Evaluation of the Myosin Denaturation Indicator of Catfish Myofibrils and Meat upon Heating

Wichulada. Thavaroj[1] and Konno. Kunihiko[2]

***Abstract*—** This study was conducted to investigate the application of myosin denaturation indicator of catfish myofibril on catfish meat instead. Myofibrils (Mf) was prepared from hybrid catfish and suspended in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5). Mf is heated in test tubes (2 ml in 15 x 105 mm). Meat samples of catfish (10g each is wrapped with polypropylene film) are heated at the same temperature, dialyzed meat against 0.1M NaCl, 20 mM Tris-HCl (pH 7.5) was also used for comparison. Ca^{2+}-ATPase inactivation was a little slower with meat than that with myofibrils. The inactivation for the dialyzed meat was almost the identical to one of myofibrils. Faster denaturation of myosin rod than S-1 was exhibited on the pattern with myofibrils, while rod denaturation was rather slower than S-1 in meat. Quick denaturation of rod found with myofibrils was not reproduced with meat. Results showed that rod denaturation in meat is restricted whereas Ca^{2+}-ATPase activity measurement is the promising index to see myosin denaturation by using Mf as a model.

***Keywords*—**myosin denaturation, ATPase inactivation, catfish

I. Introduction

HYBRID catfish meat, *Clarias macrocephalus x Clarias gariepinus*, is characterized by white and less greasy with a delicious taste. And myosin from catfish dorsal muscle is very stable similar to tilapia one among the myosin ever isolated from fish muscle [1]. High stability of tilapia myosin was explained by the adaptation to the high water temperature for its habitation [2].

Myosin is a multi-functional protein and has a unique structure constructed by double heads and connecting long coiled-coil tail. Head region contains ATP hydrolyzing site and F-actin binding site necessary for muscle contraction. As ATPase is easily assayed, its inactivation has been used as a sensitive and quantitative indicator of myosin denaturation. Long tail portion of myosin molecule is responsible for self- association to form myosin filaments in muscle cells. [3] Filaments dissociate upon addition of neutral salt such as NaCl or KCl at around 0.5 M, indicating importance of electrostatistic interaction of charged amino acid residues on myosin tail in the filament forming mechanism. As myosin is salt-soluble and neutral salt solution such as 0.5 M KCl or NaCl is used [4]. Attempts were made to cleave myosin into head and tail regions by digesting with various types of proteinases. Different enzymes produce different subfragments. Among the proteinases used, chymotrypsin is widely used for cleaving myosin molecules into head and tail regions in the absence of divalent cations [5]. As the head portion contains ATPase site, inactivation is the index for detecting denaturation of the head region. However the index does not tell the denaturation of the tail region. To detect denaturation of the tail region, chymotryptic digestion was used based on the assumption that native myosin is cleaved only at a single site of S-1/rod junction in filamentous conditions that conformational change as a result of denaturation exposes other cleavage sites [6].

Myofibrils were prepared as a model material for meat because arrangement of filamentous myosin and actin is the same as in meat. Moreover, myofibrils can be prepared easily and they require only small amounts of meat. Before the application of myofibrils in myosin study, actomyosin extracted from fish meat with neutral high salt solution was the material for the study. As quantitative handling and quantitative analysis of myosin denaturation in meat is difficult, myofibrils were used instead [7]. In employing myofibrils, it was assumed that the event occurring in myofibrils are the same as in meat during the heating process.

II. Procedure for Paper Submission

A. Fish sample

Hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*) cultured in Thailand. Fish size used in this study ranged from 30 to 42 cm in length and 500 to 1,200 g in weight. The dorsal white muscle was used. In this research two types of meat were used.

B. Preparation of catfish myofibrils

Myofibrils were prepared from catfish meat [8]. Firstly, 5 grams of chopped meat was washed buffer then homogenized on Polytron. Homogenate was centrifuged at and the precipitate was re-suspended with the same buffer. The suspension was filtered through a layer of gauze to remove connective tissue. The filtrate was used as myofibril suspension in this study.

C. Heating of catfish meat and myofibrils

Catfish meat (about 5 g) wrapped with plastic film and myofibrils (about 1 ml) were incubated in water bath at 40˚C. As the conditions of meat and myofibrils were different, dialyzed meat against 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) was also used for comparison. Meat samples heated were converted into homogenates and myosin denaturation in it was examined.

