The Beta-Turn Conformation in Wheat Gluten Proteins: Relationship to Gluten Elasticity

A. S. TATHAM, B. J. MIFLIN, and P. R. SHEWRY[1]

Cereal Chem. 62(5):405-412

ABSTRACT

A combination of circular dichroism spectroscopy and computer prediction from amino acid sequences is used to study conformations of wheat gluten proteins. The ω-gliadins are rich in β-turns, but appear to have no α-helix or β-sheet structure. β-Turns are also present in the repetitive central domain of the high molecular weight (HMW) subunits of glutenin. These β-turns are regularly distributed, reflecting the regular primary structure. The N- and C-terminal domains of the HMW subunits are probably α-helical and contain the only cysteine residues so far detected in these proteins. In the α-gliadins and probably the β- and γ-gliadins, the β-turns are concentrated in specific domains, notably a proline-rich repetitive domain close to the N-terminus. Other domains are rich in α-helix. The structure of the mammalian elastomeric protein elastin is reviewed and models for its elasticity discussed. One of these, the β-spiral model of Urry and co-workers, is used as the basis for a model for gluten elasticity. We propose that the major elastic components of gluten are the HMW subunits of glutenin. The repetitive β-turns in the central domain form an elastic β-spiral, and these elastic monomers are assembled into gluten polymers by intermolecular disulfide bonds between the cysteine residues in the α-helical domains near the N- and C-termini.

Of the cereals, only wheat and to a limited extent rye can be baked to give leavened bread. This property is determined largely by the unusual viscoelastic properties of the water-insoluble gluten proteins, which enable dough to expand by trapped fermentation gases and to be baked into bread with a light porous crumb structure. The precise molecular basis of these physical properties is not known, although a number of models have been suggested. One of the earliest of these, which has been modified subsequently, is the "linear glutenin hypothesis" of Ewart (1968, 1972, 1978). This hypothesis proposes that glutenin contains molecules joined by disulfide bonds into linear polymers, with only a limited amount of branching. These molecules are responsible for elasticity, whereas viscous flow depends primarily both on molecular slippage and on mechanical scission and disulfide interchange. This and alternative models have been discussed in more detail by Wall (1979) and Miflin et al (1983).

The gluten proteins can be classified into two groups that are present as monomers associated by hydrogen bonding and hydrophobic interactions, or as polymers linked by covalent disulfide bonds. These two groups correspond broadly to the

[1]Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ, U.K.

©1985 American Association of Cereal Chemists, Inc.

gliadins and glutenins respectively, which were defined by Osborne (1907) on the basis of their solubility or insolubility in 70–90% aqueous ethanol. This classification does not, however, reflect the chemical and genetic relationships of the component polypeptides, and we have recently proposed a new classification based on these criteria (Miflin et al 1983, Shewry et al 1984a). Three groups are recognized: the high molecular weight (HMW) prolamins (which include the HMW subunits of glutenins), the S-poor prolamins (ω-gliadins), and the S-rich prolamins (the α-, β-, and γ-gliadins, and the low molecular weight [LMW] subunits of glutenin).

Secondary structure refers to the regular arrangement of the polypeptide backbone, resulting from the formation of hydrogen bonds between the carbonyl oxygen and amide nitrogen atoms of the peptide bonds. Much early work on protein structure was carried out by X-ray crystallography on fibrous proteins, and two major structures were discovered in the early 1950s. These are the α-helix, a structure where the backbone is arranged in a helical coil, and the β-sheet, where an extended polypeptide chain forms complementary hydrogen bonds with another parallel chain (Shultz and Schirmer 1979). More recently Venkatachalam (1968), using theoretical calculations, found three favorable arrangements where the polypeptide chain could achieve a 180° change of direction. The structure, called a β-turn, is composed of four successive residues; in the β-turn, hydrogen bonding occurs between the carbonyl group of the first residue and the amide nitrogen of the fourth residue, instead of the fifth residue as in the α-helix. The formation of a hydrogen bond, however, is not a prerequisite for β-turn formation, since some 25% of turns do not contain such a bond; such a conformation is often referred to as an open reverse turn (Smith and Pease 1980). The β-turn conformation accounts for approximately one-third of the residues in globular proteins (Chou and Fasman 1977).

A number of spectroscopic techniques, including circular dichroism (CD), infra-red (IR) and nuclear magnetic resonance (NMR), can be used to estimate the amount of secondary structure in a protein. However, they cannot locate the positions of the structures along the polypeptide chain; only X-ray diffraction analysis can give an unequivocal structure determination. The number of protein sequences determined far outnumbers that of structures determined by X-ray crystallography. This has led to the development of parameters obtained from X-ray data to determine the propensity for a residue to be found in an α-helical, β-sheet, or β-turn conformation. The method of Chou and Fasman (1977, 1978), based on analysis of 29 proteins of known sequence and structure, is most widely used. For β-turn analyses the relative position of the residue is taken into consideration, as a number of residues show marked positional preferences (Chou and Fasman 1977). The authors claimed an accuracy of some 77% for their predictive method, although this has been questioned (Kabsch and Sander 1983). Also, because the method is based on globular protein data, it is not necessarily applicable to other protein types. It remains, however, the most commonly used method for predicting secondary structures of proteins. The principles of the method of Garnier et al (1978), in contrast to those of Chou and Fasman (1978), are based on information theory. The likelihood of a residue adopting a conformation is calculated by considering the neighboring residues up to a distance of eight in either direction, and compared with values which are adjusted for the best fit between predicted and observed structures. The method is, however, fully automatic and simple to use.

CD spectroscopy is the most widely used method to determine the secondary structure content of proteins. This information is calculated from the far-ultraviolet (UV) (below 250 nm) spectrum, which arises principally from the absorption of the peptide bond. In contrast, the near-UV (250–300 nm) CD spectrum arises from the absorption of aromatic and disulfide chromophores.

We have recently reported a series of studies of the conformations of wheat gluten proteins (Tatham et al 1984, Tatham and Shewry 1985) and homologous proteins from barley grain (Tatham et al 1985). Although some prolamins contain an appreciable amount of α-helix, the dominant and most widespread conformation appears to be the β-turn. We proposed a model based on elastin, in which the presence of regularly repeated β-turns in the disulfide-bonded HMW subunits conferred elasticity to gluten (Tatham et al 1984). The present paper reviews these studies, and presents further data on the distribution of β-turns in wheat gluten proteins and discusses their role in gluten elasticity.

MATERIALS AND METHODS

The α- and ω-gliadins were prepared as previously described by Tatham and Shewry (1985). HMW subunits were prepared as described by Shewry et al (1984b), but in the presence of 0.1% 2-mercaptoethanol.

CD measurements were made using a Jasco J4OCS dicograph. Variable temperature spectra were obtained using a heated cell holder and a thermocouple of a Comark electronic thermometer inserted directly into the solution. The results are calculated using an average residue weight of 105.5 for the ω-gliadin, 110.2 for the α-gliadin, and 105.3 for the HMW subunits, calculated from the amino acid compositions. The units are degrees·cm^2·mol^{-1}.

