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Comparative structures and properties of elastic proteins

Arthur S. Tatham* **and Peter R. Shewry**

Institute of Arable Crops Research, Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Bristol BS41 9AF, UK

Elastic proteins are characterized by being able to undergo significant deformation, without rupture, before returning to their original state when the stress is removed. The sequences of elastic proteins contain elastomeric domains, which comprise repeated sequences, which in many cases appear to form β-turns. In addition, the majority also contain domains that form intermolecular cross-links, which may be covalent or non-covalent. The mechanism of elasticity varies between the different proteins and appears to be related to the biological role of the protein.

Keywords: elasticity; repeat motif; rubber

1. INTRODUCTION

Elastic proteins possess rubber-like elasticity, in that they are capable of undergoing high deformation without rupture, storing the energy involved in deformation and then returning to their original state when the stress is removed. The latter phase is passive, i.e. does not require an energy input and the most efficient mechanisms return all (or nearly all) of the energy used in deformation. This latter requirement is not a prerequisite for elastomeric materials as their biological requirements for energy storage and/or dissipation may be different.

The ability of proteins to exhibit rubber-like elasticity relates to their structure. Rubber-like materials must satisfy certain criteria: the individual components must be flexible and conformationally free, so that they can respond quickly to the applied stress, and they must be cross-linked to form a network, to distribute the stress throughout the system. These cross-links can be covalent or non-covalent and examples of both types are found. Thus, the elastic properties of proteins are influenced by the nature of the elastomeric domains, their size and the degree of cross-linking.

2. SEQUENCES OF ELASTOMERIC PROTEINS

Elastomeric proteins are widely distributed in the animal kingdom, however, only a few have been characterized in detail. This is, in part, due to their chemical and physical characteristics (non-globular nature, insolubility, cross-linking etc.), which make detailed characterization difficult. More recently, gene sequences have become available that have allowed sequence comparisons to be made and structure–function relationships to be studied. Figure 1 shows the schematic structures of representative elastomeric proteins for which sequences are available. Most have distinct domain structures, with at least one domain consisting of elastomeric repeat motifs and other non-elastic domains where cross-links can be formed. Exceptions to this are resilin and abductin where cross-links occur within the elastic repeat motifs.

Abductin is present in the inner hinge ligament of bivalve molluscs, acting as an elastic pivot that antagonizes the action of the adductor muscle. It also acts as an energy store that opens the shell when the adductor muscle relaxes. In scallops this action has developed into a swimming mechanism, allowing them to swim a few meters at a time by opening and closing their shells approximately four times per second (Bowie *et al.* 1993). Cao *et al.* (1997) reported five abductin cDNA sequences from *Argopecten* (the bay scallop) (figure 1) all of which encoded proteins of 136 amino acid residues. These proteins consist of two domains. An alanine-rich N-terminal domain (residues 1–20) contains two conserved tyrosine residues that could be involved in cross-linking. This domain is thought to form a signal sequence for secretion and may be cleaved during processing. The second domain consists of 11 glycine–methionine-rich decapeptide repeats (table 1), the methionine residues of which have been reported to be converted to methionine sulphoxide (Kikuchi & Tamiya 1981). This domain also contains two conserved lysine residues and a conserved tyrosine at the C-terminus, and these residues have been proposed as possible cross-linking sites.

Byssal threads ('beards') attach mussels to hard surfaces in water. They display a gradient of mechanical properties, along the length of the fibre, from stiff to elastic, that provide sufficient flexibility to prevent brittle fracture in tidal areas (Waite *et al.* 1998). Two forms of byssus are present in the thread of *Mytilus edulis*, Col-D and Col-P (figure 1), which are predominantly present in the distal (i.e. close to the surface) and proximal (close to the shell) parts of the thread, respectively. Col-P consists of a central collagen-like domain (*ca.* 430 residues), flanked N- and C-termini by elastic domains (*ca.* 102 and 160 residues, respectively) containing a pentapeptide repeat motif and histidine-rich domains (of *ca.* 106 and 62 residues,

* Author for correspondence (arthur.tatham@bbsrc.ac.uk).

