α-Helical Structure of Fish Actomyosin Changes during Storage

Keywords: *Fish; myosin; actomyosin; α-helix; preparation; circular dichroism*

INTRODUCTION

Because myosin is so important to gel development of comminuted meat products, there have been many investigations of myosin with respect to structural changes and aggregation during heating (Wright and Wilding, 1984; Wicker and Knopp, 1988; Gill and Conway, 1989; Wu et al., 1991; Arteaga and Nakai, 1992; Sharp and Offer, 1992; Ogawa et al., 1993, 1994) and gel formation (Samejima et al., 1969, 1981; Yasui et al., 1982; Shimizu et al., 1983; Akahane et al., 1984; Sano et al., 1988, 1990; Wang and Smith, 1994). We have reported that the α-helical contents of myosin measured immediately after its preparation from the skeletal muscle differed among fish species (Ogawa et al., 1992, 1993). Bigeye tuna and stone flounder had helical contents of myosin similar to that of rabbit, while walleye pollack and sardine had far lower contents. Additionally, we have suggested (Ogawa et al., 1992) that the distinction in the α-helical content arises in the preparation process. A study was carried out to test this hypothesis. The α-helical content of natural actomyosin (AM), which can be prepared in a shorter time than myosin, was measured at different times during storage to deduce the changes in α-helical structure of myosin in the process of the preparation.

MATERIALS AND METHODS

Three species of fish with possibly different helical contents (Ogawa et al., 1992) were used in this study. New Zealand white rabbit was used as a control. The species of fish were bigeye tuna (*Thunnus obesus*), walleye pollack (*Theragra chalcogramma*), and sardine (*Sardinops melanostictus*). Bigeye tuna and sardine were purchased from Tokyo Tsukiji fish market. Walleye pollack was caught in the sea near Hakodate, Hokkaido. Sardine and rabbit were used immediately postmortem and bigeye tuna and walleye pollack after storage on ice for 1 day post-mortem.

Fish AM was prepared from the dorsal muscle according to the method described by Sano et al. (1986) and that of rabbit from the skeletal muscle according to the method of Szent-Györgyi (1947) with modifications. All preparation procedures were performed at 4 °C. Circular dichroism (CD) to determine α-helical content was carried out according to the method described by Ogawa et al. (1995), using a Jasco J-500A spectropolarimeter equipped with a water-jacketed cell holder. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) was performed using the buffer system described by Laemmli (1970). The Coomassie Brilliant Blue stained gel was analyzed using the NIH image (version 1.55) on the Macintosh computer with the Canon TV camera to determine the mobility and optical density of the bands. The above experiment was carried out twice.

RESULTS AND DISCUSSION

To discover if the α-helical content of fish myosin decreases during preparation, it is necessary to know the α-helix content at each stage of the preparation process. Thus, fish AM, which could be prepared in a shorter time than the myosin, was used as the sample. Figure 1 shows the SDS−PAGE patterns of the AM samples. The mobilities of the bands of M_r < 40 000, especially corresponding to myosin light chains (MLCs), differed among species. However, the mobilities of the major bands of myosin heavy chain (MHC) and actin were almost the same in all species. When the stoichiometry of the components of AM was estimated from the relative intensity of staining, the ratio of MHC to actin was 11:5 for each of the three species of fish.

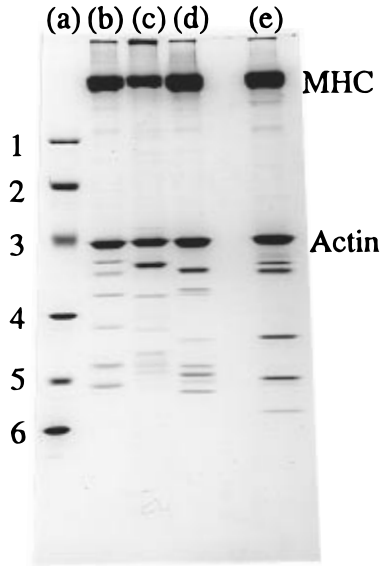

Figure 1. SDS−PAGE of AM using 7.5−20% acrylamide gradient gel: (a) molecular weight markers; (b) bigeye tuna; (c) sardine; (d) walleye pollack; (e) rabbit. The molecular weight markers used were (1) phosphorylase *b* (94 000), (2) bovine serum albumin (67 000), (3) ovalbumin (43 000), (4) carbonic anhydrase (30 000), (5) soybean trypsin inhibitor (20 100), and (6) α-lactalbumin (14 400).