A number of different approaches have been used to correlate the far-UV CD spectra of proteins with their secondary structure contents. In this study the method of Chen et al (1972) was used; this calculates the content of α-helix, β-sheet, and aperiodic structure, comprising both the β-turn and random coil conformations. Several other methods have been described, and claimed to give accurate estimates of the β-turn conformation (Bayley 1980, Smith and Pease 1980). In all cases, however, they employ an idealized β-turn reference spectrum, whereas different CD spectra for β-turns were described by Woody (1974). Also, the β-turn spectra are generally less intense than those of the β-sheet and α-helix conformations, and it is therefore often difficult to determine β-turns in the presence of high proportions of these structures. Multicomponent analysis is a useful, approximate, measure of protein conformation but the results should not be over interpreted (Bayley 1980).

The secondary structures were predicted from the available amino acid sequence data using the methods of Chou and Fasman (1978) and Garnier et al (1978). For the Chou and Fasman analysis, predictions were obtained from the N_α and N_β of the residue parameter P_α and P_β (Dufton and Hider 1977). Search distances of 6 and 5 were used for helical and sheet structures, respectively. For the β-turn analysis Chou and Fasman calculated the average probability of a turn occurrence (Pt) as 0.55×10^{-4} and selected tetrapeptides with Pt $> 0.75 \times 10^{-4}$ as probable turns. For the method of Garnier et al the unweighted prediction results were used.

RESULTS AND DISCUSSION

S-Poor ω-Gliadins

The far-UV CD spectrum of an ω-gliadin fraction in 70% (v/v) aqueous ethanol (Fig. 1A) shows none of the characteristics associated with the α-helix and β-sheet conformations (Manavalan and Johnson 1983). Although it superficially resembles the spectrum associated with random coil (Dearborn and Wetlaufer 1970) certain differences are apparent. These are notably a minimum at 204 nm rather than between 196 and 200 nm, and a broad absorbance between 220 and 240 nm. Also, when the protein was denatured in 6M urea (Fig. 1A) the absorbance above 220 nm was greatly reduced. Because urea absorbs at low wavelengths, it was not possible to determine the position of the absorption minimum. The near-UV spectrum in the same solvent (Fig. 1B) showed strong absorbances at 262 and 268 nm due to the aromatic group of phenylalanine. This indicates that these groups are present in fixed conformations, which would only occur if the secondary structure is regular. No disulfide absorbance was observed, which is consistent with the absence of cysteine in ω-gliadins (Booth and Ewart 1969, Kasarda et al 1983).

Although there is no unique CD spectrum associated with β-turns, the far-UV CD spectrum of the ω-gliadins is compatible with a β-turn rich conformation. Venkatachalam (1968) described a number of theoretical types of β-turn conformation, and Woody

(1974) calculated that these would result in four different types of CD spectra. β-Turn rich proteins usually contain mixtures of turn types, and this is reflected in the presence of "hybrid" CD spectra. It is therefore to be expected that the spectrum of ω-gliadins shows some differences from those reported for other proteins rich in β-turns (Ishizaki et al 1979, Green et al 1983), although all have minima between 202 and 208 nm and shoulders between 220 and 230 nm.

The presence of β-turns is also indicated by structural prediction, although only short N-terminal amino acid sequences of ω-gliadins have been reported (Kasarda et al 1983). These show a short N-terminal domain, which varies in length from 5 to 13 residues, followed by several pentapeptides with a consensus sequence of pro-gln-gln-pro-tyr. Prediction using the methods of Chou and Fasman (1978) (Fig. 2) and Garnier et al (1978) (not shown) suggests the presence of repetitive β-turns in ω-gliadins. The S-poor prolamin of barley, C hordein, has an N-terminus homologous to ω-gliadins (Kasarda et al 1983). Most of the rest of C hordein probably consists of repeated octapeptides (consensus pro-gln-gln-pro-phe-pro-gln-gln) (Forde et al 1985), which also show predicted probabilities for repetitive β-turns (Tatham et al 1985).

More conclusive evidence for the presence of β-turns in ω-gliadins comes from the effects of increasing temperatures on their CD spectra. When ω-gliadins were heated to 80°C in 70% (v/v) ethanol, the far-UV spectrum (Fig. 1A) showed increased absorption of the minimum at 204 nm with a concomitant decrease in the intensity of the shoulder at 228–230 nm. This indicates a conformational change on heating rather than denaturation which would occur with most globular proteins. The change appeared to be reaching completion near 60°C. The curves show two isocircular dichroic points at 211–212 nm and 198 nm, suggesting two defined conformations, one at high and one at low temperatures, with a negligible concentration of intermediates. In contrast, there was little change in the near-UV spectrum over the same temperature range (Fig. 1B), indicating that the phenylalanine side chains remain in fixed conformations. These observations are consistent with the stabilization of the conformation by hydrophobic interactions between phenylalanine residues, which are favored at higher temperatures (Tanford 1968).

A far-UV difference spectrum was obtained by subtracting the spectrum at 20°C from that at 80°C (Fig. 1A). This corresponds closely to that reported for a class B β-turn (Woody 1974), the most common class associated with the β-turn conformation, and indicates an increase in this turn type on heating. This could be caused by the introduction of additional β-turns on heating or to changes in the conformations of the β-turns already present.

HMW Subunits

The far-UV CD spectrum of a mixture of HMW subunits dissolved in 7:3 (v/v) ethanol-trifluoroethanol, a solvent which should promote a regular secondary structure by eliminating competition by water for hydrogen bonding, is shown in Figure 3A. The spectrum is similar to that of the ω-gliadins in the same solvent (Fig. 1A), implying that the HMW subunits also have a β-turn rich conformation. There was no evidence from the spectrum for extensive areas of α-helix or β-sheet. The near-UV spectrum (Fig. 3B) showed strong absorbances caused by the side chains of phenylalanine (262 and 268 nm) and tyrosine (275 nm). This shows that these groups are in fixed conformations, and is again indicative of a regular secondary structure. It was not possible to study the thermostability of the HMW subunits because of the volatility of trifluoroethanol and their insolubility in other suitable solvents.

Although no HMW subunits have been sequenced in full, a considerable amount of information is available from the direct sequencing of the N-termini of purified proteins (Shewry et al 1984b) and from the nucleotide sequences of cDNA clones (Forde et al 1983, Thompson et al 1983). These show the presence of at least three distinct structural domains. The N-terminal domain of at least thirty residues is rich in glutamate (up to 8 residues) and has two cysteines. Structural prediction (Tatham et al 1984) indicates that this region is α-helical. The distances between the two cysteine residues in the different subunits are such that they would be expected to be located one above the other on the same side of the helix, and it is extremely unlikely that they would form an intramolecular disulfide bond. The C-terminal domain of 38 residues contains one cysteine and is also fairly rich in charged residues, with two glutamate residues and one each of aspartate, lysine, and arginine. We proposed that the N- and C-terminal domains form hydrophilic α-helices that are exposed at the surface of the protein. This would facilitate the formation of intermolecular disulfide bonds, which are important in stabilizing the gluten aggregates.

