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Effect of Post-rigor Fish Storage on Ice on Physicochemical Properties of Actomyosin[a]

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Actomyosin was purified from post-rigor fish stored for various times on ice and was characterised by Mg^{2+}-ATPase activity, ATP-sensitivity and SDS-polyacrylamide gel electrophoretic patterns. It had a similar reduced viscosity to that of the myofibrillar extract and the viscosity of both extracts fell in a similar manner as the time of storage of the whole fish on ice increased. No changes in the extractability, the Mg^{2+}-ATPase activity or the electrophoretic pattern of actomyosin were detected when extracted from fish muscle held for up to 11 days in ice.

1. Introduction

It is currently accepted that myofibrillar proteins are denatured during storage of frozen fish.[1] As a consequence, a fall in the viscosity of myofibrillar proteins was observed and has been attributed to the formation of myosin and actomyosin aggregates.[1]

Muscle protein denaturation during post-mortem cold storage was also observed in unfrozen fish. Reduction in the extractability of high ionic strength muscle extracts during fish storage on ice was observed in North Sea Cod,[2] Baltic Cod and Baltic Herring.[3] Changes in ATPase activities and other properties of sardine myofibrillar proteins during ice storage were also observed.[4] However, no evident proteolysis was observed in freshwater fish stored on ice[5] and no changes in Ca^{2+}-ATPase and extractability of myofibrillar proteins, during ice storage, were reported for other species of fish.[6]

Viscosity decrease of the high ionic strength muscle extract from ice-stored fish has only been observed in freshwater fish by Baliga, *et al.*[7] and in seawater fish by Crupkin *et al.*[8] This could be missed by the workers due to the apparently aleatory behaviour of the viscosity, since it has been recently reported that the rheological properties of myofibrillar extract are influenced by the biological conditions of the fish.[9]

Results of investigation carried out to establish the myofibrillar protein responsible for the viscosity changes and the nature of this phenomenon are reported here.

2. Materials and methods

2.1. Source of fish samples

Hake (*Merluccius hubbsi*) was caught from fishing grounds on the Argentine platform in the south-east Atlantic Ocean during late summer and autumn. Fish samples were obtained from commercial vessels. The samples were kept on ice until taken to the laboratory in early post-rigor stage.

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2.2. Fish storage on ice and myofibrillar protein extract preparation

Fish was stored on ice in plastic boxes for various periods. From time to time samples were taken at random from each box and filleted. Myofibrillar protein extracts from fish muscle were prepared as previously described.[8]

2.3. Actomyosin preparation

The myofibrillar protein extract in 0.6M KCl–CO_3HNa 0.003M buffer pH7 was centrifuged at 1500*g* and actomyosin was obtained by diluting the supernatant with cold water up to a final 0.2M KCl concentration. The precipitate was solubilised in 0.6M KCl and the steps of dilution–solubilisation were repeated three times in order to obtain purified actomyosin.

2.4. Reduced viscosity

Reduced viscosity in both myofibrillar protein extract and actomyosin was measured by the previously described procedure.[8]

2.5. ATP-sensitivity

The adenosine triphosphate (ATP) sensitivity of actomyosin fraction was determined by measuring the relative viscosity before and after addition of ATP and Mg^{2+} up to 1mmol (final concentration) under the following conditions: 0.6M KCl, 0.003M CO_3HNa pH 7.0 and 2 mg ml^{-1} of protein. ATP sensitivity is defined as the change in viscosity produced by the addition of ATP and it is expressed on a percentage basis as described by others.[10]

$$\frac{\log \eta_{rel} - \log \eta_{rel,\,ATP}}{\log \eta_{rel,\,ATP}} \times 100$$

where:
η_{rel} = the relative viscosity of actomyosin.
$\eta_{rel,\,ATP}$ = the relative viscosity of actomyosin in presence of ATP 1mmol and Mg^{2+} 1mmol.

2.6. Protein determination

Protein concentration in both myofibrillar protein extract and actomyosin solution was determined by the Lowry method,[11] adapted for the determination of fish protein.[12]

2.7. Mg^{2+}-ATPase activity

Mg^{2+}-ATPase activity was measured in 0.15 mg ml^{-1} actomyosin solutions at 37°C under the following conditions: KCl, 50 mmol; Tris-maleate buffer, 30 mmol, pH 6.8; $MgCl_2$, 1mmol; ATP, 1mmol.The reaction was stopped after 5 min incubation with TCA adding up to 50 g $litre^{-1}$ final concentration. Phosphorus was measured by Chen's method.[13]

Table 1. ATP-sensitivity and Mg^{++}-ATPase activity of actomyosin fraction. Actomyosin was obtained and purified from myofibrillar extract of hake, at early post-rigor stage. Purity of the actomyosin fraction was checked by determining ATP-sensitivity and Mg^{++}-ATPase activity (see Materials and methods)

Sample	ATP-sensitivity $\left(\frac{\log \eta_{rel} - \log \eta_{rel,\,ATP}}{\log \eta_{rel,\,ATP}} \times 100\right)$	Mg^{2+}-ATPase activity (μmol Pi min^{-1} mg $protein^{-1}$)
1	100	0.60
2	91	0.57
3	99	0.60

2.8. SDS-polyacrylaimide gel electrophoresis (SDS-PAGE)

SDS-polyacrylamide gel electrophoresis of actomyosin was carried out in 10% gels according to the procedure of Porzio and Pearson[14] using a Shandon vertical gel apparatus.

