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Hydroxyl radical oxidation destabilizes subfragment-1 but not the rod of myosin in chicken myofibrils

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Abstract

The thermal denaturation process of myosin in oxidized chicken myofibrils was investigated. Exposures of myofibrils to hydroxyl radical-generation systems (HRGS) resulted in an enhanced susceptibility of myosin to thermal inactivation of Ca-ATPase and a loss of salt solubility. The chymotryptic production of subfragment-1 (S-1) from myosin in oxidized myofibrils decreased more rapidly than that in un-oxidized myofibrils upon heating, which paralleled the Ca-ATPase decay. However, the heat-induced decrease in chymotryptic production of rod from myosin was not affected by the HRGS treatment. The results suggested that free radical oxidation promoted thermal destabilization of myosin in the S-1 portion instead of the rod portion. The altered myosin denaturation pattern due to hydroxyl radical oxidation was likely a cause for functionality changes in oxidatively stressed myofibrillar proteins in meat processing.

Keywords: Myosin; Myofibrils; Oxidation; Denaturation; Ca-ATPase; Salt solubility; Chymotryptic digestibility

1. Introduction

Oxidative modifications of myofibrillar proteins during processing and storage of meat affect the quality of muscle foods through alternation of protein functionalities, including gelation, emulsification, viscosity, solubility, and water-holding capacity (Smith, 1987; Srinivasan & Hultin, 1997; Wan, Xiong, & Decker, 1993; Wang & Xiong, 1998; Xiong, Decker, Robe, & Moody, 1993). The chemical changes induced by oxidation, such as degradation and polymerization of myofibrillar proteins as well as increases in protein carbonyls, are postulated to be involved in functionality variations of myofibrillar proteins and hence, quality of muscle foods (Decker, Xiong, Calvert, Crum, & Blanchard, 1993; Liu & Xiong, 2000b; Martinaud et al., 1997; Srinivasan & Hultin, 1997).

Myosin, the most abundant myofibrillar component, is largely responsible for functionality of myofibrillar protein as a whole in muscle foods. In a previous study (Ooizumi & Xiong, 2004), we investigated the impact of hydroxyl radicals on the conformation and biochemical characteristics of myosin in chicken myofibrils. The results showed that exposure of myofibrils to hydroxyl radical-generating systems (HRGS) caused cross-linking of myosin heavy chains (MHC) via disulphide bonds. The cross-linking initially occurred inside the myosin molecules, and then between myosin molecules, leading to progressive aggregation. Myosin consists of two distinct structural domains – a globular head designated subfragment-1 (S-1) and an almost completely α-helical coiled-coil tail, referred to as rod. Thus, a subsequent study (Ooizumi & Xiong, 2006) revealed that cross-linking of myosin in HRGS-oxidized myofibrils occurred in the light meromyosin (LMM), which is the posterior part of the myosin rod including its carboxyl terminus. On the other hand, Li and King (1999) reported that malonaldehyde, a byproduct of lipid oxidation, modified the secondary structure of the globular head of myosin. These structural changes caused by oxidation may affect the mode of thermal denaturation of myosin,

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thereby affecting myosin gelation (Sharp & Offer, 1992). Liu and Xiong (2000a), using differential scanning calorimetry, had previously demonstrated that hydroxyl radicals were highly reactive and could readily destabilize myosin and induce subsequent molecular association.

Biochemical and electron microscopic studies on the thermal aggregation process of un-oxidized myosin have revealed that denaturation and aggregation of S-1 and rod (two structural domains of myosin), proceed independently (Kato, Koseki, & Konno, 1996; Konno, Yamamoto, Takahashi, & Kato, 2000; Tazawa, Kato, Katoh, & Konno, 2002; Yamamoto, 1990). On the other hand, research on oxidative myosin destabilization has so far mainly focused on the whole myosin molecule. No information is currently available concerning thermal susceptibility of different sites/parts of myosin in myofibrils exposed to an oxidative environment.

The objective of the present study was to elucidate the denaturation process of whole myosin and its subfragments (S-1 and rod) in myofibrils that were subjected to oxidation by HRGS. This was done by means of monitoring the changes in Ca-ATPase activity, salt solubility, and the gel electrophoretic patterns of chymotryptic digests of oxidized myosin.

2. Materials and methods

2.1. Preparation of myofibrils

Myofibrils were prepared from postrigor chicken breast muscle according to the method reported by Katoh, Uchiyama, Tsukamoto, and Arai (1977). The chicken carcasses (36–48 h postmortem), obtained from a local poultry processing plant, were unfrozen. Isolated myofibrils (20 mg/mL) were suspended in a Tris buffer containing 0.1 M KCl and 20 mM Tris–HCl, pH 7.5, and the myofibril stock suspension was kept at 0 °C and used within 10 days. Myofibrils, instead of purified myosin, were employed to more closely simulate the protein environment in the muscle tissue, thus, allowing a more accurate elucidation of oxidative impact on the myosin component in muscle. Although the data were not presented, preliminary assays indicated negligible changes in the Ca-ATPase activity, solubility, and chymotryptic digestibility of myosin in myofibrils within the storage time. Protein concentrations of the stock myofibril suspension as well as all assay solutions were determined by the Biuret method (Gornall, Bardawill, & David, 1949).

