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Implications of skeletal muscle creatine kinase to meat quality

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ABSTRACT

Creatine kinase (CK) is a key enzyme for the energetic metabolism of tissues with high and fluctuating energy demands *in vivo*, as it is the case of the skeletal muscle tissue, which is the most important for the meat industry. This enzyme is generally utilized as an indicator of physical stress and/or muscle damage in animal production. However, CK continues to exert important functions after the slaughter, participating in the transformation of the muscles into meat. This article considers the main aspects of the post-mortem behaviour of CK in the skeletal musculature of meat animals, and the possible implications on meat quality.

KEY WORDS: creatine kinase, skeletal muscle, post-mortem metabolism, meat quality

INTRODUCTION

Meat is the post-mortem aspect of muscles, differing from them due to a series of biochemical and biophysical modifications initiated with the slaughter of the animal. Skeletal muscles are of major economic importance in the meat industry (Hocquette et al., 1998), and the skeletal musculature is considered as a high energy demand tissue (Kunz, 2001). In this sense, creatine kinase (CK; E.C. 2.7.3.2) is a fundamental enzyme of the energy metabolism in cells and tissues with high and fluctuating energetic demands (Wallimann et al., 1992). The crucial role of CK in skeletal muscle bioenergetics raises the interest on the potential participation

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of this enzyme in the post-mortem phenomena. This article deals with the major aspects of the post-slaughter behaviour of CK in skeletal muscles of meat animals, and with the potential implications of this enzyme on meat quality.

CHARACTERISTICS AND FUNCTIONS OF CREATINE KINASE *IN VIVO*

Tissues like brain and skeletal and cardiac muscles depend on the availability of vast amounts of energy, mainly in the form of adenosine triphosphate (ATP). In these tissues, ATP is continuously and efficiently regenerated by the action of CK which catalyses the transphosphorylation reaction between phosphocreatine (PCr) and adenosine diphosphate (ADP) (Wallimann et al., 1992; Wyss et al., 1992). This reaction is illustrated below, showing the standard free-energy change is large, favouring the reaction towards PCr hydrolysis and ATP synthesis:

$$\text{Phosphocreatine}^{2-} + \text{ADP}^{3-} + \text{H}^{+} \leftrightarrow \text{Creatine} + \text{ATP}^{4-} \quad \Delta G'^{o} = -12 \text{ kJ mol}^{-1}$$

Four subunit isoforms (about 43 kDa) of CK are known in vertebrates. The isoforms M-CK (muscle) and B-CK (brain) combine and originate three dimeric cytosolic isoenzymes MM-, MB- and BB-CK. The mitochondrial subunits, sarcomeric Mi-CK and ubiquitous Mi-CK, combine to give homodimeric and homooctameric isoenzymes which are readily interconvertible (Wyss et al., 1992).

The CK isoenzymes are expressed in a specific manner, depending on the tissue and also on the developmental stage of this tissue. The MM-CK isoenzyme, for instance, is predominant in mature skeletal muscles where it replenishes intermyofibrilar ATP and acts as structural component of the myofibrillar M-band. Moreover, CK isoenzymes occupy specific locations in the cell, a phenomenon known as subcellular compartmentalization. By this concept, cytosolic CK isoenzymes are preferentially concentrated in sites of energy consumption - functionally coupled to ATPases - where they catalyse the reaction towards ATP synthesis; while mitochondrial CKs are specifically located in the mitochondrial intermembrane space where they catalyse the reaction towards the synthesis of PCr (Wallimann et al., 1992; Ventura-Clapier et al., 1998).

Two major functions are postulated for the CK/PCr system. The first function is associated with energy buffering in cells during abrupt increases in energy demand. According to this concept, cytosolic isoenzymes utilize PCr stores to maintain adequate local ATP/ADP ratios in these cells. The temporal energy buffer function is more pronounced in fast-twitch skeletal muscles which depend on the immediate availability of large amounts of high-energy phosphates to restore the ATP hydrolysed by the myofibrillar ATPases in short periods of intense activity

(Wyss and Kaddurah-Daouk, 2000). This is supported by the high proportion of the MM-CK isoenzyme, the large PCr pool and the small proportions of mitochondrial isoenzymes observed in this tissue when compared to slow-twitch skeletal muscles, cardiac muscles and brain. In fact, to accomplish this function the activity of mitochondrial isoenzymes is not required (Wallimann et al., 1992; Wyss et al., 1992). In fast-twitch skeletal muscles the mitochondria release ATP into the cytoplasm and then the cytosolic CK transfers a significant proportion of this energy through the cytoplasm as a flux of PCr; in this way CK also acts in a 'spatial' energy buffering system, which allows the equal energy distribution in these cells even if its production is localized (Saks et al., 1996).

