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European Meat Workers Conference

The Enzymic Breakdown of Collagen

by D. J. Etherington

When meat is aged for 2-3 weeks at low temperature it becomes more tender on subsequent cooking. This rather slow method of improving the quality of the meat can be hastened by holding at higher temperatures if there is adequate control to restrict the growth of micro-organisms. The involvement of the tissue proteinases in the improvement of meat quality during conditioning has been recognised for many years[1,2]. Much interest has been directed towards characterizing the changes that occur in the myofibrillar and sarcoplasmic proteins with ageing. These changes have been reviewed recently[3]. However, little alteration appears to occur in the collagen of the intra-muscular connective tissue matrix and early attempts to demonstrate the formation of soluble hydroxyproline in the post-mortem muscle proved negative[4,5]. More recently Herring et al[6], have demonstrated a small but significant increase in the amount of collagen that can be solubilized on heating the pulverized muscle to 77°C after 10 days of conditioning.

It is far from clear to what extent the increase in meat tenderness that results from ageing can be attributed specifically to enzymic fragmentation of the insoluble, cross-linked collagen of the intra-muscular connective tissue. It is possible that a significant reduction in the tensile strength of the collagen fibrils after cooking will occur with a limited number of strategic peptide bond breakages. These points of weakness would probably not become manifest until the collagen fibrils had been heated to above their shrinkage temperature. The individual filaments would then pull apart rather than contract together. Stevens[7] has shown that insoluble collagen can be degraded in such a manner with certain bacterial enzymes. On subsequent treatment with acetic acid a fine dispersion of large polymers is obtained, which can be reprecipitated into fibrils when neutralized. If conditioning operates in a manner similar to that shown by Stevens, then measurement of heat-soluble collagen will indicate only discreet changes in the integrity of the collagen matrix and for determination of the shrinkage temperatures the differences would be imperceptibly small.

Difficulties in studying the breakdown of collagen in post-mortem tissues have been a deterrent to detailed investigations in recent years. A more hopeful approach might be to examine like proteinases involved in the metabolism of collagen in the living animal. Here it is possible to examine the specificity of each proteinase *in vitro* and to extrapolate its action to the environment in post-mortem muscle.

The unique helical structure of native collagen is resistant to proteolytic degradation since when the individual molecules are cross-linked together in the fibril then the structure cannot be readily penetrated by proteolytic enzymes. In those tissues that are metabolically stable the collagen is broken down and resynthesised at relatively slow rates. Thus the half-life of muscle collagen has been estimated at 50-60 days and for tendon it exceeds 1 year[8]. In tissues, which are subject to active physiological changes or structural alterations, proteolytic enzymes cause the rapid dissolution of collagen[9]. Some examples are shown in Table 1.

Table 1

Physiological changes which are accompanied by an extensive breakdown of collagen (Taken from review by Gross[9])

1. Resorbtion of the tadpole tail at metamorphosis
2. Post-partum involution of the uterus
3. Bone and gum changes with tooth eruption
4. Tissue remodelling during embryonic development
5. Wound healing following trauma
6. Abnormal breakdown associated with certain inflammatory diseases, e.g. gingivitis, rheumatoid arthritis.

The proteinases which degrade collagen have been much studied recently. In those tissues where active breakdown of collagen is occurring a specific collagenase has been shown to exist[9]. It can only be demonstrated during the period of active collagen breakdown and it appears to be readily inhibited. The collagenase is secreted into the extracellular space and may originate from cell lysosomes. Recent evidence has indicated that it is normally present in human skin extracts, but is inhibited by complex formation with a specific serum protein[10]. The collagenase will break all three chains of the triple helix at a principal locus three-quarters of the distance from the cross-linked end of the molecule.

Investigation of the uterus during post-partum involution has suggested that this collagenase, by itself, is unable to cause the total solubilization of the uterine collagen, but that the fibrils are degraded to discrete fragments. These fragments are sufficiently small to be ingested by the macrophages that are abundant in the involuting uterus[11]. The ingested particles are then totally degraded by the enzymes of the macrophage lysosomes to small peptides and amino acids. This proposed mechanism for a two-stage breakdown is believed to operate generally for the removal of all connective tissue components[12].

