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Simultaneous process to isolate actomyosin and actin from post-rigor porcine skeletal muscle

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Abstract

A simultaneous actomyosin and actin isolation procedure from post-rigor porcine muscle was developed, based on differential solubility, gel filtration chromatography and extraction steps. The isolation process was evaluated by SDS-PAGE analysis and silver staining. Actomyosin and actin were isolated in a simultaneous process yielding 0.14 mg and 2.5 mg/g of meat, respectively, using a shorter purification process than others reported in the literature but with similar recoveries. Furthermore, actin preserves its polymerisation ability and both proteins, actomyosin and actin, could be used in further studies.

Keywords: Myofibrillar proteins; Myosin; Actin; Actomyosin; Purification processes; Polymerisation; Post-rigor porcine muscle

1. Introduction

The separation of the muscle constituents is necessary for various physiological and biochemical studies. In this sense, the isolation of muscle constituents allows their characterisation in order to relate them to meat quality (Bowker, Grant, Swartz, & Gerrard, 2004; Hidalgo, Padrón, Horowitz, Zhao, & Craig, 2001; Toldrá & Flores, 2004), to understand post-mortem changes (Toldrá, 2005), to the identification of contaminating proteins added to meat products (Toorop, Murch, & Ball, 1997) and to the study of the interaction phenomenon with other matrix components (Gianelli, Flores, & Toldrá, 2003). Myosin and actin, which are the major constituents of myofibrillar proteins, are not only important in muscle physiology, but they are also believed to be mainly responsible for important functional properties in food systems, such as water-holding, emulsifying capacity, binding ability and gelation, in various structured meat and meat products (Asghar, Samejima, & Yasui, 1985). Specifically, numerous methods for the isolation of these proteins have been developed, depending on the objective of the investigation, but many of them are very tedious as they include many extractions processes (Syrovy, 1984); nevertheless several rapids methods have been developed for application in the industry (Murch, Bruce, & Ball, 1992; Toorop et al., 1997).

Generally, the major myofibrillar proteins are isolated in separated processes (Syrovy, 1984). In particular, the extraction of myosin is usually initiated by the removal of the sarcoplasmic proteins through washes with diluted phosphate buffer. Subsequently, the extraction of myosin is carried out using modifications of Guba–Straub (Hermansson, Harbitz, & Langton, 1986) and Hasselback–Schneider (Dudziak & Foegeding, 1988) solutions. These buffers usually contain EDTA or EGTA to eliminate the heavy metals and to protect the enzymatic activity of the protein (Syrovy, 1984). In several modifications, DTT or β-mercaptoethanol are added at low concentrations, in order to avoid the oxidation of myosin. Furthermore, the elimination of contaminants was achieved by fractionation with ammonium sulphate and diverse chromatographic methods (Syrovy, 1984). The application of high pressure

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to enlarge the solubility of the myofibrillar proteins has been attempted (Macfarlane & Mckenzie, 1976) and some authors have even used HPLC to separate myosin from muscle extracts using high ionic strength solutions as mobile phase (Murch et al., 1992). On the other hand, the classical method to isolate actin is initiated with the elimination of the myosin and other myofibrillar proteins, using an extraction with a high ionic strength solution. Subsequently, the break of the intermolecular links in the F-actin is carried out using acetone or potassium iodide (Syrovy, 1984) and, finally, the G-actin is extracted. Subsequently, the protocol for actin purification could be divided into two steps: first, the preparation of the acetone powder and second, the extraction of the G-actin, where the ATP is added to the extraction buffer in order to maintain the actin functional integrity (Pardee & Spudich, 1982). After the extraction, the purification process is continued for several days with polymerisation and depolymerisation cycles to eliminate the contaminants (Pardee & Spudich, 1982). The following variations of this method were mainly focussed on the extraction solution, in the centrifugation speed and in the process utilised to obtain a greater purification degree, depending on the desired purity and the use required for the protein.

Moreover, most of these purification processes used pre-rigor muscle (Syrovy, 1984) but, in processed meat products, the majority of muscles used are in the post-rigor state. Furthermore, the separated constituents of post-rigor muscle would have characteristics different from those extracted from pre-rigor (Fukazawa, Nakai, & Yasui, 1970). It is important to evaluate, for example, the functional properties of proteins at the molecular level in processed products (Dudziak & Foegeding, 1988).

