Food Structure 15 (2018) 1–8

Contents lists available at ScienceDirect

Food Structure

journal homepage: www.elsevier.com/locate/foostr

Effect of actomyosin form extracted from skeletal fast muscle on the structural and rheological properties of heat-induced gels

Tomohito Iwasaki[a,*], Yasuyuki Masuda[a], Yasuhiro Hasegawa[a], Naoyuki Maeda[b]

[a] Department of Food Science and Human Wellness, Rakuno Gakuen University, Hokkaido, Japan
[b] Safety Research Institute for Chemical Compounds Co., Ltd., Hokkaido, Japan

ARTICLE INFO

Keywords:
Chicken breast muscle
Actomyosin
Pyrophosphate
Gel structure
Gel properties

ABSTRACT

Little is known about the mechanism of heat-induced gelation of natural actomyosin, or the influence of pyrophosphate on the morphology and physical properties of the product. In the current study, these questions were investigated using scanning and transmission electron microscopy. When using high salt concentrations (0.5 M NaCl), actomyosin lost its "arrowhead" structure after heating for two minutes, and aggregation, including interfilament binding, occurred after six minutes of heating at 60° C. The product in this case exhibited a strand-like gel structure. On the other hand, in the presence of 0.1 M NaCl, cohesion between filaments began after two minutes of heating, and an aggregate-type gel structure was formed. When pyrophosphate was added in the presence of 0.5 M NaCl, marked dissociation of actomyosin into myosin and actin occurred. In the presence of 0.1 M NaCl, the filament structure of the actomyosin bundles became difficult to detect. The addition of pyrophosphate did not improve the physical properties of the resultant heat-induced actomyosin gel, since the elasticity of the actomyosin gel decreased.

1. Introduction

The binding properties of heated meat products are a result of the thermal gelation of myosin and actomyosin from the myofibrillar proteins eluted by curing (Sigel & Schmidt, 1979a, 1979b). A previous study using synthetic actomyosin found differences in the rheological properties of the heat-induced gel depending on the ratio of myosin and actomyosin (Yasui, Ishioroshi, & Samejima, 1980; Yasui, Ishioroshi, & Samejima, 1982). By heating synthetic actomyosin with an excess of myosin with high salt concentrations, a three-dimensional strand network structure was formed when free myosin monomers crosslinked with actomyosin filaments (Yasui et al., 1982). On the other hand, under low-salt conditions, actin filaments inhibited the thermally activated interaction of myosin filaments (Ishioroshi, Samejima, & Yasui, 1983). The elution of myosin and actomyosin from myofibrils, as well as their morphology, are important for the quality of meat products. To obtain adequate water holding capacity and binding properties after heat-induced gel formation, it is necessary not only to increase the amount of eluted myosin and actomyosin, but also to control their extracted forms.

A polyphosphate is often added, together with sodium chloride, when processing meat products in order to maintain quality. The polyphosphates used exhibit different degrees of polymerization, such as pyrophosphate, tripolyphosphate, and hexametaphosphate. In particular, since pyrophosphate has a structure similar to the terminal pyrophosphate of ATP, it displays similar effects. Here, the ATP-like effect is due to the decomposition of ADP and inorganic phosphate from ATP, and relates to the dissociation of actomyosin into myosin and actin (Spicer, 1952). Furthermore, it is known that muscle fibers were solubilized by pyrophosphate or tripolyphosphate well (Xiong, Lou, Harmon, Wanhg, & Moody, 2000; Xiong, Lou, Wang, Moody & Harmon, 2000). Differences in solubility of muscle fiber type by addition of polyphosphate are investigated by Parsons and Knight (1990). They reported that red (slow) type muscle fibers are less soluble than white (fast) type, and that muscle fibers of red type require high concentrations of coexisting salts at the same pyrophosphate concentration.

It is well known that adding a polyphosphate to meat will improve its water-holding capacity and binding properties, which come from increasing the solubility of actomyosin and myosin (Bendall, 1954; Fukazawa, Hashimoto, & Yasui, 1961; Paterson, Parrish, & Stromer, 1988). Many studies have examined the heat-induced gelation of myosin and actomyosin (Acton, Hanna, & Satterlee, 1981; Hermansson, Harbitz, & Langton, 1986; Sano, Noguchi, Tsuchiya, & Matsumoto, 1988; Sano, Noguchi, Matsumoto, & Tsuchiya, 1990; O'Neill, Mulvihill, & Morrissey, 1993; Wang, Pato, Pietrasik, & Shand, 2009), but the mechanism surrounding the heat-induced gelation of natural

* Corresponding author at: Department of Food Science and Human Wellness, Rakuno Gakuen University, Ebetsu, Hokkaido, 069-8501, Japan.
E-mail addresses: iwasaki@rakuno.ac.jp (T. Iwasaki), yasu19861222@icloud.com (Y. Masuda), hasegawa@rakuno.ac.jp (Y. Hasegawa), maeda-naoyuki@ka-anken.co.jp (N. Maeda).

https://doi.org/10.1016/j.foostr.2018.01.001
Received 11 July 2017; Received in revised form 6 December 2017; Accepted 3 January 2018
Available online 09 January 2018

