Genetic Polymorphism of $\alpha$-Glycerophosphate Dehydrogenase in Saury, *Cololabis saira*—I.  
Seven Variant Forms and Genetic Control*

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Seven different $\alpha$-glycerophosphate dehydrogenase ($\alpha$-GDH) isozyme patterns were electrophoretically observed in the white muscle cell-lysates of saury, *Cololabis saira*, from off the North Pacific coast of Japan and the East China Sea. The patterns indicated that they are controlled by four codominant alleles at an autosomal locus. The occurrence of hybrid isozymes in the heterozygotes suggests that the hybrid isozymes as well as other isozymes are of dimeric structure. Additional $\alpha$-GDH isozymes which are controlled by at least two distinct loci were detected. It was found that a large scale screening of enzyme types for population analysis can be facilitated by the use of cell-lysates produced by thawing the frozen tissue. It is proposed that the dried cell-lysate-soaked filter paper and the tissue block taken in the agglutination test tray be used for population analyses of this kind because of the convenience in transporting and storing a large number of samples.

A large and rapidly increasing number of electrophoretically distinguishable genetic variants of various enzymes have recently been discovered. The variant forms provide a research tool for studying the genetic control and subunit structure of those enzymes, and are useful genetic markers in population analysis and linkage studies. Of the $\alpha$-glycerophosphate dehydrogenase (1.1.1.8, $\alpha$-GDH), two variants in *Drosophila melanogaster* attributed to alleles at a genetic locus have been described by Grell (1967)\(^1\).

In the present report, seven electrophoretic variant forms attributed to four codominant alleles at an autosomal locus are demonstrated in muscle $\alpha$-GDH of saury, *Cololabis saira*. A practical method for mass screening of enzyme types in fish species by using cell-lysates produced by freezing and thawing are described.

**Materials and Methods**

**Materials**: About 80 samples consisting of 8,000 specimens of saury caught off the Pacific coast of Japan and the East China Sea have been screened so far for muscle $\alpha$-GDH variants. Mass screening of muscle $\alpha$-GDH types was performed satisfactorily by using cell-lysates of the tissue formed by freezing and thawing process. As soon as possible after landing of fish specimens about 1.5 g each of their white muscles were taken

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in individually labelled holes of plastic trays (CF tray for agglutination test, 12 × 8 holes, Hirasawa Co., Tokyo), covered with plastic plates, sealed, and then frozen and shipped to the laboratory. In the first part of this investigation, the whole body of fish was frozen and shipped. All the specimens were kept frozen at −20 °C for the test. The frozen fish were thawed in running water, and a deep incision was made on the lateral side of the fish with a razor blade. The cell-lysates which are usually called internal drips in food refrigeration were sucked by small pieces of filter paper inserted into the incision. The blocks of muscle in each tray were thawed at room temperature, and the drips formed were sucked into the filter papers inserted at the bottom of respective holes of the tray by pushing slightly the muscle blocks. In some cases, the pieces of drip-soaked filter paper were dried on a petridish at room temperature or in a freezer, and were put together in an envelope with silica gel and sent to the laboratory. Specimens so processed could stand α-GDH typing at least for one month, without causing any difficulty in typing. The wet or dried filter papers were directly used for starch gel electrophoresis.

For analyzing tissue specific distribution of the α-GDH isozymes, live specimens caught during the cruise of KH-68-2 of the Hakuho-Maru2) were examined on board. Various tissues were homogenized by a Waring blender and/or by a Teflon homogenizer with an equal part of 1.4 mM disodium EDTA. Supernatants obtained by centrifuging the homogenates at 15,000 g for 15 min. were subjected to electrophoresis after sucking into filter paper.