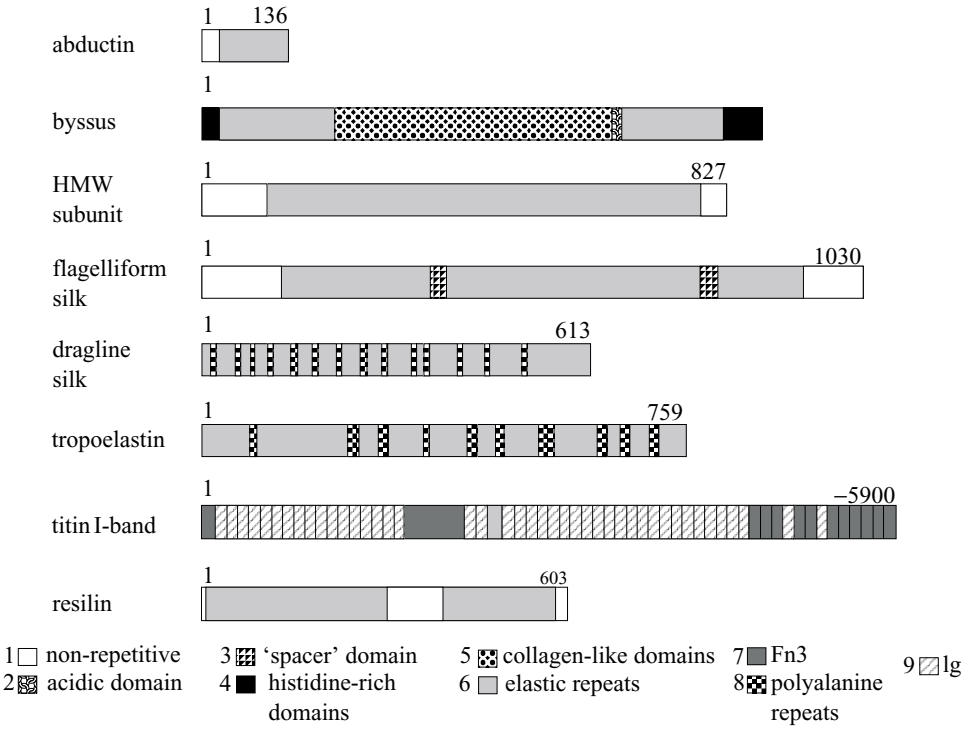

Figure 1. Domain structures of the elastomeric proteins: abductin (Cao *et al.* 1997), byssus (Coyne *et al.* 1997), HMW subunit (Shewry *et al.* 1992), flagelliform silk (Hayashi and Lewis 1998), dragline silk (Guerrette *et al.* 1996), tropoelastin (Indik *et al.* 1987), titin I-band (Labeit & Kolmerer 1995) and resilin (Ardell & Andersen 2001). Key to banding (1) non-repetitive; (2) acidic domain; (3) 'spacer' domain; (4) histidine-rich domains; (5) collagen-like domains; (6) elastic repeats; (7) Fn3; (8) polyalanine repeats; (9) Ig. Redrawn from Tatham & Shewry (2000), with permission.

Table 1. Sequences of the repeat motifs in the elastomeric domains of figure 1. X represents any amino acid. Redrawn from Tatham & Shewry (2000), with permission.

protein	repeat motif
abductin	GGFGGMGGGX
elastin	VPGG
	VPGVG
	APGVGV
byssus	GPGGG
flagelliform silk	GPGGX
dragline silks	GPGQQ
	GPGGY
	GGYGPGS
HMW subunits	PGQGQQ
	GYYPTSPQQ
	GQQ
resilin	GGRPSDSYGAPGGGN
	GYSGGRPGGQDLG
titin	PPAKVPEVPKKPVPEEKVPVPVPKKPEA

respectively) (table 1) (Qin & Waite 1995; Demming 1999). Col-D has a similar arrangement of domains, but the elastic domains are absent and replaced by alanine-rich domains, which are non-elastic (Waite *et al.* 1998). The mechanism of cross-linking is unknown, but may involve metal complexation in histidine-rich domains and/or be via tyrosine residues.

