Published in final edited form as:

Meat Sci. 2010 March 1; 84(3): 364. doi:10.1016/j.meatsci.2009.09.003.

Influence of salt and pyrophosphate on bovine fast and slow myosin S1 dissociation from actin

Qingwu W. Shen and Darl R. Swartz*

Department of Animal Science, Purdue University 901 W. State Street, West Lafayette, IN 47907-2054

Abstract

The kinetics of myosin dissociation from actin was investigated and also the impact of salt, MgPPi, and myosin heavy chain isoform on myosin subfragment 1 (S1) dissociation from actin using purified proteins and fluorescence spectroscopy. Both NaCl and MgPPi increased myosin S1 dissociation rate. When salt concentrations increased from 0.1 to 1.0 M, the dissociation rate of S1 from bovine masseter (slow) and cutaneous trunci (fast) muscle increased 38 and 78 fold, respectively. MgPPi had an even greater effect on S1 dissociation from actin. With the addition of MgPPi to the mixture of pyrene actin and S1, the fluorescence increased about 85% within the dead time of the mixing approach. Unlike salt, MgPPi had no apparent difference in its ability to dissociate slow or fast S1 isoforms from actin. The results reveal that salt and MgPPi increase myosin extraction and functionality in meat by weakening the actomyosin interaction and that some of the difference in the functionality of red and white muscle may be related to actomyosin dissociation.

Keywords

Myosin; Actin; Salt; MgPPi; Dissociation rate; Water holding capacity; Myosin isoforms

INTRODUCTION

Myosin is the major functional protein in muscle foods (Fukazawa, Hashimoto & Yasui, 1961a; Fukazawa, Yasui & Hashimoto, 1961b). It functions as the primary water and protein binding agent as well as fat holding protein in products (Acton, Ziegler & Burge, 1983; Asghar, Samejima, & Yasui, 1985; Hamm, 1986). In meat without added salt, myosin has limited functionality because it is constrained within the thick filaments of the myofibrils. It must be released and/or re-organized within the muscle matrix for its functionality to come to the fore. The addition or injection of salt and/or pyrophosphate (PPi) into meat facilitates extraction of myosin from the thick filament of the A-band, which results in the swelling of myofibrils and increased water holding capacity of meat (Offer & Trinick, 1983; Xiong, Lou, Harmon, Wang, & Moody, 2000b).

© 2009 Elsevier Ltd. All rights reserved.

*Correspondence to: Department of Animal Science, Purdue University 901 W. State Street, West Lafayette, IN 47907-2054 Tel: (765) 494-8282 Fax: (765) 494-6816 drswartz@purdue.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Since the discovery that phosphates increase meat water retention (Bendall, 1954), they have been widely used in meat and meat products, especially in reduced-fat, water-added meat products to improve quality and in place of salt for health reasons (Knipe, Olson, & Rust, 1985; Trout & Schmidt, 1983; Xiong, 2005). Previous work ascribed the increased meat hydration by salt and polyphosphates to: (1) chloride binding to the filaments to increase repulsive electrostatic forces between filaments (Offer et al., 1983), (2) ionic effects and pH alteration contributed by the polyphosphates, and (3) the dissociation of the actomyosin complex caused primarily by MgPPi (Bendall, 1954; Hamm, 1970; Trout et al., 1983; Wu & Smith, 1987). Structural studies by optical microscopy showed salt and polyphosphates induced thick filament dissolution/myosin extraction. Irrigation of myofibrils with 10 mM PPi decreased the critical salt concentration required for myofibril swelling and myosin dissolution from thick filaments (Offer et al., 1983; Xiong, Lou, Wang, Moody, & Harmon, 2000a; Xiong, 2005; Xiong et al., 2000b). Furthermore, addition of PPi changed the pattern of myosin extraction. With only salt, thick filament dissolution began from the center of A-band. In the presence of 10 mM PPi, dissolution of the thick filaments occurred at both ends of the A-band. All these studies provided valuable knowledge on meat hydration and quality. However, the molecular mechanism regarding myosin extraction from thick filament and the rationale behind the impact of salt, polyphosphate, pH and temperature on thick filament disassembly and meat quality is still poorly understood.

