Gelation of Chicken Muscle Myofibrillar Proteins Treated with Protease Inhibitors and Phosphates

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Gelation of chicken pectoralis muscle myofibrils treated with soybean trypsin inhibitor, leupeptin, antipain, egg white, ethylenediaminetetraacetic acid, and ortho- (P_i), pyro- (PP_i), tripoly- (TPP), or hexametaphosphate (HMP) was studied by dynamic rheological and differential scanning calorimetrical measurements at pH 6.0. Most myofibril samples exhibited two distinctive thermal transitions and a major rheological transition during heating from 25 to 80 °C. The abrupt loss of shear moduli upon heating from 50 to 55 °C was not prevented by protease inhibitors, indicating that gel weakening was unlikely caused by common endogenous proteases. PP_i, which destabilized myosin, interfered with myofibril gelation. TPP, which also destabilized myosin, markedly improved myofibril gelation in 0.3 or 0.4 M NaCl but decreased the gelling ability in 0.6 M NaCl. HMP, showing no effect on myosin denaturation, increased shear moduli of myofibril gels. The results may partially explain variations in functionality of poultry meat formulated with different phosphates when various levels of NaCl are used.

Keywords: *Chicken; proteins; myofibrils; gelation; protease inhibitors; phosphates*

INTRODUCTION

Heat-induced muscle protein gelation has been studied extensively because of its relevance to functionality of processed muscle foods. Much of the effort has been devoted to understanding the mechanisms of gelation as well as to alterations of gelation properties by various processing conditions to produce desirable product texture. However, controversy and ambiguity with regard to the role of some meat ingredients, e.g., phosphates, still exist. In this study, we intended to further investigate some of the processing factors.

Thermal gelation is a dynamic process. During gelation, myofibrillar proteins undergo complex changes in rheological characteristics, which are influenced by pH, protein concentration, and ionic conditions and vary among different muscle sources. When myofibrillar proteins are heated at a constant rate (e.g., 1 °C/min), they usually show transitions in shear modulus development. A temporary reduction in storage modulus (G', an elastic component) of myofibrillar protein gels has been observed for temperatures between 50 and 60 °C with different muscle sources, including chicken (Xiong and Blanchard, 1994) and fish (Sano et al., 1988). In surimi production, this phenomenon is called "modori" and was attributed by Sano et al. (1988, 1989) to the dissociation of myosin from actin and possible fragmentation of the actin filament. Egelandsdal et al. (1986) also suggested that the reduction in G' was due to denaturation of the myosin rod portion. However, some researchers argued that the gel weakening, particularly in the 50–60 °C range, was promoted by the action of alkaline proteases (Lanier et al., 1981; Hamann et al., 1990). Since muscle tissue contains many endogenous proteases, it is possible that proteases which have not been completely removed by washing can cause gel weakening and, therefore, partially account for this phenomenon. Thus, the first objective of this study was to determine possible contribution of muscle endogenous proteases to the gel weakening by comparing rheological properties of chicken myofibrils treated with or without various protease inhibitors.

Phosphates in a variety of forms (monomeric, dimeric, trimeric, and polymeric phosphates) are commonly incorporated in formulations of processed meat products to improve water- and meat-binding ability and modify textural characteristics (Trout and Schmidt, 1984). Despite these claimed benefits, several recent studies have cast doubts as to whether phosphates could really improve some of the functionalities, particularly gelation and rheological performance of whole-meat homogenates or isolated myofibrillar proteins (Robe and Xiong, 1993; Torley and Young, 1995). These later studies showed a gel-weakening effect, including the reduction in G' in the 50–60 °C temperature range, by phosphates under certain meat-processing conditions. Regardless, the effects of phosphates in processed meat were largely attributed to changes in pH and ionic strength brought about by phosphates (Trout and Schmidt, 1986). There apparently exist some other factors that influence the effectiveness of phosphates, as changes in pH and ionic strength of meat batters or protein solutions by phosphates were unable to fully account for altered protein functionalities, particularly gelation (Trout and Schmidt, 1986; Robe and Xiong, 1993). Different phosphates might affect protein gelation through different mechanisms, and their specific effects appear to be sensitive to the environmental conditions, which warrants a further investigation. Thus, the second objective of this study was to examine the effects of various phosphates on gelation properties of chicken muscle myofibrillar proteins by monitoring the dynamic gelation process, measuring the changes in solubility of myofibrillar proteins, and evaluating the quality of formed protein gels under various salt concentrations (ionic strengths).

