メバルのカタラーゼにおける電気泳動的多型

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Electrophoretic Variants of Catalase in the Black Rockfish, *Sebastes inermis**

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Three electrophoretic patterns of catalase were demonstrated in the liver extracts of the black rockfish, *Sebastes inermis*. All the types gave only single bands of catalase activity on the gel, but were distinguishable by mobility. Analysis of phenotype frequencies in the two samples consisting of 142 specimens indicated that those patterns are attributed to two alleles at an autosomal locus. The alleles were proved to be independent of the size of the fish, and to be maintained in the same frequency among the samples collected at different seasons. The catalase patterns were most clearly demonstrated by the negative iodine-starch reaction, and satisfactorily preserved in acetic acid. The method based on the enzymatic oxidation of aromatic amines was not successful for localizing the enzyme on the gel.

Electrophoretic variants of enzymes have a number of research applications in genetics and biochemistry as a natural label[1]. However, studies on the electrophoretic variants of enzymes remain to deal with only a few isozymes. Recently, the author reported the variant forms and genetic control of malate dehydrogenase[2] and α-glycerophosphate dehydrogenase[3] in *Cololabis saira*. In the present paper, variants of catalase in the black rockfish, "mebaru", *Sebastes inermis*, and genetical analysis of the distribution of the variants in two population samples are presented together with some remarks on the electrophoretic conditions and staining methods of catalase of this species.

Materials and Methods

Two samples, consisting of 142 specimens in all, of the black rockfish caught in the different seasons in 1969 by set net at Hashirimizu, Kanagawa Prefecture, were examined. All the specimens showed a blackish body color to be regarded "kuromebaru". No individuals showing a reddish or more whitish color[4] were included in the samples examined.

For screening of catalase types in the samples, livers dissected from the fresh fish or fish stored frozen at $-20°C$ were homogenized in three parts of distilled water with a Waring blender, centrifuged at 20,000 g for 15 min. The supernatants were subjected to horizontal electrophoresis in 14% starch gel, with a constant current of 1 mA/cm^2 for 13–16 hrs, using a Tris-boric acid-EDTA buffer system at pH 8.7[2]. After the electrophoresis, the gels were sliced horizontally and stained by an essentially the same method as

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that described by BECKMAN *et al.*[5] as follows:

The gels were soaked for 1 min in 0.5 percent hydrogen peroxide, washed twice with distilled water for a short time, and immersed in a 1 percent solution of potassium iodide acidified with dilute acetic acid (3:1,000). Iodine released by the peroxide gradually stained the gel into dark blue except the areas where the catalase activities had destroyed the peroxide porducing gas bubbles. Before the iodine-starch reaction fully proceeded, the gels were transferred into 0.3 % acetic acid and the solution was exchanged twice. The patterns became more distinct in the solution and were maintained in the solution for two or three days. The patterns were recorded on photographic paper of high contrast put under the stained gel, by exposure it to the light.

Results

Staining of catalase on starch gel. Liver catalase was clearly demonstrated as a broad white zone against a dark blue background by the method described (Fig. 1). Another method[6] for localization of catalase on the gel, based on the oxidation of *o*-dianisidine by the haemoglobin added in the gel with subsequent staining of the gel, gave almost the same pattern as that obtained by the negative iodine-starch reaction. An *o*-dianisidine-Teepol method[7], by which the band of catalase is said to be stained as a more restricted zone, gave only a very faint band at the site of catalase zone. The other reagents used for demonstration of peroxidatic activity, such as benzidine, *o*-toluidine, *p*-phenylenediamine, α-naphthol and leucomalachite green also gave very faint bands or nothing. It appears that peroxidatic activities catalizing the transfer of electrons from aromatic amines and leucocompound to hydrogen peroxide is very low in the liver catalase of *S. inermis*.

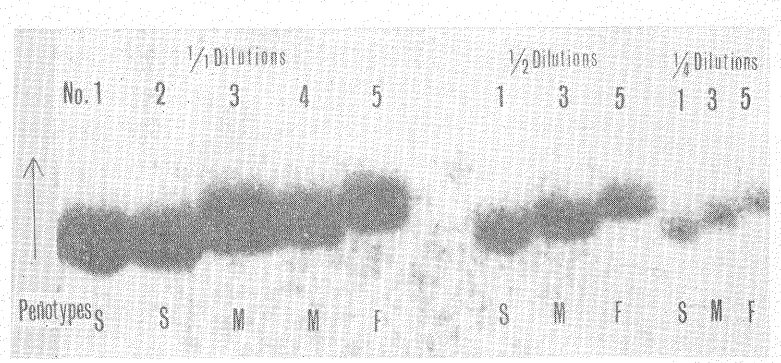

Fig. 1. Appearance of three phenotypes of liver catalase of *Sebastes inermis* in starch gel electrophoresis. Crude extracts of livers prepared with an equal part of distilled water were used as a base of doubling dilutions of samples. In this photograph directly taken by contact printing using the stained gel, the relation of light and shade is the reverse of that the original staining. The arrow indicates the direction towards the anode.