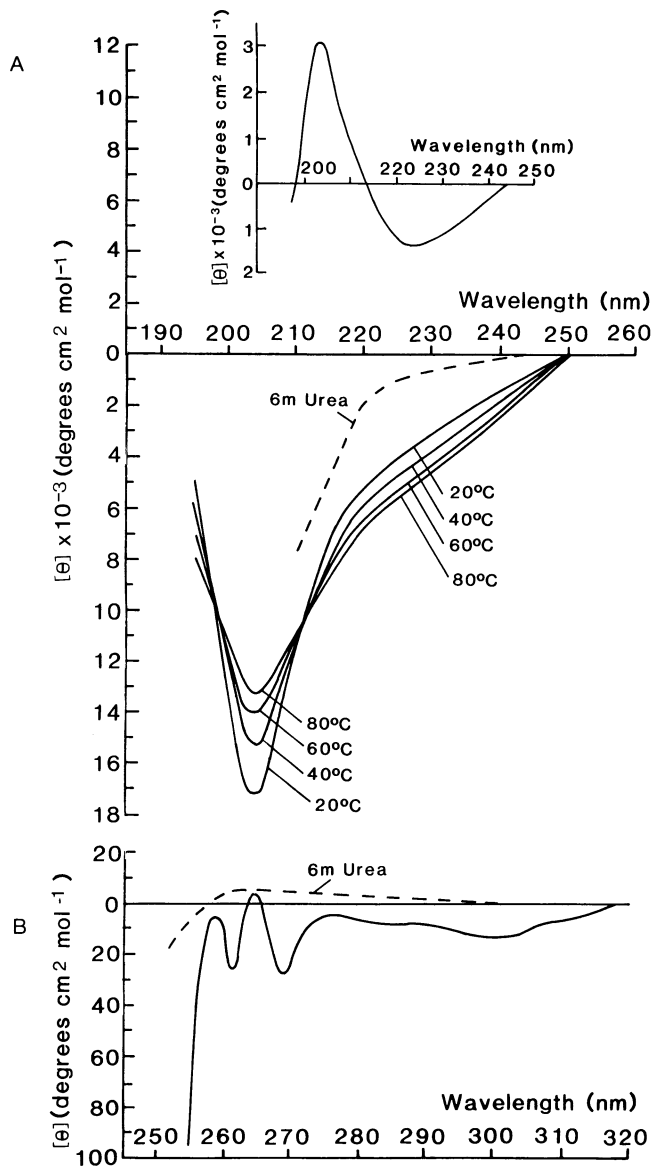

Fig. 1. Far and near-ultraviolet (UV) circular dichroism spectra of ω-gliadins in 6*M* urea at 20°C (---) and in 70% (v/v) aqueous ethanol at temperatures varying from 20 to 80°C (—)/ **A,** Far-UV spectra and far-UV difference curve (insert); **B,** near-UV spectra.

T. monococcum	ω-1	R Q L N P S D Q E L Q S P Q Q L Y P Q Q P Y P Q Q P Y
T. monococcum	ω-1	A R Q L N P S D Q E L Q S P Q Q L Y P Q Q P Y P Q Q P Y
T. aestivum	ω-2	A R E L N P S N K E L Q S P Q Q S F
T. durum	ω-1	K E L Q S P Q Q S F ? H Q Q Q P F P Q Q
T. aestivum	ω-5	S R L L S P R G K E L H T P Q Q Q F P Q Q ? ? F P

Fig. 2. N-terminal amino acid sequences of ω-gliadins. Tetrapeptides with predicted probabilities (*Pt* 0.9 to 5.5×10^{-4}) to form β-turns are underlined. The sequences are from Kasarda et al (1983).

The rest of the known sequence of HMW subunits is composed of repeated blocks of six residues with occasionally interspersed blocks of nine residues. No cysteines have been detected in this domain and charged residues are rare. The total extent of this domain is not known, but it is at least 242 residues (Thompson et al 1983). Structural predictions (Tatham et al 1984) indicate the presence of regularly repeated β-turns. Because some of the predicted turns overlap (Fig. 4A), only those with the highest probabilities may form. However, this is not necessarily the case: Isogai et al (1980) have characterized a number of multiple bends in proteins that can share pairs of residues. The distribution of the turns is summarized in Figure 4B. Their probabilities range from 1.05 to 5.29 $\times$ 10^{-4}, compared to the probability of 0.75 $\times$ 10^{-4} selected as the level of significance by Chou and Fasman (1978). Also they are very regularly distributed, spanning the junctions of the blocks and within the nine residue blocks. The high probability turns have proline or serine at the second position and glycine at the third, the most favorable positions for these residues.

S-Rich Prolamins

The S-rich prolamins include proteins classically defined both as gliadins (α, β, and γ) and as glutenins (LMW subunits). The α-gliadins, notably the aggregative A-gliadin of Bernardin et al (1967), have been studied most intensively and will be considered first.

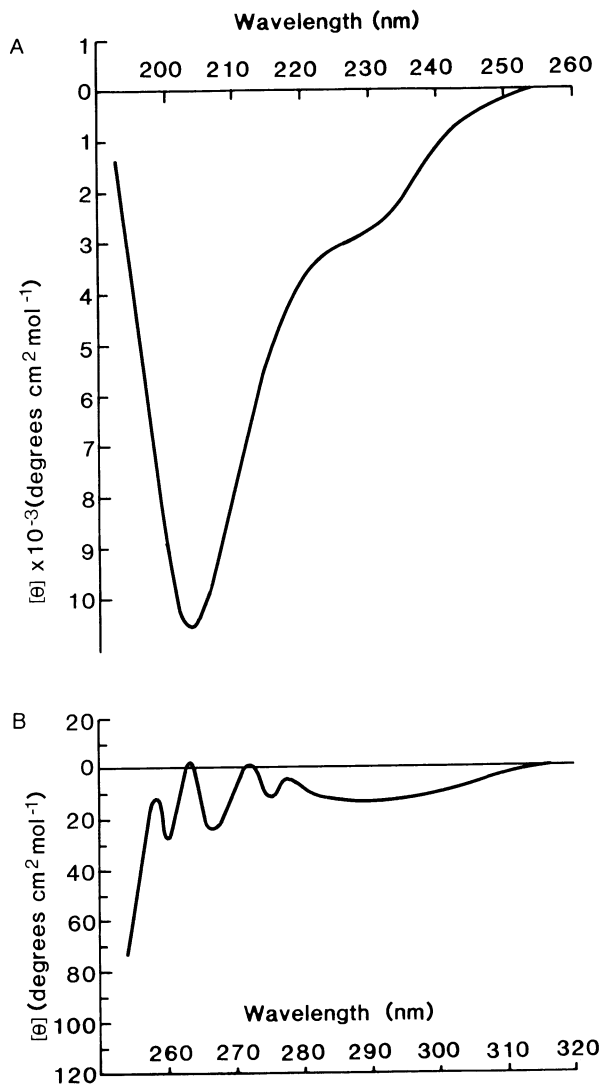

Fig. 3. Circular dichroism spectra of high molecular weight (HMW) subunits in 7.3 (v/v) ethanol:trifluoroethanol at 20°C. **A,** far-ultraviolet (UV) spectrum (redrawn from Tatham et al 1984); **B,** near-UV spectrum.

The far-UV spectrum of α-gliadin in 70% (v/v) aqueous ethanol (Fig. 5A) is typical of a protein rich in α-helix (Manavalan and Johnson 1983), with minima at 208 and 222 nm. Kasarda et al (1968) reported a similar spectrum for A-gliadin in an aqueous solvent (10^{-5} *M* HCl), indicating a similar backbone conformation.