3. Results and discussion

Fish myofibrillar proteins compared are unstable to those of mammals, which are highly stable.[1] Differences in the stability of actomyosin and myosin from several fish species had also been reported.[15] It was necessary to check the purity and properties of the fraction obtained by the

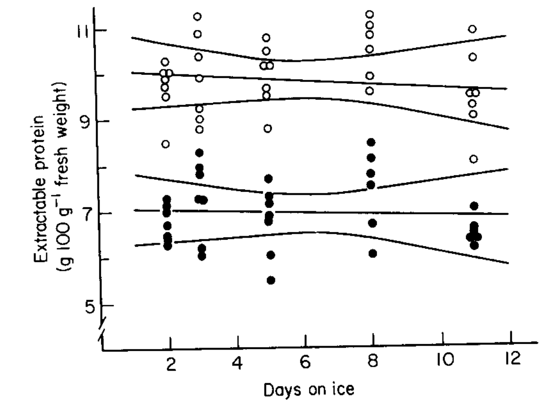

Figure 1. Extractability of total myofibrillar protein and actomyosin. Total myofibrillar protein (○) and actomyosin (●) were obtained from fish stored on ice. Protein was measured according to a modification of Lowry's method.[11,22] Each point represented the data obtained from a pool of six specimens. Superimposed full lines represented average values between confidence limits ($P < 0.001$).

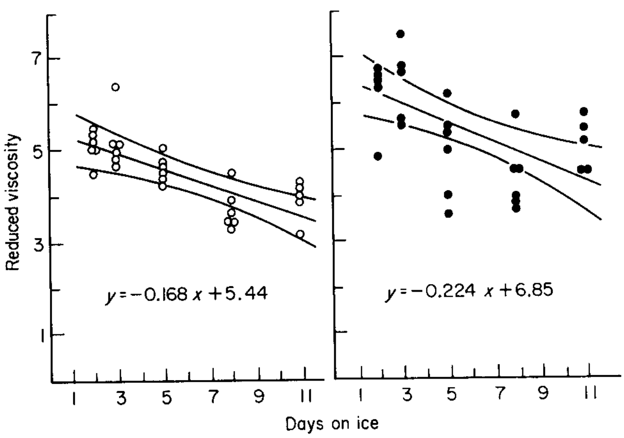

Figure 2. Reduced viscosity of total myofibrillar protein and actomyosin. Total myofibrillar protein and actomyosin were obtained from fish stored on ice. Reduced viscosity of both myofibrillar extract (○) and actomyosin (●) was measured according to previously described procedure.[8] Each point represents the data obtained from a pool of six specimens. Superimposed full lines represent average values between confidence limits ($P < 0.001$). The slopes were checked by Fisher test and values of $-0.223 \leqslant B \leqslant -0.113$ for myofibrillar protein and $-0.336 \leqslant B \leqslant -0.111$ for actomyosin were found, which indicated they were different from zero.

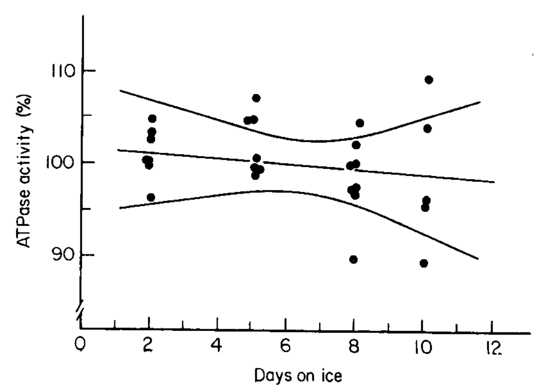

Figure 3. Mg^{++}-ATPase activity of actomyosin during fish storage on ice. Each point represents the data obtained from a pool of six specimens. Superimposed full lines represent average values between confidence limits ($P < 0.001$), 100% of activity corresponds to 0.60 μmol P min^{-1} mg $protein^{-1}$.

dilution–solubilisation method. As can be seen in Table 1, this fraction had an actomyosin-like behaviour, as judged by the high Mg^{2+}-ATPase activity and ATP sensitivity.[16]

In addition, it can be seen in Figure 4 that the SDS-PAGE pattern of purified fraction mostly shows the characteristic polypeptidic bands of actomyosin: (I) heavy myosin mol. wt 195 000; (IV) actin mol. wt 42 000 and (VI, VII) myosin light chains mol. wt 18 000 and 15 000 respectively. Two polypeptides (II, III) of mol. wt 100 000 and 70 000, respectively and tropomyosin (V) mol. wt 34 000 were also detected in the gels.