**Electrophoresis and staining of α-GDH:** The buffer system described by KRAUS and NEELY3), was used for electrophoresis with slight modification of Tris concentration in the stock solution: 0.79M Tris, 0.02M EDTA and 0.5M boric acid (pH 8.55). Starch gel was prepared at the concentration of 14% with a 1:20 dilution of the stock buffer. The electrode solutions were made of the stock solution diluting 1:20 for the anodal vessel and 1:15 for the cathodal. Electrophoresis was conducted horizontally at 5 °C at a 0.5 mA per cm for 17 hrs. After electrophoresis, the gels sliced horizontally were allowed to stand for 30 min. at room temperature in a reaction mixture of the following composition, to make visible α-GDH on the gel: 400 mg of disodium α-glycerophosphate (95–99 % α-isomer, Sigma), 30 mg of NAD, 20 mg of Nitroblue tetrazolium, 7 mg of phenazine methosulfate, 360 mg of disodium EDTA, and 100 ml of 0.1 M Tris-HCl (pH 8.7).

**Results**

**Muscle α-GDH variants.** Variant forms of α-GDH isozymes present in the white muscle of saury were surveyed by subjecting the drips or crude extracts to starch gel electrophoresis. Drips of white muscle contained 4 to 5 times the α-GDH activity of the crude extracts obtained from the tissue with an equal part of 1.4 mM disodium EDTA,
and gave clearer and more strongly stained isozyme patterns. Drips dried in filter paper gradually decreased in \(\alpha\)-GDH activity. However, 98 percent of the samples gave a clear pattern even after one month drying. No significant changes were observed in the patterns by storing the tissue in frozen state and by drying the drips either.

Seven different patterns of muscle \(\alpha\)-GDH have been detected through the screening of about 8,000 specimens of 80 samples. Among them, three patterns, in other words three phenotypes, type S, type S–N and type N (Fig. 1) were found in high frequencies in all the samples examined. The type S and type N contained single isozymes, but electrophoretic migration distance of the isozyme of type S was distinctly smaller than that of type N. The enzyme of type S–N resolved into three isozymes, the most anodal being identical in its position with that of the type N, the most cathodal corresponding to that of type S, and the middle one staining more strongly than the other two. The staining intensity of the middle isozyme of type S–N was weaker than those of the single isozymes of type N and type S. The patterns strongly indicated that the GDH isozymes of saury are dimers formed by random association of subunit molecules which are thought to be under the control of a genetic locus involving two alleles, as shown in Fig. 2. Possibly the individuals homozygous for the \(Gdh-M^X\) allele (type N) produce only the fast-moving isozyme regarded as the dimer of subunit N, and the homozygotes for the \(Gdh-M^S\) allele (type S) produce only the slow-moving isozyme regarded as the dimer of more positively charged subunit S. On the other hand the individuals heterozygous for \(Gdh-M^X\) and \(Gdh-M^S\) alleles (type S–N) seem to produce three dimers formed by the association of the subunits S and N in all possible combinations as in Fig. 2. The binomial proportions in the staining intensity of the three isozymes of type S–N, and the stronger staining of the isozymes of type N and type S are in good agreement with theoretical expectation based on the assumption of two-allele controlling system and random combination of subunit molecules into dimers.

![Fig. 1](image-url)

**Fig. 1.** Starch-gel electrophoretic patterns of seven phenotypes of muscle \(\alpha\)-glycerophosphate dehydrogenase in saury. The anode is toward the top. Refer to Fig. 2 for the details of the numbers and phenotypes.
Fig. 2. Ten phenotypes and corresponding genotypes of saury muscle α-GDH as postulated from the electrophoregrams in Fig. 1. Assumed subunit compositions of isozymes are shown at their right side. \( M^C, M^R, M^S \) and \( M^R \) indicate respectively the alleles \( Gdh-M^C \), \( Gdh-M^R \), \( Gdh-M^S \) and \( Gdh-M^R \). Three phenotypes underlined have not been detected so far, however they are presented in the theoretical expectation based on the assumption of dimeric structure for all the isozymes.

Other four types, type C-S, type C-N, type S-R and type N-R were found in very low frequencies in some, not all, samples. Those four types all presented three isozymes exhibiting binomial proportions in the intensity of enzymatic staining, and therefore, were assumed to be heterozygous. Thus, the presence of four alleles at the \( Gdh-M \) locus which make the alteration in electrical charge of the subunit, were postulated, and ten genotypes were assumed to be present in theoretical expectation. The patterns and subunit compositions for the ten genotypes, including two homozygotes and one heterozygote for the rare allele \( Gdh-M^C \) and \( Gdh-M^R \) which have not so far been detected, are presented in Fig. 2.