Dr.Wichulada Thavaroj, Dept of Food and Nutrition, Faculty of Liberal Arts, Rajamangala University of Technology Srivijaya,Songkhla, Thailand-90000 (phone: +66-846-656296; fax: +66-743-17190; e-mail: wichulada.t@rmutsv.ac.th).

Prof. Kunihiko Konno, Faculty of Fishery Science, Hokkaido University, Japan-041-0821 (e-mail: konno@fish.hokudai.ac.jp).

D. Detection of myosin denaturation in myofibrils and meat

Myosin denaturation in heated or frozen stored myofibrils was studied by monitoring several properties of myosin. The indicators employed for detecting myosin denaturation were as follows:

(i) Ca^{2+}-ATPase inactivation. Ca^{2+}-ATPase activity was assayed in a medium of 0.5 M KCl, 20 mM Tris-maleate (pH 7.0), 5 mM $CaCl_2$, and 1 mM ATP at 25 °C.

(ii) Loss of salt-solubility. Salt solubility of myofibrils was measured by the following procedures. Myofibrils were dispersed in 0.5 M NaCl and 20 mM Tris-HCl (pH 7.5), and left for 30 min in ice; then the mixture was centrifuged at 20,000 x g for 15 min for measuring solubility without ATP addition. The same mixture was added with 1 mM ATP together with 1 mM $MgCl_2$ (Mg-ATP) for getting solubility with ATP. Mg-ATP was added to remove the contribution of F-actin in solubility measurement. Myosin content in the supernatant recovered by the centrifugation of the mixture was estimated on SDS-PAGE as solubility.

(iii) Monomeric myosin content. This index was developed to distinguish monomeric myosin from salt-soluble myosin. Dissolved myofibrils was added to ammonium sulfate at 40% saturation. Fractionation was carried out in the absence or presence of Mg-ATP. The solution was centrifuged at 20,000 x g for 15 min to yield supernatant. Myosin in the supernatant was referred to as monomeric myosin. Myosin content was estimated by measuring the staining intensity of myosin HC band appearing on SDS-PAGE pattern.

(iv) Amounts of chymotryptic fragments produced and their monomeric form. Myofibrils were digested with chymotrypsin in 0.05 M NaCl, 20 mM Tris-maleate (pH 7.0), 1 mM EDTA so as to cleave myosin at S-1/rod junction. The amount of chymotryptic fragments, S-1 and rod, were estimated by measuring their staining intensity on SDS-PAGE. The ammonium sulfate fractionation with or without Mg-ATP was also conducted to separate aggregates in the pellet. The components in the supernatant were referred to as monomeric fragments.

III. RESULTS AND DISCUSSION

For a better understanding of myosin denaturation, the thermal denaturation of myosin in myofibrils was investigated by employing various indices such as ATPase, salt solubility, monomeric content, and chymotryptic digestion. As it was found that myosin and actin in meat were kept very stable [9], myosin and actin denaturation by heating of meat instead of myofibrils were further investigated. Catfish meat (about 5 g) wrapped with plastic film and myofibrils (1 ml) was incubated in water bath at 40˚C. As the conditions of meat and myofibrils were different, dialyzed meat against 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) was also used for comparison. Meat samples heated were converted into homogenates and myosin denaturation in it was examined. ATPase inactivation was a little slower with meat than that with myofibrils as shown in Fig. 1. The inactivation for the dialyzed meat was almost the identical to one of myofibrils. Therefore, there was no difference in the stability of myosin in meat and in myofibrils. The fact was completely different from the case of freeze denaturation [10]. Myosin denaturation in heated meat was also studied by monitoring several changes i.e., salt solubility, S-1 and rod decrease, monomeric myosin S-1 and rod and also actin denaturation. Faster denaturation of myosin rod than S-1 was the pattern with myofibrils, while rod denaturation was rather slower than S-1 in meat (see Fig. 2). The results clearly showed that actin in myofibrils are resistant to thermal treatment as well as meat itself.

The changes were plotted in Fig. 3. A quick denaturation of rod found with myofibrils was not the event with meat. It indicated that filament structure was damaged during heating process in which a quick loss of solubility. Thus, not only actin but also myosin filament structure was kept stable in meat. As there was practically no differences between meat itself and dialyzed meat, buffer conditions were not the reason for causing the difference between myofibrils and meat.

