EFFECTS OF SALT AND PHOSPHATES ON MYOFIBRILLAR/ CYTOSKELETAL PROTEINS OF BOVINE SKELETAL MUSCLE

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SUMMARY

The effects of sodium chloride (NaCl) and pyrophosphate (PP) were examined by treating beef *sternomandibularis* muscle tissue and isolated myofibrils with various concentrations of NaCl, with and without pyrophosphate (PP), tripolyphosphate (TPP) and polyphosphate glassy (PG). By using phase microscopy, it was observed that swelling of myofibrils increased with an increase in NaCl concentration. Less NaCl in the presence of 10 mM PP, or TPP, was required to achieve maximal swelling. Gel electrophoretograms showed that higher NaCl concentrations (1.0 M 0.7 M 0.4 M) increased the extraction of titin and other myofibrillar proteins. The addition of 10 mM PP and TPP to the NaCl solutions enhanced the extraction of these proteins. Water-holding capacity (WHC) was increased by higher NaCl concentrations and the presence of 10 mM PP and TPP. Increased myofibrillar/ cytoskeletal protein extraction, especially titin, was associated with increased myofibril swelling and increased WHC.

INTRODUCTION

Retention of moisture in meat and meat products is termed water-holding capacity (WHC), and the importance of WHC to meat quality is well-known (Hamm, 1960). Sodium chloride (NaCl) and alkaline phosphates improve WHC of meat (Hamm, 1960; 1970).

Offer and Trinick (1983) studied the disruptive effect of NaCl and pyrophosphate (PP) on isolated rabbit *psoas* myofibrils. Their results suggested that the observed changes in myofibril volume might be due to the disruption of structural constraints, such as Z-lines, M-lines and actinmyosin crossbridges, that exist within the myofibril. The two extremely large myofibrillar/ cytoskeletal proteins, titin and nebulin, however, were not investigated by them as possible constraints to myofibril volume and WHC.

Titin and nebulin make up about 15 percent of the total myofibrillar proteins (Wang et al., 1979; Wang and Williamson, 1980). Also, these proteins have been shown to be the major proteins of a third set of elastic longitudinal endosarcomeric filaments, gap filaments (LaSalle et al., 1983; Wang et al., 1984).

The objectives of this study were: to determine the effects of different concentrations of NaCl and phosphates on myofibrils and myofibrillar/ cytoskeletal proteins by using 1) phase-contrast microscopy to determine the extent of myofibril swelling and structural changes, 2) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to detect changes in myofibrillar/ cytoskeletal proteins, particularly titin and nebulin, and 3) to relate myofibril swelling and myofibrillar/ cytoskeletal protein changes to a physical measure of WHC.

EXPERIMENTAL METHODS

Beef myofibrils were prepared from beef *sternomandibularis* (STM) (Knight and Parsons, 1984). Myofibrils were irrigated as described by Offer and Trinick (1983) and observed by using a Zeiss photomicroscope. Myofibrils were irrigated with a series of increasing NaCl concentrations, 0.1 M, 0.4 M, 0.7 M and 1.0 M NaCl, with or without 10 mM tetra sodium pyrophosphate (PP), tripolyphosphate (TPP) and polyphosphate glassy (PG) in 1 mM $MgCl_2$, 10 mM Na acetate, pH 5.5.

Three protein fractions from ground STM were prepared for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The first fraction was a muscle/ NaCl/ NaCl + 10 mM of each of three phosphates homogenate (MSF). The second fraction was purified myofibrils (MFF) (Goll et al., 1974), and the third fraction was the supernatant obtained from the first wash in the purified myofibril preparation (SNF).

SDS-PAGE of the MSF, SNF and MFF fractions was performed according to the method of Paterson and

Table 1. Mean water-holding capacity (WHC) values[a] of beef muscle treated with various NaCl and NaCl + 10mM PP solutions[b]

Treatment Solutions	WHC
0.1M NaCl	95.0[u]
0.1M NaCl + 10mM PP	105.0[v]
0.4M NaCl	110.0[v]
0.4M NaCl + 10mM PP	128.0[w]
0.7M NaCl	132.0[w]
0.7M NaCl + 10mM PP	157.0[y]
1.0M NaCl	142.0[x]
1.0M NaCl + 10mM PP	171.0[z]
S.E.	3.2

[a] n = 20.

[b] 100% = Original tissue weight.

[uvwxyz] Means that bear unlike superscripts differ significantly (P<0.05).

