PROTEOLYTIC ACTIVITY OF CASPASE 3 ON MUSCLE MYOSIN HEAVY CHAIN

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Tenderness is regarded as the most important criterion for meat quality; therefore, a greater understanding of factors affecting meat tenderness would help meat producers provide consistently tender meat. Although the biochemical reactions occurring during postmortem tenderization remain controversial, it is believed that proteolysis of key myofibrillar proteins plays a major role in postmortem tenderization. In relation to this, the most studied proteolytic systems have been cathepsins, calpains and to a less extent the proteasome. However, the major peptidases of concern are not identified yet in an unquestionable way and this question is still strongly debated. We recently proposed a new way to understand postmortem muscle proteolysis by considering the hypothesis that, after animal bleeding, muscle cells will have no other alternative than to enter into programmed cell death or apoptosis. This process is mediated by a particular group of enzymes called caspases. Triggering of apoptosis in postmortem muscle cells would induce a series of biochemical and structural changes in dying cells, including hydrolysis of myofibrillar proteins. The objective of the present work was to carry out a precise characterization of the proteolytic action that caspase 3 can exert in the hydrolysis of muscle myosin heavy chain. To achieve this goal, a proteomic approach has been developed for the global identification of proteolytic cleavage sites based on selective identification of N-terminal peptides derived from caspase action. In-gel caspase incubation of muscle myosin heavy chain was followed by acetylation of free amino groups, trypsin digestion and biotinylation of the new N-terminal ends. The peptide mixture was then analysed by LC-ESI-MS/MS, concluding that caspase 3 is able to cleave muscle myosin heavy chain in the Asp residue located within the myosin head-like domain at position 221. This is the first time that a specific cleavage site is reported for this protein.