Our long-term goals are to understand the molecular mechanisms involved in salt and polyphosphate induced structural disruption of muscle tissue to form high quality and healthy muscle foods. Our hypothesis is that there are two major processes involved in myosin extraction from the myofibril (Fig. 1). Process 1 involves dissociation of myosin from actin and process 2 involves dissociation of myosin from the thick filament. Salt impacts both processes while MgPPi impacts primarily process 1. In rigor muscle, process 1 rate-limits myosin dissociation when using salt alone whereas process 2 is rate limiting when using MgPPi only or salt and MgPPi. This explains the reported different myosin dissociation patterns in the absence and presence of PPi (Offer et al., 1983).

In this study, focused is on process 1 in figure 1 and investigated the influence of salt, PPi, and myosin isoform on myosin dissociation kinetics from actin. The results show that salt concentration, MgPPi, and myosin isoforms have major effects on S1 binding to/dissociation from actin.

MATERIALS AND METHODS

Protein purification, labeling and concentration determination

Bovine cutaneous trunci (fast muscle) and masseter (slow muscle) S1 were purified from bovine market weight steers (Swartz, Greaser & Marsh, 1993) with minor modifications. To increase the yield of masseter myosin, 0.1 mM ATP was used in the extraction step; 1 mM ATP and a lower volume of (0.75 volume, rather than 1 volume) 2.7 M $(\text{NH}_4)_2\text{SO}_4$, 0.5 M KCl, 10 mM potassium phosphate (pH 7.0), and 2 mM EDTA were used to separate myosin from non-myosin protein, and more solid $(\text{NH}_4)_2\text{SO}_4$ (50% saturation) was used in the final precipitation of myosin. S1 was isolated by chymotryptic digestion of myosin and ion-exchange chromatography (Swartz & Moss, 1992). S1 was desalted on a Sephadex G-25 column into 20 mM MOPS (pH 7.0), 5 mM MgCl_2 , 1 mM NaN_3 , 1 mM DTT, stored on ice and used within two days. Actin was isolated as described by Pardee & Spudich, (1982) and was labeled with pyrene iodoacetimide (Criddle, Geeves, & Jeffries, 1985) with minor modifications (cycling from F to G-actin after conjugation, high speed centrifugation, then converting back to F-actin). Actin and pyrene actin were stored on ice and used within two weeks. The protein concentration of S1 was determined using an assumed $E^{280\text{nm}}$ of 0.75 ml/mg and MW of 120,000. Actin concentration was determined using $E^{280\text{nm}} = 1.104 \text{ ml/mg}$ and

MW = 43,000. The protein concentration of pyrene actin was determined by the BCA assay using unlabeled actin as the standard.

SDS-PAGE and gel staining

Protein purity was determined by SDS-PAGE and Coomassie blue staining. The gels, sample buffer and staining was as described by Fritz, Swartz, & Greaser, (1989). Briefly, 100 l purified bovine masseter or cutaneous trunci S1 or pyrene actin from rabbit muscle were first mixed with an equal volume of 2 SDS sample loading buffer. After heated at 95 C for 3 min, samples (4 g/lane) were loaded onto 10% SDS-PAGE, electrophoresed, and the gel was stained with Coomassie blue. After destaining, the gel was scanned with a flat-bed scanner.

S1-actin binding assay

The fluorescence intensity of labeled pyrene actin was used to monitor S1 binding to actin. After S1 binds to pyrene actin, actin changes conformation and the fluorescence decreases. The fluorescence intensity of pyrene actin was determined by fluorescence spectroscopy using a PerkinElmer Luminescence Spectrometer LS 50 B. Excitation wavelength was 365 nm. Emission scans were used for purified and labeled protein function testing. An emission wavelength of 385 nm was used for dissociation/binding studies. In all experiments, both excitation and emission slit widths were set to 10 nm. The contents of the cuvette were continuously stirred with a magnetic stirrer and held at 20 C.

