魚類筋肉からの数種解糖系酵素の溶出性

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Extractability of Glycolytic Enzymes from Fish Muscle

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Extractability of glycolytic enzymes from the ordinary muscle of red sea bream, Pacific mackerel and carp was examined in a range of 0–0.3 M KCl (pH 6.8–7.1). The amount of extracted proteins increased with an increase of KCl concentration up to 0.1 M, irrespective of fish species. The dependency on KCl concentration of the extractability of glycolytic enzymes such as aldolase (ALD), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glucosephosphate isomerase (GPI) was roughly comparable to that of total sarcoplasmic proteins. Sarcoplasmic proteins, as well as ALD, GAPDH and GPI, of Pacific mackerel were effectively extracted with 0.3% $NaHCO_3$-containing 0.15% NaCl, 0.15 M KCl, or a phosphate buffer (pH 7.5, I=0.05). The amounts of extracted ALD, GAPDH and GPI with 0.3% $NaHCO_3$-containing 0.15% NaCl were 1.5–4 times with that of distilled water. On the other hand, most of phosphoglycerate kinase and enolase was extracted in the absence of KCl.

It is well known that fish sarcoplasmic proteins consist mainly of glycolytic enzymes and their compositions are species-specific.[1,2] Shimizu *et al.*[3] examined the extractability of muscle proteins from various fish species at ionic strengths ranging from 0 to 0.3, and found that the amount of extracted protein from marine pealgic (red-fleshed) fish, sharply increased with increasing ionic strength up to 0.05, while that from marine bottom (white-fleshed) fish hardly depended upon ionic strength. They concluded that such differences between red- and white-fleshed fishes were attributable to the extractability of proteins having molecular weights of around 200,000.

In this connection, Shimizu and Ikeda[4] reported that the low extractability of sarcoplasmic proteins from red-fleshed fish muscle with water is due to their coprecipitation with myofibrillar proteins, which occurs at a low-ionic strength. Nishimoto[5] observed that a 40 K component in mackerel muscle extract, as determined by sodium dedecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), was sparingly extracted with water. The nature of the sarcoplasmic proteins remains to be elucidated.

In a previous paper,[2] we compared SDS-PAGE patterns of sarcoplasmic proteins of many fishes, and found that they can be categorized into marine white-fleshed, marine red-fleshed and freshwater fishes, by relative amounts of three major components: *i.e.*, 43 K (molecular weight 43,000),40 K and 35 K components. These components were identified as creatine kinase (EC 2.7.3.2), aldolase (EC 4.1.2.13), glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), respectively.[6] A preliminary study suggested that the difficultly extractable sarcoplasmic proteins are aldolase, glyceraldehyde-3-phosphate dehydrogenase, or analogous enzymes.

The above situation aroused us to investigate the extractability of glycolytic enzymes from the ordinary muscle of fish. This paper deals with the results obtained.

Materials and Methods

Materials

Live specimens of red sea bream *Pagrus major* and carp *Cyprinus carpio* were obtained from a commercial fish market. Fresh specimens of Pacific mackerel *Scomber japonicus* was purchased from the Tokyo Central Wholesale Market.

Extraction of Sarcoplasmic Proteins

To 2 g of a minced ordinary muscle of each species was added 20 m*l* of 1 mM EDTA-containing 0–0.3 M KCl (pH 6.8–7.1), and the mixture was stirred for 60 min and centrifuged at 10,000× g for 20 min. The resulting supernatant was used to determine the extracted protein and to assay some enzyme activities. Extractability of a given glycolytic enzyme was expressed by the ratio of

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the activity in an extract to the maximum activity under a series of experiment.

Another extraction was performed using various solvents as follows: One gram of muscle was homogenized for 5 min using mortar and pestle, with 5 volumes of distilled water, 0.3% $NaHCO_3$-containing 0.15% NaCl (pH 8.6), 10 mM Tris-HCl buffer (pH 7.5), 0.3 M mannitol,[7] 0.15 M KCl,[7] or a phosphate buffer (pH 7.5 and I=0.05).[8] The homogenate was centrifuged at 7,000×g for 20 min, and the supernatant was determined for protein and some enzyme activities.

