

# Enzymatic modification of the chicken breast myofibril proteins: effects of an oxidative enzyme and transglutaminase on gelation and thermal stability

Raija Lantto, Johanna Buchert and Karin Autio  
VTT Biotechnology, PO BOX 1500, FIN-02044 VTT, Finland,

Eero Puolanne  
Food Technology, PO Box 66, FIN-00014 University of Helsinki, Finland

The effects of transglutaminase (TG) and laccase on gelation and thermal stability of salt-soluble chicken breast myofibril proteins were studied at pH 6. Myosin heavy chain and troponin T were the most sensitive myofibril proteins to the enzyme action, whereas actin was relatively resistant to the enzymes. TG was capable of enhancing gelling of myofibrils in 0.35 M Na<sup>+</sup>, while laccase needed complete solubilisation of the proteins for enhancement of the gel formation. Extensive laccase treatment resulted in protein fragmentation and decrease in gel forming ability below the control level, although clear gel formation was observed with a lower laccase level. Thermal stability of myosin was reduced slightly with TG. Thermal stability of actin was increased by TG. Maximum transition temperatures of myosin or actin did not alter with either enzyme.

**Keywords:** myofibril proteins, protein modification, cross-linking, transglutaminase, laccase, gel formation, heat stability

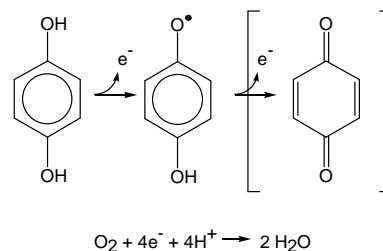
## 1 INTRODUCTION

Myofibril proteins are responsible for the formation of a gel network in meat. In addition to the type and quantity of proteins, their solubility, pH, and temperature affect gel formation and the viscoelastic properties of the gel. Myofibril proteins undergo denaturation and aggregation before forming a network.

Structure formation and water-holding capacity (WHC) of meat systems are greatly dependent on pH. Above the average isoelectric point (about pH 5.0) of the muscle proteins, the WHC increases [1] and intact myofibrils swell along with increasing pH. Gelling of isolated chicken myofibril proteins is optimal at pH 6 [2] and is dependent on extractability of the salt-soluble myofibril proteins. In addition to the pH, the functional properties of meat products are dependent on salt. NaCl is essential for the swelling of myofibrils and the solubilization of myofibril proteins. Cl<sup>-</sup> ions penetrate the myofilaments and cause them to swell [1]. The negative net charge thus formed attracts Na<sup>+</sup> ions close to the filament surfaces resulting in local concentration changes and leading to an increased osmotic pressure inside the myofibrils resulting swelling of the matrix.

Enzymes that stabilize proteins by forming additional covalent cross-links may be used to fabricate meat products with better texture. Currently, transglutaminases (TG, EC 2.3.2.13) are the only commercially available enzymes for cross-linking proteins and for improving the texture of

meat products. TG catalyzes formation of an  $\epsilon$  ( $\gamma$ -glutamyl) lysine isopeptide bond inter- or intramolecularly in myosin and actin [3]. Apart from TGs polyphenol oxidases e.g. laccase are capable of catalyzing covalent cross-links in proteins. Laccases (EC1.10.3.2) are radical-forming enzymes catalyzing oxidation of various phenol compounds, simultaneously reducing molecular oxygen to water. The role of laccases in nature is the lignin modification. As shown in Scheme 1, laccase catalyzes an electron transfer reaction of a diphenol to form an oxygen-centred free radical. A mechanism of laccase-catalyzed oxidation of tyrosine-containing peptides leading to an isodityrosine crosslink has been proposed by Mattinen *et al.* [4]. The capability of laccases to oxidize a wide variety of phenolic and other substrates has raised interest to study the application potential of laccases in modifying proteins from various sources [5-8].



Scheme 1. Laccase-catalyzed oxidation of a diphenol.

The aim of the present study was to elucidate the potential of laccase and TG as reference in modifying chicken breast muscle myofibril proteins. The consequences of the enzyme-aided modification on the gel formation of the myofibrils were monitored as storage modulus ( $G'$ ). The thermal stability of the myofibrils was monitored by differential scanning calorimetric (DSC) measurements.

## 2 MATERIALS AND METHODS

### 2.1 Enzymes and proteins

TG was fractionated from Activa<sup>®</sup>WM (Ajinomoto Inc., Japan). Laccase has been isolated and purified from the fungus *Trametes hirsuta*. The enzymes were dosed according to their activities as nkat/g of protein. Myofibrils and myofibril proteins were isolated from chicken breast muscle according to Lantto et al. [18]. The enzyme-catalysed protein modification was monitored by SDS-PAGE.