MATERIALS AND METHODS

Chemicals. Antipain, leupeptin, soybean trypsin inhibitor, sodium orthophosphate (P_i), pyrophosphate (PP_i), and tripolyphosphate (TPP) were obtained from Sigma Chemical Co. (St.

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3438 *J. Agric. Food Chem.,* Vol. 45, No. 9, 1997

Liu and Xiong

Louis, MO). Ethylenediaminetetraacetic acid (EDTA) and ethylene glycol bis(β-amino ethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) were purchased from Fisher Scientific (Springdale, NJ). Sodium hexametaphosphate (HMP) was purchased from EM Science (Gibbstown, NJ). Egg white powder (type P-110) was supplied by Henningsen Foods, Inc. (White Plains, NY). All other chemicals used in the study were of reagent grade.

Preparation of Myofibrils. Six-week-old commercially processed fresh broilers were obtained from a local retailer, stored on crushed ice, and used within 36−48 h after slaughter. All experiments were replicated three times. For each replicated trial, breast muscle (pectoralis major and minor combined) was excised from two birds. After removal of connective tissue and external fat, muscle samples (from two birds) were mixed and ground through a 4.5-mm orifice plate in a food grinder. Myofibril isolation was performed at 2 °C essentially as described by Xiong (1993). Ground muscle was washed four times using an isolation buffer (pH 7.0) containing 0.1 M KCl, 2 mM MgCl$_2$, 1 mM EGTA, and 10 mM potassium phosphate, followed by two washes using 0.1 M NaCl solution containing 1 mM NaN$_3$. Myofibril suspension was adjusted to pH 6.0 using 0.1 N HCl prior to the final centrifugation. The purified myofibril pellets were stored on ice and used within 24 h of isolation. Protein concentration of the myofibril pellet was measured with the biuret method (Gornall et al., 1949) using bovine serum albumin (Sigma Chemical Co.) as standard.

Dynamic Rheological Measurements. Myofibril suspensions (20 mg/mL protein in 0.6 M NaCl, 50 mM sodium phosphate, pH 6.0) containing protease inhibitors were prepared by adding soybean trypsin inhibitor (50 μM), leupeptin (100 μM), antipain (100 μM), EDTA (0.5, 5, or 10 mM), or egg white (0.1%) to the protein suspensions. These protease inhibitors (except EDTA) as well as their concentrations were chosen on the basis of the previous work done under similar conditions (Hamann et al., 1990; Ishida et al., 1994). Myofibril suspensions (20 mg/mL protein in 50 mM PIPES, pH 6.0, with 0.3, 0.4, or 0.6 M NaCl) containing various phosphates were made by adding stock solution of P$_i$, PP$_i$, TPP, or HMP to the protein suspensions, with a final concentration of 10 mM. Since endogenous pyrophosphatase could still be present in washed myofibrils and result in loss of PP$_i$ (Torley and Young, 1995), NaF (10 mM), a pyrophosphatase inhibitor, was added to myofibril suspensions containing either PP$_i$ or TPP with 0.6 M NaCl. MgCl$_2$ (1 mM) was also added to samples with PP$_i$. Myofibril suspensions were then stored at 0 °C for 12 h. Nondestructive, oscillatory measurements of the protein suspensions during gelation were carried out using a Bohlin VOR rheometer (Bohlin Instruments, Inc., Cranbury, NJ) as described by Xiong (1993). Thermal gelation was induced by heating myofibril suspensions from 20 to 80 °C at 1 °C/min and was monitored by shearing the sample placed between two parallel plates (upper plate, 30 mm diameter) at a fixed frequency of 100 mHz with a maximum strain of 0.02. Dynamic rheological properties of myofibril samples were described in terms of shear storage modulus (G', the elastic component) and loss modulus (G'', the viscous component).