HMM subunits of wheat gluten (also called HMW subunits) are seed storage proteins, their sole function being to act as a store of carbon, nitrogen and sulphur for the developing seedling. Their elastic properties have probably arisen as a consequence of structures that facilitate the efficient packaging and storage of the proteins in protein bodies in the endosperm. The HMM subunits are characterized by a large central repetitive domain (*ca.* 630–830 residues) consisting of hexa-, nona- and, in certain subunits, tripeptide repeats (table 1). This domain is flanked by non-repetitive N- and C-terminal domains (81–104 and 42 residues, respectively) which contain cysteine residues that may form intermolecular bonds. The elasticity of wheat dough is influenced by both the amount and type of HMW subunits present (Shewry *et al.* 1992).

Titin (or connectin) is a sarcomeric protein that is responsible for the elasticity of striated muscle myofibrils and is involved in muscle assembly. It occurs in a range of isoforms, which have different properties in different tissues. The elastic properties of titin are responsible for the force generated when passive muscle is stretched. The human cardiac form of titin comprises *ca.* 269 000 residues (Labeit & Kolmerer 1995) divided into the Z-disc (*ca.* 2000 residues), I-band (*ca.* 5900 residues), A-band (*ca.* 17 200 residues) and M-line (*ca.* 1100 residues) regions. However, only a fraction of the molecule within the I-band is functionally extensible (figure 1). The elastic domain contains repeating motifs of 26–28 amino acid residues, rich in proline, glutamate, valine and lysine residues, termed the PEVK domain (Greaser 2001) (table 1).

Resilin occurs in insect cuticles, conferring long-range elasticity to the tissue and functioning as an energy store and as a damper of vibrations in the flight systems of many

insects. Ardell & Andersen (2001) have tentatively identified a resilin gene in *Drosophila melanogaster* by searching the *Drosophila* genome for sequences related to tryptic peptides from locust (*Scistocerca gregaria*) resilin. They identified an ORF encoding a 603 residue protein (figure 1), with an N-terminal domain comprising 18 pentadecapeptide repeats, a central non-repetitive domain and a C-terminal domain comprising 11 tridecapeptide repeats (table 1). Cross-linking occurs between tyrosine residues, which are present throughout the protein.

Elastin is widely distributed in vertebrate tissues, acting both statically, as in the dermis, to resist long-term forces, and dynamically, in such tissues as arteries, where it provides an efficient energy storage system. Elastin is one of the most extensively studied elastomeric proteins. It is secreted as a soluble precursor, tropoelastin, which consists of alternating repetitive hydrophobic domains of variable length (the elastic repeats) and alanine-rich, lysine-containing domains that form cross-links (Indik *et al.* 1987) (figure 1). The elastic domains consist of tetra-, penta- and hexapeptide motifs (table 1).

Spiders produce several different silk proteins. The dragline silks have high tensile strength and will extend to *ca*. 30%, and form the dropping lines and frameworks of webs. The flagelliform silks form the capture spirals of webs and exhibit high extensibility (more than 200%), with similar properties to lightly cross-linked rubbers (Gosline *et al.* 1986). The silks are processed to produce high performance fibres with a range of mechanical properties, allowing the web to absorb the impact of the insect and capture it. The two types of silk differ in their domain structures (figure 1), the dragline silks contain polyalanine domains (which form non-covalent cross-links between proteins) separated by elastic domains comprised of pentapeptide repeats (table 1) (Guerrette *et al.* 1996). The flagelliform silks (figure 1) contain longer elastic domains, based on pentapeptide repeats GPGGX (table 1) and contain 'spacer' domains, the function of which is unknown (Hayashi & Lewis 1998, 2000). The cross-linking mechanism in the flagelliform silks is unknown, but may involve interactions between the spacer domains (Hayashi & Lewis 1998).

3. STRUCTURAL FEATURES OF ELASTOMERIC PROTEINS

The presence of regularly repeated sequences implies the formation of a regular structure. Although the direct determination of the structures of elastic proteins has proved problematic, the limited information that is available indicates that the repetitive sequences do form regular structures and that these may be important in the elastic mechanisms.

