Influence of Gonadal Stage of Hake
(Merluccius Hubbsi Marini) on Biochemical Properties of Myofibrils Stored at 2 to 4 °C

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ABSTRACT: The influence of the gonadal stage of hake on the biochemical properties of myofibrils stored at 2 to 4 °C was studied. At 0 time and during storage, both Mg2+-Ca2+-ATPase activity and Ca2+ sensitivity of myofibrils from post-spawned hake were significantly higher (p < 0.01) than those of pre-spawned fish. The profiles of SDS-PAGE gels of unstored and stored myofibrils from pre-spawned hake showed a partially denatured myosin heavy chain. The actin-myosin ratio in myofibrils from pre-spawned hake was significantly lower (p < 0.01) than the ratio in post-spawned hake. Irrespective of the gonadal condition of fish, no changes in the myosin-actin ratio of stored myofibrils were observed.

Key Words: myofibril, biochemical properties, hake, gonadal stage, cold storage

Introduction

Functional and textural characteristics of meats depend mainly on myofibrillar proteins (Goll and others 1977). This dependence is more important in fish muscle because of its low collagen content, than in mammalian muscle (Brown 1986). The study of the functional properties of fish myofibrillar proteins is necessary for determining and predicting the final quality of fishery products. Much is known about biochemical, physicochemical, and functional properties of actomyosin from iced fish (Crupkin and others 1979; Barassi and others 1982; Crupkin 1982; Roura and others 1992a, Tachibana and others 1993; Lin and Park 1996; Benjakul and others 1997). Crupkin and others (1988) reported that when comparing actomyosin from resting and post-spawning periods with actomyosin from pre-spawned hake (Merluccius hubbsi), the latter had a significantly lower relative percentage of myosin. Changes in biochemical, physicochemical, and functional properties of hake actomyosin during the reproductory cycle of fish reflect changes previously reported in the actomyosin complex (Beas and others 1988; Montecchia and others 1990; Roura and others 1990; Roura and others 1992b; Busalmen and others 1995). Beas and others (1988) reported that irrespective of heating temperature, ionic strength conditions and at pH range 5.5 to 7.5, rigidity of post-spawning hake actomyosin gels was higher than those of pre-spawning fish. Beas (1989) also observed a high setting ability in actomyosin of post-spawning hake, comparable with Alaska pollack, while that of pre-spawning fish was similar to species with lower setting ability. In addition, differential scanning calorimetry (DSC) studies on thermal denaturation of hake muscle proteins during gelling showed that myofibrillar proteins from pre-spawning hake have a lower denaturation enthalpies and were more sensitive to thermal denaturation than the post-spawning ones (Beas and others 1991). It was reported that the gonadal stage of hake influences the biochemical and functional properties either of actomyosin or of myofibrils periodically obtained from iced hake. The decrease in both reduced viscosity and Mg2+-EGTA-ATPase activity of actomyosin from post-spawned hake was higher than that from pre-spawned ones (Crupkin 1982; Roura and others 1990). Roura and Crupkin (1995) also reported that the inactivation rate of ATPase activities of myofibrils from iced post-spawning hake was lower than that corresponding to actomyosin. The biochemical behavior of isolated actomyosin during storage at 0 to 2 °C is also influenced by the gonadal stage of the fish. In that a drastic degradation of myosin in cold-stored actomyosin from hake at the pre-spawning stage occurs (Crupkin 1982). The same study also showed that during the storage of actomyosin from hake, in either resting or post-spawning period, myosin was unchanged.

Some information on proteolytic changes in cold-stored actomyosin from hake in different gonadal stage is also available (Crupkin 1982). However, information about in vitro behavior of myofibrils from hake in different gonadal conditions is lacking. The purpose of our work was to study the biochemical and physicochemical properties of myofibrils from hake in pre- and post-spawning condition, stored in vitro at 2 to 4 °C.

Materials and Methods

Fish Source
Female hake (Merluccius hubbsi) were harvested by commercial vessels in the southwest Atlantic Ocean from latitude 36° to 53° S during May to November 1998. Fish were kept on ice until they reached the laboratory in an early post-rigor condition. Fish that were 35 to 45 cm long from snout to tip of mid-caudal ray were measured to the nearest 0.1 cm. Macroscopic analysis (Christiansen and Cousseau 1971) and histology (Goldemberg and others 1987) determined the gonadal condition of the specimens. Four fish from each condition were used for myofibril extractions. From each fish, 1 sample of myofibrils was obtained. Triplicates of each sample were used in the determinations.