Measurement of dissociation rate of S1 from actin

To measure the apparent dissociation rate of S1 from actin, reaction conditions were as shown in figure 2. Unlabeled actin was added in 10-fold molar excess over pyrene actin, which forced the reaction to proceed from reaction 1 (S1 dissociation from pyrene actin) to reaction 2 (S1 association with unlabeled actin). The rate of the increase in fluorescence was thus a measure of the dissociation rate of S1. In the experiments, 0.2 M pyrene actin was first mixed with 0.3 M S1 and incubated for at least 10 min. The mixture of pyrene actin and S1 was then added to the cuvette. After about 70s, unlabeled actin (2 M) was rapidly injected via a syringe into the cuvette and the change in fluorescence monitored over time. The buffer components and concentrations are given in the figures. The fluorescence transients were fitted to a single exponential equation to obtain the apparent rate and data for PPi titration studies were fitted to a single binding site equation using GraphPad Prism (5.0a, La Jolla, CA).

RESULTS AND DISCUSSION

Protein purity and function testing

Bovine masseter (slow or type 1) and cutaneous trunci (fast, mostly type IIX) muscle were used to isolate S1. This gives the extremes in terms of myosin heavy chain isoforms so that, if needed, they can be admixed to mimic the features of other muscles containing mixtures of slow and fast myosin heavy chain isoforms. The purity of these proteins is shown in Figure 3. S1 was prepared via EDTA-chymotrypsin digestion giving only the essential light chain associated with the motor domain. As shown in the gel, the motor domain and essential light chain of cutaneous trunci S1 are of smaller than those of masseter S1. On a separate gel, only one protein band was detected for pyrene actin, showing it is pure actin.

The ability of pyrene actin to monitor S1 binding and for unlabeled actin to compete with pyrene actin for S1 binding was determined by fluorescence spectroscopy. Figure 4 shows an emission scan of pyrene-actin alone, after addition of S1, and after addition of S1 and then excess unlabeled F-actin. With pyrene actin alone, there are peaks near 389 and 410 nm. Upon addition of S1, the fluorescence decreased 70 – 80%, showing the functionality of fluorescence

labeled pyrene actin. Upon addition of a 10 fold excess of unlabeled actin followed by an incubation period, the fluorescence intensity of the S1 and actin mixture returned to the same level as prior to addition of S1. This shows that the unlabeled actin can effectively compete with pyrene actin for and release pyrene actin from S1.

To further test the function of the purified proteins, pyrene actin was titrated with S1. In this experiment, 10 μ l aliquots of cutaneous trunci S1 (aliquot concentration starting at 1.5 μ M and ending at 70 μ M) were added to 3 ml (initial) of a continuously stirred, thermo-stated cuvette containing 0.12 μ M pyrene actin and 0.3 μ M phalloidin to stabilize actin in the F-form. The S1 additions were done at 100s intervals with the spectrometer set in time mode. Figure 5 shows the fractional change in fluorescence as a function of time. Pyrene actin alone showed less than a 1% change in fluorescence over 2000s indicating that the pyrene actin is stable during the time span of the experiments. Addition of aliquots of S1 resulted in declines in fluorescence such that at the highest level of total S1, fluorescence was quenched almost 80%. In summary, pure S1 and pyrene-labeled actin can be made, the fluorescence of pyrene actin is stable and quenched by S1, and unlabeled actin can compete with pyrene actin for S1 binding.