The second function of the CK/PCr system to be considered is that of energy transport. By this concept the transport of high-energy phosphates between the sites of energy production (mainly mitochondria) and energy consumption (ATPases) is accomplished mainly by PCr and creatine (Cr). This is only possible due to the subcellular compartmentation and functional coupling of the mitochondrial CKs with oxidative phosphorylation and of the cytosolic CKs with cellular ATPases (Saks et al., 1996). The energy transport is, probably, the major function of the CK/PCr system in slow-twitch skeletal muscles, cardiac muscles and brain, which depend on a continuous ATP supply at sites of energy consumption and, thus, depend on a high rate of production and transport of high-energy phosphates. In these tissues, as compared with fast-twitch skeletal muscles, a decrease in the total activity of CK, a small PCr pool and relatively high levels of Mi-CK expression are observed (Wyss and Kaddurah-Daouk, 2000).

The CK/PCr system has other functions, but they are direct consequences of the energy buffer and energy transport functions (Wallimann et al., 1992; Wyss et al., 1992).

CREATINE KINASE IN ANIMAL PRODUCTION

Physical stress and muscle damage change the membrane permeability of the muscle cells, increasing the levels of certain sarcoplasmic enzymes, such as the MM-CK from skeletal muscles, in the blood (Amelink and Bär, 1986).

Owing to this feature, CK is currently used as an indicator of pre-slaughter physical stress/muscle damage, which are directly associated with animal welfare and meat quality (Fàbrega et al., 2002; Tuomola et al., 2002). The measurement of blood CK levels have been employed in the assessment of physical stress, or stress susceptibility, in pigs (Fàbrega et al., 2002), cattle (van de Water et al., 2003), goats (Kannan et al., 2002), poultry (Sandercock et al., 2001) and red deer (Pollard et al., 2002).

The methods used for CK measurement are generally based on evaluation of CK catalytic activity in blood serum or plasma, as in the procedure described by Rosalki (1967). However, CK assays that allow the analysis of unprocessed blood samples, such as the bioluminescent method proposed by Hwang et al. (1977) and the immunochemical method developed by Tuomola et al. (2002), seems more suitable for application in animal production and slaughterhouse environments.

IMPLICATIONS OF POST-SLAUGHTER CREATINE KINASE ACTIVITY IN SKELETAL MUSCLES

With the slaughter of the animal the blood circulation stops and the supply of oxygen (crucial for oxidative phosphorylation) and other substances that take part on the energy metabolism of the cell, like glucose, is interrupted; likewise, the removal of metabolites is ceased. Thus, the high-energy compounds available at the moment of death are employed in ATP synthesis in an attempt to continue the muscle metabolism (Pösö and Puolanne, 2005; Scheffler and Gerrard, 2007).

After slaughter ATP is replenished by PCr through the action of CK, and by glycolysis through the utilization of glucose derived from glycogen stores. PCr is the first high-energy compound degraded, and only when its concentration is reduced to approximately 10-30% of the resting value is that a significant decrease in ATP and glycogen levels and an increase production of lactate by glycolysis are observed (Henckel et al., 2002; Schäfer et al., 2002; Pösö and Puolanne, 2005; Scheffler and Gerrard, 2007). Consequently, by maintaining the ATP levels in the early post-slaughter period, CK delays the acidification of the muscle cell cytosol by (i) postponing lactate production by glycolysis; and also through (ii) proton (H^+) buffering, since the CK reaction in the direction of ATP synthesis utilizes PCr, ADP and protons. In pigs, the dietary supplementation of creatine monohydrate increased the content of PCr in loin measured immediately after bleeding, delaying the early post-mortem pH decline (Lindahl et al., 2006). The maintenance of ATP levels also delays the onset of *rigor mortis* (Scheffler and Gerrard, 2007).

In this context, the available sources for energy (ATP) production after slaughter (such as PCr and glycogen) are affected by factors such as genotype, muscle type, environmental conditions, pre-slaughter treatment, stunning method, among others. The rate of post-slaughter degradation of that sources depend on the post-mortem muscle metabolism, which is affected by the above factors and also by electrical stimulation, chilling regime of the carcass among others (Bertram et al., 2002; Henckel et al., 2002; Schäfer et al., 2002). Therefore, the post-mortem lactate formation and the subsequent pH decline are related to the muscle energy

availability and to the rate of post-mortem metabolism (Bertram et al., 2002; Henckel et al., 2002; Schaffler and Gerrard, 2007).

Nevertheless, it should be noted that at the beginning of any muscle contraction in the living animal, regardless of its intensity, the energy is provided by degradation of PCr. Thus, even a mild stress may exert an influence in the decline of muscle pH, since a reduced amount of PCr will imply in earlier glycolysis and pH decrease (Henckel et al., 2002).