The near-UV spectrum in 70% ethanol (Fig. 5B) shows strong absorbances because of the side chains of phenylalanine (at 262 and 268 nm) and tyrosine (at 275 nm) (see Strickland 1974), indicating that these groups are in fixed conformations. These results contrast with those of Kasarda et al (1968), which showed a broad absorbance between 250 and 300 nm for A-gliadin in aqueous solution. It is likely that the side chains adopt different conformations in solvents of different polarities.

The proportions of the α-helix, β-sheet, and aperiodic structure can be calculated from the far-UV CD spectra or predicted from amino acid sequences. Table I compares the results calculated from the α-gliadin spectrum in Figure 5A with those predicted from the complete amino acid sequence of A-gliadin reported by Kasarda et al (1984). Both calculations and prediction indicate that the predominant conformations of α-gliadins are the α-helix and β-turn, which each account for about 35% of the protein. Cluskey and Wu (1971) reported a similar value of 38% α-helix for gliadins determined by optical rotatory dispersion in trifluoroethanol, a solvent which promotes ordered hydrogen-bonded structures. The estimates of α-helix and β-sheet from the predictive method of Garnier et al are clearly inconsistent with those from the other two methods.

The amino acid sequences of S-rich prolamins can be divided into structural domains which have different amino acid compositions. Kasarda et al (1984) recognize five such domains in the sequence of A-gliadin; these are indicated in Figure 6A, and their amino acid compositions and predicted conformations are

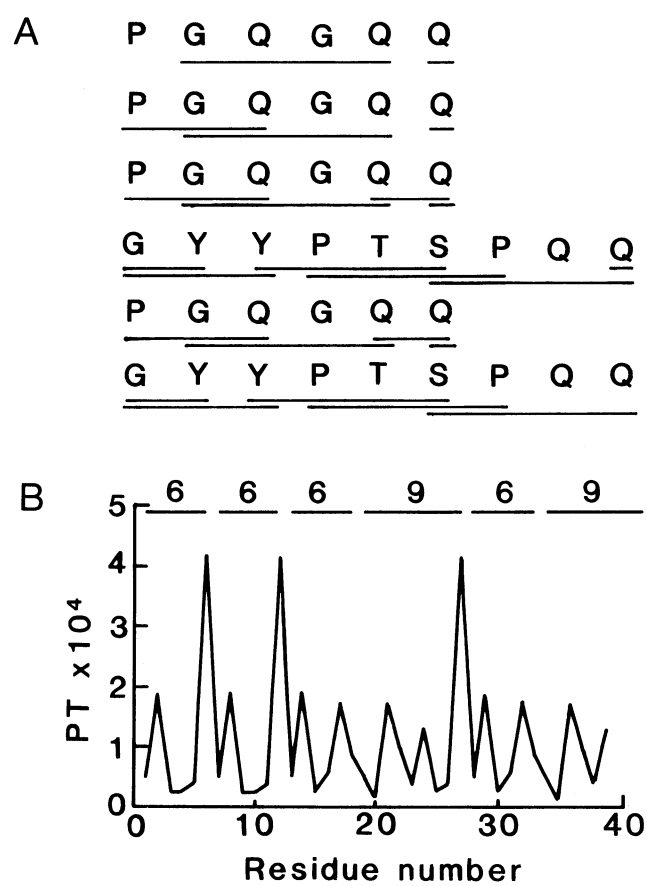

Fig. 4. A, Consensus blocks of six and nine residues from the central domain of high molecular weight subunits (based on Forde et al 1983, Thompson et al 1983). Tetrapeptides with predicted probabilities to form β-turns are underlined. **B,** Probabilities of β-turns in the series of consensus peptide sequences shown in **A**.

summarized in Table II. β-Turns are concentrated in the two proline-rich domains, the repetitive domain I (shown in detail in Fig. 6B) and the carboxy terminal domain V. However, their distribution within these domains is irregular. The high proportions of β-sheet estimated by the method of Garnier et al (1978) (Table I) result from the prediction of β-sheet rather than α-helix for the glutamine-rich domains II and IV (results not shown).

When α-gliadin in 70% ethanol was heated to 80° C, the far-UV CD spectrum (Fig. 5A) changed, indicating a montonic decrease in regular structure content. The effect was reversible, and the curves exhibited an isocircular dichroic point at 199–200 nm, indicating that the denaturation involves a helix-coil transition. The loss of α-helical content, calculated by the method of Chen et al (1974), was 6–7% over the temperature range 20–80° C (Table III). Kasarda et al (1968) also showed a small but reversible loss of α-helical content on heating A-gliadin in aqueous buffer. The near-UV spectrum showed a loss of phenylalanine band structure with increasing temperature (Fig. 5B), again suggesting a decrease in regular secondary structure, with the phenylalanine residues becoming more exposed to the solvent. Because the phenylalanine residues are concentrated in the β-turn rich repetitive domain (Table II) these results suggest that, unlike in the S-poor ω-gliadins, the β-turns are not heat stable. This may be related to their irregular distribution within the domain.

The other groups of S-rich prolamins of wheat have been less intensively studied. We have shown that γ-gliadin and β-gliadin

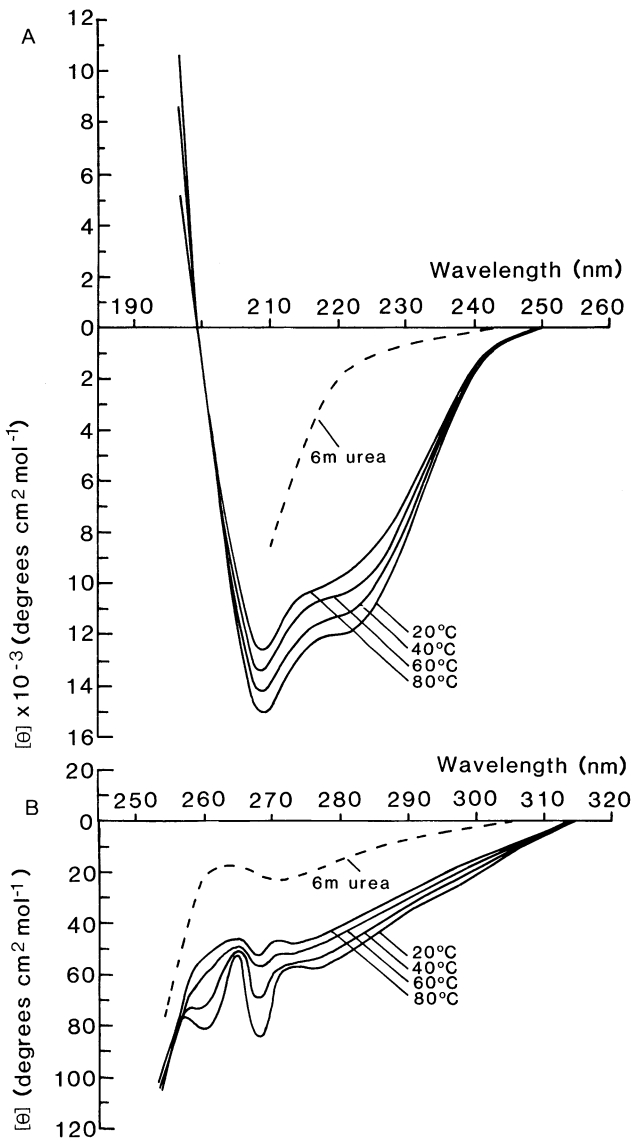

Fig. 5. Circular dichroism spectra of α-gliadins in 6 *M* urea at 20° C (---) and in 70% (v/v) aqueous ethanol at temperatures varying from 20 to 80° C (——). **A,** far-ultraviolet (UV) spectra; **B,** near -UV spectra. The spectrum in 70% (v/v) aqueous ethanol at 20° C is redrawn from Tatham and Shewry (1985).