Studies of elastin indicate that the backbone is highly mobile (Ellis & Packer 1976) and that the alanine-rich domains that contain lysine residues for cross-linking are predominantly α-helical (Foster *et al.* 1976). Urry and co-workers have carried out detailed studies of α-elastin and synthetic polypeptides corresponding to the major tetra-(VPGG), penta-(VPGVG) and hexa-(APGVGV) peptide consensus repeat motifs, demonstrating that β-turns were the predominant structural feature present. Urry and co-workers have proposed that the β-turns present in the synthetic polypentapeptide (Urry *et al.* 1984) and polyhexapeptide (Rapaka *et al.* 1978) assembled in multiple repeats, formed a β-spiral, a loose water-containing helical structure (Urry 1984). Only the polypentapeptide was found to be elastic with an elastic modulus similar to that of elastin. The proposed β-spiral formed by the polypentapeptide contained no significant hydrogen bonding between repeats, allowing flexible movement, whereas in the polyhexapeptide, intramolecular hydrogen bonding between repeats, and the interlocking of adjacent β-spirals by hydrophobic interaction, resulted in structural rigidity. The polytetrapeptide motif appeared to form a cross β-structure and exhibited little elasticity (Urry *et al.* 1986). It was proposed that the β-spiral structure formed by the polpentapeptide contributes to the elastic mechanism of elastin. However, this has been questioned by other workers who have interpreted the presence of the proline–glycine sequence in the elastin sequences as sites that can bend to form tight turns and make the backbone flexible and hence behave like a random coil (Gosline 1987).

The spider silks contain crystalline and non-crystalline regions (figure 1). The alanine-rich domains have been shown, using X-ray diffraction and NMR spectroscopy studies, to form linked anti-parallel β-sheet structures (Hijirida *et al.* 1996; Simmons *et al.* 1996), which are thought to correspond to the crystalline regions that cross-link the proteins and provide silks with their tensile strength. The structures of the glycine-rich (non-crystalline) domains are poorly understood, but β-turns have been predicted to form (table 1). In the flagelliform silks the GPGGX motif has been proposed to form β-spiral type structures composed of repetitive β-turns, which act as springs to form an elastic network (Hayashi *et al.* 1999; Hayashi & Lewis 2000). In dragline silks, the GGPGQ and YPGQQ motifs are also predicted to form β-turns (table 1) with PG as the central residues of the turns. NMR experiments have indicated the presence of 3_{10} helical structures in the glycine-rich domains, orientated in-line with the threads (Kümmerlen *et al.* 1996; Van Beek *et al.* 1999). The higher elasticity of the flagelliform silks is possibly related to their longer repetitive regions and the lower levels of cross-linking compared with the dragline silks (figure 1) and to their more hydrophobic nature.

The repetitive domains of the HMM subunits of glutenin contain three repeat motifs that are thought to be responsible for the elastic properties of wheat doughs and glutens. Structural prediction studies suggested the presence of β-turns (table 1) (Tatham *et al.* 1990), which are predicted to form within and between the repeat motifs. Similarly, spectroscopic studies of linear and cyclic peptides corresponding to the repeat motifs also indicate the presence of β-turns (Tatham *et al.* 1990; Van Dijk *et al.* 1997). Characterization of a recombinant repetitive peptide using CD and FTIR indicated the presence of β-turns in equilibrium with the poly-L-proline II-like structure in solution with less β-turns and an increase in intermolecular β-sheet structure in the hydrated solid (the environment in doughs and gluten) (Gilbert *et al.* 2000). Hydrodynamic studies indicated a rod-like structure in solution (Field *et al.* 1987) and STM images showed that the central repetitive domains adopt a helical structure with a diameter of *ca*.1.9 nm and a pitch of *ca*. 1.5 nm in

the hydrated solid state (Miles *et al.* 1991). Thus, it has been proposed that the central repetitive domains form a β-spiral type structure. Further AFM images showed rod-like molecules that aggregated side by side and end to end to form branched networks (Humphris *et al.* 2000).

Titin contains a number of repeating motifs, including two different types of 100 residue motifs with similarities to immunoglobulin and fibronectin III domains (figure 1). The unique feature of titin is the PEVK domain, which varies in length between 163 and 2174 residues, for cardiac and soleus muscles, respectively (Labeit & Kolmerer 1995). Antibody labelling of muscle at different extensions showed that the PEVK domain lengthened on stretching (Trombitas *et al.* 1998) implying that the domain has an elastic function in muscle. It was initially proposed that titin elasticity arose from an unfolding of the immunoglobulin-like domains, but it is now thought to arise from the PEVK domain (Linke *et al.* 1998, 1999). The PEVK domain is more easily stretched than the immunoglobulin-like domains and is predicted to have an unstable secondary structure, due to the high charge density and the high content of proline residues, preventing the formation of a stable tertiary structure (Labeit & Kolmerer 1995). Greaser (2001) identified repeating motifs of 26–28 amino acid residues (table 1), which contained regions rich in glutamic acid. Secondary structure prediction indicated little propensity to form α-helical or β-sheet structures in the PEVK repeats but some propensity to form α-helix in the glutamic acid regions and the presence of poly-L-proline II structure in the PEVK motifs (Greaser 2001). Structure prediction would suggest the presence of β-turns (table 1) but their presence needs confirmation by CD or FTIR spectroscopy.