Variant patterns and electrophoretic conditions for typing. All the liver extracts from 142 specimens gave only single broad zones of catalase activity on the gels. These were able to be classified into three types by the electrophoretic mobilities of catalase zones, and were termed S, M, and F as in Fig. 1. The three types were detected in various buffer systems. A Tris-boric-EDTA buffer at pH 8.7[2], and a discontinuous buffer[8] consisting of Tris-citric acid at pH 8.3 for gel and lithium hydroxide-boric acid at pH 8.1 for electode vessels, provided better resolution for type determination. In a Tris-citrate-boric acid buffer at pH 9.5[7], the differences of mobilities among the three types were somewhat smaller than those in the above two buffers. The catalase zone demonstrated in a Tris-boric acid buffer at pH 7.5 was wider and a tailing undesirable for typing was observed. In a phosphate-citric acid buffer at pH 7.0, the catalase remained near the origin at the anodal side, and the differences of mobilities among the types were very small. In the lower concentration of starch gel, a broader zone of catalase appeared. High activities of catalase were observed in liver, and crude extracts prepared with three or four volumes of water were suitable for type determination as in Fig. 1.

Table 1. Comparison of observed and expected numbers of catalase phenotypes and frequencies of the gene postulated, between the two samples of *Sebastes inermis* from Hashirimizu, Kanagawa Prefecture.

Date of Capture	Number of specimens		Phenotypes S	M	F	Gene frequencies of *Cts*	χ^2	Probability
7, May 1969	99	Obs.	24	48	27	0.485	0.295	>.500
		Exp.	23.3	49.5	26.3			
19, Oct. 1969	43	Obs.	8	22	13	0.442	0.237	>.500
		Exp.	8.4	21.2	13.3			
Total	142		32	70	40	0.472	0.442*	>.500*

* Chi square and probability for the heterogeneity of samples.

Frequencies of variant types and genetic control. The types other than the three types in Fig. 1 have never been found in the specimens examined. The distribution of three types in the two samples are shown in Table 1. The three types were found in both male and female, although most of the specimens examined were female. In the table, the expected numbers for each type on the basis of Hardy-Weinberg law for the two allele controlled inheritance of autosomal locus are presented. The

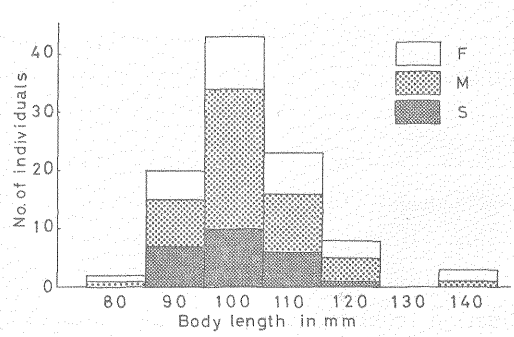

Fig. 2. Distribution of three phenotypes of liver catalase in relation to body lengths.

frequencies of the three types observed the in samples well approximated with the expectation. This suggests that the three types are under the control of two alleles on an autosomal locus: Homozygotes of allele Ct^F and Allele Ct^S have respectively the fast-moving and the slow-moving form of catalase. The intermediately moving form is produced by Ct^F/Ct^S heterozygotes. No significant excess of homozygotes and heterozygotes was observed, and the three types were found irrespective of body size in a fairly constant (Fig. 2). It appeared that the alleles coding to the catalase in *S. inermis* produce no apparent alteration in biological activity.

The aboundance of this species in the Hashirimizu waters varies from season to season, decreasing in summer and increasing in autumn due to the migration closely related to the abundance of food and their life cycle, as was suggested by HARADA[9]. Chi-square test for the heterogeneity of samples suggests no significant differences in allele frequency between the two samples collected in the different seasons from one location.

Discussion

BECKMAN *et al.*[5] have clearly shown that the heterozygous variants of catalase in maize endosperm contain three hybrid isozymes with electrophoretic mobilities intermediate between parental isozymes. On the other hand, isozymic forms of catalase have never been demonstrated in animals, though BAUR[10] and NANCE *et al.*[11] suggested that the broad zone of human-erythrocyte catalase activity on starch gels may result from the presence of isozymes. All of the variant forms of liver catalase in S. inermis presented here showed only single broad bands on the gel under a variety of staining methods and various buffer systems used for gel electrophoresis. Liver extracts from the other fish species, such as rainbow trout *Salmo gairdnerii,* chum salmon *Oncorhynchus keta,* horse mackerel *Trachurus japonicus*, and saury *Cololabis saira*, also showed single zones of catalase on starch gel. The catalase zones demonstrated in these species were wider than those of *S. inermis*, and there appeared to exist variant forms in some of those species, but isozymes were not clearly demonstrated in all the species examined. Therefore, the subunit structures of the enzymes were impossible to estimate from the patterns, and reliable estimation for the genetic control of the enzyme was hardly possible from the patterns alone.

Liver catalase of fish species is preserved by freezing the tissue without significant changes in activities and electrophoretic mobilities, and the zymograms are readily obtainable. In these points, the variants of catalase would be suitable as a genetic marker in population studies of fish species. Elucidation of the subunit structure and the examination of electrophoretic conditions making possible higher resolution of types of the enzyme must be subjects of futher investigation.

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