TABLE I
The Calculated and Predicted Proportions of α-Helix, β-Sheet, β-Turn, and Random Coil Conformations Present in α-Gliadins

Conformation	Calculated from Circular Dichroism Spectrum[a]	Predicted from Sequence[b] Chou and Fasman (1978)	Predicted from Sequence[b] Garnier et al (1978)
α-Helix	36-37	34-35	10
β-Sheet	11-12	6-7	25
β-Turn	52-53	36-37	(35-38)[c]
Random coil		24-25	30-33

[a] Calculated from the far-ultraviolet spectrum in 70% ethanol at 20° C (Fig. 5). The method used (Chen et al 1972) does not give separate estimates for the β-turn and random coil conformations.
[b] Structural predictions are based on the complete protein sequence of A-gliadin reported by Kasarda et al (1984). Similar values are obtained using the amino acid sequence of an α-gliadin deduced from the nucleotide sequence of a genomic clone determined by Rafalski et al (1984).
[c] The value for β-turns predicted by the method of Garnier et al is given in parentheses as this method does not define β-turns precisely in terms of a tetrapeptide.

TABLE II
Summary of the Domains of A-Gliadin[a] and Their Predicted Conformation

Domain	I	II	III	IV	V
Residues	1–95	96–113	114–182	183–190	191–266
Amino acids (%)					
Proline	29.5	···	1.5	···	14.5
Glutamine	36.8	100	24.6	87.5	19.7
Phenylalanine	5.3	···	1.5	···	2.6
Cysteine	···	···	5.8	···	2.6
Predicted conformation (%)[b]					
α-Helix	13	100	61	88	17
β-Sheet	···	···	9	···	8
β-Turn	58	···	24	12	51
Random coil	29	···	6	···	24

[a] The protein sequence and its division into domains are from Kasarda et al (1984). The deduced amino acid sequence of an α-gliadin reported by Rafalski et al (1984) can be divided into similar domains, which have similar predicted conformations (not shown).
[b] The conformations are predicted using the method of Chou and Fasman (1978).

TABLE III
The Conformations of α-, β-, and γ-Gliadins[a] determined in 70% (v/v) Ethanol at 20 and 80° C

	α-Gliadin		β-Gliadin		γ-Gliadin	
Conformation	20° C	80° C	20° C	80° C	20° C	80° C
α-Helix	36–37	29–30	36–37	28–29	33–34	25–26
β-Sheet	11–12	8–9	22–23	19–20	20–21	17–18
β-Turn	52–53	61–62	41–42	50–51	46–47	57–58
Random coil						

[a] The conformations are expressed as a percentage of total structure, and are calculated from the circular dichroism spectra using the method of Chen et al (1972). Data for α-, β-, and γ-gliadin at 20° C and γ-gliadin at 80° C taken from Tatham and Shewry (1985). The value for α-gliadin at 80° C is calculated from the spectrum in Figure 5. The value for β-gliadin at 80° C is calculated from unpublished data.

fractions dissolved in 70% (v/v) aqueous ethanol have similar CD spectra in the near and far-UV regions to those of α-gliadins (Tatham and Shewry 1985), indicating a similar secondary structure. They also showed similar spectral changes on heating (Table III).

Partial amino acid sequences of γ-gliadins, deduced from the nucleotide sequences of cDNA clones, have been reported recently by Okita (1984) and Scheets et al (1985). These show a similar domain structure to α-gliadins, with a proline-rich repetitive domain close to the N-terminus. Structural predictions (not shown) again indicate that the repetitive domain is rich in β-turns, but that these are distributed irregularly.

Shewry et al (1984a) reported that a mixed fraction containing LMW subunits of glutenin showed a similar CD spectrum in the far-UV region to total monomeric α-, β-, γ-, and ω-gliadins. Because the latter fraction contained predominantly the S-rich gliadins, this implies that the LMW subunits have a similar conformation to these. No extensive amino acid sequences of LMW subunits have been reported. Further detailed studies of this group are required.

Models for Protein Elasticity

Elastomeric materials are capable of undergoing large deformations under the application of stress without rupture, the deformed elastomer recovering almost completely on removal of that stress. There are two major criteria which must be satisfied for a material to be elastomeric: long polymer chains to obtain high extensibility, and a low degree of covalent cross-linking of the polymer to provide the internal mobility which is required during the rearrangement of chain configurations under stress and recovery. In a so-called idealized rubber system, the fundamental elastomeric mechanism is purely entropic because of the reduced conformational probability of stretched, random-coil chains with negligible enthalpic contributions.

The most well-known elastomeric protein is mammalian connective tissue, elastin. Although this has been studied intensively, there is no generally accepted model for its molecular organization and how this determines its physical properties. Partridge (1966) suggested a globular cross-linked structure, and this model was extended by Weis-Fogh and Andersen (1970), who suggested that the exposure of hydrophobic residues contributed to the elastomeric mechanism. Urry (1971) showed that elastin contained repetitive β-turns and suggested that these formed spirals (see below). This formed the basis of the "oiled coil" model of Gray et al (1973), which suggested that each monomer is fibrillar and made up of alternating segments of cross link regions and "oiled coils" of β-spirals; these are linked to other monomers to form a three-dimensional network. More recently Hoeve and Flory (1974) and Dorrington and McCrum (1977) have suggested that elastomeric behavior is caused purely by entropic considerations of random polymer chains, and invoke no structural models.

The most elegant and in our opinion most convincing model for the elasticity of elastin comes from the studies of Urry and co-workers. The amino acid sequence of elastin contains repeats of