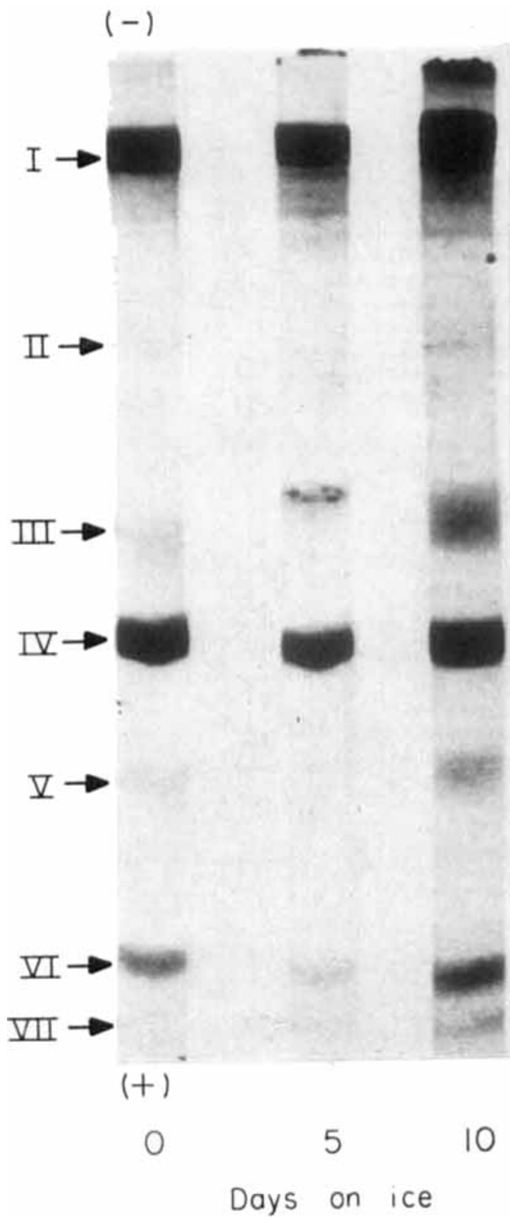

Figure 4. SDS-PAGE of actomyosin. Actomyosin was obtained from fish stored on ice. SDS-PAGE of actomyosin was performed according to the procedure of Porzio and Pearson (see Materials and methods). Zero day means start of the experiment. It was obtained after 48 h of initial fish storing on ice on board.

Tropomyosin is a tenacious contaminant of the actomyosin fraction. The presence of this protein has been reported in actomyosin, even after Sepharose 2B gel filtration of natural actomyosin from the striated adductor of scallop.[17] No attempt has been made to identify polypeptide bands (II) and (III), because they accounted for only 5% of total purified actomyosin.

Fish actomyosin represents nearly 65% of the total protein of muscle and, compared with the other proteins which comprise the myofibrillar extract, it has the highest intrinsic viscosity.[18] Accordingly, the reported viscosity decrease in myofibrillar extracts during storage of whole fish on ice[8] can be due to changes in the relative amount of actomyosin in the extracts or to changes in the properties of the actomyosin during storage time. Figure 1 shows that no changes in the extractability of both total myofibrillar proteins and actomyosin were observed up to 11 days of fish storage on ice.

The reduced viscosity of actomyosin is very similar to that obtained from the myofibrillar extracts and the viscosity of both actomyosin and myofibrillar extracts decreases in a similar manner during storage of whole fish on ice (Figure 2). These results suggest that the viscosity changes described earlier in myofibrillar extracts are reflecting changes in their actomyosin behaviour. However, further research is necessary to determine if other muscle proteins play some role in the observed viscosity decrease of actomyosin.

It is widely accepted that changes in the Mg^{2+}-ATPase activity occur when changes in the actin–myosin interaction are produced. As can be seen in Figure 3, the initial ATPase activity of actomyosin remained unchanged up to 10 days of storage on ice. This result is in agreement with that obtained by Hay *et al.*[19] and Wolfe and Samejima,[20] who reported essentially unchanged levels of actomyosin ATPase activity up to 7 days of post-mortem cold storage of chicken or hens' carcasses. The described results on Mg^{++}-ATPase activity of actomyosin of post-rigor hake stored on ice are in accordance with those of Seky *et al.*[4] who reported only a minor decrease in Mg^{2+}-(EGTA) ATPase activities of sardine myofibrils and myosin B prepared after rigor mortis development from ice stored muscles.

It has been reported that the SDS-PAGE patterns of myofibrils obtained from rabbit skeletal muscle stored at 4°C show little difference when compared to fresh ones.[21] Proteolysis signals in myofibrillar proteins of rattail fish (*Coryphalmoides acrolepis*) ice-stored for 15 days were not detected by SDS-polyacrylamide gel electrophoresis.[22] This seems to be the same for hake, as shown in Figure 4.

The results presented here are compatible with the idea of actomyosin as responsible for the viscosity fall of myofibrillar extracts obtained from fish stored on ice and that other factors instead of merely dissociation or fragmentation of actomyosin are involved in the viscosity decrease.

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