Fig. 1. 3.2% SDS-PAG electrophoretogram of beef muscle/NaCl/PP after treatment with various NaCl and NaCl + PP solutions

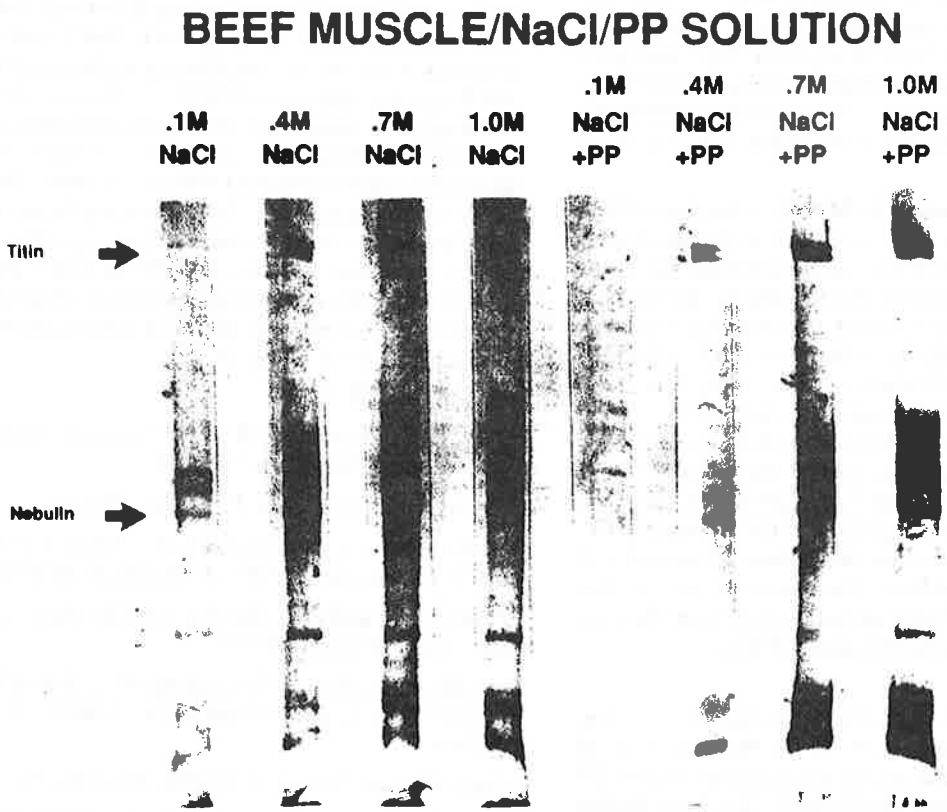
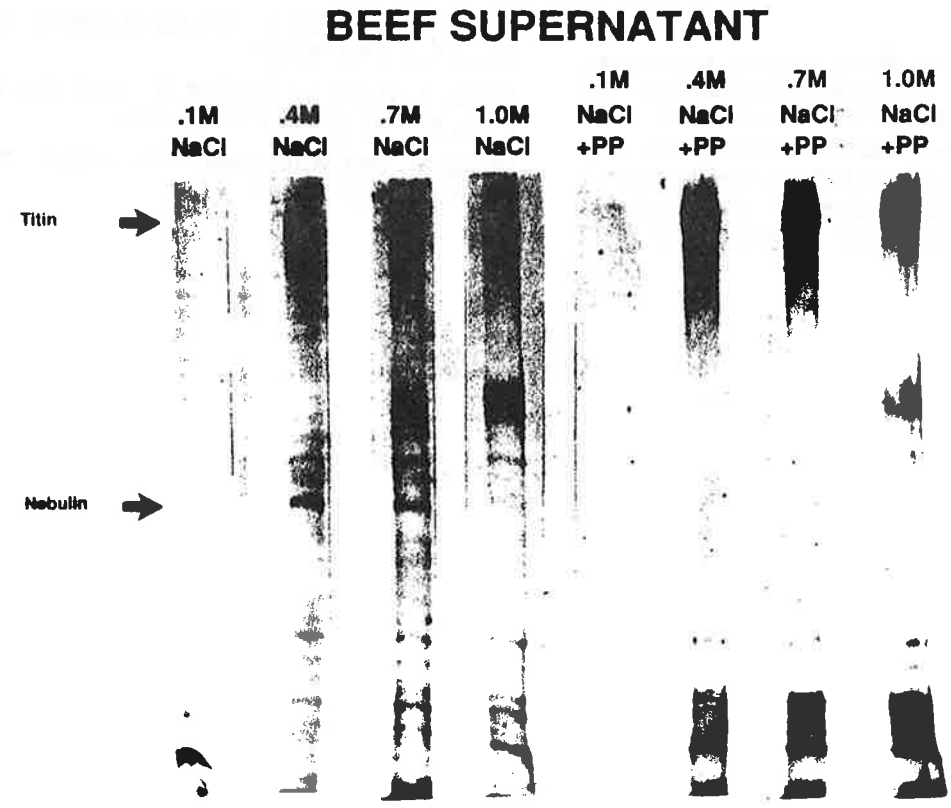


Fig. 2. 3.2% SDS-PAG electrophoretogram of beef supernatant after myofibrils were treated with various NaCl and NaCl + PP solutions



Parrish (1986) using 3.2% polyacrylamide (acrylamide/bisacrylamide, 37:1) slab gels (pH 8.0). Gels were silver stained by the procedure of Heukeshoven and Dernick (1985).

