A new basis for meat tenderness, in terms of gap filaments

R. H. LOCKER

Meat Industry Research Institute of New Zealand, P.O. Box 617, Hamilton, New Zealand.

Introduction

Muscle is a composite structure, of contractile machinery enveloped in connective tissue. The morphology and biochemistry of each have developed to the point where meat science has begun to interprete properties of cooked meat, such as tenderness (or toughness), in terms of the fine structure of these two components. This paper is concerned largely with the myofibrillar component, but will attempt finally to put the two together. It pays much attention to tensile properties, which are a major factor in shear force, although not the sole determinant. The novel feature of this account is the role of the enigmatic third set of filaments in the myofibril, the "gap filaments". The germ of these ideas, published five years ago (Locker et al., 1977), has so far produced a negligible response. New results now lend support to the theory.

Morphology of the gap filaments

My model (Fig. 1) for the connections of gap filaments (G-filaments) (Locker and Leet 1976a), proposes that each G-filament forms a core to an A-filament, but emerges at one end only, passing between the I-filaments, through the Z-line, and between the I-filaments of the next sarcomere, to terminate as a second core to a thick filament. In this unusual symmetry, centred on the Z-line, G-filaments link half the A-filaments in an A-band to those in one neighbouring sarcomere and half to those in the other. G-filaments are continuous through the Z-line, but not through the sarcomere, and therefore depend on adequate anchoring of their ends to maintain structural continuity under stress. They are resistant to a variety of powerful protein solvents, but dissolve in some, in the presence of thiol reducers. They are vulnerable to indigenous or exogenous proteases (Locker et al., 1977). It seems that the protein of G-filaments is an unusual one, of very high molecular weight (of the order of a million) and can be identified with the "connectin" of Maruyama et al.,(1980) or the "titin" of Wang et al. (1979). The prime biological function of G-filaments is unclear. An organising role in embryonic development seems possible for a "core" protein. An elastic role in contraction is also possible. The one definable role is that G-filaments support the N_2-line structure which probably serves to guide I-filaments from a square array at Z-line into a hexagonal array in the A-band (Locker and Leet, 1976b). G-filaments plus N_2-line are probably responsible for the re-entry of thin filaments into the A-band, in muscles stretched beyond the "no over-lap" point.

Gap filaments in raw meat

Since pre-rigor muscle offers such slight resistance to stretch, G-filaments must be readily extensible in this state. With onset of rigor, myosin to actin bridges impose a low extensibility, but on loading a muscle strip, to near 1 kg/cm^2, it suddenly reaches a point where it continues to extend without further loading (Locker and Wild, 1982a, 1982b). This "yield point" we interprete from electron micrographs (Locker and Wild, 1982a) as the tearing of I-filaments out of the Z-line, leaving the G-filaments to stretch until the collagen net (Rowe, 1974), takes up the strain. Ageing causes a dramatic fall in yield point, to about 0.1-0.2 kg/cm^2 (Locker and Wild, 1982a, 1982b), and I-bands then snap, mainly along the A-I junction. It seems that both I- and G-filaments have been rotted by catheptic action.

Fig. 1 Each G-filament (dotted) begins as core to one A-filament and terminates as core to another in the adjacent sarcomere.

The modification of myofilaments by cooking

Tenderness usually has meaning only in cooked meat. The term "cooked" covers many degrees of heat denaturation. The various myofilaments react to this in different ways, with big shifts in their relative significance for response to tensile stress. I will first discuss the stability of the various filaments and then the question of "cooked character" as a whole.

Myosin in solution flocculates readily at 53°C (Locker, 1956) or in the myofibril becomes inextractable (Davey and Gilbert, 1974). The thick filaments appear stable at 50°C, but lose their identity at 60°C (Giles, 1968; Schmitt and Parrish, 1971). Giles (1968) found I-filaments losing fine structure after 20 min at 60°C and breaking at the A-I junctions after 20 min at 70°C. Schmitt and Parrish (1971) found I-filaments were well defined on reaching 60°C, but had disintegrated by 70°C.

Connective tissue, strained from homogenised beef sternomandibularis muscle, undergoes thermal shrinkage at 62-68°C (Davey and Gilbert, 1974). Giles (1968) showed the striations of collagen were stable during 140 min at 60°C, but gradually disappeared at 70°C. Macrarlane et al. (1981), using differential calorimetry, found a thermal transition peak for connective tissue at 63°C. They also found peaks for myosin in solution at 48°C (52°C in muscle) and 63°C, and for F-actin at 73°C.