Differential Scanning Calorimetry (DSC). Myofibril suspensions (40 mg/mL protein in 50 mM PIPES, pH 6.0, containing 0.3 M, 0.4 M, or 0.6 M NaCl, either with or without phosphates) were prepared and stored at 2 °C for 12 h before analysis. Samples (14−17 mg) of myofibril suspensions were accurately weighed into aluminum pans and hermetically sealed. DSC was performed on a TA Instruments Model 2920 differential scanning calorimeter (TA Instruments, Inc., New Castle, DE). Samples were thermally scanned from 10 to 90 °C at 10 °C/min. Most myofibril samples exhibited one or two thermal transitions, and the maximum transition temperature (T_m), which corresponds to 50% denaturation of the protein(s) involved in the transition, was recorded. All DSC scanning was performed with at least triplicate samples. Distilled water was used as reference sample. Enthalpy was expressed in watts per gram of sample.

Protein Solubility. Myofibril suspensions (5 mg/mL protein in 50 mM PIPES, pH 6.0, containing 0.3 M, 0.4 M, or 0.6 M NaCl, either with or without phosphates) were prepared
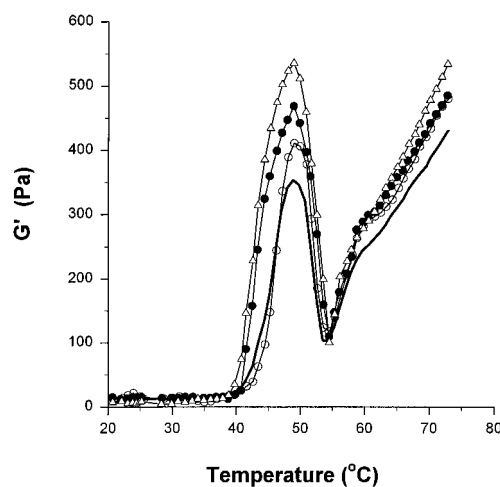
Figure 1. Changes in storage modulus (G') during thermal gelation of chicken breast myofibrils treated with protease inhibitors. (−) Control, soybean trypsin inhibitor (50 μM), antipain (100 μM), leupeptin (100 μM), or egg white (0.1%); (○) 0.5 mM EDTA; (●) 5 mM EDTA; (△) 10 mM EDTA.

and stored at 2 °C for 12 h. The suspensions were then centrifuged at 5000g for 15 min to determine protein solubility, which was defined as the protein concentration in the supernatant divided by that in the original suspension and then multiplied by 100.

Gel Strength. Myofibrils were suspended (20 mg/mL) in 50 mM PIPES buffers (pH 6.0) containing 0.6 M NaCl with different phosphates. Gels were formed in 16.5-mm (inner diameter) × 80-mm (length) glass vials by heating the myofibril suspensions (about 5 mL) from 20 to 70 °C at 0.8 °C/min in a water bath. After cooling to 0 °C in an ice slurry and subsequently equilibrating at room temperature for 1 h, gels were penetrated with a stainless steel probe (12.5 mm diameter) attached to a Model 4301 Instron universal testing instrument (Instron Corp., Canton, MA). The penetration force, defined as the force required to rupture the gel, was expressed as gel strength (Xiong, 1993).

Water-Holding Capacity (WHC). WHC of cooked gels was measured essentially according to the method of Foegeding and Ramsey (1987). Myofibril gels were prepared in the same way as described above for gel strength measurement. Gel samples, 1.5 ± 0.3 g, were placed onto three layers of preweighed filter paper and then centrifuged at 30000g for 15 min at 4 °C. After removal of the expressed gel pellet, the filter paper was reweighed to determine water gain. WHC was calculated as: (total grams of water − grams of water lost during centrifugation)/grams of protein, with total water determined from moisture analysis of gel samples (AOAC, 1984).

Statistical Analysis. Data were analyzed using the general linear model procedure of the Statistix 3.5 software package (Statistix 3.5, Analytical Software Inc., St. Paul, MN). Analysis of variance (ANOVA) was conducted to determine the significance of main effects (protease inhibitors, phosphates, salt concentrations, etc.). Significant ($P < 0.05$) differences between means were identified using the least significant difference procedure (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Effect of Protease Inhibitors. Heating induced complex changes in the storage modulus (G') of myofibrillar protein suspensions (Figure 1). All samples, with or without protease inhibitors, exhibited the same rheological pattern. The initial change in G' was detected at about 39 °C. The G' increased rapidly from 45 to 49 °C, indicating the transformation of the viscous sol to an elastic network. This increase was followed by a sharp decline that reached a minimum at about 54 °C. The G' then increased again and showed a shoulder transition at around 60 °C. These rheological

Gelation of Myofibrillar Proteins

*J. Agric. Food Chem.,* Vol. 45, No. 9, 1997 **3439**

characteristics were essentially the same as have been reported by Xiong and Blanchard (1994).