Fish AM was permitted to stand at 4 °C over a period of about 4 days after its preparation, and its α-helicity was measured at different times during the period. The results of the measurements are shown in Figure 2. Here, time zero corresponds to the initiation of the extraction from the muscle. Bigeye tuna's AM indicated only small changes in α-helicity for the period of the measurements. On the other hand, walleye pollack's and sardine's AM showed an abrupt decrease in α-helicity for the period, especially in the first 24 h. We also measured rabbit AM (data not shown); there was no observable change over the whole time range. Possibly, the α-helicity of fish AM decreased largely during extraction and purification. Thus, α-helicity was extrapolated to time zero by a dashed line. The values at time zero were 53% for bigeye tuna, 52% for walleye pollack, and 50% for sardine. Thus, α-helicity values extrapolated to time zero were not greatly different among fish species. Ratios of the main components of AM also differed little among fish species (see Figure 1). Judging from these two results, we think the AM which was included in the muscle will have approximately the same helical content among fish species.

An abrupt decrease in α-helicity with the passage of time caused no less than 10% difference after 100 h between bigeye tuna and walleye pollack or sardine. Thus, purified fish myosins had different helical contents: 66% for bigeye tuna, 47% for sardine, and 38% for walleye pollack (Ogawa et al., 1992). The α-helix content of AM is mostly accounted for by myosin. About 70% of α-helix of AM was from the myosin when calculated from the ratio and α-helicity of each constituent of AM. Accordingly, the decrease in α-helicity of

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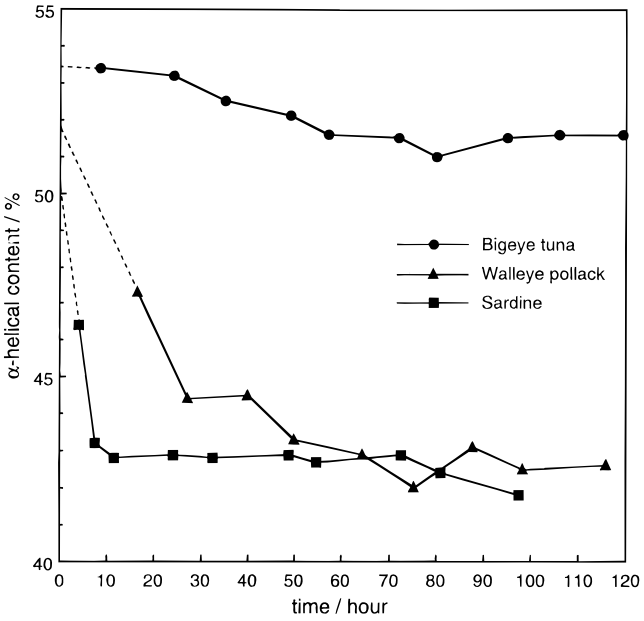

Figure 2. Changes in α-helical content of AM during storage. Time zero corresponds to the initiation of extraction of AM. After the preparation, the AM in 0.6 M KCl−20 mM potassium phosphate buffer (pH 7.0) including 0.02% NaN_3 was kept at 4 °C. The protein concentration was 0.2 mg/mL. The data are the mean of the two measurements. Duplicates agreed to within 5% from the mean.

AM is thought to be due to the unfolding of the myosin molecule during its preparation. Johnston and Goldspink (1975) reported that myosin isolated from cold water fish was characterized by a decline of the ATPase activity to zero, within a few hours of preparation. Additionally, such cold water fish as walleye pollack and sardine were found in this study to undergo the structural changes of myosin during preparation. From these two results, it can be stated that the cold water fish myosins lost the active sites of ATPase with the unfolding of secondary structure during preparation owing to the extreme instability.

From the above results we concluded that AM from cold water fish muscle, which has a helical content similar to that of warm water species, was gradually unfolded once extraction was started. Accordingly, when fish AM or myosin is prepared, it is preferable to minimize the preparation time and handle the sample as quickly as possible.

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