Genetic Controls of MDH and LDH Isozymes in the Goldfish

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Genetic Controls of MDH and LDH Isozymes in the Goldfish

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Summary

Genetic controls of malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) isozymes in eight races of the goldfish (*Carassius auratus*) evolved from tetraploid fish were examined by electrophoretic analysis. The tissue specificity revealed that MDH isozymes of this fish are coded by six separate *Mdh* loci (*Mdh-A1*, *Mdh-A2*, *Mdh-B1*, *Mdh-B2*, *Mdh-C1* and *Mdh-C2*), suggesting the existence of a duplicated gene controling s-MDH-A, s-MDH-B and m-MDH-C isozymes.

The tissue specificity of LDH indicated LDH-A (heart predominant), LDH-B (skeletal muscle predominant), LDH-E (brain specific) and LDH-F (liver specific) isozymes. Out of these isozymes, LDH-A, -E and -F could not be demonstrated through gene duplication for a fixed single banded phenotype, although a polymorphism of LDH-B suggested gene duplication and that the isozyme is coded by two separate loci (*Ldh-B1* and *Ldh-B2*). The experiments of heat stability and pairmating for this isozyme supported the above suggestion and revealed a polymorphism of this isozyme based on *A* and *B* alleles at *Ldh-B1* locus.

Cyprinid fish have been demonstrated to be in a diploid-tetraploid relationship in analysis of genome with respect to the DNA content per cell and the number of chromosomes (1, 2). The number of gene loci of malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) isozymes may be determined by extensive gene duplication.

In most fish species, MDH isozyme patterns have been found to contain two separate soluble-form (s-form) *Mdh* loci (*Mdh-A* and *Mdh-B*) and a single mitochondria-form (m-form) *Mdh* locus (*Mdh-C*) which code for four dimeric bands: A_2, AB, B_2 and C_2 (3, 4). Of the s-MDH isozymes, the B_2 isozyme generally is the most anodal, and the A_2 isozyme has the highest activity in all tissues.

Five-banded LDH isozyme patterns in heart and skeletal muscle have been found to contain two separate loci, *Ldh-A* and *Ldh-B*, which code for A and B

subunits. A- and B-subunits form five tetrameric bands: A_4, A_3B_1, A_2B_2, A_1B_3 and B_4. In addition to these two loci, there are three other loci: the *C* locus functions only in primary spermatecytes in mammals and birds, the *E* locus functions in eye and brain tissues, and the *F* locus codes for the liver-specific isozyme (5).

The extensive gene duplication complicates an interpretation of electrophoretic pattern. Complexities of interpretation arise when subunits of active proteins encoded by different loci have similar or identical electrophoretic mobility. Actual mating data, where the phenotypic ratios of progeny for a particular enzyme are consistent with the known phenotypes of the parents, are needed for studies of population genetics. The purpose of this work is to point out the restricted existence of MDH and LDH isozymes in highly differentiated tissues in goldfish and to elucidate the genetic control of their electrophoretic variants.

Materials and Methods

The Goldfish (*Carassius auratus*) were purchaced from a breeder in Yatomi Town, Aichi Prefecture and were kept in our laboratory pond. Isozyme patterns of MDH and LDH were determined by means of horizontal starch gel electrophoresis. The methods of electrophoresis were based on Fujio (6). Two buffer systems adopted for gel and electroding were as follows: TC-7 (tris-citric acid, pH 7.0) (6) and CAPM (citric acid, 4-3-aminopropylmorpholine, pH 6.0) (7). The staining procedures were based on Fujio (6). In all fish, the brain, eye, heart, skeletal muscle and liver were analyzed.

For examining heat stability of LDH isozymes, the gel (after the electrophoresis) was subjected to heat treatment in a water bath. The procedure of heat treatment of the gel was described in the previous paper (8).