Egelandsdal et al. (1986) and Wu et al. (1991) found that purified myosin also exhibited a reduction in G' between 50 and 60 °C and suggested that high temperatures weakened protein−protein interactions of myosin gels. A recent study by Smyth et al. (1996) also suggested that gel weakening in this temperature range might be related to the denaturation of myosin light chains. Assuming that the myosin model system is free of muscle endogenous proteases, the rearrangement of intra- and intermolecular bonds resulting from unfolding of light meromyosin may be a possible explanation for the G' decline. However, the situation might be different for myofibrils, which are prepared by repeatedly washing muscle mince and which represent a composite system. Surimi (minced and washed fish muscle), for example, has been reported to contain heat-stable proteases that are responsible for gel weakening in an intermediate temperature range, e.g., around 55 °C (Lanier et al., 1981; Chang-Lee et al., 1989). Thus, incorporation of protease inhibitors in the myofibril suspension would help determine whether or not endogenous proteases were responsible for the G' reduction.

The protease inhibitors used in this study are theoretically effective against a broad range of proteases in muscle tissue. Soybean trypsin inhibitor is specific for trypsin-like serine-proteases, while leupeptin and antipain inhibit cysteine−proteases such as cathepsins. Egg white contains several inhibitors, including ovomucoid and ovoinhibitor, which are serine−protease inhibitors, and cystatin, which is a cysteine−protease inhibitor (Stevens, 1991). EDTA is a metal chelator that can inhibit metalloproteases. Nevertheless, addition of these protease inhibitors did not prevent the occurrence of the gel weakening in the temperature range of 50−60 °C. Since these inhibitors have been shown to be effective under similar conditions in previous studies (Hamann et al., 1990; Ishida et al., 1994), the reduction of G' of chicken myofibrillar proteins in this temperature range was unlikely caused by endogenous proteases in the muscle tissue. Instead, it might be caused by some intrinsic physicochemical factors as suggested by Egelandsdal et al. (1986) and Wu et al. (1991).

EDTA (5 mM), however, significantly increased ($P < 0.05$) G' at the peak (49 °C) and at the end of heating (72 °C) (Figure 1), compared to control sample (without any protease inhibitor) and samples treated with other inhibitors. In addition, EDTA also augmented G'' at the peak (48 °C) (results not shown). Because of the apparent effect by EDTA, further experiments were performed to measure G' and G'' of myofibrils at other EDTA concentrations, i.e., 0.5 and 10 mM (Figure 1). It was found that G' and G'' values generally increased with the EDTA concentration. Considering that the concentrations of EDTA used should be sufficient to inhibit metalloprotease(s) through the removal of possible metal ion cofactors (Auld, 1995), the increase in G' and G'' could be explained by the negative charges introduced by EDTA, since EDTA tends to bind nonspecifically to proteins (Auld, 1995).

Effect of Phosphates. Although phosphates are widely used in processed muscle foods and their effects on functionalities of muscle proteins have been extensively examined, their mechanisms of action on protein gelation are still inconclusive. Discrepant, sometimes conflicting, results have been obtained, partly due to differences in experimental conditions (e.g., pH, ionic strength, heating) and protein systems used (e.g.,
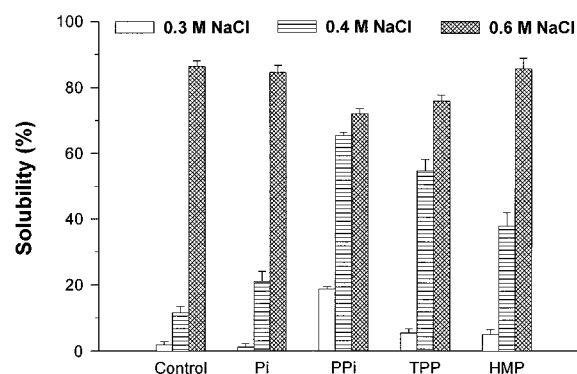

Figure 2. Effects of various phosphates on solubility of chicken breast muscle myofibrillar proteins at different salt concentrations.

myosin, actomyosin, myofibrils, muscle). In this study, the effects of various phosphates were investigated under controlled pH and ionic strength conditions to facilitate data interpretation.