Analytical Methods

Activities of glycolytic enzymes such as glucose-phosphate isomerase (GPI, EC 5.3.1.9), aldolase (ALD, EC 4.1.2.13), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) and phosphoglycerate kinase (PGK, EC 2.7.2.3) were measured by the method of Scopes.[9] Enolase (ENO, EC 4.1.2.11) activity was measured by the method of Pietkiewicz *et al.*[10]

The determination of protein concentration and SDS-PAGE were performed as described in a previous paper.[6]

Gel filtration on a Sephadex G-150 column (2.6×70 cm) which was equilibrated with 50 mM triethanolamine-HCl buffer (pH 7.5) containing 5 mM $MgCl_2$, was performed at a flow rate of 10 ml/h for determination of protein composition.

Results and Discussion

The effect of ionic strength on the extractability of sarcoplasmic proteins was examined on the ordinary muscle of the three fish species (Fig. 1). SDS-PAGE patterns of the extracted proteins are shown in Fig. 2. Regardless of fish species, the extractability of sarcoplasmic proteins increased with the increase of KCl concentration in a range from 0 to 0.1 M, beyond which the extractability remained almost constant. Extractability patterns of ALD and GAPDH were essentially comparable to those of sarcoplasmic proteins. The extractability of GPI from Pacific mackerel muscle was extremely low in a range from 0–

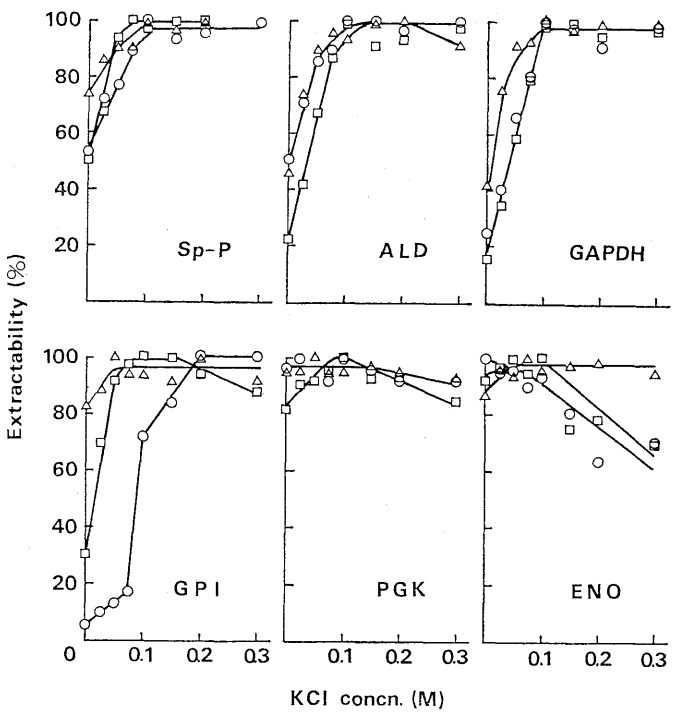

Fig. 1. Effect of KCl concentration on extraction of sarcoplasmic proteins (Sp-P) and several glycolytic enzymes from the ordinary muscle of red sea bream (□), Pacific mackerel (○) and carp (△).

Abbreviations used were ALD for aldolase, GAPDH for glyceraldehyde-3-phosphate dehydrogenase, GPI for glucosephosphate isomerase, PDK for phosphoglycerate kinase and ENO for enolase.