Influence of [NaCl] and S1 isoform on rigor S1 dissociation from actin

High salt in food causes hypertension and cardiovascular disease. Understanding of the mechanism of muscle hydration will help to lower salt use in meat without changing product quality. Structural studies using isolated myofibrils showed that salt increases myofibril hydration by dissolving thick filaments (Offer et al., 1983; Xiong et al., 2000a; Xiong et al., 2000b). Based on these previous studies, we hypothesize that two steps are involved in myosin extraction from myofibrils: 1) dissociation of myosin from actin and 2) breakage of thick filament into myosin monomers (Fig. 1). Both steps can be rate-limiting during myosin extraction from myofibrils under different conditions.

To measure the apparent dissociation rate of S1 from actin and the influence of salt and myosin isoforms on S1 dissociation from actin, reaction conditions were as shown in figure 2. Unlabeled actin was rapidly added to pyrene actin and S1 mixture to force the reaction to proceed from reaction 1 (S1 dissociation from pyrene actin) to reaction 2 (S1 association with unlabeled actin) as shown in figure 2. The rate of increase in fluorescence was thus used to estimate the apparent dissociation rate of S1 from actin. An example is shown in figure 6 where a 10 fold excess of unlabeled actin was added to either cutaneous trunci S1-pyrene actin or masseter S1-pyrene actin. Upon addition of unlabeled actin, there was an increase in the fluorescence resulting from S1 dissociating from pyrene actin and associating with unlabeled actin. Comparison of the fluorescence transients for cutaneous trunci S1 with masseter S1 shows: (1) The masseter S1 initial fluorescence level was lower than that of cutaneous trunci. Since pyrene actin and S1 were mixed for at least 10 min before the addition of unlabeled actin, the binding of S1 to pyrene actin reached equilibrium prior to the addition of unlabeled actin. Thus the lower initial fluorescence level of masseter suggests that the dissociation constant (K_d) of slow S1 is lower than fast S1. This suggests that under rigor conditions and low salt (100 mM NaCl in figure 6), there are more strong crossbridges in slow muscle than in fast muscle. (2) The final fluorescence level after addition of unlabeled actin was similar for both S1 isoforms, indicating 10 fold excess of unlabeled actin can similarly replace pyrene actin independent of the S1 isoform. (3) Cutaneous trunci S1 dissociation was much faster than masseter. Single exponential fits were used to obtain the apparent dissociation rate of S1 from actin and the influence of salt concentration and S1 isoform on the apparent dissociation rate of S1 from actin are shown in figure 7. Again, it is apparent that cutaneous trunci S1 dissociation from actin is much faster than masseter S1 at all salt concentrations. The slope factors to an empirical second order polynomial fit are 5 - 10 times greater for cutaneous trunci than masseter. Also, figure 7 shows that NaCl has a greater influence on cutaneous trunci S1

dissociation from actin than masseter S1. When the NaCl concentration increased from 50 to 1000 mM, the dissociation rate of cutaneous S1 increased 78 fold while masseter S1 only increased 38 fold. All these data show that there is a difference in the kinetics of S1 binding to or dissociation from actin between fast and slow S1. The greater influence of salt on fast S1 dissociation from actin and the lower apparent dissociation rate of the slow S1 isoform indicate that S1 domain dissociation from thin filaments in myofibrils could limit myosin dissociation more for slow than fast myosin.