Protein Solubility. To form an ordered elastic gel network, myofibrillar proteins must first be extracted or solubilized. Thus, any change in protein solubility caused by phosphates would likely lead to an alteration in gelation properties of the myofibrils. For this reason, protein solubility of myofibrils in the presence of various phosphates was examined at different ionic strengths. As expected, the protein solubility of myofibrils increased ($P < 0.05$) with increasing salt concentrations (Figure 2). However, the protein solubility was markedly affected by phosphates only at low salt concentrations. At 0.3 M NaCl, PP$_i$ increased ($P < 0.05$) the protein solubility about 10-fold compared to the control sample or the sample treated with P$_i$, while TPP and HMP increased ($P < 0.05$) the protein solubility about 3-fold. When the salt concentration was increased to 0.4 M, all phosphates, particularly PP$_i$ and TPP, improved protein solubility. When the salt concentration reached 0.6 M, the effects of phosphates diminished. It is well-known that within certain ionic strength ranges, addition of salt increases protein−water interaction and, hence, solubilization of myofibrillar proteins. This is generally referred to as "salting-in," which is presumably the case for the salt concentrations used in this study. The different effects of phosphates on protein solubility are probably due to the different amounts of negative charges they carry. PP$_i$ is a fluidizing agent that can be used to dissociate actomyosin (Wang and Smith, 1994). Hence, it is not surprising that PP$_i$ was the most effective phosphate in increasing the protein solubility. Furthermore, phase contrast microscopy showed that the extraction of proteins from myofibril filaments in the presence of phosphates occurred in different patterns: PP$_i$ and TPP extracted myosin from both ends of the A-band, while P$_i$, HMP, and control (without phosphate) extracted myosin from the center of the A-band (unpublished data). The different myosin (actomyosin) extraction processes could also contribute to the variation in protein solubility caused by these phosphate types.

Protein Denaturation. Protein gelation is generally described as a two-step process involving unfolding (denaturation) of proteins and aggregation of denatured proteins into three-dimensional gel networks (Ferry, 1948). Protein unfolding is essential to the gel formation because aggregation is achieved through cross-linking between exposed reactive groups. Figure 3 and Table 1 show the thermal stability of myofibrillar proteins under different experimental conditions as
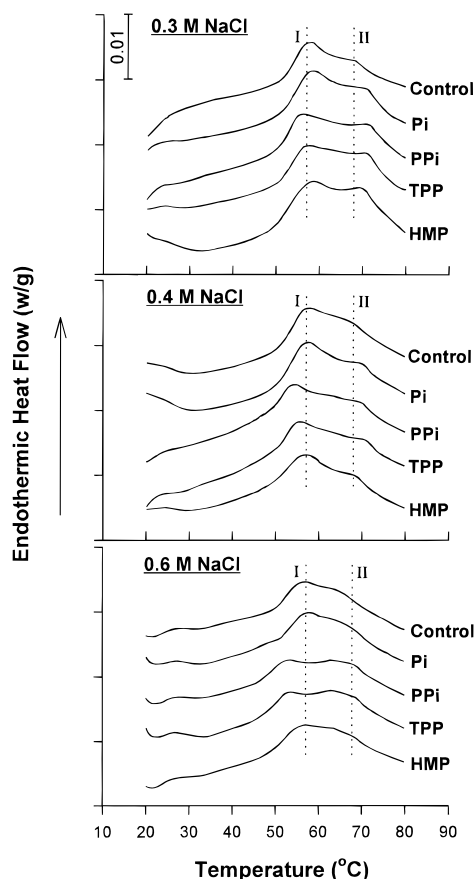
Figure 3. DSC endotherm of myofibril suspensions (40 mg/mL protein in 50 mM PIPES, pH 6.0, containing 0.3, 0.4, or 0.6 M NaCl, either with or without phosphates), heated from 10 to 90 °C at 10 °C/min.