For the determination of genetic control of LDH isozymes, five pairs of goldifish were employed. In the skeletal muscles of the fishes, the isozyme patterns of LDH were determined for obtaining the offspring. The offspring from individual pairs were kept in a separate small pond. They were raised for more than 12 months, until they had reached a size large enough to permit the electrophoretic determination of their isozyme patterns for LDH in the skeletal muscles.

Results

Isozyme patterns of MDH and LDH

Tissue distribution of MDH in the goldfish is shown in Fig. 1. The total number of the observed bands was nine in the heart. The six anordal bands are s-MDH and the three least anordal bands are m-MDH. Among the s-MDH isozymes, the slowest band has the highest activity in all tissues, except for

skeletal muscle. It suggests that the three most anordal bands of skeletal muscle and the three least anordal bands of the other tissues consist of B- and A-subunits, respectively. The former bands as observed in skeletal muscle are labeled $B1_2$, B1B2 and $B2_2$ from most to least anordal, suggesting two separate loci, *Mdh-B1* and *Mdh-B2*. On the other hand, the latter bands were observed in brain, eye and liver and are labelled $A2_2$, A1A2 and $A1_2$ from the most to least anordal, suggesting two separate loci, *Mdh-A1* and *Mdh-A2*. The six anodal bands of heart were interpreted as the result of equivalent expression by these four *Mdh* loci in this tissue. The estimated composition of subunits for those bands is shown in Fig. 2. These are labeled $B1_2$, B1B2+A2B1, $A2_2$+A2B2, A1A2+A1B2 and $A1_2$ from the most to least anordal.

Among the m-MDH isozymes, the slowest band has the highest activity in brain, heart and skeletal muscle. The activity of their most anordal band was weak. These bands are labeled $C2_2$, C1C2 and $C1_2$ from the most to least anordal. This suggests that two separate loci, *Mdh-C1* and *Mdh-C2* for m-MDH.

Tissue distribution of LDH in the goldfish is shown in Fig. 3. The total number of bands appearing in the dominant type was five in the skeletal muscle. Such a five-banded pattern was observed in all other tissues : brain, eye, heart and liver. The slowest migrating band within the five bands was observed in skeletal muscle and was observed weakly in the heart. This pattern is typical in almost fish, suggesting that LDH is a tetrameric molecule and controlled by two separate loci (*Ldh-A* and *Ldh-B*) which code for A- and B-subunits, respectively. A- and B-subunits indiscriminately associate and form five tetrameric isozymes (A_4, A_3B_1, A_2B_2, A_1B_3 and B_4) which can be visualized as five distinct bands. Three or

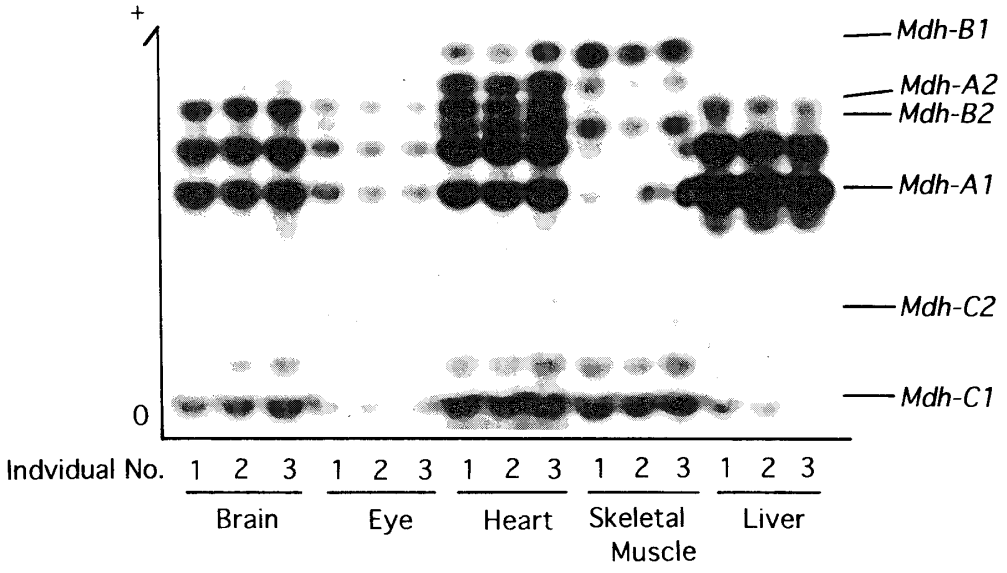

FIG. 1. Tissue specificity of MDH isozyme patterns in the goldfish. The buffer for the gel and electroding was TC-7 (tris-citric acid, pH 7.0).