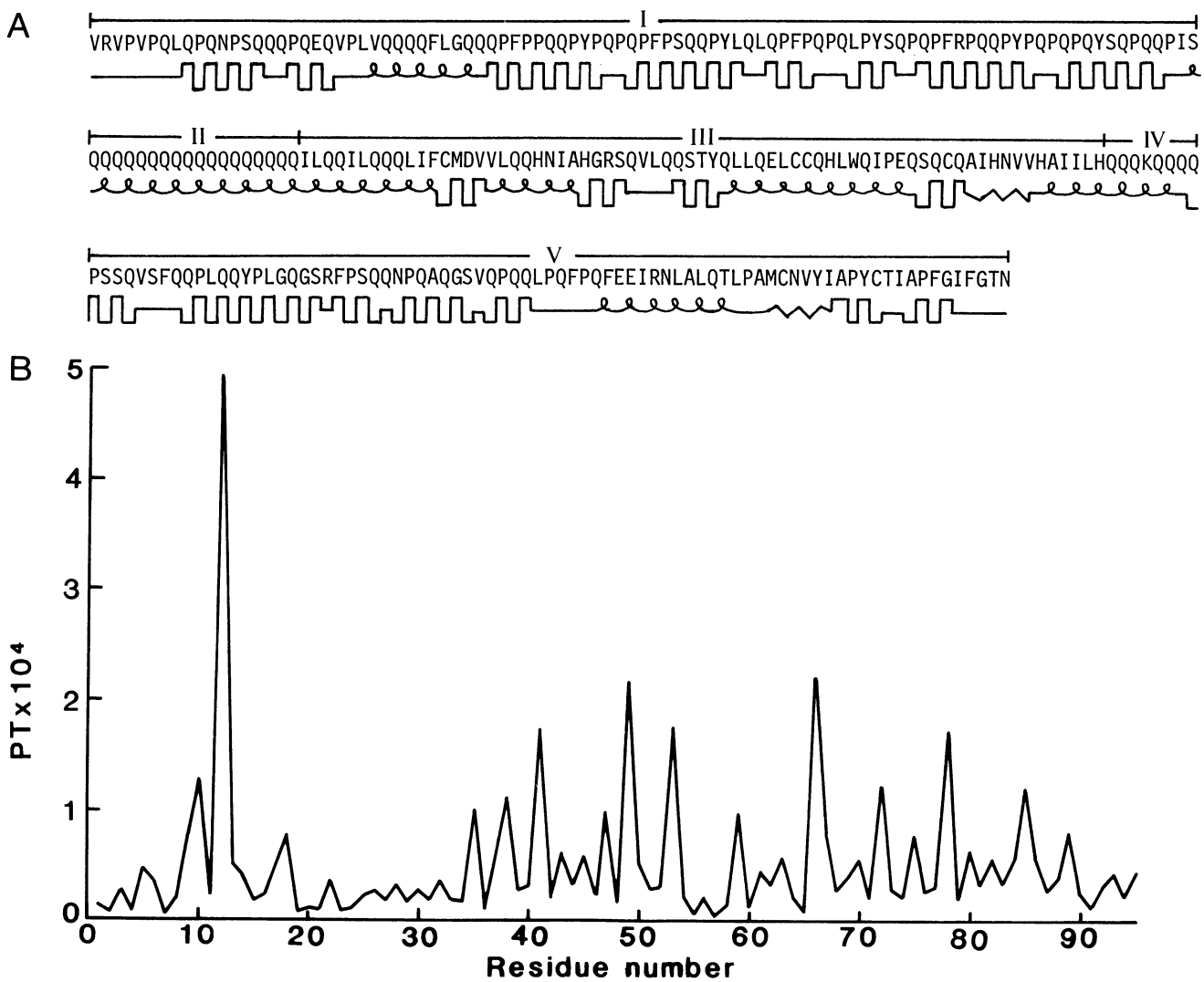

Fig. 6. Structure prediction of the A-gliadin sequence of Kasarda et al (1984). **A,** complete sequence, key: (⌇⌇) α-helix, (∿) β-sheet, (⊓) β-turn, and (—) random coil. **B,** β-turn probabilities for the proline-rich domain I.

three peptides (Urry 1982, Urry et al 1983); a hexapeptide ala-pro-gly-val-gly-val, a pentapeptide ala-pro-gly-val-gly, and a tetrapeptide val-pro-gly-gly. All three peptides contain the dipeptide pro-gly-, which has a high probability as the central dipeptide of a β-turn (Chou and Fasman 1977). Urry and co-workers suggest that the repetitive pentapeptide and hexapeptide regions form β-spirals, which are helical structures consisting of repetitive β-turns. This model is supported by data from crystallography (Cook et al 1980), model building based on the pentapeptide repeat (Venkatachalam and Urry 1981), and NMR spectroscopy (Urry and Long 1976). The β-spiral differs from the α-helix with 13.5 rather than 3.6 residues per turn, and a translation per turn of 9.45 rather than 5.4Å.

In addition to the repetitive regions, elastin also contains shorter regions which are rich in alanine. These are thought to be α-helical, and are covalently cross-linked via lysine residues. Urry (1982) showed that the covalently cross-linked synthetic polypentapeptide forms fibrils which behave as an anisotropic elastomer with an elastic modulus which, dependent on water content, can be the same as that of fibrous elastin. In contrast, the cross-linked synthetic polyhexapeptide and polytetrapeptide are not elastic. The synthetic polyhexapeptide forms a β-spiral, but this is rigid due to intramolecular hydrogen-bonding between repeats (Rapaka et al 1978). In the polypentapeptide the hydrogen bonds are predominantly intermolecular (between adjacent spirals), and the spiral can extend some 130% of its original length (Urry et al 1983). The polytetrapeptide does not form a β-spiral but a cross-β-sheet, a conformation where the turns are separated by short β-strands. Proteins with this conformation are not elastomeric but may form elongated sheets; examples are the adenovirus coat protein (Green et al 1983) and the chrysopa silks (Geddes et al 1968). The synthetic polytetrapeptide of elastin is also not elastomeric (Urry and Long 1976).

The β-spiral conformation in the polypentapeptide region would represent an energetically favorable state. Stretching would disrupt this conformation, deforming the peptide backbone, the hydrogen bonds, and the hydrophobic interactions into a less stable state. On the removal of stress the stable conformation would reform, resulting in elastic recoil. Several workers have suggested that the energetically unfavorable exposure of the hydrophobic, aromatic amino acids to the aqueous solvent in the stretched state is especially important in determining recoil (Weis-Fogh and Andersen 1970, Gray et al 1973, Gosline 1978).

In summary, the studies of Urry and co-workers indicate that the elasticity of elastin monomers is due to the presence of β-turns in β-spirals. Not all β-spirals, however, are elastic. This depends on the extent of hydrogen bonding between the turns. Finally, the elastic monomers are combined to form a polymer by covalent cross-links between the α-helical regions. The extent of cross-linking would be expected to affect the rigidity of the polymer.

Elastin is the only elastic protein which has been studied in detail. However, we consider that the model proposed by Urry can be applied to other elastic proteins including gluten (see below). One interesting type of elastic protein is the giant salivary gland protein of *Chironomus thummi*. This has an M_r of over 1×10^6 (Serfling et al 1983), which is sufficiently long to form an elastic mass without covalent cross-linking. It contains multiple repeats of the consensus sequence pro-lys-thr-ser-lys-his-ser-gly- (Baumlein et al 1982). The predictive method of Chou and Fasman (1978) suggests that these repeats form repetitive overlapping β-turns (not shown), as in elastin.

A Model for Gluten Elasticity

Although gluten is not a simple polymer such as elastin, aspects of the elastin model can be applied to gluten elasticity.

