Meat Science 84 (2010) 248–256

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Review

Tenderness – An enzymatic view

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ARTICLE INFO

Article history:
Received 5 April 2009
Received in revised form 2 June 2009
Accepted 3 June 2009

Keywords:
Post-mortem proteolysis
Meat tenderisation
Calpains
Calpastatin
Caspases

ABSTRACT

One of the most common causes of unacceptability in meat quality is toughness. Toughness is attributed to a range of factors including the amount of intramuscular connective tissue, intramuscular fat, and the length of the sarcomere. However, it is apparent that the extent of proteolysis of key proteins within muscle fibres is significant determinant of ultimate tenderness. The objective of this manuscript is to describe the main endogenous proteolytic enzyme systems that have the potential to be involved in muscle post-mortem proteolysis and whether the experimental evidence available supports this involvement.

Contents

1. Introduction

Of all the meat traits, tenderness is considered to be the most important with regard to eating quality (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). It appears that the main determinant of ultimate tenderness is the extent of proteolysis of key target proteins within muscle fibres (Koohmaraie & Geesink, 2006; Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995a). Given the recognised importance of proteolysis in the tenderisation process this review has focused on the biochemistry of enzyme systems that have a potential role in the process, and some of the experimental evidence either rejecting or supporting their involvement. Our research in this area has focused on the fundamental biochemical aspects of the potential proteolytic systems involved tenderisation in pigs therefore the majority of the research described in this review focuses on this species, however, the scope of this review has been extended to cover fundamental observations made in other species.

2. Post-mortem proteolysis and candidate proteolytic systems

The final tenderness of meat depends on the degree of alteration of the muscle structural and associated proteins (Hopkins & Taylor, 2002). Specific myofibrillar, myofibril cytoskeleton and costamere proteins, such as titin, desmin and vinculin respectively, are subjected to cleavage, with some cleavage of the major myofibrillar proteins such as actin, myosin (Fig. 1; Goll, Thompson, Taylor, & Christiansen, 1992; Hopkins & Thompson, 2002; Koohmaraie & Geesink, 2006; Lametsch et al., 2003; Taylor et al.,

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0309-1740/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.meatsci.2009.06.008

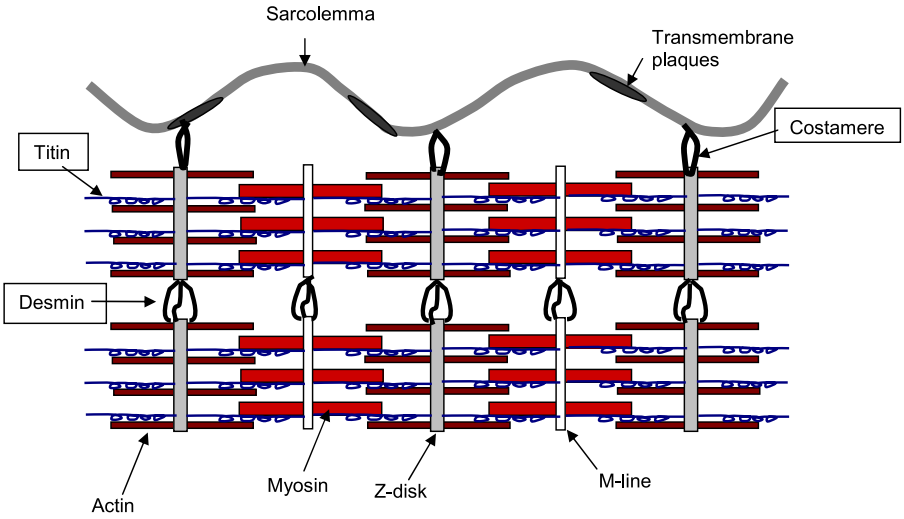

Fig. 1. Schematic representation of muscle myofibrillar proteins showing the major components of the sarcromere. Boxes indicate the cytoskeletal structures and proteins susceptible to post-mortem cleavage (adapted from Taylor et al., 1995a).

1995a). For a protease system to be considered to be involved in post-mortem proteolysis and meat tenderisation it must meet a certain basic criteria as defined by Koohmaraie (1988): firstly, the protease must be endogenous to skeletal muscle cells; secondly it must be able to mimic post-mortem changes in myofibrils *in vitro* under optimum conditions and finally it must have access to myofibrils in tissue. In this review the candidate proteolytic systems considered as having a potential role in the degradation of proteins post-mortem are described and the experimental investigations that have been carried out to determine whether they have a role are discussed.