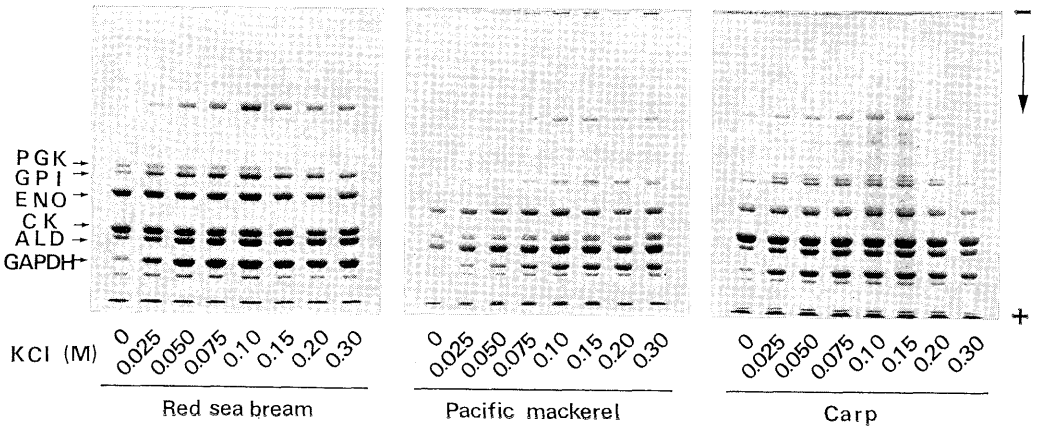

Fig. 2. SDS-gel electrophoretic patterns of sarcoplasmic proteins extracted from the ordinary muscle of the three species at various KCl concentrations. Solvents used were 1 mM EDTA-containing 0–0.3 M KCl (pH 6.8–7.1). Refer to the legend to Fig. 1 for abbreviations of enzymes. CK: creatine kinase.

0.75 M KCl (Figs. 1 and 2).

The extractability patterns of PGK and ENO clearly differed from those of sarcoplasmic proteins and the above enzymes. Eighty to 100% of PGK and END were extracted in a range of 0–0.5 M KCl. The extractability of carp ENO was not influenced by KCl concentration in the range covered, whereas those of red sea bream and Pacific mackerel tended to decrease with the increasing KCl concentration beyond 0.05 M. Species-specificity in ENO extractability was not clear electrophoretically (Fig. 2). In this connection, it was reported that ENO activity of rainbow trout muscle was enhanced at 0.2–0.3 M KCl, beyond which the activity was depressed.[11] The above phenomenon might have been caused by this reason. The extractability of creatine kinase, one of the major sarcoplasmic proteins,[6] was not influenced in a range of 0–0.3 M KCl as was that of ENO (Fig. 2).

From these results, it was concluded that the differences in extractability of sarcoplasmic proteins between the three species were mainly due to those of ALD, GAPDH and GPI.

Another aspect of the extractability of sarcoplasmic proteins was examined with Pacific mackerel. As shown in Fig. 3, the extractability pattern of sarcoplasmic proteins differed depending on the solvent used. A 0.3% $NaHCO_3$-containing 0.15% NaCl, which often is used as a washing solvent for red-fleshed fish, showed an effective extraction of sarcoplasmic proteins. A comparable extraction was attained by 0.15 M KCl or a phosphate buffer (I=0.05, pH 7.5). Extraction efficiency of other solvents such as distilled water, 10 mM Tris-HCl buffer or 0.3 M mannitol was much lower.