2.2. Oxidation of myofibrils

Myofibrils (5 mg/mL) were treated with non-enzymatic, HRGS consisting of 0.1 mM ascorbic acid, 0.01 mM $FeCl_3$, and 0.1–5 mM of H_2O_2 at 0 °C for 18 h, as described previously (Ooizumi & Xiong, 2004). To terminate oxidation, HRGS was removed by washing the myofibrils with 0.1 M KCl including 20 mM Tris–HCl (pH 7.5).

2.3. Heat treatment

Aliquots of the oxidized myofibril suspensions in 0.1 or 0.5 M KCl placed in a series of test tubes were heated in waterbaths at 45, 47 or 49 °C. These temperatures were chosen because denaturation of myosin S-1 and rod in myofibrils could be detected at the same time, as determined in preliminary experiments. At appropriate time intervals, samples were drawn and immediately cooled in an ice slurry before analysis. The sample without heating was also submitted to analysis (0 heating time).

2.4. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was carried out using a 3% stacking gel and a 10% separating gel of polyacrylamide according to Laemmli (1970). The protein samples were treated with 5% 2-mercaptoethanol.

2.5. Ca-ATPase activity

The enzyme assay, which is indicative of structural integrity of the active site of the S-1 portion (globular head) of myosin, was performed at 25 °C with 0.2 mg/mL of myofibrils suspended in 0.5 M KCl, 5 mM $CaCl_2$, 25 mM Tris–maleate (pH 7.0), and 1 mM ATP, by colorimetrically measuring the liberated inorganic phosphate, as described by Katoh et al. (1977).

2.6. Salt solubility of myosin

The concentration of KCl in the heated myofibril suspension with or without HRGS treatment was adjusted to 0.5 M by adding 2 M KCl with 20 mM Tris–HCl, pH 7.5, and the solution was set at 0 °C for 2 h. Thereafter, 1 mM ATP-Mg was mixed into the solution, which was immediately centrifuged at 5000*g* for 30 min at 4 °C to separate the salt-soluble fraction (supernatant) from the insolubles (Yamamoto, Takahashi, Kato, & Konno, 2002). SDS–PAGE was performed on the salt-soluble protein fraction and the relative amount of myosin was estimated by measuring the density of the MHC band using an ULTROSCAN XL densitometer (Pharmacia LKB Biotechnology Inc., Piscataway, NJ, USA). Salt solubility of myosin under the condition reflects the extent of its aggregation (Konno & Ueda, 1989).

2.7. Chymotryptic digestibility of myosin

The enzyme digestion was conducted by incubating myofibrils (5 mg/mL) with α-chymotrypsin (Sigma Chemical Co., St. Louis, MO, USA) in a medium containing 0.1 M KCl, 1 mM EDTA, and 20 mM Tris–HCl (pH 7.5) at 25 °C for 20 min. The enzyme to substrate (myofibrils) ratio was 1/500 (w/w). The production of S-1 and rod was monitored by determining their respective SDS–PAGE

band intensities in the myofibril digests with densitometric scan, as aforementioned. Although chymotrypsin exclusively cleaves native myosin into S-1 and rod portions in the presence of 0.1 M KCl with 1 mM EDTA (Ooizumi & Xiong, 2006; Weeds & Pope, 1977), various fragments from both portions are also produced from denatured myosin due to the exposure of hydrophobic amino acid side chains (Konno et al., 2000). Therefore, the percentage of S-1 or rod produced by the enzyme can serve as an index of their denaturation under oxidative or heat conditions.

2.8. Denaturation rate constant

Although the details are described in Section 3, the loss of Ca-ATPase activity and myosin solubility, and the production of S-1 and the rod proceeded as first-order reactions as reported by Takahashi, Yamamoto, Kato, and Konno (2005). Therefore, the denaturation rate constants (k_D) for these indices were estimated according to the following equation:

$$k_D = (\log C_2 - \log C_1)/(t_2 - t_1),$$

where C_1 and C_2 are the relative values of Ca-ATPase activity, salt solubility of myosin, or production of S-1 or rod, after t_1 and t_2 seconds of incubation. The data collected at 45, 47, and 49 °C were used to calculate the reaction rate constants.

2.9. Statistics

HRGS treatment was performed at least in triplicate on different myofibril preparations from different chickens for the different experiments. To establish significant effects of H_2O_2 concentration in HRGS, ANOVA was run to analyze the enzyme activity and the relative staining intensity of protein bands from different sample treatments. Scheffe's test was used to compare means when a significant ($P < 0.05$) treatment effect was identified.