Preparation of Myofibrils
Myofibrils were prepared as described by Yasui and others (1975) as previously reported (Roura and others 1992c). Triton X-100 was not used for further purification of hake myofibrils because it inactivates contractile proteins (Roura and others 1992c).
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Myofibrillar ATPase Activity

The enzymatic activity of myofibrils was measured at 37 °C in a 30 mM Tris-maleate buffer (pH 7.0). Specific conditions for each enzyme were 0.25 mg.mL⁻¹ of protein, 0.75 mM ATP, 60 mM KCl, 2 mM MgCl₂, and 0.5 mM of ethyleneglycol-bis-(beta-aminoethyl ether) N, N'-tetraacetic acid (EGTA) for the Mg²⁺(EGTA)-ATPase activity; 0.12 mg.mL⁻¹ of protein, 0.75 mM ATP, 60 mM KCl, 0.1 mM CaCl₂, and 2 mM MgCl₂ for the Mg²⁺-Ca²⁺-ATPase activity. Incubation times were 5 min for Mg²⁺(EGTA)-ATPase and 4 min for Mg²⁺-Ca²⁺-ATPase. Reactions were stopped by addition of a cold 40% trichloroacetic acid solution (at 10% final concentration). Liberated phosphorus was determined according to the method of Chen and others (1956). Ca²⁺ sensitivity was calculated according to the method previously described (Seki and Narita 1980).

\[ \text{Ca}^{2+} \text{ sensitivity} = \left(1 - \frac{\text{Mg}^{2+}-(\text{EGTA})-\text{ATPase activity}}{\text{Mg}^{2+}-\text{Ca}^{2+}-\text{ATPase activity}}\right) \times 100 \]

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE of actomyosin was performed in 10% gels using a MicroSlab (Sigma Chemical Co., St. Louis, Mo., U.S.A.) according to the procedure described by Laemmli (1970). The protein loaded on the gel was varied to check linearity of myosin heavy chain, actin, and myosin light chains. A linear response was obtained with 30 µg of protein. The mobility-molecular weight curve was calibrated with standards of molecular weights (MW wide range; Sigma Chemical Co., St. Louis MO).

Quantitative myofibril composition was determined by scanning gels at 600 nm with a Shimadzu dual-wavelength chromatogram scanner (model CS 910) equipped with gel-scanning accessory (Shimadzu, Kyoto, Japan). The myosin-actin ratio was calculated by dividing myosin heavy chain plus myosin light chain areas by actin.

Statistical Analysis

Analysis of variance (ANOVA) and the Duncan’s range test were performed using the statistical analysis package Statistica/Mac (Statistica/MAC 1994).

Results and Discussion

The behavior of the solubility of myofibrils obtained from post- and pre-spawned hake during storage at 2 to 4 °C are shown in Fig. 1. The solubility values for myofibrils obtained from post- and pre-spawned hake at 0 time of storage were 11.20 ± 1.60 and 15.00 ± 1.60 mg.mL⁻¹, respectively. This difference was significant (p < 0.01) and could be due to additional lability of myofibrils of hake in pre-spawning conditions, which led to an increase in the solubility of its
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components. Roura and Crupkin (1995) noted, by SDS-PAGE determinations, a partially denatured myosin heavy chain (MHC) and polypeptide bands with lower molecular weight that probably represent proteolytic fragments produced by in vivo degradation of MHC. About 73% of the myofibrils from post-spawned hake and 56% of those from pre-spawned fish could be solubilized after 2 d of cold storage (Fig. 1). This result suggests a higher loss of functional components by centrifugation in myofibrils of pre-spawned hake than those from post-spawned fish. Similar results were obtained during the purification of natural actomyosin from pre- and post-spawned fish (Roura and others 1990). No significant changes in the solubility of myofibrils from either post- or pre-spawned hake were observed thereafter up to the end of the storage period.

Myofibrillar ATPase activities have been widely used as a measure of actomyosin integrity and to monitor postmortem changes in fish during iced or frozen storage (Seki and others 1979; Roura and others 1990; McDonald and Lanier 1994; Montecchia and others 1997; Paredi and Crupkin 1997). Mg$^{2+}$ and Mg$^{2+}$-Ca$^{2+}$-ATPase activities of myofibrils are indicative of the integrity of the actin-myosin complex in the presence of endogenous or exogenous Ca$^{2+}$ ions, respectively (Roura and others 1990). Mg$^{2+}$-(EGTA)-ATPase activity indicates the integrity of the tropomyosin-troponin complex (Ebashi and Endo 1968; Ouali and Valin 1981; Watabe and others 1989). The changes in Mg$^{2+}$-Ca$^{2+}$-, Mg$^{2+}$-(EGTA)-ATPase activities and Ca$^{2+}$ sensitivity of myofibrils from pre- and post-spawned hake stored at 2 to 4 °C, are shown in Fig. 2. At 0 time and during the storage period, Mg$^{2+}$-Ca$^{2+}$-ATPase activity of myofibrils from post-spawned hake was significantly higher (p < 0.01) than that of myofibrils from pre-spawned ones. Similar results were obtained with myofibrils periodically obtained from iced hake (Roura and Crupkin 1995). No significant differences were observed in the Mg$^{2+}$-(EGTA)-ATPase activity of myofibrils by either storage or the gonadal condition of the fish.