CREATINE KINASE AND MEAT QUALITY

Post-mortem acidification is a prerequisite in the conversion of muscles into high quality meat (Pösö and Puolanne, 2005). The rate and extent of the post-mortem pH decline affect the colour and water holding capacity (WHC) of the meat, which are major quality traits and, thus, of main importance for both processors and consumers (Bertram et al., 2002; van de Wiel and Zhang, 2007).

The effect of post-slaughter pH fall on meat colour and WHC is mainly related to changes in protein solubility and/or denaturation of muscle proteins, affecting the structure and the ability of these proteins to bind water molecules. However, the degree of muscle protein solubility and/or denaturation are not only dependent on pH but are rather determined by both pH and temperature, where the combination between low pH and high temperature values generally favours protein denaturation (Den Hertog-Meischke et al., 1997; Schäfer et al., 2002; Scheffler and Gerrard, 2007). The development of PSE (pale, soft and exudative) or PSE-like meat, for instance, is associated with the pronounced denaturation of muscle proteins due to a rapid post-mortem pH fall while the carcass temperature is still high (about body temperature). Nevertheless, if the carcass chilling rate is fast enough, the extent of protein denaturation is generally lower, resulting in higher WHC (Den Hertog-Meischke et al., 1997).

Sarcoplasmic proteins, the soluble proteins of the sarcoplasma, correspond to approximately 30% of the total muscle protein (Tornberg, 2005), and are generally more susceptible to denaturation than myofibrillar proteins (Farouk et al., 2002). CK is found among the denatured sarcoplasmic proteins in PSE pork (van de Wiel and Zhang, 2007). In the same direction, the electrophoretic pattern of sarcoplasmic protein fractions from fast-glycolysing porcine *Longissimus dorsi* muscles ($pH_{45\ min}$ <5.8) pointed that various proteins, including CK, were found as fainter bands when compared to normal-glycolysing samples ($pH_{45\ min}$ ≥ 5.8), indicating the denaturation and deposition of these proteins on myofibrils due to low pH conditions (Ryu et al., 2005). Similarly, O'Halloran et al. (1997) found increased intensity of CK band in electrophoretic profiles of myofibrillar

proteins from fast glycolysing in comparison to slow glycolysing bovine muscle. Likewise, Rathgeber et al. (1999) reported the increased appearance of CK and glycogen phosphorylase on extracts of myofibrillar proteins from turkey carcasses with faster post-mortem glycolysis ($pH_{15\ min} \leq 5.8$) than on controls ($pH_{15\ min} > 6.0$), indicating an increased association of these proteins with the myofibrillar fraction and/or a reduction in extractability due to denaturation.

Specifically, the low WHC of PSE meat is attributed to the denaturation of myofibrillar proteins, mainly myosin (Offer, 1991). However, the denaturation of sarcoplasmic proteins may also be involved in the reduction of meat WHC, since negative correlations between sarcoplasmic protein solubility and drip loss were observed in bovine (Den Hertog-Meischke et al., 1997) and porcine (van de Wiel and Zhang, 2007) meat samples. Likewise, the WHC decrease in pork was associated to the precipitation of sarcoplasmic proteins, and CK was indicated as a major protein involved. The increased drip in conventionally chilled pigs coincided with a decreased sarcoplasmic protein concentration (including CK) on the exudate, suggesting that the denaturation of these proteins may be a possible explanation for this event (Ryu et al., 2005).

The denaturation of CK and glycogen phosphorylase was also observed in RSE (reddish-pink, soft and exudative) pork. However, the protein solubility (total, sarcoplasmic and myofibrillar) of this quality class was not significantly different than that of normal meat and, therefore, it is proposed that the high drip loss in RSE pork is caused by a lower final pH, with the associated deposition of CK and glycogen phosphorylase onto to the myofibrils (Ryu et al., 2005).

The colour of pork also appears to be related with the denaturation of sarcoplasmic proteins and deposition over the myofibrillar proteins. A negative correlation between the pale colour of pork and the sarcoplasmic protein solubility is observed (Ryu et al., 2005; van de Wiel and Zhang, 2007), indicating that CK is among the major denatured proteins. In pork muscles submitted to supercritical carbon dioxide treatment, the pale colour of the samples was associated with the denaturation of sarcoplasmic proteins, and the denaturation of CK was identified (Choi et al., 2008). Eikelenboom and Smulders (1986) reported that the effect of electrical stimulation on colour and WHC of veal was partially explained by a certain degree of denaturation of sarcoplasmic proteins resulting from the induced rapid post-mortem pH decline in the treated carcasses.