3. Results and discussion

3.1. Myosin Ca-ATPase

The relative changes in myosin Ca-ATPase activity in oxidized and un-oxidized myofibrils upon heat treatment at 49 °C are shown in Fig. 1a. As reported previously (Ooizumi & Xiong, 2004), HRGS treatment without heating enhanced the enzyme activity of myofibrils (from 0.197 to 0.238 μmol Pi/min/mg). Sekine and Yamaguchi (1963) reported that blocking of the two reactive thiol groups located around ATPase active site of myosin with SH reagents caused Ca-ATPase enhancement and K-ATPase decay. The ATPase changes caused by HRGS treatment strikingly similar to those observed when myosin was treated with SH reagents (Ooizumi & Xiong, 2004), suggesting that the same thiol groups located around the active site of myosin were modified. In addition, our previous study (Ooizumi & Xiong, 2004) also revealed that myosin in myofibrils cross-linked predominantly inside the myosin molecules via disulphide bonds under this oxidation condition. The heat-induced loss of Ca-ATPase in oxidized myofibrils was more rapid than that in un-oxidized control. For instance, after heat treatment for 30 min, 75% of Ca-ATPase activity was retained for un-oxidized myofibrils, compared to about 50% for oxidized myofibrils. Although the standard deviations of the ATPase activities were not shown in Fig. 1a, they were in the range from 0.00% to 3.87%. These results suggested that oxidation rendered the structure of the myosin globular head (S-1) around the ATPase active site more susceptible to heat denaturation.

3.2. Salt solubility of myosin

The amount (%) of myosin present in the salt-soluble fraction for both the control and oxidized myofibril samples gradually reduced with heating time (Fig. 1b). The solubility decrease for both samples proceeded faster than the

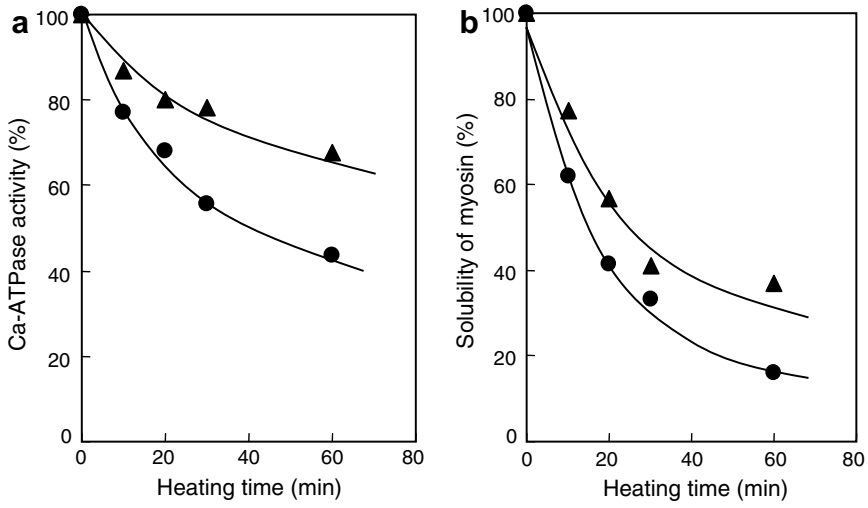

Fig. 1. Influence of oxidation of myofibrils by HRGS (containing 1 mM H_2O_2) on thermal inactivation of Ca-ATPase (a) and loss of salt solubility (b) of myosin in myofibrils. Oxidized myofibrils (●) and un-oxidized control (▲) were heat-treated in 0.1 M KCl at 49 °C for different time periods.

respective Ca-ATPase activity losses. Irrespective of the heating time, the solubilities of oxidized myosin sample were somewhat lower than those of un-oxidized control although the difference was not necessarily significant, indicating that the HRGS treatment of myofibrils promoted thermal aggregation of myosin. The standard deviations of the data in Fig. 1b were less than 6.43%.

3.3. Chymotryptic digestibility

The aforementioned results indicated that HRGS treatment destabilized myosin globular head (S-1) and promoted thermal aggregation of myosin. In order to understand the effect of HRGS treatment on the thermal stability of myosin rod, chymotryptic digestion, which separates myosin into S-1 and rod fragments, was performed. It is worth noting that formation of cross-links in myosin through disulphide bonds by the same HRGS treatment did not alter the susceptibility of myosin to chymotryptic digestion, i.e., the digests were composed mostly of S-1 and rod (Ooizumi & Xiong, 2006).