We consider that the major elastic components of gluten are the HMW subunits. These have been implicated in determining baking quality by correlative studies (see review by Payne et al 1984). Also, they are present only in large gluten aggregates (Field et al 1982), the amount of which is also related to baking quality (Huebner and Wall 1976; Bietz and Huebner 1980). The CD spectra and the structural predictions indicate that the repetitive central domains of the subunits form repetitive β-turns, and we suggest that these form a β-spiral as in elastin. Several molecules may aggregate to form elastic fibrils, stabilized by hydrogen bonding between carbonyl and amino functional groups present in the peptide backbone and glutamine side chains, and by hydrophobic interactions between aromatic residues (notably tyrosine). The studies with elastin suggest that there must be little or no hydrogen bonding between the adjacent turns of the individual subunit spirals. As in elastin, stretching would disrupt the stable conformation, notably exposing the aromatic residues to the solvent. On the removal of stress the stable conformation would reform.

The individual HMW subunits of gluten are assembled into elastic polymers by covalent disulfide bonds. The cysteine residues appear to be located close to the N- and C-termini of the subunits, with few if any in the repetitive domain (Field et al 1982, Shewry et al 1984b, Thompson et al 1983, Forde et al 1983). This would result in mainly linear polymers, as proposed for "glutenins" in the "linear glutenin hypothesis" of Ewart (1968, 1972, 1978). However the presence of two cysteines in the N-terminal domain of all subunits (Shewry et al 1984b) would permit some branching, cross-linking and interaction with LMW subunits of glutenin. There is also evidence for additional cysteine residues in the central domain of the 1Dy subunits (Shewry et al 1984a). The presence of covalent disulfide bonds between the α-helical regions of the HMW subunits is strikingly similar to the presence of covalent cross-links, via lysine residues, between the alanine-rich, α-helical regions of elastin.

β-Turns are also present in the ω-gliadins, where they exhibit unusual stability to heating. Their regular distribution, probably throughout the polypeptide chain, could also result in the formation of a β-spiral. However, the ω-gladins could not form an elastic polymer by themselves because of their inability to form covalent cross-links.

In the S-rich α-gliadins the β-turns are concentrated in the repetitive proline-rich domain and the C-terminal domain. Their distribution within these domains is irregular, making it unlikely that β-spirals would form. It is probable that the other S-rich monomeric gliadins (β and γ-gliadins) have a similar overall structure, although they may vary in detail. Too little is known about the LMW subunits of glutenin to draw any firm conclusions about their secondary structure.

We consider that the results described here provide a working hypothesis for the elastomeric properties of gluten. This is being tested by detailed physical studies of whole gluten and its component polypeptides.

ACKNOWLEDGMENTS

The authors would like to thank A. F. Drake for discussion and provision of CD facilities, S. Parmar for electrophoretic analysis of protein fractions used in the study, R. White for the statistical analysis, and D. D. Kasarda for discussions.

LITERATURE CITED

BAUMLEIN, K. H., WOBUS, U., GERBI, S., and KAFATOS, F. C. 1982. Characterisation of a 249-bp tandemly repetitive, satellite-like repeat in the translated portion of Balbiani ring c of *Chironomus thummi*. EMBO J. 5:641.

BAYLEY, P. 1980. Circular dichroism and optical rotation. Page 148 in: An Introduction to Spectroscopy for Biochemists. S. B. Brown, ed. Academic Press: New York.

BERNARDIN, J. E., KASARDA, D. D., and MECHAM, D. K. 1967. Preparation and characterization of α-gliadin. J. Biol. Chem. 242:445.

BIETZ, J. A., and HUEBNER, F. R. 1980. Structure of glutenin: achievements at the Northern Regional Research Center. Ann. Technol. Agric. 29:249.

BOOTH, M., and EWART, J. A. D. 1969. Studies on four components of wheat gliadins 181:226.

CHEN, Y. H., YANG, J. T., and MARTINEZ, H. M. 1972. Determination of secondary structures of proteins by circular dichroism and optical rotatory dispersion. Biochemistry 11:4120.

CHOU, P. Y., and FASMAN, G. D. 1977. β-Turns in proteins. J. Mol. Biol. 115:135.