What is the fate of the G-filaments during cooking? Various pieces of evidence, none individually conclusive, point to the steady denaturation of G-filaments within an hour at 60°C (for a fuller discussion see Locker, 1982). They become less extensible than in the raw state, but remain strong and elastic. Curves for shear force versus cooking time have flattened out by 100 h at 80°C and 5 h at 100°C, leaving residual values in the tender range (Davey et al., 1976; Davey and Niederer, 1977). Since collagen has been destroyed, these values can only be attributed to some highly heat-stable component within the myofibril and gap filaments seem the only candidate.

From the above it appears that the first hour at 60°C is a critical stage in the emergence of "cooked character". A-filaments have dissociated and coagulated, I-filaments appear to survive, and G-filaments have denatured. The myofibril may be regarded as "cooked". One characteristic of cooked meat, a clean bite in a tooth-type tenderometer, has just become evident (Locker and Carse, 1976). By a narrow margin the collagen net remains undenatured.

After an hour at 70°C, emergence of cooked character is largely completed. The steepest changes in the hydration and length have occurred, although there are further substantial changes up to 80°C (ibid.). The collagen net (Rowe 1974) has undergone thermal shrinkage to a taut "crossed diagonal" configuration (Locker and Daines, 1975) and the previously inelastic collagen fibres have become elasticised. Tensile strains now involve the stretching in unison of elastic gap filaments and pre-tensioned elastic connective tissue.

Continuity of the I-band

The first clear pointer to the significance of gap filaments in tenderness came from ageing studies by Davey and Graafhuis (1976). In muscle stretched beyond the point where A- and I-filaments overlap, then allowed to go into rigor and cooked while still restrained at this extension, G-filaments could be seen surviving intact within the "gap". If the meat had previously been well aged, G-filaments disappeared from the gap, leaving no structural continuity. When such experiments were repeated without ageing but with extreme cooking to destroy collagen (4 h at 100°C) the gap filaments again survived, along with most of the tensile strength. Final breakage occurred exclusively in the gap and the broken ends of G-filaments were clearly visible (Locker et al., 1977). We concluded that gap filaments (as discussed earlier) are rather stable to heat and determine the tensile strength of such abnormally stretched muscle after cooking.

But what of filaments in the short I-bands of muscles at near excised length? If actin filaments have disintegrated by 70°C it is surprising that on one has asked what holds the sarcomere together. In fact a sparse array of filaments with adhering coagulum survives in the I-band (Locker et al., 1977). These are about half as numerous as were the A-filaments, and we identify them as G-filaments.

Continuity of the A-band

Current theories of tenderness (Voyle, 1969; Marsh and Carse, 1974) regard the A-band as the strong unit in cooked meat and the I-band as the weak link. The disappearance of the latter accounts for toughness in well shortened meat, which is held to consist of a continuum of coagulated but still strong A-filaments. Our recent results challenge such a fate for the A-band. If muscle at excised length (with I-bands) or cold shortened (without I-bands) is cooked under restraint for 40 min at 80°C, stretched to near its limit, and then fixed at 50-70% extension, the A-filaments disappear. Instead, there remains an array of fine filaments, embedded in fragments of coagulum (Locker and Wild, 1982c). Our interpretation is that A-filaments have been dissociated by heat, the myosin then forming an actomyosin gel with the actin from disintegrated I-filaments. Only the gap filament cores remain intact on stretch, extending elastically while the gel cracks up.

A basis for cold shortening

When a sarcomere shortens to 1.6 μm, the A-filaments touch the Z-line, and on further shortening may penetrate it. Voyle (1969) explained the steep rise in shear force during shortening by 20-40% (Marsh and Leet, 1966) as a statistical increase in the population of sarcomeres without I-bands. This idea of progressive elimination of the weak link is compatible with my model (Locker and Leet, 1976a) which has twice as many G-filaments in the A-band as in the I-band. The G-filaments in the A-band are also reinforced to a degree by the actomyosin coagulum.

The notion of an A-filament continuum through the Z-line depends solely on lateral adhesion by coagulation of overlapping A-filaments. The G-filaments would form a stronger continuum, since although half depend on overlap, the other half are truly continuous through the Z-line (for additional evidence see Locker and Leet, 1976b). It must be pointed out that in my model, gap filaments pass through only one Z-line, so cannot form a true long-range continuum, but again depend on lateral adhesion for continuity within the A-band. Such a restraint is very evident near the M-line in grossly overstretched pre-rigor fibres, in which the A-filaments become dislocated but never separate, retaining an overlap of 0.6 μm even in an 11 μm sarcomere (Locker and Leet, 1976a).

Marsh and Carse (1974) proposed that a short I-band is stronger than a long one. Although their reasons are to me unconvincing, this could be fact, since a G-filament might be weaker if heat denatured in a stretched, and therefore thinner state.