Densitometric scan of the SDS–PAGE revealed that the heat treatment gradually decreased the S-1 and rod band intensity in both myofibril samples (Fig. 2), indicating that unfolding of myosin with exposure of hydrophobic amino acid side chains had occurred in the S-1 and the rod regions upon heating at 49 °C. Comparing the decreasing rate of the production of S-1 with that of rod from un-oxidized myosin, the rod production decreased slightly faster than S-1 production. This trend was similar to the denaturation process of carp myosin though the denaturation temperature of carp myosin was 9 °C lower than that of chicken myosin (Yamamoto et al., 2002).

The amount of S-1 produced from oxidized myosin was reduced ($P < 0.05$) when compared to that from un-oxidized myosin at equal heating times (Fig. 2). The decrease in the production of S-1 essentially paralleled the Ca-ATPase decay (Fig. 1a), which, for a comparison purpose, is shown again in Fig. 2a. In contrast, the decrease in the production of rod from oxidized myosin almost coincided with that from un-oxidized one, suggesting that the same HRGS treatment did not affect the decrease of chymotryptic production of rod from myosin by heat treatment (Fig. 2b). Although standard deviations of the data were not shown in Fig. 2, they were in the range from 2.14% to 7.26% for S-1 production and from 0.50% to 6.72% for rod production, respectively.

These results further supported the hypothesis that the HRGS treatment of myofibrils destabilized the myosin S-1 portion, notably at the Ca-ATPase active site. Furthermore, they were an indication of insensitivity of the helical rod to HRGS modification despite the formation of the intramolecular cross-links in LMM portion of myosin rod via disulphide bond under these oxidation conditions (Ooizumi & Xiong, 2004, 2006). In other words, the intramolecular cross-linking in myosin rod through disulphide bonds formed by HRGS treatment probably caused minor changes in the conformation of the rod. However, the details remain obscure and further studies to examine the microstructure of the heated and oxidized rods are needed.

3.4. H_2O_2 concentration effect

The results described above were obtained by following the thermal denaturation process of myosin in myofibrils oxidized by HRGS with a fixed concentration of H_2O_2 (1 mM). To determine whether the process was H_2O_2 dose dependent, myofibrils were treated with HRGS at different concentrations of H_2O_2 (0–5 mM) before subjecting to heat

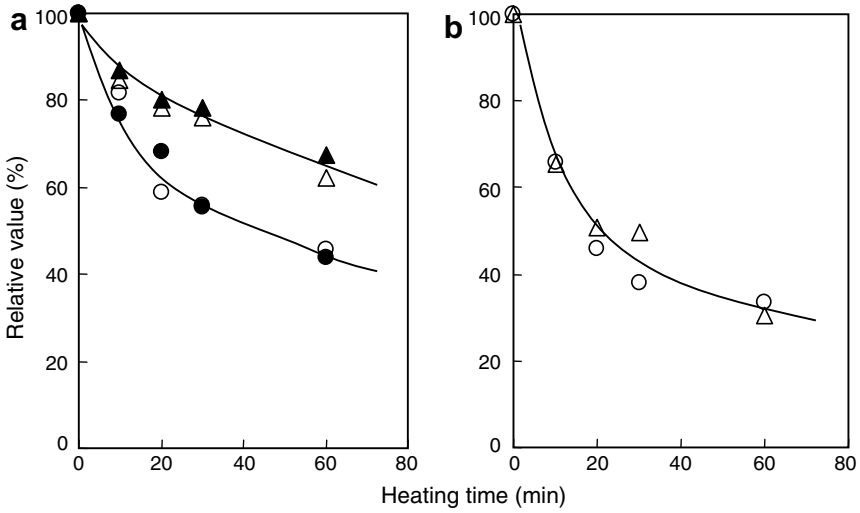

Fig. 2. Influence of oxidation of myofibrils by HRGS on changes in chymotryptic production of S-1 or rod from myosin due to heat treatment. The relative amounts of S-1 (a) or rod (b) produced from myosin in myofibrils with (○) or without (△) HRGS treatment were measured by densitometric scan of SDS–PAGE. The Ca-ATPase activity changes were also plotted with the same but enclosed symbols.

at 49 °C for 20 min. Changes in Ca-ATPase, myosin salt solubility, and chymotryptic production of S-1 and rod were examined. Both the Ca-ATPase activity (Fig. 3a) and myosin solubility (Fig. 3b), which dropped to 89% and 38%, respectively, after heating (0 mM H_2O_2) were further reduced ($P < 0.05$) with increasing levels of H_2O_2 for oxidation. The decrease in chymotryptic production of S-1 by heating was not affected by the oxidation with 0.1 mM H_2O_2, but was promoted (by an additional 30%) with a 0.5 mM H_2O_2 treatment (Fig. 3c). No further decrease ($P > 0.05$) in S-1 production was seen beyond 0.5 mM H_2O_2, suggesting that the H_2O_2 effect probably reached a maximal or saturation level. However, the production of rod (Fig. 3d) was not affected by oxidation in the 0.1–5 mM H_2O_2 concentration range, indicating that the HRGS treatment of myofibrils did not increase the susceptibility of the rod to heat.