It is also doubtful whether myofibrils are the model material for meat in thermal denaturation. Thermal denaturation of myosin and actin in catfish meat and myofibrils were compared. Ca^{2+}-ATPase inactivation was a little slower when heated as meat than as myofibrils but the difference was not great as found in freeze denaturation. A great difference found in thermal denaturation between myofibrils and meat was a very slow denaturation of myosin rod in meat. A quick rod denaturation as found with myofibrils was not reproduced with meat. Slow denaturation of rod in meat explained a slow decrease of salt solubility.

IV. CONCLUSIONS

Myofibrils are very useful material for studying myosin denaturation. Thermal denaturation of myosin and actin of myofibrils and meat were also different, especially with rod portion. The events occurring in meat are not reproduced in myofibrils. Practically, rod denaturation in meat is restricted; however, Ca^{2+}-ATPase activity measurement is the promising index to see myosin denaturation.

ACKNOWLEDGMENT

The author thanks Ministry of Science and Technology of Thailand for providing financial assistance and support to carry out all of my research.

REFERENCES

[1] W. Thavaroj and K. Konno, "Thermal denaturation properties of catfish myosin as compared with those of tilapia", ***Proceedings of*** *the 41st Annual West European Fish Technologists Association (WEFTA),* Gothenburg, Sweden. p.85, September 27-30, 2011

[2] R. Takashi, T. Murozuka, K. Arai, "Purification and properties of tilapia myosin from dorsal muscle", Nippon Suisan Gakkaishi 40: 1155-1161, 1974.

[3] Y. Azuma and K. Konno, "Freeze denaturation of carp myosin subfragment-1 as compared with thermal denaturation". Fisheries Sci. 65: 455-458, 1999.

[4] M. Matsuura and K. Arai, "Filament forming ability of tilapia myosin in association with biochemical activities". Nippon Suisan Gakkaishi 51: 1697-1704, 1985.

[5] A. Persechini and, A. J. Rowe, "Modulation of myosin filament conformation by physiological levels of divalent cations". J. Mol. Biol. 172: 23-39, 1984.

[6] M. Takahashi, T. Yamamoto, S. Kato, K. Konno. "Species-specific thermal denaturation pattern of fish myosin when heated as myofibrils as studied by myosin subfragment-1 and rod denaturation rates". Fisheries Sci. 71: 405-403, 2005.

[7] N. Katoh, H. Nozaki, K. Komatsu, K. Arai, "A new method for evaluation of the quality of frozen surimi from Alaska pollack. Relationship between myofibtillar ATPase activity and Kamaboko forming ability of frozen surimi". Nippon Suisan Gakkaishi 45: 1027-1032, 1979.

[8] Y. Azuma and K. Konno, "Freeze denaturation of carp myofibrils compared with thermal denaturation". Fisheries Sci. 64: 287-290, 1998.

[9] K. Arai, H. Takahashi, T. Saito, "Studies on muscular proteins of fish-III. Inhibition by sorbitol and sucrose on the denaturation of carp actomyosin during frozen storage". Nippon Suisan Gakkaishi 36: 232-236, 1970.

[10] W. Thavaroj and K. Konno, "Myosin and actin denaturation of catfish and tilapia myofrbrils upon frozen storage as compared with heating", *Proceedings of The Japanese Society of Fisheries Science (JSFS),* p.166, March 27-30, 2012.

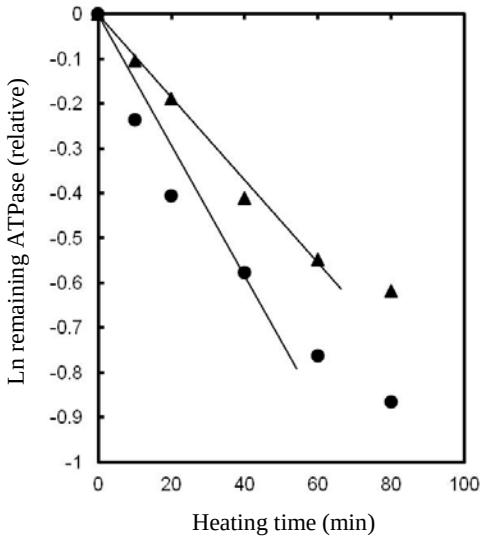

Fig. 1 Thermal inactivation of ATPase when heated in myofibrils and in meat of catfish
Myofibrils (circles) and meat (triangles) were heated at 40˚C, and ATPase inactivation in the samples was compared.