The mechanism of ageing

It is now accepted that ageing is predominantly an attack on the myofibrils in indigenous proteases. The relative roles of the various cathepsins and neutral proteases are far from clear, but between them they weaken the already weaker I-band. The disappearance of the Z-line and subsequent breakage at this point has been recorded by Davey and Gilbert (1969) and by others, and claimed to be the key to the drop in shear force.

Ageing produces a drastic drop in the "yield point" of raw muscle, and instead of myofilaments failing while G-filaments stretch, both snap together (Locker and Wild, 1982a). Since only the gap filaments survive in the cooked myofibril, their erosion seems likely to be the real mechanism of ageing. The experiments of Davey and Graafhuis (1976) on ageing of highly stretched meat (described earlier), support this view. It may be noted that despite extreme ageing (3 days at 15°C) the Z-lines remained intact in their muscle. Fading of the Z-line, although an indicator of advanced ageing, is in my view, largely irrelevant to tenderisation. We have repeated the experiments of Davey and Graafhuis using highly stretched hypercontracted muscle, incubated in a crude muscle protease preparation before cooking. A similar destruction of G-filaments occurred (Locker et al., 1977). The vulnerability of G-filaments to proteases has also been demonstrated by irrigating over-stretched fibres with dilute solutions of trypsin or crude muscle protease. The fibres snap within minutes (ibid.). Residual filaments, identified as connectin, remaining in myosin-extracted chicken and rabbit myofibrils, are no longer seen after prior ageing for 7 days at 5°C (Takahashi and Saito, 1979).

In passing from the morphological to the protein level, we find only a limited number of proteins known to undergo a clear-cut degradation during ageing. Troponin-T is destroyed (Cheng and Parrish, 1979), and although its destruction appears to be a good indicator of degree of autolysis (Penney and Ferguson-Pryce, 1979) and of tenderisation (Penney and Dransfield, 1979), the protein itself is unlikely to have a role in tenderness. More significant is the progressive disappearance during ageing of desmin (Young et al., 1981) which has a structural role in a transverse sense.

Of most interest to the present theory is the finding that connectin, apparently the protein of the G-filaments (Maruyama et al., 1980), disappears rapidly in muscle homogenates at pH 5.5 and 55°C, or in whole muscle at 50-70°C (King et al., 1981), more rapidly than other muscle proteins, and apparently due to a carboxyl protease (King and Harris, 1982). The disappearance is more rapid at 60°C than at lower or higher temperatures (King and Kurth, 1980) in exact analogy to ageing, which is maximal at 60°C (Penfield and Meyer, 1975; Davey and Gilbert, 1976). The denaturation of G-filaments at 60°C might also facilitate the process. An awkward fact for my theory is the disappearance of connectin from SDS electrophoresis gels when meat has been heated at 80°C (King and Kurth, 1980; King et al., 1981). This can hardly be an enzymic process. If connectin is so easily destroyed by heat alone and is indeed the substance of G-filaments, clearly these could not contribute to the strength of cooked meat. However, I believe the explanation lies in the failure of this unusual protein either to dissolve in SDS, or to enter the gels, after a certain degree of heat denaturation.

The reluctance of cold shortened meat to age (Davey et al., 1967) may be re-interpreted in terms of gap filaments. The current view is that the vulnerable zones, the I-band or normal Z-line, have been removed by shortening. While in my view this is still true, it is because only in the I-band, are the G-filaments exposed and vulnerable to proteases. At a sarcomere length of 1.5 μm or below, the G-filaments exist only as cores to A-filaments and are completely sheathed in myosin "tails" (known to be resistant to proteolysis). No chink remains by which a protease can gain access.

Pressure-heat treatment

Australian workers (Macfarlane et al., 1981) have shown that rigor meat may be markedly tenderised by high pressure treatment (150 MPa 50-60°C). Unlike ageing this is particularly effective on cold shortened meat. We have currently been studying this effect and have found that after only 20 min. at 55°C (60 MPa), the G-filaments snap on stretching in the raw state but remain intact in a temperature control. It requires more rigorous treatment to modify shear force significantly (1 h at 60°C) and in such meat, when cooked, the G-filaments survive morphologically, but are more extensible and break at lower loads than in a temperature control (Locker and Wild, 1982d).

Conclusion

Our results provide reasonable explanations for most of the basic facts of tenderness. Enough evidence has accumulated to demand a rather drastic revision of current ideas about the basis of tenderness in meat, or more accurately the contribution of the myofibril. This contribution is important as the one capable of being greatly modified for better or for worse by post mortem treatment of meat.