The texture of meat and meat products may as well be affected by the denaturation of sarcoplasmic proteins. The firmness and cohesiveness of mackerel meat increased concomitantly with the increase in insolubilization of sarcoplasmic proteins, including CK, after salt-vinegar curing, suggesting that the precipitation of these proteins might contribute to the texture change caused by the curing process (Toyohara et al., 1999). Precipitation of sarcoplasmic proteins might

also have a role in the consistency of cooked meat and meat products (Tornberg, 2005). For instance, Farouk et al. (2002) reported that sarcoplasmic proteins are important in determining the cohesiveness of cooked sausage batter. Results from Kim et al. (2005) showed that the inclusion of sarcoplasmic proteins positively contributed to the gelation properties of myofibrillar proteins (surimi).

Tenderness is the most important meat attribute, except for fish meat, and it has been proposed that the proteolysis of myofibrillar and associated proteins plays a significant role in the tenderization process. The major proteases associated with meat tenderization are calpains and cathepsins (Koohmaraie, 1996; Hocquette et al., 1998). Proteasomes may also contribute to tenderization processes (Lamare et al., 2002) but it appears not to hydrolyse sarcoplasmic proteins (Matsuishi and Okitani, 1997). CK was degraded by m-calpain (Purintrapiban et al., 2001) and also by cathepsins (Delbarre-Ladrat et al., 2004). In general, native CK (41-43 kDa) is largely resistant to *in vitro* proteolysis; however, some nonspecific proteases, such as proteinase K, cleave this enzyme at restricted site(s) (Leydier et al., 1997). Muscle CK consists of two folding domains (a large C terminal domain of about 250 amino acid residues, and a minor N terminal domain of about 100 residues), and the primary site of proteolytic cleavage is postulated to be at a putative domain linker of about 30 amino acid residues (Webb et al., 1997). In structural studies CK is partially unfolded/denatured at first to render susceptible to various other proteases, such as trypsin, chymotrypsin and pepsin, which could therefore cleave - mainly at connectors between elements of secondary structure - the enzyme into several fragments (Webb et al., 1997; Mazon et al., 2005).

During post-mortem storage of porcine muscles (4ºC for up to 48 h), both structural and metabolic proteins were degraded; however, the accumulation of fragments from metabolic proteins, including CK fragments of 29, 31 and 34 kDa, were the most abundant changes observed (Lametsch et al., 2002). Laville et al. (2005) also reported the appearance of 31 and 34 kDa protein fragments in stored pig muscle (4°C for 72 h), suggesting that these fragments correspond to products of CK proteolysis. Similar results were reported by Morzel et al. (2004). During ageing of bovine muscle (4°C for up to 15 days), one peptide (~5.7 kDa) was isolated and identified as a product of CK proteolysis (Stoeva et al., 2000). On one-dimension SDS-PAGE, four bands corresponding to CK fragments had their intensities increased after ageing of pork (4°C for 7 days) (Park et al., 2007). Additionally, Hughes et al. (2002) observed the appearance of one peptide as a result of CK proteolytic degradation during ripening of semi-dry fermented sausages.

The proteolysis of metabolic proteins has probably no direct influence on meat texture; however, the resulting fragments might be useful markers of proteolytic activity on meat and may, thus, function as meat quality indicators (Stoeva et al., 2000; Lametsch et al., 2002; Park et al., 2007).

Also, CK may serve as a marker protein for WHC of pork. Recently, higher CK levels in pig muscle shortly after slaughter were related with meat showing high drip loss after 5 days of storage at 4°C (van de Wiel and Zhang, 2007). According to these authors the high CK levels rapidly degraded PCr, shortening the delay phase of rigor and this, in turn, caused a more rapid pH decline and muscle contraction, leading to higher drip loss.

CONCLUSIONS AND PERSPECTIVES

Creatine kinase (CK) is a crucial enzyme for the *in vivo* energy metabolism of skeletal muscles from meat animals, and continues to possess significance after the slaughter, hindering the muscle pH decline and the onset of *rigor mortis*. CK is one of the most abundant sarcoplasmic proteins in skeletal muscles and its denaturation and/or insolubilization, resulting from post-slaughter conditions of pH and temperature, may negatively affect both meat colour and water holding capacity. Additionally, sarcoplasmic proteins may play a role on texture of meat and meat products submitted to heat treatment. Furthermore, CK is substrate of proteases associated with the meat tenderization process; the resulting fragments may be employed as markers for proteolytic activity and thus, could be useful as meat quality indicators.

However, the role of metabolic proteins such as CK on the post-mortem metabolism of meat animals has not received the appropriate attention. In this sense, future research must approach this topic as it may be relevant to meat quality.

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