The lysosomal proteinases are optimally active under weakly acidic conditions and within each digestive vacuole an acid micro-environment is believed to be generated[12]. At death, the tissues become acid due to an accumulation of lactic acid, but the lysosomal enzymes must diffuse through the cellular membranes in order to come into contact with the connective tissue collagen. The importance of the lysosomal proteinases in the autolysis of tissue components generally has been recognized for many years[13].

Table 2

Proteolytic enzymes found in mammalian lysosomes

Enzyme	Substrate
Cathepsin A	Peptides
Cathepsin B1	Proteins and peptides
Cathepsin B2	Peptides
Cathepsin C	Peptides
Cathepsin D	Proteins
Cathepsin E	Proteins
Collagenolytic cathepsin	Proteins
Aminopeptidase	Peptides
Dipeptidase	Peptides
(? Collagenase	Collagen)

In Table 2 are listed those proteinases and peptidases that have been shown to exist in mammalian lysosomes. From the known modes of action of these enzymes it is possible to predict what effect they may have upon collagen in the post-mortem situation. The structure of native tropocollagen can be divided into a helical region, forming the body of the molecule and a non-helical telopeptide. The helical region can be broken, by definition, only by a true collagenase and all other proteinases are restricted in their action to the small telopeptide region of the molecule[8].

The true collagenase has already been described, but this enzyme is normally strongly inhibited. Furthermore it appears to be inactive at pH 5.5. Cathepsin D has been shown to have no measurable effect in degrading acid soluble or insoluble collagen[14] and in this laboratory cathepsin D isolated from bovine longissimus dorsi muscle failed to remove the intramolecular cross-link from the telopeptide of tropocollagen[15]. The inability of cathepsin D to degrade the telopeptide may reflect a steric hindrance to the enzyme rather than a lack of susceptible bonds in this region of the collagen molecule. The telopeptides contain several hydrophobic residues, including a single phenylalanyl residue[16], which in other proteins, are known to form peptide bonds sensitive to cleavage by cathepsin D[17]. Cathepsin B has a specificity similar to that of cathepsin D[17], but it has never been shown to exist in muscle. Cathepsin B was considered for many years to be a peptidase which could hydrolyse the synthetic substrate

N-α-benzoyl-L-arginineamide (BAA). Recently it has been found that two enzymes can be distinguished, B1 and B2, and which both deamidate BAA. Cathepsin B2 may be a true peptidase, but cathepsin B1 has been shown to extensively degrade the B-chain of oxidized insulin and at more bonds than cathepsin D. Cathepsin B1 does not solubilize insoluble collagen and there is no indication of any demonstrable change in soluble collagen. However from its specificity it should be able to cleave some bonds in the telopeptide if these are accessible to the enzyme.

A lysosomal enzyme known to degrade insoluble collagen[18] has been studied in our laboratory. This 'collagenolytic cathepsin' at 37°C degraded insoluble collagen optimally at pH 3.5[18]. We have found that it can effectively remove the crosslink from the telopeptide of tropocollagen and the soluble material derived from insoluble collagen consists almost exclusively of the individual α chains of tropocollagen. This cathepsin has little solubilizing effect on insoluble collagen above pH 4.5. It is not known whether it can still remove the crosslink from tropocollagen at pH 5.5. The lysosomal peptidases would degrade only the large peptides obtained from digestion of the collagen by these proteinases.

The effect of the tissue proteinases upon the intramuscular connective tissue during post-mortem conditioning is small. This most probably represents the limit of breakdown to be expected when the proteinases are operating at an unfavourable pH and in combination with a reduced temperature. To what extent the collagen must be degraded in order to reduce the toughness of meat to a desirable level is not known at the present time.