CHOU, P. Y., and FASMAN, G. D. 1978. Empirical predictions of protein conformations. Ann. Rev. Biochem. 47:251.
CLUSKEY, J. E., and WU, Y. V. 1971. Optical rotatory dispersion, circular dichroism, and infrared studies of wheat gluten proteins in various solvents. Cereal Chem. 48:203.
COOK, W. J., EINSPAHR, H. M., TRAPANE, T. L., URRY, D. W., and BUGG, C. E. 1980. Crystal structure and conformation of the cyclic trimer of a repeat pentapeptide of elastin, cyclo-(L-valyl-L-prolylglycyl-L-valylglycyl). J. Am. Chem. Soc. 102:5502.
DEARBORN, D. G., and WETLAUFER, D. B. 1970. Circular dichroism of putative unordered polypeptides and proteins. Biochem. Biophys. Res. Comm. 39:314.
DORRINGTON, K., and McCRUM, N. G. 1977. Elastin as a rubber. Biopolymers 16:1201.
DUFTON, M. J., and HIDER, R. C. 1977. Snake toxin secondary structure predictions. J. Mol. Biol. 115:177.
EWART, J. A. D. 1968. A hypothesis for the structure and rheology of glutenin. J. Sci. Food Agric. 19:617.
EWART, J. A. D. 1972. A modified hypothesis for the structure and rheology of glutelins. J. Sci. Food Agric. 23:687.
EWART, J. A. D. 1978. Glutenin and dough tenacity. J. Sci. Food Agric. 29:551.
FIELD, J. M., SHEWRY, P. R., MIFLIN, B. J. and MARCH, J. F. 1982. The purification and characterisation of homologous high molecular weight storage proteins from grain of wheat, rye and barley. Theor. Appl. Gen. 62:329.
FORDE, J., FORDE, B. G., FRY, R. P., KREIS, M., SHEWRY, P. R., and MIFLIN, B. J. 1983. Identification of barley and wheat cDNA clones related to the high-M_r polypeptides of wheat gluten. FEBS Lett. 162:360.
FORDE, B. G., KREIS, M., WILLIAMSON, M. S., FRY, R. P., PYWELL, J., SHEWRY, P. R., BUNCE, N. A. C., and MIFLIN, B. J. 1985. Short tandem repeats shared by B- and C-hordein cDNAs suggest a common evolutionary origin for two groups of cereal storage protein genes. EMBO J. 4:9.
GARNIER, J., OSGUTHORPE, D. J., and ROBSON, B. 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. 120-97.
GEDDES, A. J., PARKER, K. D., ATKINS, E. D. T., and BRIGHTON, E. 1968. Cross-β-conformation in proteins. J. Mol. Biol. 32:343.
GOSLINE, J. M. 1978. Hydrophobic interaction and a model for the elasticity of elastin. Biopolymers 17:677.
GRAY, W. R., SANDBERG, L. B., and FOSTER, J. A. 1973. Molecular model for elastin structure and function. Nature 246:461.
GREEN, N. M., WRIGLEY, N. G., RUSSELL, W. C., MARTIN, S. R., and McLACHLAN, A. D. 1983. Evidence for a repeating cross-β-sheet structure in the adenovirus fibre. EMBO J. 2:1357.
HUEBNER, F. R., and WALL, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chem. 53:258.
HOEVE, C. A. J., and FLORY, P. J. 1974. The elastic properties of elastin. Biopolymers 13:677.
ISOGAI, Y., NEMETHY, G., RACKOVSKY, S., LEACH, S. J., and SCHERAGA, H. A. 1980. Characterisation of multiple bends in proteins. Biopolymers 19:1183.
ISHIZAKI, H., McKAY, R. H., NORTON, T. R., and YASUNOBU, K. T. 1979. Conformational studies of the peptide heart stimulant anthopleurin A. J. Biol. Chem. 254:9651.
KABSCH, W., and SANDER, C. 1983. How good are predictions of protein secondary structure? FEBS Lett. 155:179.
KASARDA, D. D., AUTRAN, J. C., LEW, E. J-L., NIMMO, C. C., and SHEWRY, P. R. 1983. N-Terminal amino acid sequences of ω-gliadins and ω-secalins: Implications for the evolution of prolamin genes. Biochim. Biophys. Acta 747:138.
KASARDA, D. D., BERNARDIN, J. E., and GAFFIELD, W. 1968. Circular dichroism and optical rotatory dispersion of α-gliadin. Biochemistry 7:3950.
KASARDA, D. D., OKITA, T. W., BERNARDIN, J. E., BAECKER, P. A., NIMMO, C. C., LEW, E. J-L., DIETLER, M. D., and GREENE, F. 1984. Nucleic acid (cDNA) and amino acid sequences of α-type gliadins from wheat (*Triticum aestivum*). Proc. Natl. Acad. Sci. USA 81:4712.
MANAVALAN, P. and JOHNSON, W. C., Jr. 1983. Sensitivity of circular dichroism to protein tertiary structure class. Nature 305:831.
MIFLIN, B. J., FIELD, J. M., and SHEWRY, P. R. 1983. Cereal storage proteins and their effect on technological properties. Page 255 in: Seed Proteins. J. Daussant, J. Mosse, and J. Vaughan, eds. Academic Press: London.
OKITA, T. W. 1984. Identification and DNA sequence analysis of a γ-type gliadin cDNA plasmid from winter wheat. Plant Mol. Biol. 3:325.
OSBORNE, T. B. 1907. The proteins of the wheat kernel. Carnegie Inst. Washington Publ., 84. Washington, DC.
PARTRIDGE, S. M. 1966. Diffusion of solutes in elastin fibres. Biochim. Biophys. Acta 140:132.
PAYNE, P. I., HOLT, L. M., JACKSON, E. A., and LAW, C. N. 1984. Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Philos. Trans. R. Soc. London Ser. B 304:359.
RAFALSKI, J. A., SCHEETS, K., METZLER, M., PETERSON, D. M., HEDGCOTH, C., and SOLL, D. G. 1984. Developmentally regulated plant genes: the nucleotide sequence of a wheat gliadin genomic clone. EMBO J. 3:1409.
RAPAKA, R. S., OKAMOTO, K., and URRY, D. W. 1978. Non-elastomereic polypeptide models of elastin. Int. J. Peptide Prot. Res. 11:109.
SCHEETS, K., RAFALSKI, J. A., HEDGCOTH, C., and SOLL, D. G. 1985. Heptapeptide repeat structure of a wheat γ-gliadin. Plant Sci. Lett. 37:221.
SCHULZ, G. E. and SCHIRMER, R. H. 1979. Chapter 5 in: Principles of Protein Structure. Springer-Verlag: New York.
SERFLING, E., MEYER, L., RUDOLPH, A., and STEINER, K. 1983. Secretory proteins and Balbiani ring gene activities in salivary glands of *Chironomus thummi* larvae. Chromosoma 88:16.
SHEWRY, P. R., TATHAM, A. S., FORDE, J., MIFLIN, B. J., and KASARDA, D. D. 1984a. The primary structures, conformations and aggregation properties of wheat gluten proteins. Page 51 in: Proc. 2nd Internat. Wheat Gluten Workshop TNO: Wageningen, The Netherlands.
SHEWRY, P. R., FIELD, J. M., FAULKS, A. J., PARMAR, S., MIFLIN, B. J., DIETLER, M. D., LEW, E. J-L., and KASARDA, D. D. 1984b. The purification and N-terminal amino acid sequence analysis of the high molecular weight gluten polypeptides of wheat. Biochim. Biophys. Acta 788:23.
SMITH, J. A., and PEASE, L. G. 1980. Reverse turns in peptides and proteins. CRC Crit. Rev. Biochem. 8:315.
STRICKLAND, E. 1974. Aromatic contribution to circular dichroism spectra of proteins. CRC Crit. Rev. Biochem. 2:113.
TANFORD, C. 1968. Protein denaturation. Adv. Prot. Chem. 23:121.
TATHAM, A. S., SHEWRY, P. R., and MIFLIN, B. J. 1984. Wheat gluten elasticity: a similar molecular basis to elastin? FEBS Lett. 177:205.
TATHAM, A. S., DRAKE, A. F., and SHEWRY, P. R. 1985. A conformational study of a glutamine and proline-rich cereal seed protein, C hordein. Biochem. J. 226:557.
TATHAM, A. S., and SHEWRY, P. R. 1985. The conformation of wheat gluten proteins, The secondary structures and thermal stabilities of α, β, γ and ω-gliadins. J. Cereal Sci. 3:103.
THOMPSON, R. D., BARTELS, D., HARBERD, N. P., and FLAVELL, R. B. 1983. Characterisation of the multigene family coding for HMW glutenin subunits in wheat using cDNA clones. Theor. Appl. Genet. 67:87.
URRY, D. W. 1971. Neutral sites for calcium ion binding to elastin and collagen. Proc. Natl. Acad. Sci. USA 68:810.
URRY, D. W. 1982. Characterisation of soluble peptides of elastin by physical techniques. Meth. Enz. 82:673.
URRY, D. W., and LONG, M. M. 1976. Conformations of the repeat peptides of elastin in solution: an application of proton and carbon-13 magnetic resonance to the determination of polypeptide secondary structure. CRC Crit. Rev. Biochem. 4:1.
URRY, D. W., VENKATACHALAM, C. M., LONG, M. M., and PRASAD, K. U. 1983. Dynamic β-spirals and a librational entropy mechanism of elasticity. Page 11 in: Conformations in Biology. R. Srinvasan and R. M. Sarma, eds. Adenine Press: New York.
VENKATACHALAM, C. M. 1968. Stereochemical criteria for polypeptides and proteins. V. Conformation of a system of three linked peptide units. Biopolymers 6:1425.
VENKATACHALAM, C. M., and URRY, D. W. 1981. Development of a linear helical conformation from its cyclic correlate. β-Spiral model of the elantin polypentapeptide (UPGVG)n. Macromol. 14:1225.
WALL, J. S. 1979. The role of wheat proteins in determining baking quality. Page 275 in: Recent Advances in the Biochemistry of Cereals, D. L. Laidman and R. G. Wyn Jones, eds. Academic Press: London.
WEIS-FOGH, T., and ANDERSEN, S. O. 1970. New molecular model for the long range elasticity of elastin. Nature 227:718.
WOODY, R. W. 1974. Studies on theoretical circular dichroism of polypeptides, contribution of β-turns. Page 338 in: Peptides, Polypeptides and Proteins. E. R. Blout, F. A. Bovey, M. Goodman, and N. Lotan, eds. John Wiley & Sons: New York.

[Received December 17, 1984. Revision received February 11, 1985. Accepted February 12, 1985.]