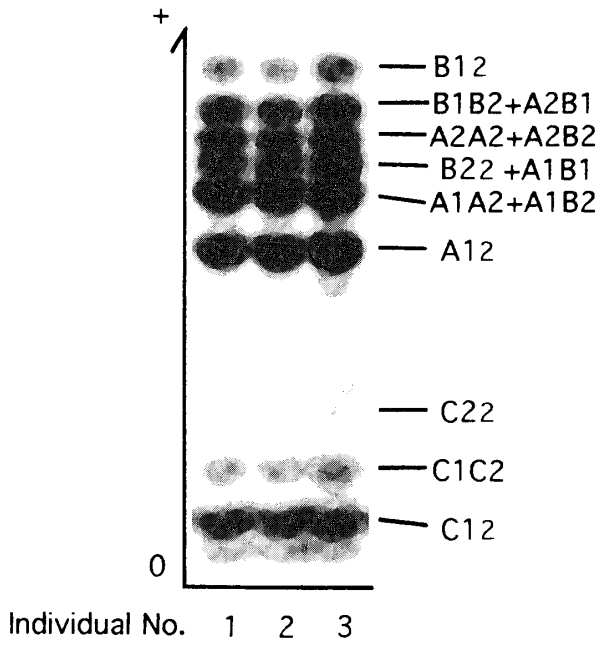

FIG. 2. MDH isozyme pattern of heart in the goldfish. The presumed constitutions of subunits in each isozyme bands are shown on the right. The buffer for the gel and electroding was TC-7 (tris-citric acid, pH 7.0).

two additional bands were found in the brain and heart toward the cathodal side on the gel, suggesting the brain-specific LDH-E isozyme. Furthermore, four additional bands were observed in the liver. They appeared toward the highly cathodal side in the gel and the last band showed strong activity. This suggests the liver-specific LDH-F isozyme. The LDH isozyme patterns in the goldfish were tissue specific.

Variant B subunits of LDH

The isozyme patterns of MDH and LDH were surveyed for 289 specimes of the eight races of the goldfish. The results revealed that the isozyme pattern of MDH showed no variation among any specimens but three different patterns of LDH were observed in heart and skeletal muscle. Each of the three patterns was designated as a types (I, II and III, respectively) as shown in Fig. 4. Type I showed five bands, and types II and III showed nine bands. The difference between type II and type III was observed in the five bands from the most to least anordal, that is, type II showed strong staining in the most anordal band, while type III was weak or not at all observed. Three different phenotypes suggest the existence of two separate loci, *Ldh-B1* and *Ldh-B2*, and the difference between them is presumed to be reflected by two alleles, *A* and *B*.

To distinguish clearly between types II and III, the isozymes were subjected to heat treatment. Activity of the LDH-B isozymes was observed at 72°C for 10