Much interest has been generated in the use of added enzymes to accelerate the conditioning process. Those enzymes, which are commercially available act best during the cooking process. The discovery of a heat-labile enzyme that could degrade the collagen mainly during the hanging period would offer distinct advantages to the meat trade. From the foregoing it would appear that such an enzyme must act only upon the telopeptide region of collagen; a true collagenase would cause too much damage to the collagen network.

References

1. Hoagland, R., McBryde, C.N. and Powick, W.C. (1917) U.S. Dept. Agric. Bull., No. 433.

2. Fearson, W.R. and Foster, D.L. (1922) Biochem. J., 16, 564.

3. Goll, D.E., Arakawa, N., Stromer, M.H., Busch, W.A. and Robson, R.M. (1970) in The Physiology and Biochemistry of Muscle as a Food, 2, edited by Briskey, E.J., Cassens, R.G. and Marsh, B.B. p. 755, The University of Wisconsin Press, Wisconsin.

4. Prudent, I. (1947) Ph.D. Thesis, Iowa State University, Ames, Iowa.

5. Sharp, J.G. (1963) J. Sci. Fd. Agric., 14, 468.

6. Herring, H.K., Cassens, R.G. and Briskey, E.J. (1967) J. Fd. Sci., 32, 534.

7. Stevens, F.S. (1964) Ann. Rheum. Dis., 23, 300.

8. Bailey, A.J. (1968) in Comprehensive Biochemistry, edited by Florkin, M. and Stotz, E.H. Vol. 26B, p. 297, Elsevier, Amsterdam.

9. Gross, J. (1970) in Chemistry and Molecular Biology of the Intercellular Matrix, edited by Balazs, E.A., Vol. 3, p. 1623, Academic Press, London.

10. Eisen, A.Z., Bauer, E.A. and Jeffrey, J.H. (1971) Proc. Nat. Acad. Sci., 68, 248.

11. Parakkal, P.F. (1969) J. Cell. Biol., 41, 345.

12. Fell, H.B. (1969). Ann. Rheum. Dis., 28, 213.

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13. Fruton, J.S. (1960) in The Enzymes, edited by Boyer, P.D., Lardy, H. and Myrbäck, K., Vol. 4, p. 233, Academic Press, New York.

14. Bazin, S. and Delauney, A. (1970) in Chemistry and Molecular Biology of the Intercellular Matrix, edited by Balazs, E.A., Vol. 3, p. 1727, Academic Press, London.

15. Etherington, D.J. Unpublished observations.

16. Piez, K.A., Bladen, H.A., Lane, J.M., Miller, E.J., Bornstein P., Butler, W.T. and Kang, A.H. (1968) Brookhaven Symposia In Biology, 21, 345.

17. Barrett, A.J. (1969) in Lysosomes in Biology and Pathology, edited by Dingle, J.T. and Fell, H.B., Vol. 2, p. 245. North Holland, Amsterdam.

18. Anderson, A.J. (1969) Biochem. J., 113, 457.

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Progress in the study of connective tissues and some interactions with muscle during growth.

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Summary

Since the work of Neuberger and others on the turnover of collagen in the early 1950s it has become increasingly clear that the insoluble fibrous elements of connective tissue, collagen and elastin, are synthesized by cells as soluble proteins; the morphological stability and metabolic inertia of connective tissue structures are owed to the in situ formation of covalent crosslinkages.

During the linear growth phase of a young animal the amount of soluble collagen precursor which can be extracted from a tissue such as the skin can be related mathematically to the collagen growth rate and the time (T) required for the establishment of a sufficient number of intermolecular crosslinks per molecule to form an insoluble polymer. For elastin T can be estimated independently by the use of isotopically labelled lysine.

The growth of muscle and the connective tissue system (including bone) are known to interact but the control mechanisms are largely independent. The importance of out-of-phase differences in the development of muscle and bone to the visible conformation of the animal will be discussed in relation to our present sparse knowledge of factors controlling development of the hard and soft skeletons.