Ca$^{2+}$ sensitivity in myofibrils from post-spawned hake was 2 to 3 times higher than that corresponding to myofibrils from pre-spawned ones (Fig. 2). The Ca$^{2+}$ sensitivity of myofibrillar protein is attributed to the activity of native tropomyosin (Ebashi and others 1968). It was also reported that the troponin-tropomyosin complex is necessary to the control by Ca$^{2+}$ ions of the actin-myosin interaction in vertebrate striated muscles (Huxley 1972). In addition, the loss of Ca$^{2+}$-sensitivity is considered to be due to the filamentation of myofibrils caused by hydrolysis of proteases (Tokiwa and Matsumiya 1969). However, according to the studies by Seki and Hasegawa (1978), Seki and Iwabuchi (1978), Shitamura and Seki (1978), and Seki and others (1979), the loss in calcium sensitivity is caused by the modification of the actin-myosin interaction by oxidation of the thiol groups of the myosin moiety. Roura and Crupkin (1995) reported that the loss of Ca$^{2+}$ sensitivity in myofibrils from pre-spawned hake could be related to an increment in proteinase activity, which selectively degrades myosin during gonadal maturation. A previous study also reported a decreased affinity between myosin and actin in actomyosin from pre-spawned hake (Roura and others 1992b). These results indicate that in myofibrils obtained from hake before spawning, a decrease in Ca$^{2+}$ sensitivity could be caused by a degraded myosin that produces modifications in actin-myosin interactions. However, from our results, the possibility that it is caused by oxidation of thiol groups of the myosin moiety should still be considered.

The electrophoretic patterns in 10% SDS-PAGE of myofibrils from post- and pre-spawned hake stored at 2 to 4 °C
are shown in Fig. 3 and 4, respectively. At 0 time and at different 6 d of storage, the characteristic polyepitopic bands of post-spawned hake myofibrils can be observed (Fig. 3). These results agree with those previously reported on myofibrils periodically obtained from iced hake (Roura and Crupkin 1995). In addition, similar patterns were obtained with stored myofibrils before and after centrifugation (Fig. 3 lines D-E, F-G, H-I). This result suggests that decreased solubility is not caused by changes in the relative composition of functional myofibrils during storage. The electrophoretic patterns of myofibrils from pre-spawned fish show the presence of partially degraded MHC with a molecular weight of 180 kDa. Crupkin and others (1988) reported low myosin-ac- tin ratios in actomyosin from pre-spawned hake. The presence of less myosin in this phase of the gonadal cycle was explained by the hypothesis that myosin was more sensitive in vivo to proteolysis than actin (Crupkin and others 1988). A partially degraded myosin heavy chain with a molecular weight of 180 kDa and polypeptide bands under this component, probably representing proteolytic fragments produced by degradation of MHC in vivo, was also shown in the profiles of myofibrils from hake in pre-spawned conditions (Roura and Crupkin 1995). Although no significant differenc- es could be observed in the electrophoretic pattern of myo- fibrils before and after centrifugation (Fig. 4 lines D-E, F-G, H-I), 20-25 kDa polypeptide bands could be detected in the gels after the 2nd d of storage (Fig. 4). These bands could be proteolytic fragments produced by degradation of some myo- fibrillar protein during the storage. However, further inves- tigations are necessary to clarify the nature of this proteolyt- ic activity present in myofibrils from hake caught before the spawning. The relative percentages of the major myofibrillar proteins and myosin-actin ratios of stored myofibrils from post- and pre-spawned hake are shown in Table 1. Myofibrils from pre-spawned hake had lower relative percentages of myosin and myosin-actin ratios than those of post-spawned ones. However, irrespective of the gonadal condition of the fish, no changes (p > 0.05) in the relative percentage of the major myofibrillar proteins or in myosin-actin ratios were observed during storage. The results shown in Table 1 suggest that no major changes occur in myofibrillar proteins during in vitro storage of myofibrils from pre- and post- spawned hake. Similar results were obtained with myofibrils periodically obtained from pre- and post-spawned hake stored on ice (Roura and Crupkin 1995).

Conclusions

The results of this work indicate that the gonadal stage of the hake also influences the biochemical properties of myofibrils stored in vitro at 2 to 4°C. They also em- phasize the importance of knowing the possible influence of the gonadal stage of fish when carrying out experiments with myofibrils as a model system.

References


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Figure 4—10% SDS-PAGE patterns of myofibrils from pre- spawned hake during storage at 2-4 °C. Line A: Molecular weight standard; B and C unstored myo- fibril; D, F, and H: myofibril before centrifugation; E, G, and I: myofibril after centrifugation. For other details, see Fig. 3.
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