### 2.2 Viscoelastic measurements

Gel formation of the enzyme-treated and control myofibril samples was measured using a Bohlin VOR rheometer in oscillatory mode (Bohlin Reologi Ab, Sweden) to determine the storage modulus ( $G'$ ) at constant temperatures. 4 % myofibril suspension was treated with enzymes in 50 mM Na-phosphate buffer, 0.35 M or 0.60 M NaCl, pH 6, for 3 h.

### 2.3 DSC

Changes in the thermal stability of the enzymatically cross-linked and control myofibrils were measured using a Mettler Toledo DSC820 differential scanning calorimeter. After the enzymatic treatment myofibrils were heated from 40°C to 100°C at a scan rate of 10°C/min. Pre-treatments were carried out to 7 % myofibril suspension in 50 mM Na-phosphate buffer, 0.60 M NaCl, pH 6, for 3 h.

## 3 RESULTS AND DISCUSSION

### 3.1 Modification of myofibril proteins by the enzymes

The major modifications of the myofibril proteins caused by TG and laccase were tentatively determined by comparing the relative mobilities and staining intensities of the enzyme-treated proteins on SDS-PAGE. Under the given conditions the following electrophoretic changes were observed:

(1) progressive disappearance of myosin heavy chain (MHC) and (2) troponin T with both enzymes. In addition, laccase when dosed to excess caused fragmentation of at least MHC and troponin T.

### 3.2 Gel formation of myofibrils

The  $G'$  of the samples was monitored at the constant temperature of 40°C during 3 h of treatment. At the lower ionic strength (0.35 M Na<sup>+</sup>)  $G'$  increase was very weak with the control and the laccase - treated myofibrils (Fig 1 a, b). With TG  $G'$  increased along with the increasing dosage (Fig 1 a).

Only when the ionic strength was raised to 0.60 M Na<sup>+</sup> also laccase was able to enhance the gel formation (Fig 1 c). The optimum dosage of laccase to increase gel formation in the used conditions was 200 nkat/g of protein. The lower dosage of 100 nkat/g of protein was not sufficient to induce gel formation. A further increase of laccase dosage (1000 nkat/g) caused a decrease in gel formation below the control level. With the dosage of 1000 nkat/g protein fragmentation was observed also on SDS-PAGE (data not shown).

It can be concluded that laccase caused protein fragmentation in addition to protein polymerisation. The action of TG was more straightforward. The gel formation increased along with increased dosage of both TG and tyrosinase.

### 3.3 Heat stability of myofibrils

Myofibril samples treated with TG and laccase showed different thermal behaviour (Fig. 2). Two major endothermic transitions were observed with peak temperatures ( $T_{max}$ ) at about 59°C (myosin) and about 66°C (actin). TG decreased the thermal stability of myosin whereas laccase did not have an effect (Fig 2 a). However, TG increased the thermal stability of actin. Laccase was also capable of increasing the  $\Delta H$  of actin after 1 h of pre-treatment (data not shown), but after 3 h of the treatment the  $\Delta H$  of actin with laccase was reduced back to the level of the control.  $T_{max}$  of myosin or actin was not affected by the enzyme treatments. Decreased thermal stability of myosin in the TG-treated myofibrils may be a consequence of the polymerization of the myosin molecules to larger but less thermostable structures. Additional intramolecular bonds may lead to more stable protein structures. A significant increase of the actin transition was observed with TG, which may be a result of a stabilizing intramolecular cross-linking of actin by these enzymes. On SDS-PAGE actin seemed more resistant to the enzyme-catalysed modification than e.g. MHC (data not shown).