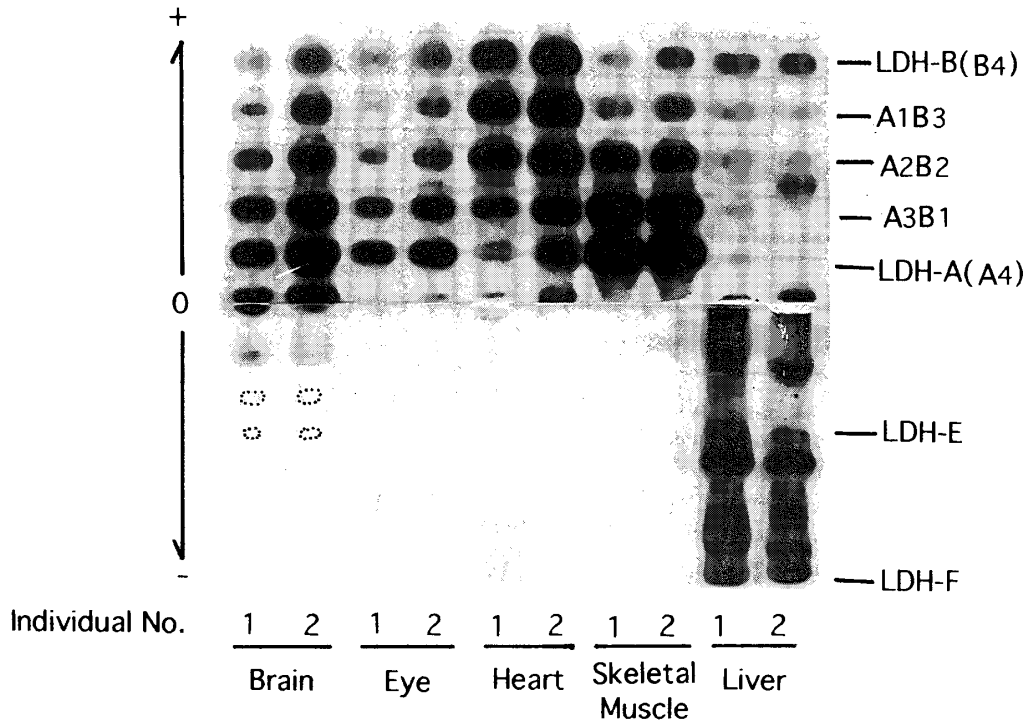

FIG. 3. Tissue specificity of LDH isozyme patterns in the goldfish. The buffer for the gel and electroding was CAPM (citric acid, 4-3-aminopropylmorpholine, pH 6.0). The bands of LDH-E isozyme (brain specific) and a hybrid band between LDH-E and LDH-A which were not presented completely in this photograph were indicated by the dotted circles.

minutes but was not observed in the LDH-A isozyme. This indicates that the heat stability is different between LDH-A and LDH-B isozymes. Fig. 5 shows the zymogram heated at 72°C for 10 minutes in skeletal muscle. Type I showed only one band in the most anordal side. In type II, the most anordal band strongly stained and the least anordal band showed weakly or not at all. In type III, the most anordal band showed weakly or not all and the least anordal band showed strongly staining. This indicates that the most anordal band was a homotetramer of B1 (A/A) and B2 subunits in type I, the most anordal band was a homotetramer of B2 subunit and the least anordal band was a variant of B1 subunit (B/B) in type III, and that type II was a hybrid between type I and type III.

Mating experiment and polymorphism at Ldh-B1 *Locus*

The distribution of type I, II and III were examined for the eight races of the goldfish. The results is shown in Table 1. Type I was observed in Wakin, Comet, Ryukin, Sanshoku-demekin but not in Kuro-demekin, Aka-demekin, Oranda-shishigashira and Ranchu. Type II and III were observed in all races examined.

