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Tripolyphosphate hydrolysis by bovine fast and slow myosin subfragment 1 isoforms

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ABSTRACT

Polyphosphates are used in the meat industry to increase the water holding capacity of meat products. Tripolyphosphate (TPP) is a commonly used polyphosphate and it is metabolized into pyrophosphate and monophosphate in meat. The enzymes responsible for its metabolism have not been fully characterized. The motor domain of myosin (subfragment 1 or S1) is a likely candidate. The objectives of this study were to determine if bovine S1 hydrolyzes TPP, to characterize the TPPase activity of the fast (*cutaneous trunci*) and slow (*masseter*) isoforms, and to determine the influence of pH on S1 TPPase activity. S1 hydrolyzed TPP and in comparison with ATP as substrate, it hydrolyzed TPP 16–32% more slowly. Fast S1 hydrolyzed both substrates faster compared to slow S1 and the difference between the isoforms was greater with TPP as the substrate. The V_{max} was 0.94 and 5.0 nmol Pi/mg S1 protein/min while the K_m was 0.38 and 0.90 mM TPP for slow and fast S1, respectively. Pyrophosphate was a strong inhibitor of TPPase activity with a K_i of 88 and 8.3 μM PPi for fast and slow S1 isoforms, respectively. Both ATPase and TPPase activities were influenced by pH with the activity being higher at low pH for both fast and slow S1 isoforms. The activity at pH 5.4 was 1.5 to 4-fold higher than that at pH 7.6 for the different isoforms and substrates. These data show that myosin S1 readily hydrolyzes TPP and suggest that it is a major TPPase in meat.

1. Introduction

Inorganic polyphosphates are used in the meat industry to increase the water holding capacity (WHC) of pork, poultry and beef products (Bernthal, Booren, & Gray, 1991; Gault, 1985; Knipe, Olson, & Rust, 1985; Siegel & Schmidt, 1978, 1979). An increase in WHC improves cook yield and tenderness of the resulting cooked product (Cheng & Sun, 2008). Polyphosphate use has also been linked to a decrease in lipid oxidation (Akamittath, Brekke, & Schanus, 1990; Craig, Bowers, Wang, & Seib, 1996; Tims & Watts, 1958). Polyphosphates were initially used mainly in the poultry (Schwall, Rogers, & Corbin, 1968) and fish industry (Swartz, 1970), with recent applications in pork and current studies on beef in terms of near intact muscle cuts (McGee, Henry, Brooks, Ray, & Morgan, 2003; Vote et al., 2000). Polyphosphate use in comminuted meat products improves yield and is particularly useful for production of low salt products (Barbut, Maurer, & Lindsay, 1988).

The mechanism of action of polyphosphates to increase WHC is multi-factoral, and some of their actions are poorly understood. One mechanism is by altering the pH of the meat. Alkaline polyphosphates increase the pH away from the isoelectric point of proteins, which increases the net charge of myofibrillar proteins thus decreasing filament packing (Cheng & Sun, 2008; Martin, Atkinson, & Merrifield, 2002; Trout & Schmidt, 1983). Another mechanism that is less understood is their action on actomyosin. Polyphosphates, or mainly pyrophosphate (PPi), acts as an ATP analogue and aids in actomyosin dissociation and myosin extraction from the thick filament (Bernthal et al., 1991; Hamm & Neraal, 1977a; Parsons & Knight, 1990; Shen & Swartz, 2010; Xiong, Lou, Harmon, Wang, & Moody, 2000). The dissociation of the actomyosin rigor linkage allows for salt to more effectively extract myosin from the thick filaments. This extraction also enhances the binding and gelation properties of the meat.