The repeat motif of abductin has not been studied in detail, but prediction indicates that the domain adopts a random-coil conformation (Cao *et al.* 1997).

Resilin contains two repeat motifs of 15 and 13, termed the A and B repeats, respectively. Both contain conserved PG motifs associated with β-turns and an additional turn over PS was predicted in the A motif (Ardell & Andersen 2001). An additional turn can be predicted in both motifs (table 1). Ardell & Andersen (2001) suggested that both repeats may form β-spirals, but with larger and more irregular loops than in other elastic proteins. Spectroscopic evidence is required to confirm the presence of β-turns and/or other structures.

4. ELASTIC MECHANISM AND FUNCTION

The sequences and structures of the different elastomeric proteins would imply that there may be differences in their elastic mechanisms.

Although elastin is the most highly characterized elastomeric protein, a number of different mechanisms have been proposed to account for its elasticity. One such mechanism is based on classical rubber theory. Elastin is regarded as a network of random chains of high entropy, stressing orders the chains and decreases the entropy of the system by limiting the conformational freedom of the chains. This decrease in entropy provides the restoring force to the initial state (Hoeve & Flory 1974). Urry and co-workers have proposed an alternative model, in which elasticity arises from a regular structure, a β-spiral comprising repetitive β-turns (Urry 1988). In the polypentapeptide repeat, the β-turns are repeated regularly and act as spacers between the turns of the spiral, suspending chain segments in a kinetically free state (conformationally free). On stretching, this conformational freedom is reduced, resulting in a reduction in entropy, this loss of entropy providing the restoring force (Urry *et al.* 1986). In both the random-chain and β-spiral models the restoring force is entropic. Gosline (1978) has proposed that hydrophobic interactions account for some of the restoring force. Stretching elastin exposes hydrophobic side chains to an aqueous environment, decreasing the entropy of the surrounding water molecules, the restoring force arising from the re-establishment of the hydrophobic interactions. Thus, whereas it is agreed that the driving force for elastic recoil is entropic in origin, the precise mechanism (and structures involved) is not known.

The elastic domains of the byssal proteins have repeat motifs similar to elastin (table 1) and an entropic mechanism can also be invoked to account for the elasticity of the protein PreCol-P. However, the byssal threads themselves do not show instant recoil, due to energy dissipation, a mechanism that stops the mussel being dashed against the rocks after the wave has passed (Bell & Gosline 1996).

There have been limited studies on abductin, but the amino acid sequence indicates a hydrophobic character. Jimenez *et al.* (2000) synthesized a pentapeptide (FGGMG) mimic of abductin and proposed a hydroelasticity model, in which elastic recoil results from a hydrophobic mechanism and peptide self-assembly to produce an aggregate with rubber-like properties, even in the absence of covalent cross-links.

Resilin behaves as an entropic elastomer (Weis-Fogh 1960), and was initially proposed to consist of randomly coiled protein chains linked by stable covalent cross-links, the elastic force being accounted for by a decrease in conformational entropy when the material was strained (Weis-Fogh 1961). Unlike elastin, the repetitive domains of resilin are not hydrophobic, but dominated by hydrophilic residues, suggesting that the domains will tend not to interact by hydrophobic interactions. Ardell & Andersen (2001) suggested that the presence of the β-turns results in a β-spiral, as proposed for other proteins, and that the structure can act as a readily deformed spring. At low extension disruption between the loops (which are stabilized by hydrophobic interactions, hydrogen bonds and/or electrostatic attractions) occurs, while at higher extensions elastic recoil is mainly due to a decrease in conformational entropy.

