor of hydrogen bonding with the solvent, this being facilitated by the loose nature of the spiral structures. Consequently, all the models were well hydrated with approximately three intermolecular hydrogen bonds per residue.

DISCUSSION

Three models have been developed that show good correlation with available experimental data. Field et al (1987) reported a length of 50–60 nm for subunit Bx20, Thomson et al (1999) using SAXS on the same subunit determined a length of ≈ 69 nm. Although the precise sequence of this subunit is not known, it has a similar M_r by SDS-PAGE to subunit 1Bx7 which consists of 770 residues including a repetitive domain of 647 residues (Anderson and Greene 1989). The dimensions determined for subunit 20 can be compared

TABLE I
Predicted Turn Location and Turn Types Within Repeat Motif of HMW Glutenin Subunits

Motif	Probability of Turn Formation ^a	Predicted Turn Type ^b
QPGQ	4.15	II
QGYI	0.90	II
QQGY	1.72	I/III
SPQQ	1.28	I/III
YPTS	1.70	I/III
GQGQ	1.86	II
PTSP	0.93	I/III

^a Predictive method of Chou and Fasman (1978).

^b Predictive method of Wilmott and Thornton (1988).

TABLE II
Calculated Energy Differences for Turn Stability in Isolated Turns and Solvated Turns^a

	Isolated (kcal mol ⁻¹)		Solvated (kcal mol ⁻¹)	
	I	II	I	II
QPGQ	1.0	0.0	3.9	0.0
QGYI	0.0	1.6	0.0	2.5
QQGY	0.0	7.3	0.0	9.8
SPQQ	0.0	5.0	0.0	4.3
YPTS	0.0	5.8	0.0	14.3
GQGQ	2.8	0.0	4.5	0.0
PTSP	0.0	4.7	0.0	9.2

^a I and II refer to turn types. Only type I turns were considered for turn calculations due to the similarity of type I and III turns.

TABLE III
Calculated Turn Types^a for Model Peptides Containing Consecutive β -Turns

Model	Peptide	Turn Location	Calculated Turn Type	
			Isolated	Solvated
A	QGYIPTS	QGYI	II	I
		YPTS	I	II
B	SPQQPGQ	SPQQ	I	I
		QPGQ	II	I
C	YPTSPQQ	YPTS	II	II
		SPQQ	I	II
D	QPGQGQQ	QPGQ	II	II
		GQGQ	II	I

^a I and II refer to turn types. Only type I turns were considered for turn calculations due to the similarity of type I and III turns.

TABLE IV
Intramolecular Hydrogen Bond Types^a

Model	<i>i, i+2</i>	<i>i, i+3</i>	<i>i, i+4</i>
1	6(4)	19(5)	13(9)
2	5(3)	24(5)	9(7)
3	26(6)	5(5)	5(6)

^a In vacuo and in solution (parentheses).

TABLE V
Solvation Energies and Hydrogen Bonding in Solution (energies in kcal)

Model	Intramolecular		Intermolecular	
	Energies	Hydrogen Bonds	Energies	Hydrogen Bonds
1	-38.3	23	-680	186
2	-57.9	21	-769	203
3	-89.9	19	-740	206

with those for a repetitive domain of 650 residues based on models 1, 2 and 3. Lengths calculated for models 1 and 3 (53.5 and 54.5 nm, respectively) are in good agreement with the hydrodynamic data, considering the lengths determined by hydrodynamic analysis were for the whole subunit (including N- and C-terminal domains). The 37.7 nm length calculated for model 2 clearly agrees less well.

Dimensions for the diameter (2 nm) of subunit 20 and the pitch of the spiral structure formed by the repetitive domain (1.5 nm) have also been determined from STM images (Miles et al 1991). Although these dimensions may be affected by adsorption of the proteins to the substrate and should therefore only be considered as approximate, they nevertheless show good agreement with the hydrodynamic and modeling data. In particular, pitch of 1.5 nm agrees well with those calculated for all three models (1.6, 1.3, and 1.5 nm for models 1, 2 and 3 respectively).

Matsushima et al (1992) also calculated similar lengths for the HMW-GS (56–78 nm) based on SAXS. Although this method gave large diameters (6–8 nm), calculation of the partial specific volumes from the data reported indicates that errors may have occurred in the dimension determinations, most probably in R_c (the radius of gyration of the cross section) due to aggregation.