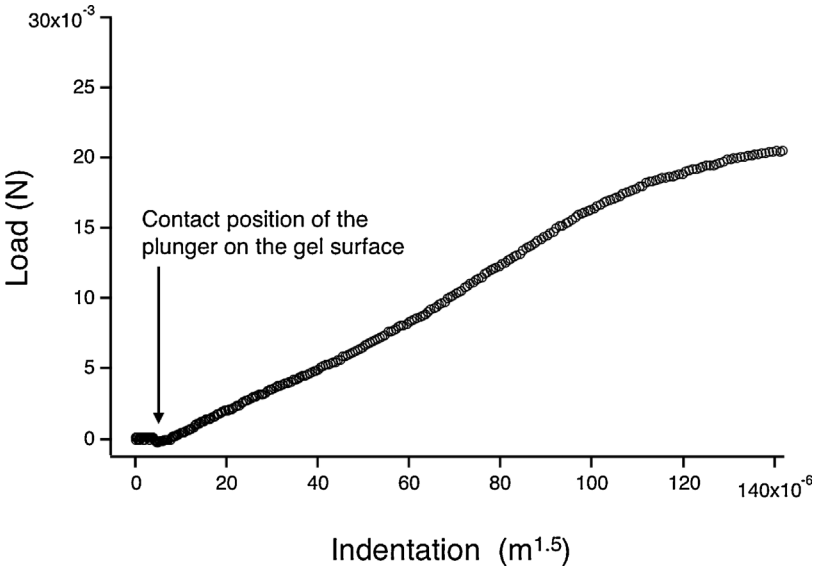

Fig. 1. The typical curve of the load (N) versus indentation ($m^{1.5}$) of heat-induced actomyosin gel. Actomyosin dissolved in 0.5 M NaCl at 20 mg/mL protein concentration. The sample solution was heated at 60 °C for 30 min.

Table 1
Effect of NaCl concentration on the apparent elasticity of heat-induced actomyosin gels.

Elastisicity (kPa)			
NaCl (M)	mean		SD
0.1	2.76[a]	±	0.70
0.2	4.72[a]	±	1.06
0.3	6.24[a]	±	1.60
0.4	17.0[b]	±	1.30
0.5	25.4[c]	±	2.90

Twenty mg/mL of actomyosin was dissolved in 10 mM Bis-Tris (pH 6.0) with 0.1–0.5 M NaCl. Thermal treatment was applied at 60° C for 30 min. Values not sharing a letter differed significantly using $p < 0.05$, n = 5.

actomyosin, which is important for the quality of meat products, has not been elucidated yet. In order to maintain the quality of processed meat products, such as ham and sausage, it is important to understand the influence of added polyphosphate on natural actomyosin form, along with the effects on the shape of the protein upon gelation. However, the existing literature on the influence of polyphosphate on the morphology of natural actomyosin, and its effects on the heat-induced gel product, is surprisingly scant.

In this study, the effect of pyrophosphate and salt on the shape of natural actomyosin extracted from chicken breast (fast) muscle was clarified using electron microscopy. In addition, the influence of the shape of this protein on the rheological properties and structure of the heat-induced gel, and the mechanism of its gelation, were investigated. The overall effect of pyrophosphate on improving the physical properties of the gel was also considered.

2. Material and methods

2.1. Preparation of actomyosin from chicken pectoralis major muscles

The animals in this study were cared for in accordance with the Guide for the Care and Use of Experimental Animals (Experimental Animals Committee of Rakuno Gakuen University, Japan). Six hens (live weight 2–3 kg) were slaughtered. Culled chicken pectoralis major muscles were obtained from the carcasses immediately after slaughter, and minced with a meat chopper following the removal of fat and connective tissue. Natural actomyosin was extracted using a Weber-Edsall solution (Haga, Maruyama, & Noda, 1965). Protein concentrations were measured by biuret methods using a bovine serum albumin standard. The actomyosin was dissolved in a solution containing 0.5 M NaCl before use.

2.2. Measurement of the apparent elasticity of the gel

Twenty mg/mL of actomyosin was dissolved in solutions with 10 mM Bis-Tris (pH 6.0) involved various NaCl concentrations (0.1–0.5 M) and pyrophosphate (PPi) concentrations (10–40 mM), along with 1 mM $MgCl_2$. The actomyosin solutions were put into 12 mm diameter glass tubes with 30 mm depths and sealed, then put into a water bath at 60° C for 30 min to gel the samples. Then, the samples were cooled in an ice bath. A spherical 5-mm diameter plunger then penetrated each gel at a rate of 0.5 mm/min using a creep meter RE–33005 (YAMADEN, Japan). Since the plunger used here was spherical and the contact area increased with the load, the apparent modulus of elasticity in the micro deformation region was calculated using the Eq. (1) based on the Hertz's theory which developed Hooke's law. (Lee & Radok, 1960; Iwasaki, Washio, Yamamoto, & Nakamura, 2005):

$$F = \left(\frac{16\sqrt{R}}{3}\right) \times G \times h^{\frac{3}{2}} \quad (1)$$

where R was the radius of plunger (0.0025 m), F was the load (N), h was indentation (0.0005 m), and G was the apparent elasticity (Pa). The typical curve of the load (N) versus indentation ($m^{1.5}$) was shown in Fig. 1.

2.3. Scanning electron microscopy

The gels were cut into 1 − to 2 mm cubes, fixed with 2.5% glutaraldehyde containing 0.1 M sodium phosphate (pH 7.3), and then dehydrated in a graded series of ethanol (50%, 70%, 90%, and 100%). The dehydrated samples were transferred into 2-methyl-2-propanol to replace the ethanol and then freeze-dried. The samples were vacuum-coated with platinum-palladium in an evaporator. The specimens were observed using a Hitachi S-2460N scanning electron microscope (Hitachi High-Technologies co., Tokyo, Japan) with an accelerating voltage of 10 kV.

2.4. Transmission electron microscopy

A drop of actomyosin suspension (0.1 to 0.2 mg/mL) was applied to a carbon-coated grid, which was glow-discharged just before use. The grid was negatively stained with 2% aqueous uranyl acetate. The