The elastic repeat motifs of flagelliform spider silks, which form the capture part of spider webs, show sequence similarity with elastin (table 1), are highly extensible and are covered in droplets of glue that capture the insect and hold it. The high extensibility gives the insect no solid structure to push against and an entropic mechanism is conducive to such a function (Vollrath & Edmonds 1989). The sequences of dragline silks, which form the dropline and radial threads of the web, contain glutamine residues (table 1). The mechanism of elastic recoil is not entirely entropic, but this accounts for *ca.* 85% of the total force (Gosline *et al.* 1984). Part of

the energy of an insect hitting the web is absorbed and dissipated as heat, the energy is, therefore, not available to break the silk threads and the insect is not catapulted out of the web. In view of their content of glutamine residues, which have a capacity to hydrogen bond, non-covalent cross-linking between elastic repeats, via hydrogen bonding may act to modulate the elastic mechanism. Both flagelliform and dragline silks have been proposed to contain β-turns and to form β-spirals which account for their elasticity.

HMM subunits of glutenin that have been cross-linked using γ-radiation show a similar modulus of elasticity to the cross-linked polypentapeptide of elastin, however, the mechanism of elasticity appears complex (Tatham *et al.* 2001). The high content of glutamine residues in the repetitive domains suggests that elastic recoil, in part, may be associated with extensive hydrogen bonding within and between subunits. Thus, entropic and energetic mechanisms contribute to the observed elasticity of the HMM subunits. The elastic properties have no known role, so there has not been selective pressure to attain mechanisms that are energy efficient. The proteins contain repetitive β-turns which may form β-spirals, as proposed for other elastic proteins, but the spirals can interact via hydrogen bonding to form cross-links, perhaps making their behaviour more similar to dragline silks and resilin than to elastin.

Titin elasticity has been proposed to arise from completely different mechanisms than in other protein elastomers. Repeating motifs of 26–28 residues, termed PPAK, have been identified in the PEVK domain, where they occur in groups of 2–12 motifs separated by regions rich in glutamic acid. It has been suggested that the difference in charge between the PPAK motifs and the glutamic acid-rich regions infers that ionic interactions between the positively charged PPAK regions and negatively charged glutamic acid-rich regions are involved in the elastic mechanism (Greaser 2001).

5. CONCLUSIONS

The advantage of an entropic mechanism of elasticity is that it depends on the decrease in numbers of accessible states on extension and can be considered ideal for biological systems. Entropic elasticity provides for a durable elastomer, with elastic fibres that can last the lifetime of an individual. Several protein systems (i.e. elastin and titin) have evolved elastic proteins, which have similar entropic mechanisms, where energy is required to stress the proteins and relaxation is non-energy consuming. In other systems, however, it would be detrimental to restore all the energy to the system (i.e. byssal threads, dragline silk) as the function of the material, is in part, to dissipate the energy. Thus, similarities and differences in the sequences, structures and mechanisms of elasticity show that precise biological requirements for elastic behaviour can be satisfied with different mechanisms.

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REFERENCES

Ardell, D.H. & Andersen, S. 2001 Tentative identification of a resilin gene in *Drosophila melanogaster*. *Insect Biochem. Mol. Biol.* **31**, 965–970.

Bell, E. C. & Gosline, J. M. 1996 Mechanical design of mussel byssus: material yield enhances attachment strength. *J. Exp. Biol.* **199**, 1005–1017.

Bowie, M. A., Layes, J. D. & Demont, M. E. 1993 Damping in the hinge of the scallop *Placopecten magellanicus*. *J. Exp. Biol.* **175**, 311–315.

Cao, Q., Wang, Y. & Bayley, H. 1997 Sequence of abductin the molluscan 'rubber protein'. *Curr. Biol.* **7**, R677–R678.

Demming, T. J. 1999 Mussel byssus and biomolecular materials. *Curr. Opin. Chem. Biol.* **3**, 100–105.

Ellis, G. E. & Packer, K. J. 1976 Nuclear spin-relaxation studies of hydrated elastin. *Biopolymers* **15**, 813–832.

Field, J. M., Tatham, A. S. & Shewry, P. R. 1987 The structure of a high M_r subunit of durum wheat (*Triticum aestivum*) gluten. *Biochem. J.* **247**, 215–221.