Matsushima et al (1990) attempted to model the nonapeptide repeat of the HMW-GS but concluded that, as the repeat contained two proline residues, there were many degrees of freedom and, hence, many models could be constructed. They reported no detailed models but concluded that models with diameters of 1.4–1.6 nm could be built with two or three β -turns per repeat. Kasarda (Kasarda 1994; Kasarda et al 1994) developed models based on the hexapeptide repeat and nonapeptide consensus repeats and a 15-mer (i.e., 9+6 residues) fitted to the template of the hexapeptide sequence. The polypeptide chain formed a helical structure that was energy-minimized and subject to molecular dynamics. The resulting structures were again subject to energy minimization. β -Turns of different types were tried, giving a spiral. However, it was distorted and energies after minimization were poor. When inverse γ -turns were used, a more stable spiral structure was obtained. Modeling gave a diameter of ≈ 2.4 nm and pitch of ≈ 0.9 nm. The spiral was stabilized by an extensive network of hydrogen bonding. We cannot rule out the formation of γ -turns, but we consider the models reported here based on β -turns are more consistent with data from other studies.

Van Dijk et al (1996a) reported evidence for β -turns in the repetitive sequences using 2D-NMR, infra-red (IR), and circular dichroism (CD) spectroscopy of synthetic and heterologously expressed peptides. CD and IR indicated β -turns with no evidence of γ -turns. 2-D NMR of cyclic peptides, cyclo-[PGQGQQPGQGQQ], cyclo-[GYPTSPQQGA] and cyclo [PGQGQQGYPTSPQQ], and the linear peptide PGQGQQ_n corresponding to the repeat sequences in the HMW-GS indicated β -turns. There was a type II β -turn structure at QPGQ in both the cyclic and linear peptides; type I β -turns at YPTS and SPQQ with an additional β -turn of either type I or II at QQGY. The proline residue in YPTS showed considerable *cis/trans* proline isomerism with $\leq 50\%$ of the population in the *cis*-conformation, the other proline residues were $>90\%$ in the *trans*-conformation. The conversion from *trans* to *cis* destroys the type I β -turn at YPTS but leads to an increase in stability of the β -turn at SPQQ or QQGY. These β -turns are in agreement with those used in the models described here. Model 2 corresponded most closely with the NMR data. This contains a type II turn a QGY, whereas the NMR data indicates the presence of a type I or II turn at QQGY. Van Dijk et al (1996b) also examined the solution structures of two HMW-GS and an M_r 16,802 peptide corresponding to part of the central repetitive domain expressed in *E. coli*. They reported that the CD and IR spectra of the domain were compatible with β -turns, stabilized by hydrogen bonds both within and between turns.

The M_r 16,802 peptide expressed in *E. coli* by van Dijk et al (1996b) was soluble in water. This behavior contrasts with that of whole HMW-GS proteins that are insoluble in water, but it is consistent with the models reported here. All three models predict

that the repetitive domain would be water-soluble, with the successive breaking of hydrogen bonds during hydration. FT-IR studies of the HMW-GS indicate that the structure is distorted in the dry state due to protein-protein hydrogen bonds and that, as the subunits are hydrated, these are partially replaced by protein-water hydrogen bonds (Belton et al 1995). Nevertheless, the molecules remain insoluble even when fully hydrated. IR studies show this may be due to protein-protein interaction, with the whole subunits forming extended intermolecular β -sheet structures at higher levels of hydration. Model 3 has a degree of intramolecular β -sheet structure that could presumably form intermolecular β -sheet structures with the central repetitive domains of other HMW-GS molecules. This model may, therefore, resemble most closely the structure of the HMW-GS at a higher level of hydration.

There has been speculation as to whether the secondary structure of the HMW-GS contributes to elastomeric properties in glutenin polymers stabilized by interchain disulfide bonds. Urry et al (1976) showed that the β -spiral structure formed by a synthetic polypentapeptide based on a repeat motif of elastin is intrinsically elastic and that this may be relevant to the mechanism of elastin synthesized in vivo (Urry 1982). They proposed an entropic mechanism, where the elastic force results from a decrease in entropy on stretching. However, NMR studies of a HMW-GS indicate that this mechanism does not apply to gluten elasticity (Belton et al 1994). The elastomeric properties of the unusual spiral structure described here remain to be established.

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