Influence of MgPPi on S1 dissociation from actin

Pyrophosphate (or in the form of tripolyphosphate) is widely used in combination with salt to increase meat water holding capacity and production yield. Previous studies suggest that PPi increases muscle hydration by dissociating myosin from actin (Bendall, 1954; Hamm, 1970). Here the influence of PPi on S1 binding to actin was investigated using pyrene actin. 0.2 M pyrene actin and 0.3 M cutaneous trunci S1 were first mixed and incubated for at least 10 min and then added to the spectrometer. After about 70s, 4 mM (final) MgPPi was added to the mixture of pyrene actin and S1. The change in fluorescence was monitored. Figure 8 shows that, after addition of 4 mM MgPPi, the fluorescence increased rapidly by about 85%. This indicates that PPi splits the actomyosin complex that restrains myofibril swelling and inhibits muscle hydration (Offer et al., 1983; Xiong et al., 2000b). To further characterize the impact of PPi on S1 binding to actin, pyrene actin-S1 was titrated with PPi. 0.2 M pyrene actin and 0.3 M cutaneous trunci or masseter S1 were first mixed and incubated for at least 10 min and then added to the spectrometer. After about 70s, 10 μ l aliquots of PPi (initially 10 μ M progressing to 100 mM) were added over 100 s intervals, and the fluorescence transient was monitored. Figure 9 shows that with the addition of PPi, the fluorescence increases for both fast and slow S1, with estimated Kds of 6.0 and 7.9 μ M for cutaneous trunci and masseter S1, respectively.

Factors influencing myosin-actin interactions

To maximize its functionality, myosin needs to be released from filaments. In this process, the dissociation of myosin from thin filament actin can be rate limiting. The influence of salt, PPi, and myosin isoforms on the kinetics of S1 dissociation from actin using purified proteins and fluorescence spectroscopy was investigated. In agreement with previous observations (Offer et al., 1983; Xiong et al., 2000b), the data showed that salt increases the S1 dissociation rate (Figure 7) from actin. All these data suggest that addition of salt to meat increases myosin dissociation from actin, and thus, the extractability and functionality of myosin in meat.

MgPPi also increased the dissociation of S1 from actin, which contributes the large effect of PPi on myofibril swelling and thick filament dissolution (Offer et al., 1983; Xiong et al., 2000b). This is reasonable because the binding of the myosin motor domain to actin depends on the nucleotide or nucleotide analogue bound to S1. Without bound nucleotide, the myosin motor domain binds to actin strongly. After MgPPi binds to myosin, the myosin interaction with actin becomes weak (Gordon, Homsher & Regnier, 2000; Spudich, 2001). Stiffness studies (a measure of the “strength” of myosin interaction with actin) by Brenner, Chalovich, Greene, Eisenberg, and Schoenberg (1986) showed that the stiffness of skinned single muscle fibers was reduced to about 10% of rigor at low strain rates when 4 mM MgPPi was present. In another study, Ishiwata, Muramatsu and Higuchi (1985) found that 5 mM MgPPi reduced the critical KCl concentration for significant stiffness change in skinned muscle fibers and the apparent rate of thick filament disassembly from 0.5 to 0.1 M. Thus MgPPi increases myofibril swelling and myosin extraction in meat by increasing myosin dissociation from actin. Another finding is that, although NaCl has a greater effect on fast isoforms of S1 dissociation from actin, MgPPi has no apparent difference in its ability to dissociate fast and slow S1 from actin (Figure 9). The binding rate of MgPPi to S1 is so fast (Figure 8) that it is difficult to measure the rate without the use of a stopped-flow device.

The myosin heavy chain isoform contributes to differences in final meat product quality due to difference in extraction and gelation properties (Xiong, 1994). Xiong and Brekke (1989) showed a greater extraction of proteins with NaCl for white compared to red muscle and the time course of the change in protein solubility and viscosity suggested that the rates were slower for red than white. After irrigation for 3 min, swelling of myofibrils from chicken white muscle was also greater than that of myofibrils from red muscle at the same NaCl concentration in the absence of PPi (Xiong et al., 2000b). Without PPi, the extraction of rigor myosin from myofibrils is rate-limited by the dissociation of the actomyosin complex. These previous studies indicate that the greater and faster myosin extraction from myofibrils of white muscle is at least partially mediated by faster dissociation of myosin from actin.