Foster, J. A., Bruenger, E., Rubin, L., Imberman, M., Kagan, H., Mecham, R. & Franzblau, C. 1976 Circular dichroism studies of of an elastin crosslinked peptide. *Biopolymers* **15**, 833–841.

Gilbert, S. M., Wellner, N. K., Belton, P. S., Greenfield, J. A., Siligardi, G., Shewry, P. R. & Tatham, A. S. 2000 Expression and characterisation of a highly repetitive peptide derived from a wheat seed storage protein. *Biochim. Biophys. Acta* **1479**, 135–146.

Gosline, J. M. 1978 Hydrophobic interaction and a model for the elasticity of elastin. *Biopolymers* **17**, 677–695.

Gosline, J. M. 1987 Structure and mechanical properties of rubberlike proteins in animals. *Rubber Chem. Technol.* **60**, 417–438.

Gosline, J. M., Denny, M. W. & Demont, M. E. 1984 Spider silk as rubber. *Nature* **309**, 551–552.

Gosline, J. M., Demont, M. E. & Denny, M. W. 1986 The structure and properties of spider silk. *Endeavour* **10**, 37–43.

Greaser, M. 2001 Identification of new repeating motifs in titin. *Proteins Struct. Funct. Genet.* **43**, 145–149.

Guerrette, P. A., Ginzinger, D., Weber, B. & Gosline, J. 1996 Silk properties determined by gland-specific expression of a spider fibroin gene family. *Science* **272**, 112–115.

Hayashi, C. Y. & Lewis, R. V. 2000 Molecular architecture and evolution of a modular spider silk protein gene. *Science* **287**, 1477–1479.

Hayashi, C. Y. & Lewis, R. V. 1998 Evidence from flagelliform silk cDNA for the structural basis of elasticity and modular nature of spider silks. *J. Mol. Biol.* **275**, 773–784.

Hayashi, C. Y., Shipley, N. H. & Lewis, R. V. 1999 Hypotheses that correlate the sequence structure and mechanical properties of spider silk proteins. *Int. J. Biol. Macromol.* **24**, 271–275.

Hijiridia, D. H., Do, K. G., Michal, C., Wong, S., Zax, D. & Jelinski, L. W. 1996 C_{13} NMR of *Nephila clavipes* major ampullate silk gland. *Biophys. J.* **71**, 3442–3447.

Hoeve, C. A. J. & Flory, P. J. 1974 The elastic properties of elastin. *Biopolymers* **13**, 677–686.

Humphris, A. D. L., McMaster, T. J., Miles, M. J., Gilbert, S. M., Shewry, P. R. & Tatham, A. S. 2000 Atomic force microscopy (AFM) study of interactions of HMW subunits of wheat glutenin. *Cereal Chem.* **77**, 107–111.

Indik, Z., Yeh, H., Ornstein-Goldstein, N., Sheppard, P., Anderson, N., Rosenbloom, J. C., Peltonen, L. & Rosenbloom, J. 1987 Alternative splicing of human elastin mRNA indicated by sequence analysis of cloned genomic and complementary DNA. *Proc. Natl Acad. Sci. USA* **84**, 5680–5684.

Jimenez, F., Banco, M., Goddard, W. A. & Sandberg, L. 2000 Scallop abductin translates hydrophobicity to elasticity. See http://www.wag.caltech.edu/anmeeting/2000/presentations/mario2/.

Kikuchi, Y. & Tamiya, N. 1981 Methionine sulfoxide in the resilin of surf clams. *J. Biochem.* **89**, 1975–1976.

Kümmerlen, J., Van Beek, J., Vollrath, F. & Meier, B. 1996 Local structure in spider dragline silk investigated by two-dimensional spin-diffusion nuclear magnetic resonance. *Macromolecules* **29**, 2920–2928.

Labeit, S. & Kolmerer, B. 1995 Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* **270**, 293–296.

Linke, W. A., Ivemeyer, M., Mundel, P., Stockmeier, M. R. & Kolmerer, B. 1998 Nature of PEVK–titin elasticity in skeletal muscle. *Proc. Natl Acad. Sci. USA* **95**, 8052–8057.

Linke, W. A., Rudy, D. E., Centner, T., Gautel, M., Witt, C., Labeit, S. & Gregorio, C. C. 1999 I-Band titin in cardiac muscle is a three element molecular spring and is critical for maintaining thin filament structure. *J. Cell Biol.* **146**, 631–644.