3. Cathepsins and other muscle peptidases

Cathepsins are a group of enzymes comprised of both exo- and endo-peptidases and are categorised into cysteine (cathepsins B, H, L and X), aspartic (cathepsins D and E) and serine (cathepsins G) peptidase families (Sentandreu, Coulis, & Ouali, 2002). Many research groups have discarded the contribution of cathepsins to meat tenderisation on the basis of a number of observations. Firstly, there is not large scale actin and myosin degradation in the post-mortem conditioning period; these being primary substrates for cathepsins (Koohmaraie, Whipple, Kretchmar, Crouse, & Mersmann, 1991). Secondly, cathepsins are located in the lysosomes and must therefore be released for them to have access to myofibril proteins and to add to meat tenderness (Hopkins & Taylor, 2002). However, low pH levels and high carcass temperature can enhance the disruption of the lysosomal membrane (O'Halloran, Troy, Buckley, & Reville, 1997) and failure of ion pumps in the membranes as the carcass enters rigor, consecutively to ATP depletion, could overcome this (Hopkins & Thompson, 2002). Thirdly, there is little association between cathepsins' activities and the variation in tenderness in meat samples (Whipple et al., 1990). However, cathepsins B and L activities at 8 h post-mortem have been found to positively correlate with tenderness in beef (O'Halloran et al., 1997). Cathepsin L hydrolyses the largest number of myofibrillar proteins, including troponin T, I and C, nebulin, titin and tropomyosin; which are degraded during the post-mortem conditioning period as well as myosin and actin, in rabbit, beef and chicken myofibrils (Mikami, Whiting, Taylor, Maciewicz, & Etherington, 1987).

More recently it has been reported that activity of serine peptidases inhibitors was a good predictor of meat toughness (Zamora et al., 2005). Serine peptidases form the largest group of peptidases in mammalian systems. The most best characterised are those associated with digestion (trypsin and chymotrypsin) and blood clotting (thrombin). There are reports of serine peptidases being expressed in skeletal muscle, although there is debate whether the activity identified in muscle homogenates originates from muscle itself or the associated cells (for a review see Sentandreu et al., 2002). In the study by Zamora et al. (2005), the activity of semi-purified serine peptidase inhibitor was found to be positively related to toughness, and when combined with 6 other variables, which included micro-calpain activity, was predictive of cattle meat toughness after 6 days post-mortem storage. Such reports re-enforce the observations that inhibitors of the peptidases have a better predictive value of meat quality than the enzyme directly involved in the proteolysis, an example of this is calpastatin, the calpain proteolytic enzyme inhibitor (see below).

4. Proteasomes

The proteasome is a multicatalytic protease complex involved in the regulation of a number of basic cellular pathways, by their degradation of proteins in the cytosol and nucleus (Coux, Tanaka, & Goldberg, 1996). Proteasomes are ubiquitously expressed in the body and are abundant in skeletal muscle (Robert, Briand, Taylor, & Briand, 1999). The proteasome (26S) consists of a 19S regulatory subunit and the 20S multicatalytic structure containing the proteolytic enzyme activities. The 20S proteasome, also known as the multicatalytic proteinase complex (MCP), is the catalytic core of these proteasome complexes (Dahlmann, Ruppert, Kloetzel, & Kuehn, 2001). Proteolysis by the proteasome is an ubiquitin-dependent process, at least four ubiquitin proteins must attach to the lysine residue of the target substrate. The poly-ubiquitinated proteins are subsequently recognised by the proteasome, which removes the ubiquitin chain and degrades the substrate (Taillandier et al., 2004). This process is ATP dependent and once this is depleted the 26S proteasome dissociates into the 19S subunit and the 20S proteasome, the latter not requiring ATP or ubiquitin (Peters, Franke, & Kleinschmidt, 1994). This latter observation along, with its relatively high level of expression in skeletal muscle, has led several groups to examine the potential role of the 20S proteasome in post-mortem proteolysis. Initial studies reported that proteasome extracted from ovine muscle was not involved in myofibril proteolysis (Koohmaraie, 1992). However, a number of subse-