The main polyphosphates used in the meat industry are hexametaphosphate (HMP), tripolyphosphate (TPP), and pyrophosphate (PPi) with TPP being the most commonly used polyphosphate. The rank order of the effectiveness of these polyphosphates to improve WHC is PPi > TPP > HMP (Trout & Schmidt, 1984). Of these HMP does not likely interact directly with myosin. However, TPP interacts with myosin but likely does not dissociate actomyosin or facilitate myosin extraction (Yasui, Sakanish, Hashimoto, Fukazawa, & Takahashi, 1964). Studies using PPi found that it influences actomyosin dissociation and myosin extraction from the thick filament (Brenner, Chalovich, Greene, Eisenberg, & Schoenberg, 1986; Hamm & Neraal, 1977a; Ishiwata, Muramatsu, & Higuchi, 1985; Yasui et al., 1964). Studies on the action of TPP

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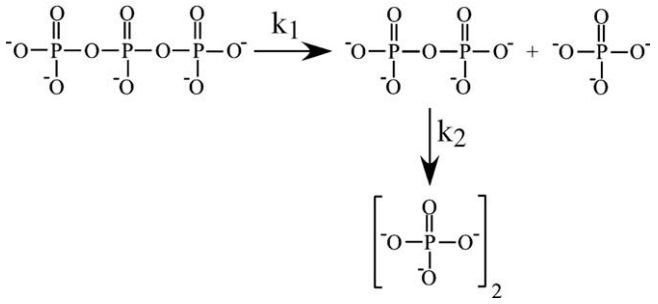
on crude actomyosin preparations (Yasui et al., 1964) demonstrated that TPP influences actomyosin interactions only after hydrolysis to PPi, and other studies showed that TPP is hydrolyzed in meat homogenates (Neraal & Hamm, 1977b). Recent NMR studies on polyphosphate metabolism in marinated chicken breast showed that, while PPi was completely hydrolyzed to monophosphate in 1.5 h, it takes twice as long for TPP to be completely hydrolyzed (Li, Kerr, Toledo, & Teng, 2001). Therefore, the part of the functionality of TPP is dependent upon its metabolism to PPi and the lifetime of PPi in the muscle.

The likely reaction scheme for TPP metabolism in meat is (adapted from Sutton (1973) and Weilmeier and Regenstein (2004):



where k_1 involves TPP hydrolysis by tripolyphosphatase (TPPase) and k_2 involves hydrolysis by pyrophosphatase. The enzymes and the kinetics involved in TPP and PPi hydrolysis in meat systems are partially understood. The pyrophosphatase activity in muscle has been characterized (Morita & Yasui, 1985; Nakamura, Yamaguchi, Morita, & Yasui, 1969) and the enzyme recently isolated and characterized from fish muscle (Goa et al., 2008). The major TPPase in meat is likely myosin (Xiong, 2005), as early studies using crude actomyosin suggested (Friess & Morales, 1955; Yasui et al., 1964). However, this point needs to be confirmed using purified myosin preparations and specifically S1. If TPP is hydrolyzed by myosin, it likely follows the same pathway as the myosin ATPase (Ferenczi, Homsher, Simmons, & Trentham, 1978):

$$M + TPP \rightleftarrows M{-}TPP \rightleftarrows M{-}PPi{-}Pi \rightleftarrows M{-}PPi + Pi \rightleftarrows M + PPi$$

where M = myosin, TPP = tripolyphosphate PPi = pyrophosphate and Pi = monophosphate.

Myosin binds TPP, forming a complex. The TPPase of myosin hydrolyzes TPP into PPi and Pi, with both products still bound to myosin. Pi is released first, then PPi dissociates. Relative to TPP, the binding constant of PPi is higher (Schaub, Watterson, Loth, & Foletta, 1983). Considering this fact, PPi is likely a competitive inhibitor for the myosin TPPase and PPi has been shown to inhibit TPP hydrolysis in meat homogenates (Neraal & Hamm, 1977a).