3.5. Rate constant of myosin denaturation

The results described so far were obtained by analyzing the extent of heat denaturation process of myosin in oxidized myofibrils by HRGS. Subsequent experiments were run to elucidate the effects of HRGS treatment on rate constant of myosin denaturation. For that purpose, the logarithmic values of the relative Ca-ATPase activity, myosin solubility, and the production of S-1 as well as rod as shown in Figs. 1 and 2 were plotted against heating time (data not presented). There were linear relationships between the logarithmic values for respective denaturation indices and the heating time with high regression coefficients (0.88–0.99). Thus, denaturation rate constants were estimated from the slope of these linear relationships.

First, to elucidate the effect of temperature on denaturation process of oxidatively modified myosin, myofibril

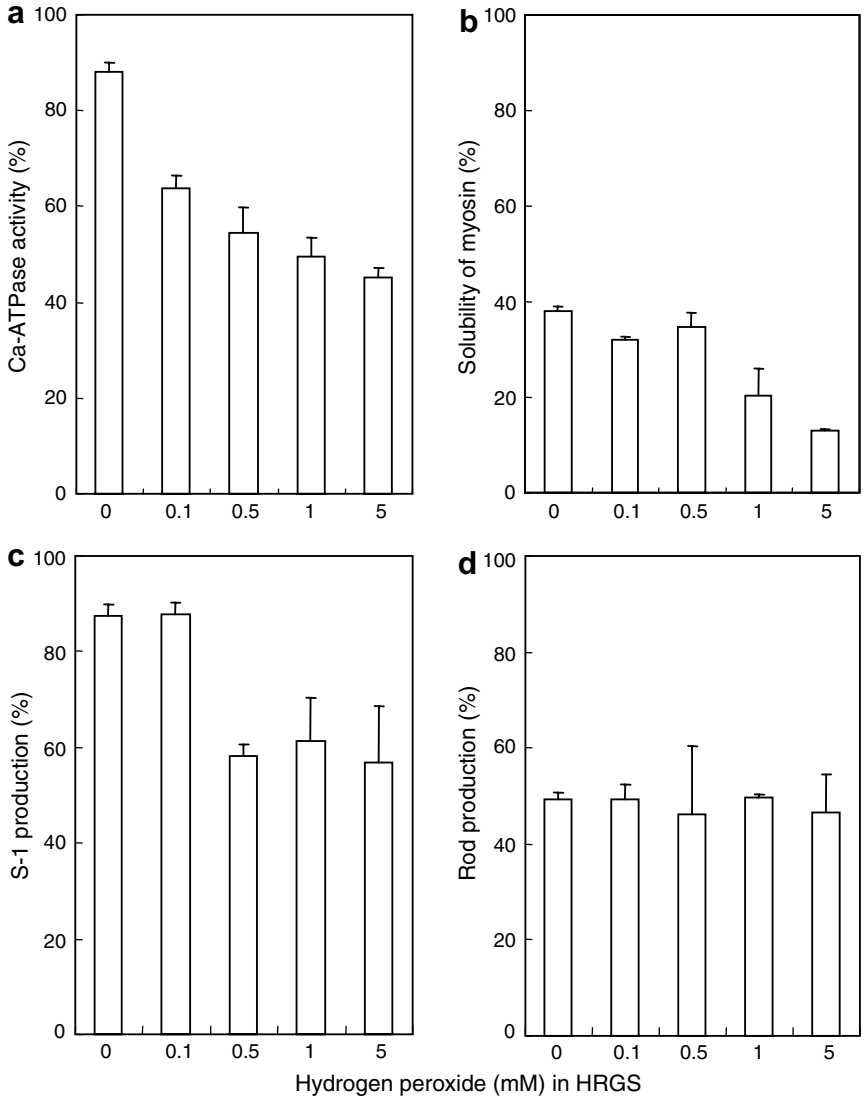

Fig. 3. Effect of H_2O_2 concentration on heat-induced denaturation of myosin. Myofibrils were first oxidized with HRGS containing various concentrations of H_2O_2 and then heat-treated at 49 °C for 20 min. Ca-ATPase activity (a), solubility of myosin (b), S-1 production (c), and rod production (d), determined in the same manner as in Figs. 1 and 2, were expressed as relative values (%) to those without heating.

samples treated with or without HRGS containing 1 mM H_2O_2 were heated in 0.1 M KCl at 45, 47, or 49 °C for various durations. The rate constants for Ca-ATPase destruction, myosin solubility loss, and reduction in producing S-1 and rod were presented in Fig. 4. To clearly demonstrate the changes in the rate constants, the scales of the abscissas in Fig. 4 were adjusted depending on the heating temperature. The rate constants estimated from each of myosin denaturation indices increased with rising temperature. For un-oxidized myofibrils, the rate constants for myosin solubility loss and for rod production loss were larger ($P < 0.05$) than those for Ca-ATPase activity and S-1 production, when compared at the same heating temperatures.