All the seven types were found in both male and female specimens. This would suggest that the locus coding to muscle α-GDH is located on an autosome.

Additional α-GDH isozymes. Homogenates of various tissues from live saury were analyzed for α-GDH isozymes. Tissues examined were white muscle, red muscle, heart muscle, liver, intestine, gill, whole eye, brain, testis, and blood. One to three isozymes different from the muscle α-GDH isozymes were detected in eye, brain, and testis, as shown in Fig. 3. These isozymes were featured by more faintly staining and far more rapid migration towards the anode than the muscle α-GDH isozymes: Their migration distances were 4.7 to 5.5 cm under the condition used here. Under the same condition, even the fastest band of the muscle α-GDH of type S-N migrated by only 2.9 cm (Fig. 3). In red muscle, heart muscle, liver, intestine, and gill, α-GDH isozymes could not be detected on
the gel under the condition described. No tissues have been found exhibiting both the muscle $\alpha$-GDH and those additional isozymes. Those additional $\alpha$-GDH isozymes should be considered to be at separate genetic loci from the locus for muscle $\alpha$-GDH.

On the genetic control of the additional isozymes, further investigation is now in progress, but it appears that there are at least two distinct loci, one coding to the isozyme of brain, and the other coding to the isozyme of blood. Three isozymes of eye might be produced by combination of two subunits produced by the above two loci. The middle isozyme of eye and the anodal of blood may be considered as a hybrid isozyme composed of the two subunits. At present, it cannot be said with certainly if the isozyme of testis is the same as that of brain.

Discussion

Grell\textsuperscript{1) }surveyed inbred strains and a wild population of \textit{Drosophila melanogaster} and found three types of $\alpha$-GDH patterns. The three patterns are very similar to the patterns of type N, type S–N and type S in saury presented in this paper. In the case of \textit{D. melanogaster}, two slower-moving and more faintly staining bands of $\alpha$-GDH activity were found in all the three types. In saury, two bands of $\alpha$-GDH activity were also observed, though very faintly, at the anodal side of the fastest-isozyme of each type shown in Fig. 1 when the gel was incubated in the reaction mixture for a longer period. The staining intensity of the said two bands did not change during the storage of tissue or drip. Whether these multiple forms are present in the living tissues of saury or are artifacts is not clear at this stage.

In this paper, at least two isozyme systems of $\alpha$-GDH which are under the control of three genetic loci, are assumed to be present in saury. The existence of multiple loci for one enzyme has been well known in lactate dehydrogenase\textsuperscript{4,5) }from various sources, especially from fish species.\textsuperscript{5) }This, however, is the first report suggesting the presence of multiple loci for $\alpha$-GDH. Whether the existence of multiple loci concerned for the enzyme is a rare or common phenomenon, and what its biochemical significance is, remains unknown.
Now, since the presence of multiple alleles at the locus for the muscle $\alpha$-GDH was demonstrated, the enzyme could be used as a valuable genetic marker in the studies of saury population. Generally, variant types of an enzyme contained in tissues have usually been screened by electrophoresis of the supernatants of their homogenates. However, preparation of such supernatants is often too laborious to screen a large number of samples enough to analyze population. In the present paper, simple routine methods for sampling and transportation of specimens, and preparation of samples for electrophoresis were established for population analysis. It is well known that fish meat is apt to form drips which is a serious problem in its refrigeration. In the present study, this character of fish meat was utilized profitably for preparing samples for electrophoresis. Neither homogenizing of the tissue nor centrifuging the homogenate is needed. In the process of enzyme-typing by using the drip, freezing of the tissue serves for both maintenance of enzyme activity and preparation of the sample for electrophoresis. The trays as used in the present study are very useful to transport and preserve a large number of specimens. In the tray, specimens can be arranged systematically in numerical order, making it easy to relate them to various data kept elsewhere and to pick out immediately the specimens for re-testing, whenever necessary. The dried drip-soaked filter paper will be also very useful in transportation of many samples in large scale studies on the population analysis of fish species.

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References