Table 1. T_m **of Thermal Transitions of Chicken Breast Myofibrils Treated with Various Phosphates at Different Salt Concentrations As Analyzed by DSC**[a]

	T_m (°C)					
	0.3 M NaCl		0.4 M NaCl		0.6 M NaCl	
	peak I	peak II	peak I	peak II	peak I	peak II
control	57.2[b]	67.9[c]	57.2[a]	ND	57.1[a]	ND
P_i	57.9[a]	70.5[b]	57.4[a]	69.4[b]	57.6[a]	ND
PP_i	55.4[c]	70.9[a]	53.9[c]	69.8[b]	53.5[c]	ND
TPP	57.0[b]	71.0[a]	55.4[b]	71.1[a]	54.4[b]	ND
HMP	57.6[a]	68.4[c]	57.0[a]	68.3[c]	57.0[a]	ND

[a] ND, nondistinctive. Data are means of three replicated experiments. Means within the same column bearing different letters differ significantly ($P < 0.05$).

monitored by DSC. All myofibril samples, with or without phosphates, showed two major transitions, which were tentatively ascribed to myosin (53–58 °C) and actin (67–72 °C) (Stabursvik and Martens, 1980). Increasing the salt level did not change ($P > 0.05$) the denaturation temperature (T_m) of myosin in the control sample, whereas in another study (Kijowski and Mast, 1988) T_m was markedly lowered by the presence of NaCl. On the other hand, the enthalpy of denaturation showed a tendency to decrease with increases in the salt concentration, as indicated by the reduced size of thermal transition peaks (Figure 3). This suggests that addition of salt decreased the stability of myosin and actin. An increase in ionic strength would modify the distribution of surface charges of protein molecules and, hence, intramolecular electrostatic interactions that stabilize the native protein structure. In this case, the conformational changes resulting from the addition of

salt probably favored the unfolding of proteins thermodynamically.

Apart from the effect of salt concentration, phosphates had a major influence on both T_m and enthalpy of denaturation. PP_i and TPP destabilized myosin (decreasing T_m), with PP_i being more effective. In addition to their nonspecific interactions with surface charges of myosin molecules, P_i and PP_i are capable of binding to the myosin S1 subunit at the same binding sites (Muhlrad et al., 1991). Wang and Smith (1994) suggested that binding of PP_i to the myosin molecules, particularly the S1 subunit of the myosin heavy chain, resulted in structural changes which facilitated protein unfolding. Moreover, dissociation of actomyosin by PP_i might also be part of the reason the T_m value of myosin decreased, since actin was reported to stabilize myosin upon binding (Wang and Smith, 1995). Because interactions of phosphates with protein are of electrostatic nature, effects of phosphates obviously would be influenced by ionic strength. It was shown in this study that the destabilizing effect of PP_i and TPP was further enhanced by the addition of NaCl (Table 1), indicating synergistic effects of salt with phosphates. Variations in the effect of phosphates with ionic strength were also shown in the study by Kijowski and Mast (1988), in which PP_i and TPP were found to stabilize myosin in the absence of salt and to destabilize it at 2% NaCl (0.34 M). Disparities between phosphates might be attributed to differences in the number of negative charges as well as binding constants of phosphates for myosin (Muhlrad et al., 1991). HMP, for example, essentially had no effect ($P > 0.05$) on the denaturation temperature of myosin and actin, probably due to steric hindrance. Being a large cyclic molecule, HMP may be restrained from binding strongly to myosin. P_i, PP_i, and TPP increased the T_m of actin at 0.3 M NaCl (Table 1). A similar trend was also observed at 0.4 and 0.6 M NaCl (Figure 3). The results could not be easily explained.

Protein Gelation. The evolution of viscoelastic properties during thermal gelation of the myofibrils in the absence or presence of various phosphate treatments is shown in Figure 4. At 0.6 M NaCl, myofibril samples treated with PP_i and TPP produced a G' transition peak at the average temperature of 46 and 47 °C, respectively, while control sample and samples treated with P_i and HMP all produced a peak around 49 °C. At lower salt concentrations (0.3 and 0.4 M NaCl), G' peaks had rather irregular shapes and inconsistent peak temperatures among different samples. Thus, peak temperatures are not reported for these conditions. It is difficult to directly compare the gelation results with those from DSC analysis. For example, protein unfolding (endothermic heat flow) and aggregation (exothermic heat flow) shown in Figure 3 were somewhat insensitive to ionic strength, while gelation of the protein shown in Figure 4 had a great dependence on ionic strength due to its solubility requirement, among other factors. However, some relationship did seem to exist between DSC and G' changes. For example, the effects of phosphates on the "onset temperature" (i.e., the temperature at which thermal transitions began to occur) and the peak temperature for DSC and G' transitions were similar. Both the G' peak temperature (shown in Figure 4) and the denaturation temperature (DSC) (Table 1) for myosin in samples treated with PP_i and TPP were lower than control sample and samples treated with P_i and HMP at 0.6 M NaCl. As previously discussed, binding of phosphates which introduced a large number of negative charges to myofibrillar proteins would alter the process of protein unfolding,