Water-holding capacity (WHC) was determined on homogenates 1:3 w/v (meat to NaCl or NaCl + 10 mM of each of three phosphates) as a percentage of contents retained after heating to 80°C.

RESULTS AND DISCUSSION

Myofibril swelling and extraction

Maximal beef myofibril swelling was achieved by irrigation with 1.0 M NaCl or with 0.7 M NaCl + 10 mM of each of three phosphates. The addition of 10 mM PP and TPP did not affect maximal myofibril swelling, but it did lower the NaCl concentration necessary to achieve it from 1.0 M to 0.7 M NaCl.

Irrigating solutions containing just NaCl preferentially extracted myofibrillar protein from centers of myofibril A-bands; whereas, irrigating solutions containing both NaCl and 10 mM PP extracted myofibrillar proteins from the ends of A-bands. Our results on myofibril swelling and extraction closely agree with those reported by Ishiwata (1981), Offer and Trinick (1983), and Knight and Parsons (1984).

The 3.2% SDS-gel electrophoretogram of the MSF protein fraction showed as NaCl concentrations increased from 0.1 M to 1.0 M NaCl, more of the titin doublet was extracted (increased intensity of bands) from beef STM (Fig.1). The addition of lOmM PP or TPP to NaCl solutions extracted more titin than did solutions with NaCl alone. Nebulin was more effectively extracted with higher NaCl concentrations as was titin; however, unlike titin, NaCl solutions extracted more

nebulin than NaCl + 10 mM PP or TPP solutions (Fig.1).

The electrophoretogram of the SNF fraction was similar to the MSF fraction inasmuch as more titin was present as the NaCl concentration increased (Fig.2). Also, the titin protein bands were more pronounced when 10 mM PP was combined with NaCl than when NaCl was used alone. The small amount of nebulin detected by the 3.2% SDS-gels of the SNF fraction appeared more prominently at the higher NaCl concentrations in the absence of 10 mM PP.

The electrophoretogram of the MFF had decreased titin band intensity at the higher NaCl concentrations, especially when 10 mM PP was combined with NaCl (not shown). This result indicates that the greater amounts of titin detected in the MSF and SNF fractions were actually due to the extraction effects of NaCl or NaCl + 10 mM PP rather than to the presence of more protein in those samples. Nebulin protein bands also showed decreased intensity at the higher NaCl concentrations, but only when 10 mM PP was absent. Offer and Trinick (1983) examined the effect of NaCl and pyrophosphate on myofibrillar proteins by using SDS-PAGE; however, they studied only those proteins of molecular weights of myosin heavy chain and less. Therefore, we are the first to study the effect of NaCl and phosphates on the high molecular weight proteins titin and nebulin.

WHC

WHC for beef STM increased significantly ($P < 0.05$) as NaCl concentration increased from 0.1 M through 1.0 M NaCl (Table 1). In addition, the presence of 10 mM PP combined with NaCl had significantly ($P < 0.05$) higher WHC values at each NaCl concentration. Our results agree with those reported by Voyle et al. (1984) in which they attributed increased WHC to greater myofibrillar protein extraction.

CONCLUSIONS

By uniquely combining phase contrast microscopy, SDS-PAGE and WHC tests to show the effects of NaCl and NaCl + 10 mM PP on beef muscle tissue, it was demonstrated that higher NaCl concentrations increased both beef myofibril swelling and myofibrillar protein extraction, and improved tissue WHC. The addition of lOmM PP to NaCl solutions decreased the NaCl concentration required for maximal myofibril swelling. The presence of 10 mM PP or TPP also increased myofibrillar protein extraction, especially of titin and myosin heavy chain, and substantially improved tissue WHC. Most importantly, the results of our study suggest that the extraction of titin with NaCl and PP is an important event in regulating increased myofibril swelling and improved WHC. Increased myofibril swelling and improved WHC are presumably due to removal of structural restraints of titin containing structures (gap filaments) within the three dimensional array of the myofibril, thus allowing more space for myofibrillar proteins to swell. Consequently, our work supports the mechanism of WHC of Offer and Trinick (1983) in that the removal of structural constraints within the myofibril seem to be the most important mechanism of improved WHC in meat.

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