Muscle contraction, due to myosin interaction with actin, which finally leads to muscle contraction and force generation, is initiated by Ca^{2+} binding to troponin C (TnC), which leads to the movement of tropomyosin (Tm) away from myosin binding site on actin. Myosin then interacts with actin and muscle contracts (Haselgrove, 1973; Huxley, 1973; Parry & Squire, 1973). These studies suggest that Ca^{2+} and muscle thin filament regulatory protein could regulate myosin interaction with actin and myosin extractability in rigor meat. This was confirmed by single muscle fiber stiffness studies. Brenner et al. (1986) showed that the salt and PPi induced declines of skinned muscle fiber stiffness were reduced in the presence of Ca^{2+} . Ishiwata et al. (1985) studied stiffness and apparent disassembly of thick filaments using optical diffraction in skinned muscle bundles. They found the skinned muscle fiber bundle was stiffer in the presence of Ca^{2+} when the other conditions were the same. Calcium also altered KCl and MgPPi induced thick filament disassembly. Higuchi and Ishiwata (1985) measured the apparent rate of thick filament disassembly in skinned muscle fiber bundles as a function of environmental conditions. The apparent rate in the presence of 5 mM MgPPi increased with increasing KCl and pH but decreased with Ca^{2+} and an increase in temperature. Removal of Ca^{2+} increased the rate of thick filament disassembly 5 – 10 fold at 0.3M KCl. This Ca^{2+} effect was sarcomere-length dependent being less as the extent of thin and thick filament overlap decreased (i.e fewer actin-myosin interactions). In consideration of the high Ca^{2+} concentration in rigor meat (about 0.1 mM since it is no longer sequestered by the sarcoplasmic reticulum), it is probable that Ca^{2+} plays an important role in myosin extraction and functionality in meat. The above studies and others (Xiong et al., 2000b) also suggest that pH and temperature play a role in myosin extraction and thick filament disruption. Limited work has been done investigating the effects of Ca^{2+} , pH, and temperature on myosin extraction in meat. Detailed studies are needed to determine the mechanisms involved so that processes can be enhanced to maximize muscle protein functionality.

Reference

- Acton JC, Ziegler GR, Burge DL. Functionality of muscle constituents in the processing of comminuted meat-products. *Crc Critical Reviews in Food Science and Nutrition* 1983;18:99–121. [PubMed: 6337783]
- Asghar A, Samejima K, Yasui T. Functionality of muscle proteins in gelation mechanisms of structured meat-products. *Crc Critical Reviews in Food Science and Nutrition* 1985;22:27–106. [PubMed: 3899516]
- Bendall J. The swelling effect of polyphosphate on lean meat. *Journal of the Science of Food and Agriculture* 1954;5:468–475.
- Brenner B, Chalovich JM, Greene LE, Eisenberg E, Schoenberg M. Stiffness of skinned rabbit psoas fibers in mgatp and mgppi solution. *Biophysical Journal* 1986;50:685–691. [PubMed: 3022835]
- Criddle AH, Geeves MA, Jeffries T. The use of actin labeled with n-(1-pyrenyl)iodoacetamide to study the interaction of actin with myosin subfragments and troponin tropomyosin. *Biochemical Journal* 1985;232:343–349. [PubMed: 3911945]

