Scanning Calorimetric Behavior of Tilapia Myosin and Actin due to Processing of Muscle and Protein Purification

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ABSTRACT
The thermal behavior of tilapia muscle proteins was investigated by differential scanning calorimetry at various stages in the processing of surimi and during purification of myosin and actin. A shift in the thermal transition of actin to lower temperature was observed and the enthalpies of denaturation for both actin and myosin decreased with further processing. Salt addition also induced shifts in denaturation transitions to lower temperatures and decreased enthalpies of denaturation.

INTRODUCTION
In attempting to relate the eating quality of cooked meat and processed meat products to denaturation of the myofibrillar proteins (particularly actin and myosin), many investigators have studied the thermotability of isolated proteins and extrapolated their findings to the intact muscle (Hamm, 1977; Wright et al., 1977; Quinn et al., 1980; Akahane et al., 1985). However, it has not been documented that the behavior of the proteins is the same both in the intact tissue and in isolated preparations. The present study was designed to follow the changes in Differential Scanning Calorimeter (DSC) thermograms of the fish myofibrillar proteins actin and myosin as these were progressively purified from whole muscle and as the muscle was comminuted and processed into surimi and a surimi sol.

MATERIALS & METHODS
Preparation of myosin and actin
Myosin and actin were prepared immediately post-mortem from live tilapia (Arochromis aureus), obtained from S. C. Cooper Corp. (Georgetown, SC), by the method of Martone et al. (1986) with modifications as shown in Fig. 1. The composition of solutions used in the preparation was as follows: solution A: 0.1M KCl, 0.02% NaN3, and 20 mM Tris-HCl buffer, pH 7.5; solution B: 0.45M KCl, 5 mM beta-mercaptoethanol, 0.2M Mg(CH3COO), 1mM chelate glycobals N, N', N'-tetraacetic acid (EGTA), 20 mM Tris-malate buffer, pH 6.8; solution C: 0.5M KCl, 5 mM beta-mercaptoethanol, 20 mM Tris-HCl buffer, pH 7.5; solution D: 0.8M KCl, 5 mM beta-mercaptoethanol, 0.2M Mg(CH3COO). All chemicals and molecular weight standards were obtained from Sigma Chemical Co. (St. Louis, MO). Samples were obtained during the purification as illustrated in Fig. 1: myofibrillar proteins (2), crude myosin (3), myosin (7), crude actin (9), and actin (11).

Processing of muscle into surimi and its sol
Samples were obtained at different stages of surimi processing as illustrated in Fig. 1: prerigor whole muscle (1), coarsely ground muscle (II), surimi (washed, partially deaerated mince) (III), surimi sol (IV).

Analysis by differential scanning calorimeter (DSC)
Thermal denaturation was studied in a Perkin-Elmer DSC System 4. Samples were weighted (60.0 ± 0.5 mg) and sealed in stainless steel large volume capsules. As reference, a pan containing 50 mg water was used. The scanning temperature was 15-100°C at a heating rate of 10°C/min.

Electrophoretic analysis
The purity of myosin and actin at each stage of purification was assessed by sodium dodecyl sulfate acrylamide gel electrophoresis (SDS-PAGE) using 10% acrylamide gels and the method of Laemmli (1970). Samples in solution were taken at the different stages of purification from whole muscle to myosin or actin as illustrated in Fig. 1: myofibrillar proteins (3), very crude myosin (4), crude myosin (6), myosin (8), very crude actin (10), crude actin (12), actin (13). Forty micrograms protein were loaded in each well of a slab gel. Molecular weights were determined using molecular weight standards: rabbit myosin (200,000 daltons), beta-galactosidase (116,250 daltons), phosphorylase B (92,500 daltons), bovine serum albumin (66,200 daltons), egg albumin (45,000 daltons), carbonic anhydrase (31,000 daltons), soybean trypsin inhibitor (21,500 daltons). The standards used were about 2 months old. They were kept in a freezer (~18°C).

Protein concentration was determined with o-phthalaldehyde (OPA) by the method of Park et al. (1987).

RESULTS & DISCUSSION
Effects of surimi processing
The pattern of the DSC thermograms were dependent upon the state of the muscle (Fig. 2). Prerigor intact muscle exhibited a prominent exothermic peak at 50.5°C. Two endothermic peaks were clearly discernible. The T_max of the component transitions were 58.3°C and 78.6°C. These peaks have been assigned to the denaturation of myosin and actin, respectively (Wright et al., 1977; Starbusvik et al., 1984). Park and Lanier (1988) attributed the large exothermic peak of prerigor tilapia muscle at 50.0°C to rapid ATP hydrolysis induced with rising temperature, while Wright et al. (1977) supposed that the exotherm observed for prerigor rabbit was a result of muscle contraction.