Fig. 4 also showed that regardless of heating temperatures, the HRGS treatment increased ($P < 0.05$) the rate constants for the ATPase activity decay, myosin solubility loss, and drop in S-1 production. However, the rod production did not seem to be affected by the HRGS. The marked increase in the rate of reducing myosin salt solubility by oxidation was likely a result of an enhanced denaturation of S-1, which was in line with the Ca-ATPase activity change. The relative decreasing rate constants for ATPase activity and S-1 production to those for rod production became larger by oxidation at every temperature, indicating that denaturation mode of myosin was modified by HRGS treatment. This may be a cause of altered gel formation of oxidized myofibrillar proteins as reported elsewhere (Liu & Xiong, 2000b; Xiong et al., 1993).

Ionic strength of the medium also affects denaturation mode of myosin (Yamamoto et al., 2002). This is considered to be associated with the filament formation of myosin. Myosin forms thick filament in myofibrils under physiological ionic strength (<0.15), but myosin filaments dissolve with an increase in ionic strength in the solution (>0.3). Therefore, the effects of ionic strength on denaturation constants of myosin in myofibrils subjected to HRGS were examined at 49 °C. As depicted in Fig. 5, the rate constants of un-oxidized myosin for Ca-ATPase decay, salt solubility loss, and reduction in S-1 production were greater ($P < 0.05$) at 0.5 M KCl than at 0.1 M KCl, but the decreasing rate constant for rod production was minimally affected by the increase in KCl concentration. As a result, the rate constants for heat-induced Ca-ATPase inactivation and the S-1 production loss of un-oxidized myofibrils tended to be greater, albeit non-significant ($P > 0.05$), than that for the decrease in rod production in the presence of 0.5 M KCl. The results indicated that denaturation of S-1 preceded that of rod at 0.5 M KCl, while the reverse was true in 0.1 M KCl. Under both ionic strength conditions, oxidation increased the rate of Ca-ATPase decay, promoted loss of myosin salt solubility, and suppressed S-1 production, but it barely affected the change in the rate constant for rod production. The results suggested that the heat-induced denaturation of oxidatively modified myosin head was sensitive to ionic strength shifts, but that of oxidatively modified tail portion was independent of the ionic strength regardless of the filament formation of the myosin.

As aforementioned, the thermal stability of myosin rod was insensitive to HRGS treatment despite the formation of the intramolecular cross-links of myosin through disulphide bond in LMM portion of myosin rod. These results imply the intramolecular cross-linking of myosin barely affected the progress of the heat-induced unfolding of α-helical structure of myosin rod.

On the other hand, myosin S-1 was significantly destabilized by HRGS treatment. Murozuka (1979) demonstrated that *N*-ethylmaleimide (a SH-blocking reagent) also caused an increase of Ca-ATPase activity as well as promotion of thermal inactivation. Therefore, destabilization of myosin S-1 seemed to be associated with the modification of the reactive thiol groups located around ATPase active site, which are involved in the ATPase activity changes. It was

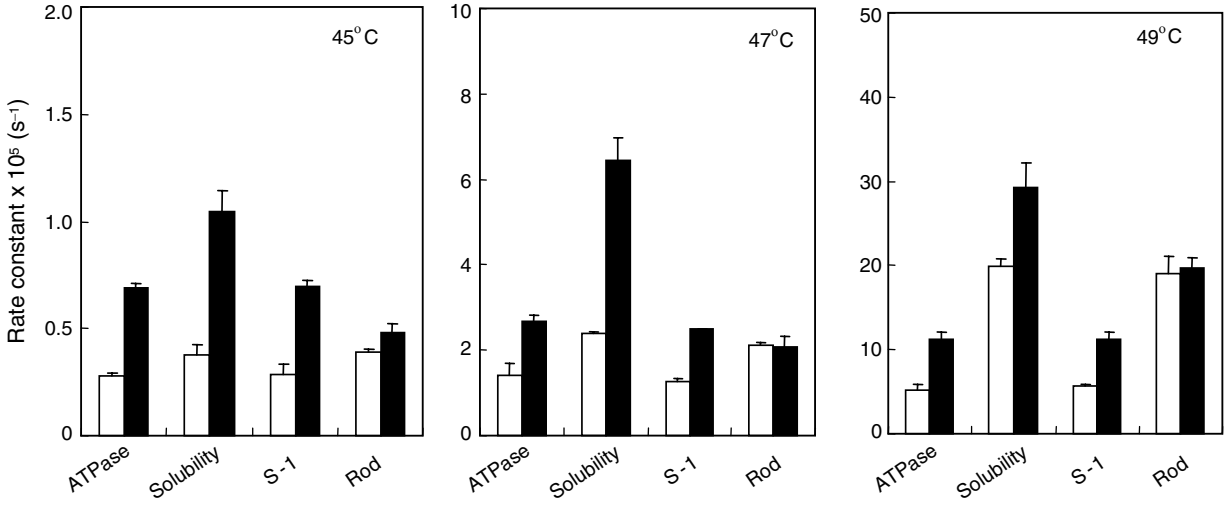

Fig. 4. Effect of temperature on denaturation rate constants of myosin in myofibrils oxidized by HRGS. Oxidized myofibrils were heat-treated in 0.1 M KCl at 45, 47, or 49 °C. The black and white columns stand for, respectively, myofibril samples with or without HRGS treatment under the same condition as in Fig. 1.