- Fritz JD, Swartz DR, Greaser ML. Factors affecting polyacrylamide gel electrophoresis and electroblotting of high-molecular-weight myofibrillar proteins. *Analytical Biochemistry* 1989;180:205–210. [PubMed: 2817350]
- Fukazawa T, Hashimoto Y, Yasui T. The relationship between components of myofibrillar protein and the effect of various phosphates that influence the binding quality of sausage. *Journal of Food Science* 1961a;26:550–555.
- Fukazawa T, Yasui T, Hashimoto Y. Effect of some proteins on binding quality of an experimental sausage. *Journal of Food Science* 1961b;26:541–&.
- Gordon AM, Homsher E, Regnier M. Regulation of contraction in striated muscle. *Physiological Reviews* 2000;80:853–924. [PubMed: 10747208]
- Hamm, R. Interaction between phosphates and meat proteins. In: DeMann, JM.; Melnychyn, P., editors. *Phosphates in Food Processing*. AVI Publishing Co.; Westport, CT: 1970. p. 65-82.
- Hamm, R. Functional properties of the myofibrillar system and their measurement. In: Bechtel, PJ., editor. *Muscle as Food*. Academic Press; New York: 1986. p. 135-199.
- Haselgrove JC. X-ray evidence for a conformational change in actin-containing filaments of vertebrate striated-muscle. *Cold Spring Harbor Symposia on Quantitative Biology* 1973;37:341–352.
- Higuchi H, Ishiwata S. Disassembly kinetics of thick filaments in rabbit skeletal muscle fibers - effects of ionic strength, Ca^{2+} concentration, pH, temperature, and cross-bridges on the stability of thick filament structure. *Biophysical Journal* 1985;47:267–275. [PubMed: 2983792]
- Huxley HE. Structural changes in actin-containing and myosin-containing filaments during contraction. *Cold Spring Harbor Symposia on Quantitative Biology* 1973;37:361–376.
- Ishiwata S, Muramatsu K, Higuchi H. Disassembly from both ends of thick filaments in rabbit skeletal-muscle fibers - an optical diffraction study. *Biophysical Journal* 1985;47:257–266. [PubMed: 2983791]
- Knipe CL, Olson DG, Rust RE. Effects of sodium-hydroxide and selected inorganic phosphates on the characteristics of reduced sodium meat emulsions. *Journal of Food Science* 1985;50:1017–1020.
- Offer G, Trinick J. On the mechanism of water holding in meat - the swelling and shrinking of myofibrils. *Meat Science* 1983;8:245–281.
- Pardee JD, Spudich JA. Purification of muscle actin. *Methods in Cell Biology* 1982;24:271–289. [PubMed: 7098993]
- Parry DAD, Squire JM. Structural role of tropomyosin in muscle regulation - analysis of x-ray-diffraction patterns from relaxed and contracting muscles. *Journal of Molecular Biology* 1973;75:33–55. [PubMed: 4713300]
- Spudich JA. The myosin swinging cross-bridge model. *Nature Reviews Molecular Cell Biology* 2001;2:387–392.
- Swartz DR, Greaser ML, Marsh BB. Structural studies of rigor bovine myofibrils using fluorescence microscopy .1. Procedures for purification and modification of bovine muscle proteins for use in fluorescence microscopy. *Meat Science* 1993;33:139–155.
- Swartz DR, Moss RL. Influence of a strong-binding myosin analog on calcium-sensitive mechanical-properties of skinned skeletal-muscle fibers. *Journal of Biological Chemistry* 1992;267:20497–20506. [PubMed: 1400367]
- Trout G, Schmidt GR. Utilization of phosphates in meat products. *Reciprocal Meat Conference Proceedings* 1983;36:24–27.
- Wu FY, Smith SB. Ionic Strength and Myofibrillar Protein Solubilization. *Journal of Animal Science* 1987;65:597–608. [PubMed: 3624103]
- Xiong YL, Brekke CJ. Changes in protein solubility and gelation properties of chicken myofibrils during storage. *Journal of Food Science* 1989;54:1141–1146.
- Xiong YL, Lou X, Wang C, Moody WG, Harmon RJ. Protein extraction from chicken myofibrils irrigated with various polyphosphate and nacl solutions. *Journal of Food Science* 2000a;65:96–100.
- Xiong YLL. Myofibrillar protein from different muscle-fiber types - implications of biochemical and functional-properties in meat processing. *Critical Reviews in Food Science and Nutrition* 1994;34:293–320. [PubMed: 8068202]

- Xiong YLL. Role of myofibrillar proteins in water-binding in brine-enhanced meats. *Food Research International* 2005;38:281–287.
- Xiong YLL, Lou XQ, Harmon RJ, Wang CZ, Moody WG. Salt- and pyrophosphate-induced structural changes in myofibrils from chicken red and white muscles. *Journal of the Science of Food and Agriculture* 2000b;80:1176–1182.