The T_max of the first peak remained fairly constant, in the range 58.7 ± 0.5°C, during reduction in particle size and subsequent leaching to produce surimi (Fig. 2A, B, C, D). However, the T_max of the second peak shifted to a lower temperature; from 78.6°C to 74.0°C.

The effect of salt, commonly used to extract the myofibrillar proteins in the further processing of surimi into food products, is clearly shown by curves D and E of Fig. 2. Following the addition of 3% salt to the surimi, the T_max values shifted to lower temperatures and thermal denaturation occurred in three transition stages as evidenced by T_max values at 42.9°C, 54.3°C, and 63.2°C. The heat capacities (the amount of heat required to raise the temperature of 1g of the substance by 1°C) are ΔH/ΔT, Rawn, 1983; or simply the peak height from base line, Findlay et al., 1986) at the transition temperature for the first two peaks (myosin; Wu et al., 1985) dropped to about half their former values, while that for the third peak (actin)
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Effects of protein purification

DSC thermograms of isolated myofibrils, myosin, and actin are shown in Fig. 2. Three transitions were obtained for myofibrillar proteins (curve F) at 47.8°C, 57.7°C, and 68.0°C. Crude myosin (curve G), pure myosin (H), crude actin (I), and pure actin (J) evidenced single peaks at 52.8°C, 51.6°C, 59.3°C, and 61.4°C, respectively. Purification of myosin (curves G, H) and actin (curves I, J) almost doubled the enthalpies at denaturation (see areas under curves), while the peak temperatures for denaturation changed little. In studies by Wright et al. (1977) and Wright and Wilding (1984), myosin displayed a single transition at pH 7.0 and ionic strength 0.05. Similar conditions were applied in this experiment; myosin was washed with solutions of pH 6.8 to 7.5 and ionic strength near zero. A very broad single peak was obtained for all of the myosin and actin fractions: crude myosin (curve G: 39.0°C to 60.5°C), pure myosin (H: 30.6°C to 65.1°C), crude actin (I: 40.9°C to 70.2°C), and pure actin (J: 43.5°C to 71.1°C). This indicates that thermal denaturation of these proteins does not occur suddenly at a specific temperature but, rather, gradually over a wide temperature range.

As illustrated by electrophoretic patterns of each fraction in Fig. 3, purified myosin (8) and actin (13) were obtained. Some minor bands appeared in the electrophoretogram of the high molecular weight standard (S). These were probably due to partial proteolysis of the standard rabbit myosin during storage. However, all major bands including rabbit myosin (200,000 daltons) and egg albumin (45,000 daltons) were clearly shown to match the purified fish myosin and actin. By this electrophoretic evidence, the DSC endothermic peaks at about 52°C and 60°C were verified as being derived from denaturation of myosin and actin, respectively.

CONCLUSION

SOME DESTABILIZATION of myofibrillar proteins, evidenced more by decreased enthalpies of denaturation rather than by shifts in the temperature of transitions, was noted due to mincing of whole muscle. Solubilization of these proteins by salt addition effected shifts in T<sub>max</sub> values to lower temperature, causing myosin to exhibit two denaturation peaks under the present preparatory conditions. Purification of actin and myosin yielded thermograms evidencing a single peak, whose enthalpy of denaturation increased with further purification.

REFERENCES


Hamm, R. 1977. Changes in muscle proteins during the heating of meat. In "Physical, Chemical and Biological Changes in Food Caused by Ther-
Fig. 2—Changes in DSC thermogram during processing of muscle into surimi and its sol and during isolation of myofibrillar proteins, myosin, and actin. (A) pre-rigor intact muscle(I); (B) 24 hr postmortem muscle coarsely ground(II); (C) mince muscle(III); (D) surimi(IV); (E) surimi with 3% NaCl(V); (F) myofibrillar proteins(II); (G) crude myosin(III); (H) myosin(IV); (I) crude actin(V); (J) actin(II). Numbers in parentheses denote different stages during processing and isolation as illustrated in Fig. 1.

Fig. 3—Electrophoretic patterns of myofibrillar proteins, myosin, and actin: (3): myofibrillar proteins; (4), (6), (8): myosin; (10), (12), (13): actin. (3) - (13) denote different stages during isolation as illustrated in Fig. 1. S1 and S2 denote high and low molecular weight standards, respectively.