The concept of a denatured collagen net stretching in parallel with denatured G-filaments is admittedly an oversimplified view of meat tenderness. Tensile strength although probably the major factor, is not the only one involved in the complex parameter of toughness, whether measured by human molars or by machine. Lateral adhesion is undoubtedly important. It is now known that a strong lateral network integrates all the myofibrils within a fibre by means of collars around the Z-line. The protein desmin of which these are made, has been shown in this laboratory to be readily destroyed by ageing (Young et al., 1981). The advancing science of adhesions between cell surfaces may open new avenues on tenderness.

However in spite of the over-simplification, I believe the ideas put forward here are a significant step towards a basic understanding of a much valued quality in a favoured food.

References

Cheng, C.S. and Parrish, F.C. (1979). J. Fd Sci., 44, 22.
Davey, C.L. and Gilbert, K.V. (1969). J. Fd Sci., 34, 69.
Davey, C.L. and Gilbert, K.V. (1974). J. Sci. Fd Agric., 25, 931.
Davey, C.L. and Gilbert, K.V. (1976). J. Sci. Fd Agric., 27, 244.
Davey, C.L. and Graafhuis, A.E. (1976). J. Sci. Fd Agric., 27, 301.
Davey, C.L., Kuttel, H. and Gilbert, K.V. (1967). J. Fd Technol., 2, 53.
Davey, C.L. and Niederer, A.F. (1977). Meat Sci., 1, 271.

Davey, C.L., Niederer, A.F. and Graafhuis, A.E. (1976). J. Sci. Fd Agric. 27, 251.
Giles, B.G. (1968). Proc. 14th Congr. of Europ. Meat Workers, 289.
King, N.L. and Harris, P.V. (1982). Meat Sci., in press.
King, N.L. and Kurth, L. (1980). In Fibrous Proteins, 2, 57. (see Maruyama et al.)
King, N.L., Kurth, L. and Shorthose, W.R.(1981). Meat Sci., 5, 389.
Locker, R.H. (1956). Biochim. Biophys. Acta, 20, 514.
Locker, R.H. (1982). Proc. 35th Ann. Recipr. Meat Conf., Blacksburg, Virginia.
Locker, R.H. and Carse, W.A. (1976). J. Sci. Fd Agric., 27, 891.
Locker, R.H. and Daines, G.J. (1975). J. Sci. Fd Agric., 26, 1711.
Locker, R.H., Daines, G.J., Carse, W.A. and Leet, N.G. (1977). Meat Sci., 1, 87.
Locker, R.H. and Leet, N.G. (1975). J. Ultrastruct. Res., 52, 64.
Locker, R.H. and Leet, N.G. (1976a). J. Ultrastruct. Res., 55, 157.
Locker, R.H. and Leet, N.G. (1976b). J. Ultrastruct. Res., 56, 31.
Locker, R.H. and Wild, D.J.C. (1982a). Meat Sci., in press.
Locker, R.H. and Wild, D.J.C. (1982b). J. Text. Studies, in press.
Locker, R.H. and Wild, D.J.C. (1982c). Meat Sci., in press.
Locker, R.H. and Wild, D.J.C. (1982d)., in preparation.
Macfarlane, J.J., McKenzie, I.J. and Turner, R.H. (1981). Meat Sci., 5, 307.
Marsh, B.B. and Carse, W.A. (1974). J. Fd Technol., 9, 129.
Maruyama, K., Kimura, S., Toyota, N. and Ohashi, K. (1980). in Fibrous Proteins: Scientific Industrial and Medical Aspects (eds. Parry, D.A.D. and Creamer, L.K.) Acad. Press, London, 2, 33. (See also Locker, R.H. and Daines, G.J., ibid., 43; King, N.L. and Kurth, L., ibid., 57).
Penfield, M.P. and Meyer, B.H. (1975). J. Fd Sci., 40, 150.
Penny, I.F. and Dransfield, E. (1979). Meat Sci., 3, 135.
Penny, I.F. and Ferguson-Pryce (1979). Meat Sci., 3, 121.
Rowe, R.W.D. (1974). J. Fd Technol., 9, 501.
Schmitt, J.G. and Parrish, F.C. (1971). J. Fd Sci., 36, 110.
Takahashi, K. and Saito, H. (1979). J. Biochem., 85, 1539.
Voyle, C.A. (1969). J. Fd Technol., 4, 275.
Wang, K., McClure, J. and Tu, A. (1979). Proc. Nat. Acad. Sci., 76, 3698.
Young, O.A., Graafhuis, A.E. and Davey, C.L. (1981). Meat Sci., 5, 51.