Miles, M. J., Carr, H. J., McMaster, T. J., I'Anson, K. J., Belton, P. S., Morris, V. J., Field, J. M., Shewry, P. R. & Tatham, A. S. 1991 Scanning tunneling microscopy of a wheat storage protein reveals details of an unusual supersecondary structure. *Proc. Natl. Acad. Sci. USA* **88**, 68–71.

Qin, X. & Waite, J. H. 1995 Exotic collagen gradients in the byssus of the mussel. *Mytilus edulis*. *J. Exp. Biol.* **198**, 633–644.

Rapaka, R. S., Okamoto, K. & Urry, D. W. 1978 Non-elastomeric polypeptide models of elastin. *Int. J. Peptide Protein Res.* **11**, 109–127.

Shewry, P. R., Halford, N. G. & Tatham, A. S. 1992 The high molecular weight subunits of wheat glutenin. *J. Cereal Sci.* **15**, 105–120.

Simmons, A., Michal, C. & Jelinski, L. 1996 Molecular orientation and two component nature of the crystalline fraction of spider dragline silk. *Science* **271**, 2920–2928.

Tatham, A. S. & Shewry, P. R. 2000 Elastomeric proteins: biological roles, structures and mechanisms. *Trends. Biol. Sci.* **299**, 567–571.

Tatham, A. S., Drake, A. F. & Shewry, P. R. 1990 Conformational studies of peptides corresponding to the repetitive regions of the high molecular weight (HMW) glutenin subunits of wheat. *J. Cereal Sci.* **11**, 189–200.

Tatham, A. S., Hayes, L., Shewry, P. R., Urry, D. W. 2001 Wheat seed proteins exhibit a complex mechanism of protein elasticity. *Biochim. Biophys. Acta.* **1548**, 187–193.

Trombitas, K., Greaser, M., Labeit, S., Jin, J. P., Kellermeyer, M., Helmes, M. & Granzier, H. 1998 Titin extensibility *in situ*: entropic elasticity of permanently folded and permanently unfolded molecular segments. *J. Cell Biol.* **140**, 853–859.

Urry, D. W. 1984 Protein elasticity based on conformations of sequential polypeptides—the biological elastic fibre. *J. Protein. Chem.* **3**, 403–436.

Urry, D. W. 1988 Entropic elastic processes in protein mechanisms. 1. Elastic structures due to an inverse temperature transition and elasticity due to internal chain dynamics. *J. Prot. Chem.* **7**, 1–34.

Urry, D. W., Henze, R., Harris, R. D. & Prasad, K. U. 1984 Polypentapeptide of elastin: temperature dependent correlation of elastomeric force and dielectric permittivity. *Biochem. Biophys. Res. Commun.* **125**, 1082–1088.

Urry, D. W., Harris, R. D., Long, M. M. & Prasad, K. U. 1986 Polytetrapeptide of elastin: temperature correlated elastomeric force and structure development. *Int. J. Peptide Protein Res.* **28**, 649–660.

Van Beek, J. D., Kümmerlen, D., Vollrath, F. & Meier, B. H. 1999 Supercontracted spider dragline silk: a solid state NMR study of the local structure. *Int. J. Biol. Macromol.* **24**, 173–178.

Van Dijk, A., DeBoef, E., Bekkers, A., Van Wijk, L. L., Van Swieten, E., Hamer, R. J. & Robillard, G. T. 1997 Structure characterization of the central repetitive domain of high molecular weight gluten proteins. II. Characterization in solution and the dry state. *Protein Sci.* **6**, 649–656.

Vollrath, F. & Edmonds, D. T. 1989 Modulation of the mechanical properties of spider silk by coating with water. *Nature* **340**, 305–307.

Waite, J. H., Qin, X. X. & Coyne, K. J. 1998 The peculiar collagens of mussel byssus. *Matrix Biol.* **17**, 93–106.

Weis-Fogh, T. 1960 A rubber-like protein in insect cuticle. *J. Exp. Biol.* **37**, 889–907.

Weis-Fogh, T. 1961 Molecular interpretation of the elasticity of resilin, a rubber-like protein. *J. Mol. Biol.* **3**, 648–667.