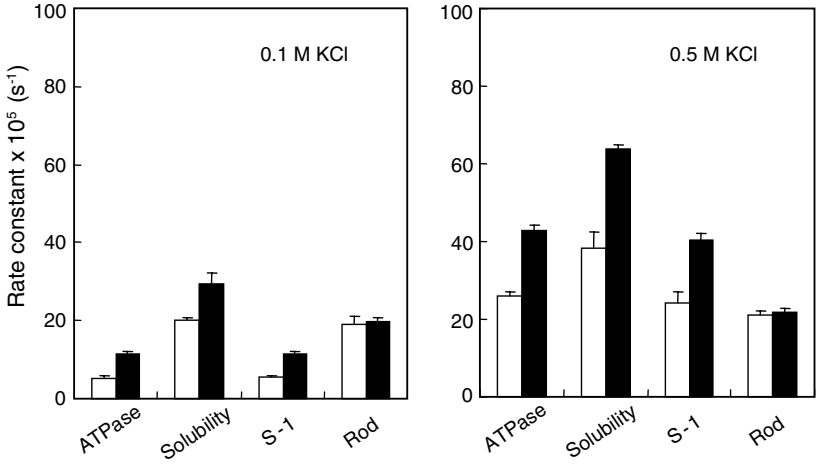

Fig. 5. Effect of KCl concentration on denaturation rate constants of myosin in myofibrils oxidized by HRGS. Oxidized myofibrils were heat-treated in 0.1 M or 0.5 M KCl at 49 °C. The black and white columns stand for, respectively, myofibril samples with or without HRGS treatment under the same condition as in Fig. 1.

already confirmed that these SH reagents bonded to the thiol groups in myosin active site, but not necessarily caused intra- or intermolecular cross-linking of myosin S-1. These observations coincided with the fact that HRGS treatment did not cause cross-linking of myosin S-1.

As well known, myosin is stabilized by bonding to actin in myofibrils (Yasui, Kawakami, & Morita, 1968). Thus, a possibility seemed to exist that destabilization of myosin by HRGS treatment was caused by weakening of the interaction of myosin to actin. To investigate this possibility, oxidized and un-oxidized myofibrils were heated in the medium containing 1.72 M KCl, where myosin mostly dissociates from action (Wakameda, Nozawa, & Arai, 1983), and then submitted to Ca-ATPase activity measurement. Although the data were not presented, rapid inactivation of myosin in oxidized myofibrils was still observed even in higher concentration of KCl when compared with un-oxidized myofibrils. It was, thus, suggested that destabilization of myosin S-1 was caused by its structural changes, not by weakening of the interaction between myosin and actin.

In conclusion, the results from this study demonstrated that oxidative stress from exposures to HRGS produced different effects on thermal susceptibilities of two distinct domains of myosin (S-1 and rod) in myofibrils. Destabilization of myosin occurred mainly in the S-1 globular head region instead of the rod portion, which changed the kinetics of myosin thermal denaturation. The alteration in myosin denaturation pattern as induced by oxidation as well as intra- and intermolecular cross-linking of myosin as presumably causative factor for changing the functionality of myofibrillar proteins (gelation, water-binding, emulsification, etc.) under similar meat processing conditions.

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References

Decker, E. A., Xiong, Y. L., Calvert, J. T., Crum, A. D., & Blanchard, S. P. (1993). Chemical, physical, and functional properties of oxidized turkey white muscle myofibrillar proteins. *Journal of Agricultural and Food Chemistry, 41*, 186–189.

Gornall, A. G., Bardawill, C. S., & David, M. N. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry, 177*, 751–766.

Kato, S., Koseki, H., & Konno, K. (1996). Oligomerization of carp myosin which retains its ATPase activity. *Fisheries Science, 62*, 985–989.

Katoh, N., Uchiyama, H., Tsukamoto, S., & Arai, K. (1977). A biochemical study on fish myofibrillar ATPase. *Nippon Suisan Gakkaishi, 43*, 857–867.

Konno, K., & Ueda, Y. (1989). Mg-ATPase enhancement of carp myofibrils upon thermal treatment. *Nippon Suisan Gakkaishi, 55*, 1457–1462.

