THE Fe-H_2O_2 OXIDATION PROMOTES MYOFIBRILLAR PROTEIN GELATION BY ENHANCING MYOSIN TAIL-TAIL INTERACTION

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Introduction

It is now established that protein oxidation is involved in the development of structural characteristics of muscle foods during processing and the changes in their textural and water-binding properties during storage. Our recent studies have shown that hydroxyl radicals generated in meat processing could shift the dynamics and equilibrium of head-head, tail-tail, and head-tail interactions of myosin; and disulfide bonds were the primary cross-links formed between myosin subunits (Ooizumi and Xiong, 2006). The oxidant-induced myosin cross-linking was preceded by the modifications of certain amino acid residue side chain groups as well as myosin structure, and the formation of protein carbonyls (Park et al., 2006; Park and Xiong, 2007). Further investigations showed that the extent of myosin cross-linking, and the specific sites involved, tended to vary, depending on the type and concentration of the specific oxidants, i.e., iron, metmyoglobin, or linoleic acid (Park et al., 2007). The objective of the present study was to determine how protein oxidation, induced by iron-ascorbate with various concentrations of H_2O_2, would change the dynamic gelling properties of myofibrillar proteins.

Materials and Methods

Myofibrillar protein isolate (MPI), prepared from porcine *Serratus ventralis* muscle (intermediate fiber type) and suspended in 0.6 M NaCl in a 15 mM PIPES buffer (pH 6.0), was oxidized by incubation with a hydroxyl radical-generating system (10 μM $FeCl_3$, 0.1 mM ascorbic acid, 0.1–10.0 mM H_2O_2) at 4 °C for 24 h. Oxidation was stopped by adding propyl gallate/Trolox C/EDTA (1 mM each). Oxidized MPI pellets were digested with chymotrypsion (1:500) for 60 min at 21 ºC in: a) 0.12 M NaCl and 1mM EDTA (for S-1 and rod), and b) 0.6 M NaCl and 1mM $CaCl_2$ (for heavy meromyosin, HMM and light meromyosin, LMM). Digestion was terminated by treating with 0.5 mM phenylmethylsulfonyl fluoride (PMSF). SDS-PAGE with a 10% polyacrylamide resolving gel with or without 5% β-mercaptoethanol was run to identify cross-linked myosin subunits (Laemmli, 1970). Losses of proteins and myosin fragments due to oxidation were quantified by digitizing the protein bands with a pixel-counting software.

Protein gelation was induced by heating MPI samples (30 mg/mL in 15 mM PIPES buffer, pH 6.0) from 20 to 73 ºC, and the gel storage modulus (rigidity) was measured using an oscillatory mode of dynamic rheological testing with a 0.1 Hz frequency and a 0.02 strain. Data from 5–6 replicated trials were subjected to analysis of variance assuming a general linear model. Differences between sample means for significant treatment effects (H_2O_2 concentration; temperature) were identified by the least significant difference procedure.

Results and Discussion

Oxidation resulted in the disappearance of myosin heavy chain (MHC); essentially all MHC were lost in 5 mM H_2O_2, where considerable amounts of actin remained intact (Figure 1). Analysis of the chymotryptic digests in 0.60 M NaCl solution showed that the loss of the LMM subfragment at increasing H_2O_2 concentration was more drastic and extensive than that of HMM (Figure 2). Consistent with the result, the MPI digests in 0.12 M NaCl revealed more rapid loss of rod than loss of S-1 (data not shown), indicating that hydroxyl radicals generated in the Fe-H_2O_2 oxidizing system preferentially attacked the cysteine residues in the tail portion of myosin. Intermolecular disulfide cross-linking ostensibly led to the aggregation of myosin polymers insoluble in the aqueous solution, because treatment with β-mercaptoethanol recovered most myosin and actin. The same oxidizing condition was previously shown to induce aggregation of myosin from chicken *Pectoralis* muscle (white fiber type) almost exclusively via tail-tail cross-linking (Ooizumi and Xiong, 2004). The discrepancy may be attributed to myosin isoforms that are specific to muscle types or animal species.