Figure 1. Model for myosin extraction from muscle

The two major processes involved in myosin dissociation from the myofibril. Process 1 involves dissociation of actomyosin and process 2 involves dissociation of myosin from the thick filament. Salt impacts both processes while PPi impacts primarily process 1.

Figure 2. Reaction scheme and conditions to measure dissociation (k_{off}) of S1 from actin
 Reaction 1 is dissociation of S1 from pyrene actin to give the high fluorescence state of pyrene actin. Reaction 2 is S1 association with unlabeled actin. Reaction conditions are designed to give a pseudo first order reaction.

Figure 3. SDS-PAGE of S1 and actin

Masseter (ma) and cutaneous trunci (ct) S1 were isolated from their respective bovine myosins. Actin was purified from rabbit muscle and labeled with pyrene iodoacetimide. Samples (4 μ g/lane) were loaded onto 12% SDS-PAGE, electrophoresed, stained and scanned.

Figure 4. Emission spectra of pyrene-labeled F-actin alone, after addition of S1, and after addition of unlabeled actin

Pyrene actin (0.2 M) was added to the cuvette and an emission scan performed. Cutaneous trunci S1 was added (0.3 M final) and the sample scanned again. Finally, unlabeled F-actin was added to 2.0 M final concentration and after 20 min, the sample was scanned. Buffer conditions: 100 mM NaCl, 20 mM MOPS (pH 7.0), 5 mM MgCl₂, 1 mM NaN₃, 1 mM DTT. Excitation wavelength was 365 nm with a 10 nm slit width.

Figure 5. Titration of pyrene actin with S1

Ten μ l aliquots of cutaneous trunci S1 were titrated into pyrene actin (0.12 M) at 100 s intervals and the fluorescence emission continuously monitored over 2000s. Upper data trace shows the change in fluorescence of pyrene actin alone. Lower traces show sequential decline in fluorescence upon each addition of S1. Buffer conditions were as in Fig. 4. Excitation and emission wavelengths were 365 and 385 nm. Both excitation and emission slit widths were 10 nm.

Figure 6. Fluorescence transients used to estimate S1 dissociation rate

Pyrene actin (0.2 M) and S1 (0.3 M) were mixed and incubated for > 10 min, then added to the spectrometer cuvette. After about 70s, unlabeled actin (2 M) was “rapidly” injected into the cuvette and the change in fluorescence monitored over time. Buffer and spectrometer settings are the same as in figure 5. Protein concentrations are final.

Figure 7. Impact of [NaCl] on S1 dissociation rate

Experiments were done as in Figure 6 with the exception of NaCl concentration and use of higher S1 (about 10 fold) at 1.0 M NaCl to give higher saturation of pyrene actin with S1. Data points represent the mean of 3-4 observations for each S1 at each [NaCl]. Smooth lines are from second order polynomial fits giving: $Y = -0.017 + 0.46 * X + 0.35 * X^2$, $r = 0.98$ and $Y = 0.0016 + 0.039 * X + 0.086 * X^2$, $r = 0.99$ for cutaneous trunci and masseter S1.

Figure 8. Dissociation of S1-pyrene actin by MgPPi

Cutaneous trunci S1 was mixed with pyrene actin as in Figure 6, incubated, and at the arrow, 150 μ l of PPi (4 mM final) was injected into the cuvette. Buffer conditions and spectrometer settings conditions were as in Figure 5.

Figure 9. Dissociation of S1 from actin by MgPPi

Pyrene actin and either bovine cutaneous trunci or masseter S1 were mixed as in Figure 6. Aliquots of PPi were added and fluorescence intensity measured as in Figure 5. Data were corrected for dilution and fitted with a one binding site hyperbola. Buffer conditions and spectrometer settings were as in Figure 5.