Gelation of Myofibrillar Proteins

J. Agric. Food Chem., Vol. 45, No. 9, 1997 **3441**

Table 2. Effects of Various Phosphates on G' and G'' of Chicken Breast Myofibrils at Different Salt Concentrations[a]

	G' at peak (Pa)			final G' (Pa)			G'' at peak (Pa)		
	0.3 M NaCl	0.4 M NaCl	0.6 M NaCl	0.3 M NaCl	0.4 M NaCl	0.6 M NaCl	0.3 M NaCl	0.4 M NaCl	0.6 M NaCl
control	ND	114[c]	266[b]	67[b]	156[c]	421[c]	ND	19[c]	58[a]
P_i	ND	191[b]	326[a]	23[d]	223[b]	409[c]	ND	34[b]	70[a]
PP_i	55[b]	ND	160[c]	37[c]	77[d]	458[b]	16[a]	16[c]	55[b]
TPP	72[a]	437[a]	259[b]	102[a]	386[a]	493[b]	13[a]	105[a]	78[a]
HMP	22[c]	288[b]	389[a]	30[d]	317[a]	579[a]	7.0[b]	45[b]	76[a]

[a] ND, nondistinctive. Data are means of three replicated experiments. Means within the same column bearing different letters differ significantly ($P < 0.05$).
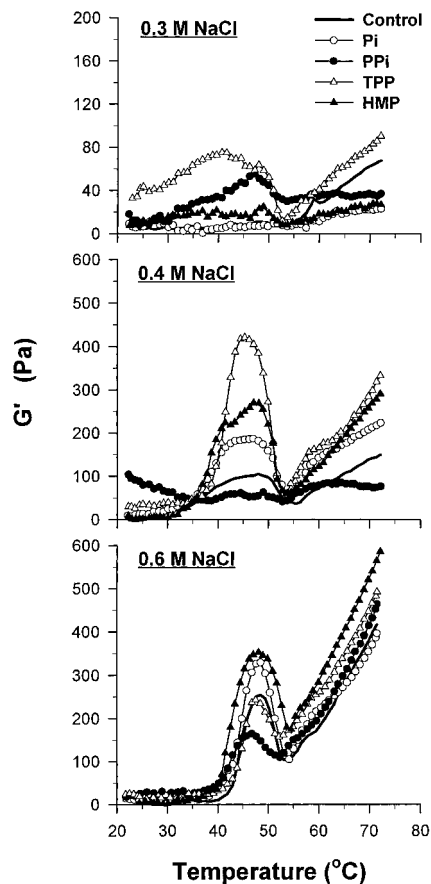
Figure 4. Changes in storage modulus (G') during thermal gelation of chicken breast myofibrils treated with various phosphates at different salt concentrations.

change the protein electric double layer, and, hence, affect interactions between denatured protein molecules. Thus, the effects of phosphates on storage moduli of myofibril gels were most likely achieved through modification of protein denaturation and aggregation, both of which are integral parts of the gelation process.

The magnitudes of peak G', final G', and peak G'' of chicken breast myofibrils are shown in Table 2. The G' and G'' values generally increased with the salt concentration, except for the sample treated with TPP, which had the highest peak G' and G'' at 0.4 M NaCl. The increase in the G' and G'' values upon the addition of salt corresponded well, in most cases, with the increase in protein solubility shown in Figure 2. This is expected because more protein cross-links can be formed when an increased number of myofibrillar proteins are extracted. However, it should be noted that protein solubility did not always coincide with shear moduli. For example, myofibrils containing PP_i with 0.4 M NaCl had lower G' and G'' values than the control myofibrils at 0.4 M NaCl, although protein solubility was greatly increased by PP_i at this salt level. This suggested that protein solubility alone could not fully

account for gelation properties of myofibrillar proteins treated with phosphates.