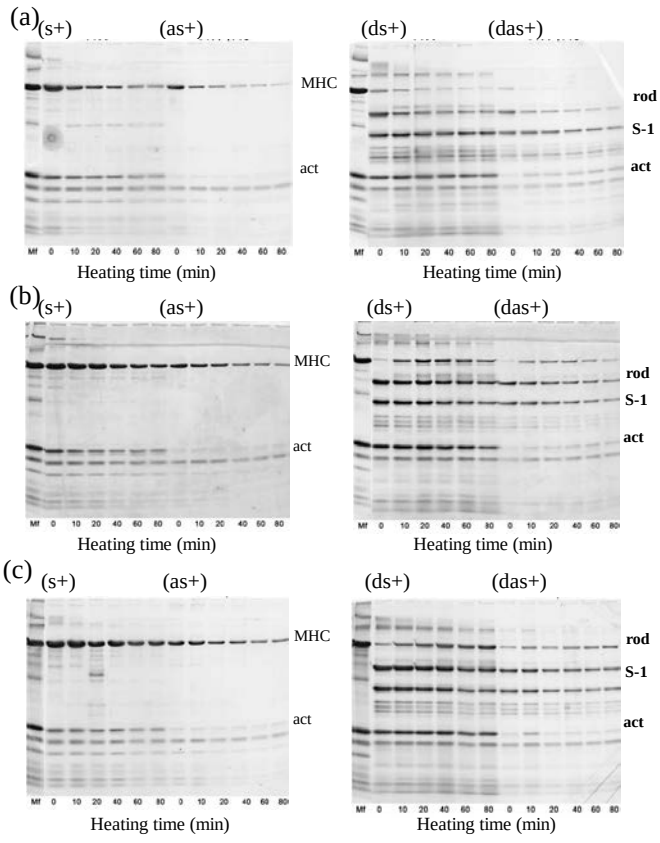

Fig. 2 Thermal denaturation of myosin and actin in myofibril, washed meat, and intact meat of catfish
Myofibrils (a) and dialyzed meat against 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) (b) and intact meat (c) were heated at 40˚C, and myosin denaturation was analyzed. Solubility in the presence of Mg-ATP (s+), monomeric myosin (as+), soluble fraction in the presence of Mg-ATP (ds+) and monomeric fragments (das+) were measured.

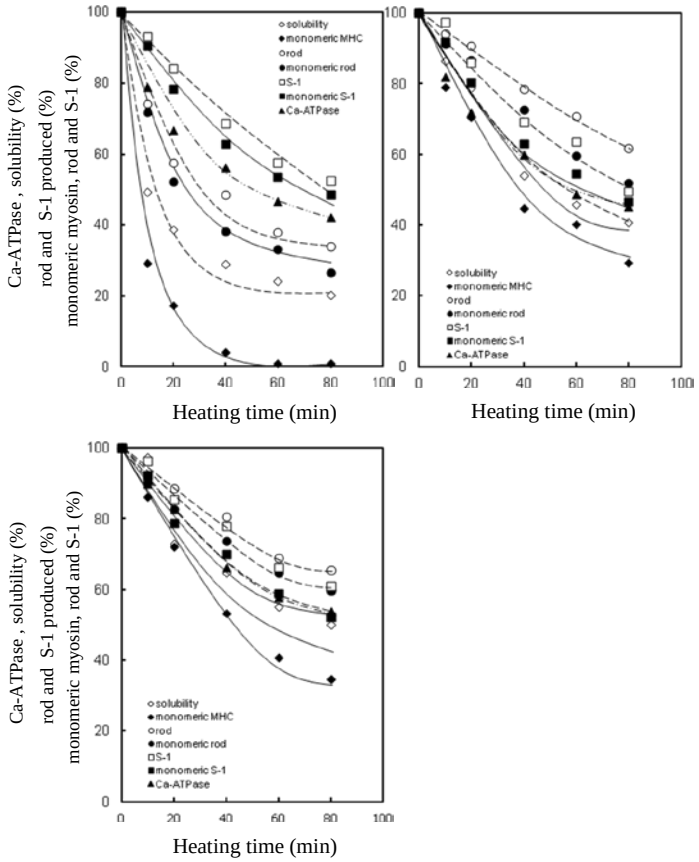

Fig. 3 Myosin and actin denaturation in meat of catfish upon heating
The results in Fig. 1, 2 were used to construct the graph. (a), (b), and (c) were the same as in Fig. 2. ATPase (closed triangles), salt-solubility in the presence of Mg-ATP (open diamonds), momomeric myosin (closed diamonds), S-1 produced (open squares), monomeric S-1 (closed squares), rod produced (open circles), and monomeric rod (closed circles) were measured.