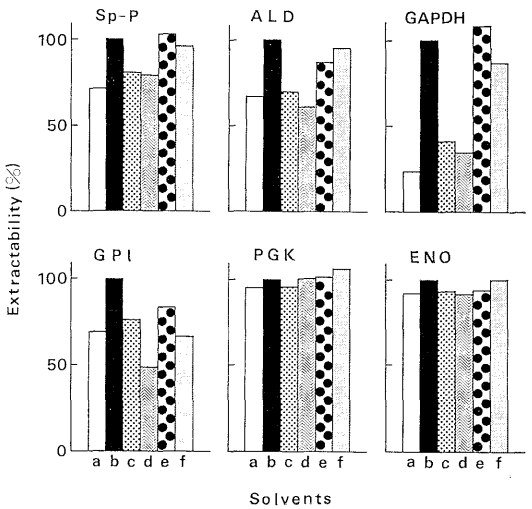

Fig. 3. Extraction of sarcoplasmic proteins (Sp-P) and several glycolytic enzymes from the ordinary muscle of Pacific mackerel with distilled water (a), 0.3% $NaHCO_3$-containing 0.15% NaCl (pH 8.6) (b), 10 mM Tris-HCl buffer (pH 7.5) (c), 0.3 M mannitol (d), 0.15 M KCl (e), and phosphate buffer (pH 7.5 and I=0.05) (f).

The amount of sarcoplasmic proteins or each enzyme extracted with solvent (b) was taken as 100%. Refer to the legend to Fig. 1 for abbreviations of enzymes.

The extractability of ALD, GAPDH and GPI was widely different depending on the solvent used, while that of PGK or ENO was not. These results were also confirmed by electrophoresis (data not shown).

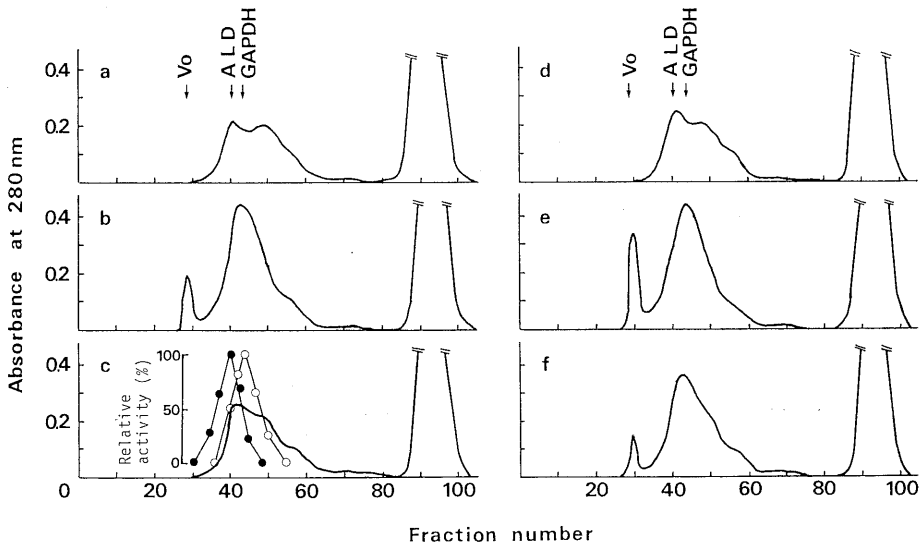

Fig. 4. Sphadex G-150 gel filtration of sarcoplasmic proteins extracted from Pacific mackerel ordinary muscle with six solvents (a-f). Refer to the legends to Figs. 1 and 3 for symbols. Vo: void volume. ALD (●) and GAPDH (○) activity peaks are indicated by arrows. Fractions of 4 m*l* were collected.

The composition of sarcoplasmic proteins thus extracted was studied by gel filtration on a Sephadex G-150 column. As shown in Fig. 4, gel filtration of the extracts obtained with 0.3% $NaHCO_3$-containing 0.15% NaCl, 0.15 M KCl and the phosphate buffer (I=0.05) demonstrated the presence of proteins eluted at the void volume, whereas the extracts with distilled water, 10 mM Tris-HCl buffer and 0.3 M mannitol did not. As shown in Fig. 4c for example, ALD and GAPDH activity peaks appeared at around fraction No. 40, suggesting that both enzymes were to less extent extracted with the latter three solvents. In this connection, the extractability of GAPDH from kokanee salmon muscle clearly differed from those of other glycolytic enzymes.[12] The increase of extracted proteins could partly be caused by solubilization of myofibrillar proteins at high-ionic strengths.

It was concluded from these data that the glycolytic enzymes binding to myofibrils at low-ionic strengths be accounted for by ALD and GAPDH.

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