Because muscles have variations in their flavor characteristics and textural properties due to the proportion of muscle fiber types within the structure (Xiong, 1994), it is important to determine whether the TPPase activities also differ between muscle fiber types assuming myosin is the major TPPase. Differences in ATPase activities between muscle fiber types have been observed (Seidel, Thompson, Gergely, & Sreter, 1964), with fast muscle fiber types having a higher activity than slow. Differences in the amino acid sequence in the loop 1 region of S1 is primarily responsible for variations in ATPase rates and shortening velocity between the isoforms (Sweeney et al., 1998). Comparisons of fast and slow bovine myosin heavy chain sequences showed that the loop 1 region of fast myosin had a longer amino acid sequence than slow (Chikuni, Muroya, & Nakajima, 2004).

The purpose of this study was to determine if S1 is a TPPase and if it is likely the major TPPase in meat, and whether myosin isoform, PPi and pH have an influence on its TPPase activity.

2. Methods

2.1. Myosin isolation

Bovine fast (*cutaneous trunci*) myosin was purified as described in Swartz, Greaser, and Marsh (1990). Bovine slow (*masseter*) myosin was purified as described in Swartz et al. (1990) with modifications. The *masseter* muscle was excised less than 1 h after harvest, cooled on ice for 1 h then the surface fat and connective tissue were removed. The muscle was ground through a 3/8 in. plate. ATP was added to the phosphate/pyrophosphate extraction buffer to give a concentration of 0.1 mM and the ground muscle was extracted for 2 h with occasional stirring. The pH of the extract supernatant was adjusted to 6.8–7.0 with 5 M H_3PO_4 then it was diluted with nine volumes 1 mM EDTA. The crude myosin was collected by centrifugation and washed twice with 20 mM KH_2PO_4 (pH 7.0) and 1 mM EDTA. The pellets were weighed, and suspended in an equal volume of 1 M KCl, 10 mM KH_2PO_4 (pH 7.0) and 1 mM EDTA to give a final concentration of 0.5 M KCl. ATP was added to give a final concentration of 1 mM ATP then 0.75 volumes (relative to solution volume) of 2.7 M $(NH_4)_2SO_4$, 0.5 M KCl, 10 mM KH_2PO_4 (pH 7.0) and 2 mM EDTA were slowly added with constant stirring. The solution was stirred for another 10–20 min, centrifuged at 10,000×*g* for 20 min and the supernatant was collected by filtering through glass wool. Myosin was precipitated by adding 120 g/L solid ammonium sulfate then mixing for 10–20 min before centrifugation at 10,000×*g* for 20 min. The resulting pellets were dissolved in a minimal volume of 0.6 M NaCl, 10 mM $NaHPO_4$ (pH 7.0), 1 mM EDTA and 1 mM DTT and dialyzed four times against 20 volumes (relative to dissolved pellet volume) of 0.6 M NaCl, 10 mM $NaHPO_4$ (pH 7.0), 1 mM EDTA and 1 mM DTT. The absorbance at 280 nm was measured and protein concentration estimated using an extinction coefficient of 0.56 ml/mg. For long-term storage, an equal volume of glycerol was added plus DTT to bring the latter's final concentration to 4 mM final and the protein was stored at −20 °C.

2.2. Bovine S1 purification

Bovine fast and slow S1 were isolated and purified as described in Swartz and Moss (1992) and Weeds and Pope (1977). This procedure separates out the A1 and A2 essential light chain isoforms of rabbit S1 as well as removing other peptide fragments. For bovine S1s, there was either little (fast) or no A2 isoforms so the S1 elutes as mostly one peak during the salt gradient (data not shown). The S1 was desalted on a Sephadex G-25 column equilibrated with the "base buffer" (see below) and diluted at least 5-fold in the final assay.

2.3. SDS–PAGE analyses of fast and slow S1

SDS–PAGE of samples from the columns was used to analyze the elution profile and to assess the purity of the pooled protein peaks. Gels and samples were prepared and electrophoresed, stained and de-stained as described in Fritz, Swartz, and Greaser (1989). Comparison of the pooled protein samples of the isoforms by SDS–PAGE showed that both the slow S1 motor domain and the essential light chain had higher apparent MWs than the fast (data not shown) as shown in a previous study (Shen & Swartz, 2010).