The phosphates used affected protein gelation differently at the three salt levels (Table 2). At 0.6 M NaCl, the myofibril sample treated with PP_i had the smallest ($P < 0.05$) peak G' and G'' values of all myofibril samples, including the control (Figure 4). At 0.4 M NaCl, the PP_i sample did not form a distinctive peak and, again, had the lowest G' around 46 °C. When the NaCl concentration was further reduced to 0.3 M, the PP_i-treated myofibrils unexpectedly produced a transition around 55 °C, while the P_i-treated and control samples did not show a G' transition. Addition of NaF (pyrophosphatase inhibitor) did not effect any change ($P > 0.05$) in G' and G'' (results not shown) of PP_i-treated sample, indicating that pyrophosphatase was unlikely present in the myofibril preparations. At 0.6 M NaCl, addition of TPP did not change ($P > 0.05$) the peak G' of myofibril samples when compared to control sample, while TPP increased the G' value at lower salt concentrations. These results were in partial agreement with the study by Robe and Xiong (1993), who reported that under similar experimental conditions (0.6 M NaCl, pH 6.0), addition of PP_i and TPP reduced G' of porcine muscle salt-soluble proteins.

As shown in Figure 4 and Table 2, the effects of other phosphates also depended on the salt concentration. The variations among these phosphates in their effects on myofibrillar protein gelation might be explained by their different abilities to bind to myosin, which obviously were also affected by ionic strength. PP_i had the additional effect on gelation because of its capability of dissociating actomyosin. Furthermore, phosphates extract proteins from myofibril filaments in different patterns as mentioned previously. Thus, the myosin to actin ratio in the solution and in the unextracted myofibrils would be different for samples treated with various phosphates. This would create more complexity in the gelation process since the myosin to actin ratio was shown to affect the protein gelation (Wang and Smith, 1995).

The physical quality of final gels formed from myofibrils treated with different phosphates was evaluated by the gel penetration and water-holding tests. HMP increased ($P < 0.05$) gel strength (gel penetration force) of myofibril samples (Figure 5), while PP_i reduced ($P < 0.05$) gel strength. P_i and TPP did not change ($P > 0.05$) gel strength of myofibril samples. The gel strength data did not necessarily agree with the findings of the final G' in dynamic rheological measurements, probably because the final G' was the measurement of elasticity of myofibril gels when the sample was still "hot", while gel strength was measured after the formed gels were cooled down. Also, dynamic rheological measurements are nondestructive, whereas gel penetration force measurement is destructive. They represent different aspects of the rheological profile of protein gels and, hence, may not be simply related. WHC of myofibril gels was
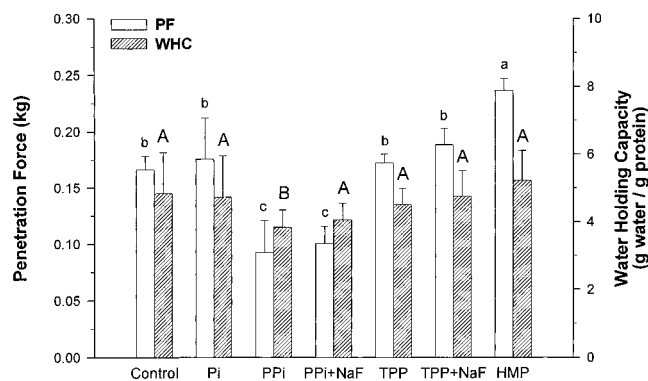
Figure 5. Effects of various phosphates on gel strength (white bar) and water-holding capacity (slashed bar) of chicken breast muscle myofibrillar protein gels containing 0.6 M NaCl. Samples bearing different letters differ significantly ($P < 0.05$).

reduced ($P < 0.05$) by PP_i, but not changed by other phosphates (Figure 5). The ability of a protein gel to take up water depends on two factors: hydration of protein and capillary effect. WHC of myofibril gels was probably more affected by the gel structure in this case since the effects of phosphates on protein hydration would be minimized at the high salt concentration (0.6 M). Myofibril gels treated with PP_i might have a more coarse structure than other samples and, hence, less water uptake through capillary forces.

Conclusion. The reduction in storage modulus (G'), or gel weakening at temperatures around 50–60 °C, is unlikely caused by endogenous protease in muscle tissue. The mechanism of this phenomenon may involve the intrinsic factors of myosin or actomyosin molecules. P_i, PP_i, TPP, and HMP exert various effects on protein solubility, thermal transition, and gelation properties of myofibrillar proteins, apparently through different mechanisms. These effects can be further complicated by other factors, such as ionic strength. The information obtained in this study may be used to minimize variations in functionality of poultry meat formulated with different phosphates.

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