Konno, K., Yamamoto, T., Takahashi, M., & Kato, S. (2000). Early structural changes myosin rod upon heating of carp myofibrils. *Journal of Agricultural and Food Chemistry, 48*, 4905–4909.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Li, S. J., & King, A. J. (1999). Structural changes of rabbit myosin subfragement 1 altered by malonaldehyde, a byproduct of lipid oxidation. *Journal of Agricultural and Food Chemistry, 47*, 3124–3129.

Liu, G., & Xiong, Y. L. (2000a). Electrophoretic pattern, thermal denaturation, and in vitro digestibility of oxidized myosin. *Journal of Agricultural and Food Chemistry, 48*, 624–630.

Liu, G., & Xiong, Y. L. (2000b). Thermal transitions and dynamic gelling properties of oxidatively modified myosin, β-lactoglobulin, soy 7S globulin and their mixtures. *Journal of the Science of Food and Agriculture, 80*, 1728–1734.

Martinaud, A., Mercier, Y., Marinova, P., Tassy, C., Gatellier, P., & Renerre, M. (1997). Comparison of oxidative process on myofibrillar proteins from beef during maturation and by different model oxidation systems. *Journal of Agricultural and Food Chemistry, 45*, 2481–2487.

Murozuka, T. (1979). Comparative study on the enzymatic properties and thermal stabilities of *N*-ethylmaleimide modified myosins from fish dorsal and rabbit skeletal muscles. *Nippon Suisan Gakkaishi, 45*, 1503–1512.

Ooizumi, T., & Xiong, Y. L. (2004). Biochemical susceptibility of myosin in chicken myofibrils subjected to hydroxyl radical oxidizing systems. *Journal of Agricultural and Food Chemistry, 52*, 4303–4307.

Ooizumi, T., & Xiong, Y. L. (2006). Identification of cross-linking site(s) of myosin heavy chains in oxidatively stressed chicken myofibrils. *Journal of Food Science, 71*, C196–C199.

Sekine, T., & Yamaguchi, M. (1963). Effect of ATP on the binding of *N*-ethylmaleimide to SH groups in the active site of myosin ATPase. *Journal of Biochemistry, 54*, 196–198.

Sharp, A., & Offer, G. (1992). The mechanism of formation of gels from myosin molecules. *Journal of the Science of Food and Agriculture, 58*, 68–73.

Smith, D. M. (1987). Functional and biochemical changes in deboned turkey due to frozen storage and lipid oxidation. *Journal of Food Science, 52*, 22–27.

Srinivasan, S., & Hultin, H. O. (1997). Chemical, physical, and functional properties of cod proteins modified by a nonenzymic free-radical-generating system. *Journal of Agricultural and Food Chemistry, 45*, 310–320.

Takahashi, M., Yamamoto, T., Kato, S., & Konno, K. (2005). Species-specific thermal denaturation pattern of fish myosin when heated as myofibrils as studied by myosin subfragment-1 and rod denaturation rates. *Fisheries Science, 71*, 405–413.

Tazawa, T., Kato, S., Katoh, T., & Konno, K. (2002). Role of neck region in the thermal aggregation of myosin. *Journal of Agricultural and Food Chemistry, 50*, 196–202.

Wakameda, A., Nozawa, S., & Arai, K. (1983). Effect of neutral salts on thermal denaturation of myofibrillar Ca-ATPase of Fish. *Nippon Suisan Gakkaishi, 49*, 237–243.

Wan, L., Xiong, Y. L., & Decker, E. A. (1993). Inhibition of oxidation during washing improves the functionality of bovine cardiac myofibrillar protein. *Journal of Agricultural and Food Chemistry, 41*, 2267–2271.

Wang, B., & Xiong, Y. L. (1998). Functional stability of antioxidant-washed, cryoprotectant – treated beef heart surimi during frozen storage. *Journal of Food Science, 63*, 293–298.

Weeds, A., & Pope, B. (1977). Studies on the chymotryptic digestion of myosin. Effect of divalent cations on proteolytic susceptibility. *Journal of Molecular Biology, 111*, 129–157.

Xiong, Y. L., Decker, E. A., Robe, G. H., & Moody, W. G. (1993). Gelation of crude myofibrillar protein isolated from beef heart under antioxidative conditions. *Journal of Food Science, 58*, 1241–1244.

Yamamoto, K. (1990). Electron microscopy of thermal aggregation of myosin. *Journal of Biochemistry, 108*, 896–898.

Yamamoto, Y., Takahashi, M., Kato, S., & Konno, K. (2002). Denaturation mode of carp myofibrils when affected by temperature and salt concentration. *Fisheries Science, 68*, 627–633.

Yasui, T., Kawakami, H., & Morita, F. (1968). Thermal inactivation of myosin A adenosine triphosphatase in the presence of F-actin. *Agricultural Biological Chemistry, 32*, 225–233.