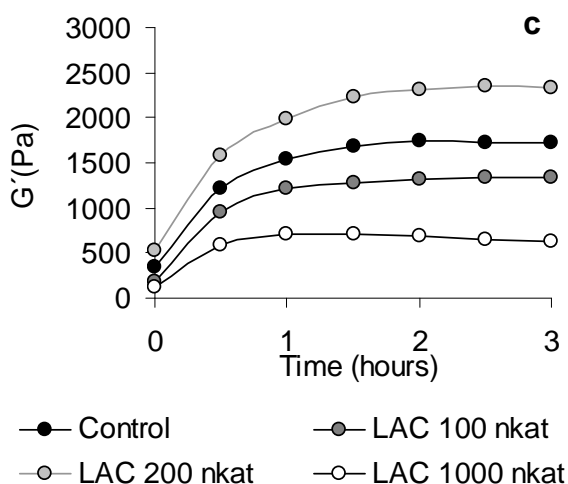
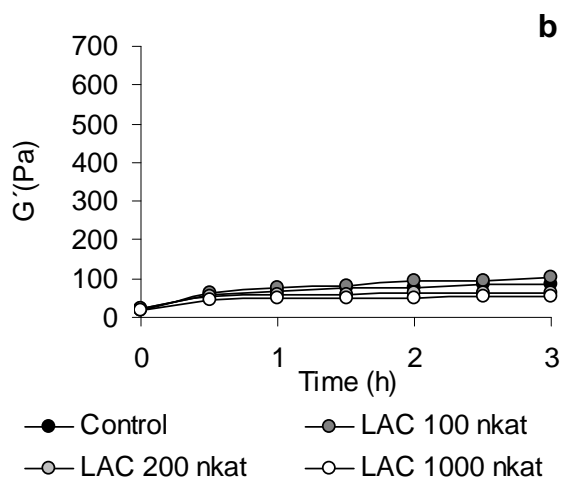
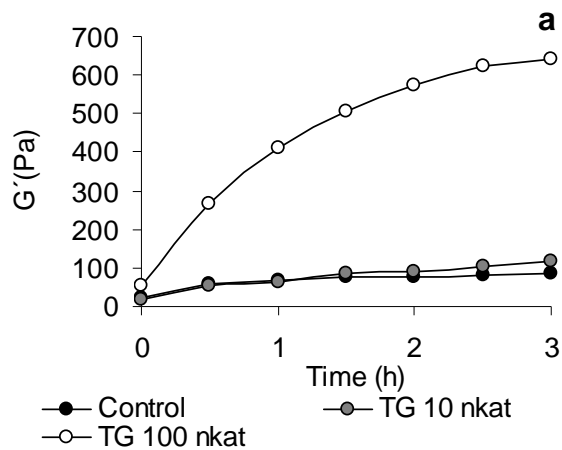


Figure 1: Storage modulus ( $G'$ ) of myofibrils measured at a constant temperature of  $40^{\circ}\text{C}$ . Treatment with (a) TG in  $0.35\text{ M NaCl}$ , (b) laccase in  $0.35\text{ M NaCl}$  and (c) laccase in  $0.60\text{ M NaCl}$ . Control was treated similarly but without enzymes.

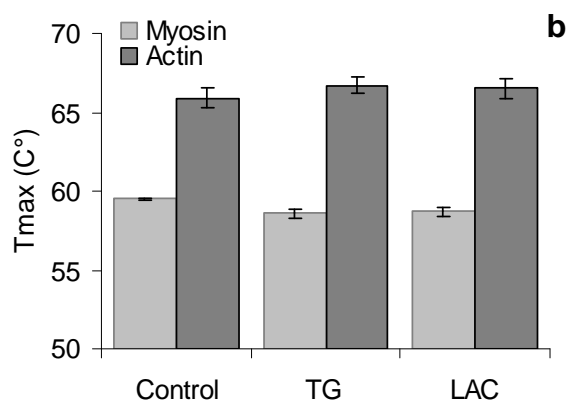
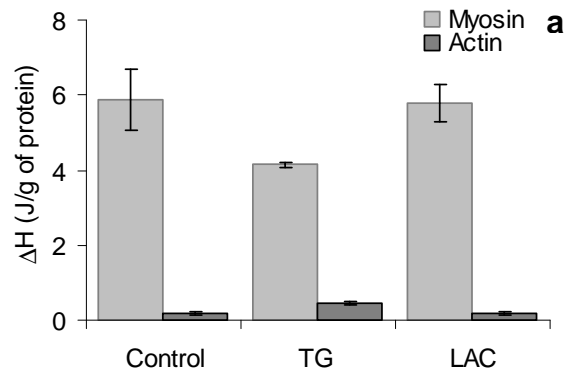


Figure 2: (a) Heat transition ( $\Delta H$ ) and (b) peak temperature ( $T_{\text{max}}$ ) of the TG and laccase treated myofibrils. Control was treated similarly but without enzymes.

#### 4 CONCLUSIONS

TG and laccase were capable of polymerizing chicken breast myofibril proteins. The most susceptible proteins were MHC and troponin T. In addition to polymerization, laccase caused protein fragmentation in extensive treatment conditions.

With TG gel forming was enhanced along with the increasing enzyme dosage. TG was also capable of gel forming in a reduced salt concentration, in which myofibril proteins are not completely soluble. Laccase was capable of increasing gel forming only in the conditions where proteins were soluble. Laccase decreased the gel forming below the control level when an elevated laccase dosage was used.

TG caused slight decrease in thermal stability of myosin possibly due to formation of protein polymer structures with reduced thermostability. TG and laccase treatments resulted in an increase in actin thermostability possibly because of intramolecular cross-linking.

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