Within the 0 to 5 mM H_2O_2 concentration range, rigidity of heat-induced MPI gels increased ($P < 0.05$) with the H_2O_2 application level except that the rheological transition in 40–50 ºC was diminished (Figure 3). Since this transient gel rigidity increase typically results from the association of myosin head (Egelandal et al., 1986), the lack of this transition in oxidized MPI samples was explainable because oxidation promoted myosin tail-tail association. The pre-formation of tail-tail myosin oligomers would predispose MPI to aggregation through oligomer-oligomer association, leading to a viscoelastic gel network structure. The rapid increase in gel rigidity at temperatures > 60 °C was widely attributed to the interaction between myosin tails (light meromyosin), which was accentuated by oxidation. No apparent off-flavor could be detected from these gel products.

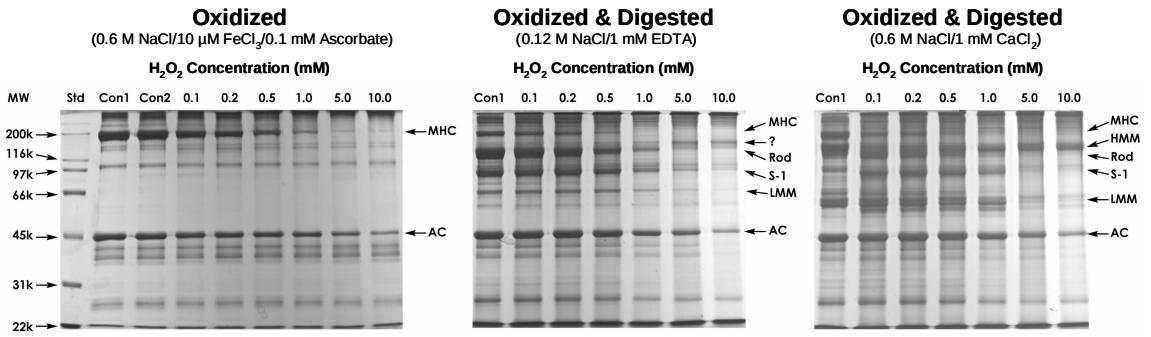

Figure 1. SDS-PAGE of chymotrypsin-digested oxidized myofibrillar protein isolate (no β-mercaptoethanol). The bands showed more extensive disappearance of myosin tail (rod; LMM) than myosin head (S-1; HMM) with increasing H_2O_2 concentrations. MHC = myosin heavy chain; AC = actin.

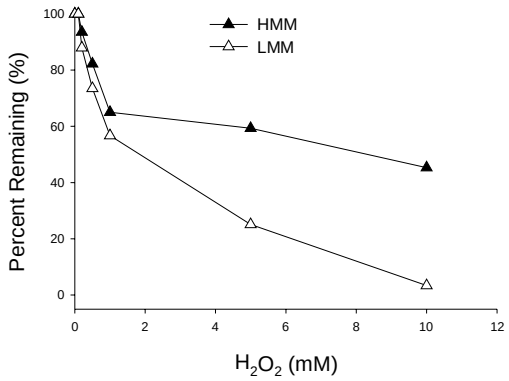

Figure 2. Remaining myosin subunits after oxidation with Fe-H_2O_2. HMM = heavy meromyosin; LMM = light meromyosin.

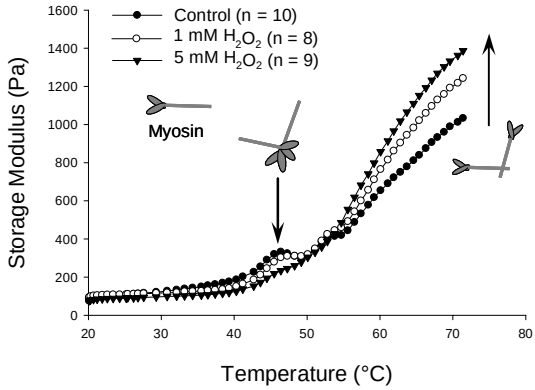

Figure 3. Gel rigidity (storage modulus) of MPI oxidatively modified by Fe-H_2O_2.

Conclusions

The results demonstrated the positive effect of mild oxidation to improve the thermal gelation of porcine myofibrillar proteins. Such gelling property improvement resulted from the shift from the predominant head-head association of myosin to its tail-tail cross-linking through disulfide bridges. The findings could explain, in part, variability in textural quality of processed muscle foods, and at the same time, challenge the conventional notion that in food and food products, oxidation is always undesirable, regardless of the level of protein modification.

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