Summing up, the methods for isolating myofibrillar proteins are long and tedious. Therefore, the aim of this investigation was the isolation of actomyosin and actin, in an unique extraction process, from post-rigor porcine muscle, based on differential solubility, gel filtration chromatography and extraction steps, in order to obtain fractions that could be used for further studies.

2. Materials and methods

2.1. Materials

Salts, ($MgCl_2$, KCl, NaCl, NaN_3 and NaH_2PO_4) were purchased from Panreac (Barcelona, Spain), expect $CaCl_2$ (Sharlau, Barcelona, Spain) and $Na_4P_2O_7 \cdot 10H_2O$ (Riedel-de Haën, Seelze). EGTA (ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), β-mercaptoethanol and $ATPNa_2$ (adenosine 5′-triphosphate disodium salt) were purchased from Sigma (St. Louis, MO, USA) and EDTA (ethylenediaminetetraacetic acid disodium salt 2-hydrate) from Panreac (Barcelona, Spain). Buffers Tris (Tris(hydroxymethyl)-aminoethane) and KH_2PO_4/K_2HPO_4 were purchased from Panreac (Barcelona, Spain). All the chemicals used were reagent grade (purity >98%).

Molecular weight standards of broad range for SDS-PAGE were purchased from Bio-Rad Laboratories (Richmond, CA) and include: myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase-b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa) and carbonic anhydrase (31 kDa). Molecular weight standards for gel-chromatography were: myosin (450 kDa) and carbonic anhydrase (31 kDa) purchased from Sigma (St. Louis, MO, USA) and bovine serum albumin (66.2 kDa) from Roche (Mannheim, Germany).

2.2. Sample

Porcine muscle *Longissimus dorsi*, 2 d post-mortem, was acquired from local butchery. Fat and connective tissue were remove and the meat was cut in portions, packaged in vacuum bags and stored at −20 °C.

2.3. Protein purification processes

2.3.1. Simultaneous purification process

The simultaneous purification process (Fig. 1) started by washing 50 g porcine of post-rigor muscle three times with 0.1 M Tris–HCl at pH 7.0 containing 20 mM EDTA (wash buffer) in order to eliminate the sarcoplasmic proteins. Afterwards, the myofibrillar proteins were extracted with Hasselbach–Schneider solution, consisting of: 0.1 M KH_2PO_4/K_2HPO_4 at pH 6.4 with 0.6 M KCl, 10 mM $Na_4P_2O_7 \cdot 10H_2O$, 1 mM $MgCl_2$ and 20 mM EGTA (Dudziak & Foegeding, 1988) and were precipitated by diluting 1/20 with deionised water. The last pellet (M4p) was submitted to a further purification step using gel chromatography in a XK column (Ø 2.6 cm × 66.5 cm. Amersham Pharmacia Biotech, Uppsala, Sweden) filled with Sephacryl S-300 with a 10–1500 kDa fractionation range. The sample was eluted with 20 mM KH_2PO_4/K_2HPO_4 at pH 7.0 and 0.5 M NaCl as elution buffer and 80 fractions were recovered with a flow of 18 ml/h. The elution pattern was monitored by measuring the absorbance at 280 nm in a spectrophotometer Ultrospec 3000 (Pharmacia Biotech, England). The combination of the six fractions that gave the maximum absorbance constituted the fraction M8. Previously, the column was calibrated using 1 mg/ml of myosin (450 kDa), bovine serum albumin (66.2 kDa) and carbonic anhydrase (31 kDa) that were eluted in fractions 12, 30 and 39, respectively (data not shown).

In the second part of the process, the pellet obtained after the extraction with Hasselbach–Schneider solution (M3p, Fig. 1) was used to prepare the acetone powder by three successive extractions with acetone (20 vol/g pellet) for 20 min and further filtration through Whatman paper. Afterwards, the acetone powder was used for the extraction of G-actin with buffer A (20 ml/g acetone powder) containing 2 mM Tris–HCl at pH 8.0 and 0.2 mM $ATPNa_2$, 0.5 mM β-mercaptoethanol, 0.2 mM $CaCl_2$ and 0.005% NaN_3 at different extraction times. Then the fractions were