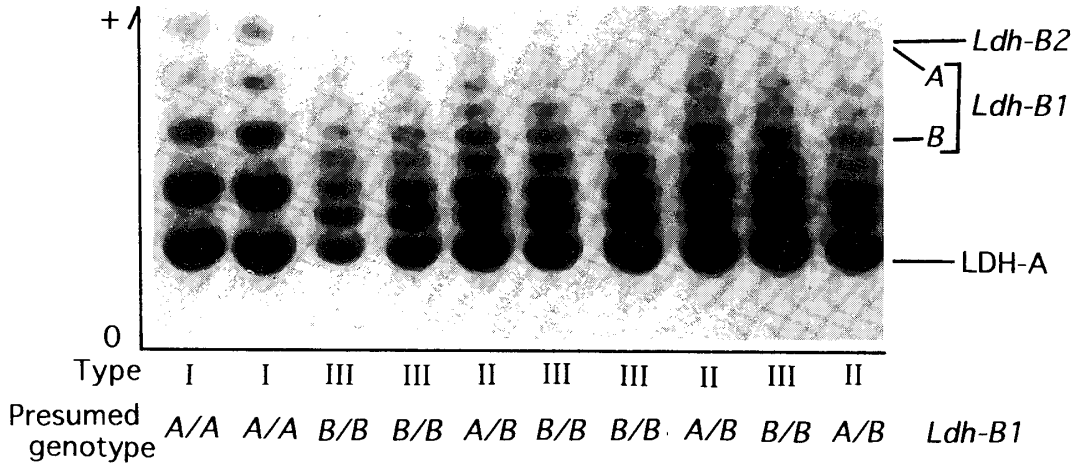

FIG. 4. Three LDH isozyme patterns (I, II and III) of skeletal muscle in the goldfish. The buffer for the gel and electroding was TC-7.

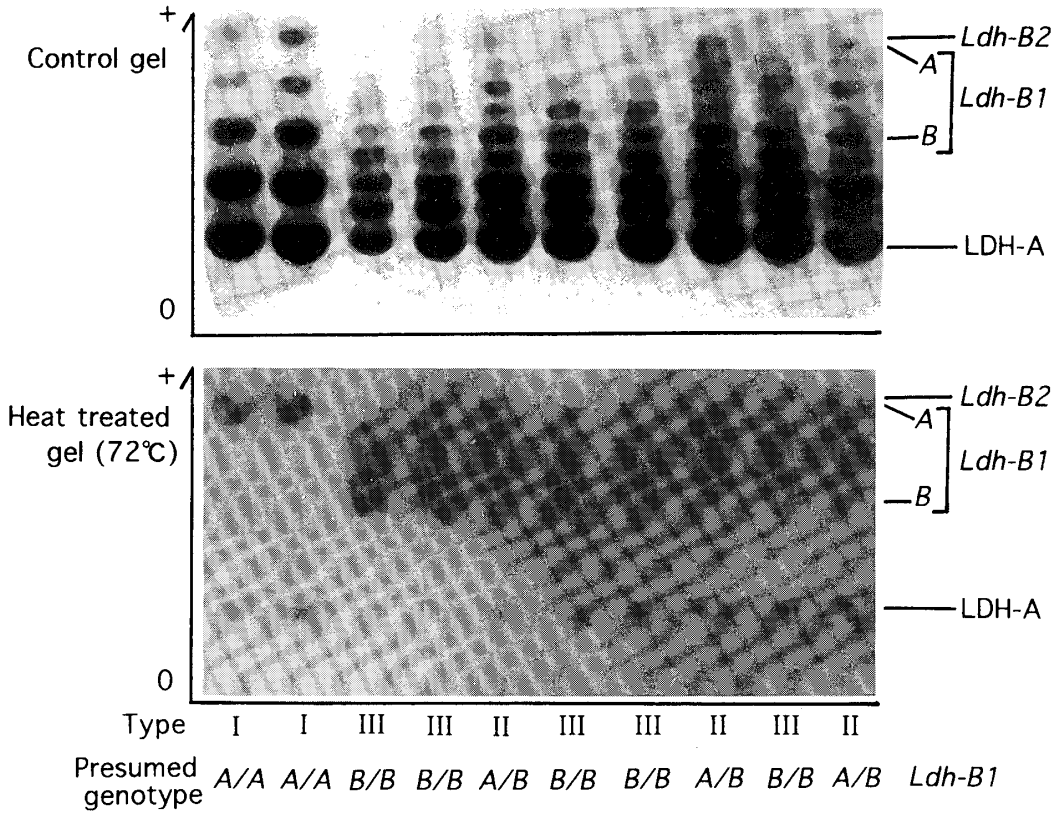

FIG. 5. Heat treatment of LDH isozyme of skeletal muscle in the goldfish. After electrophresis using TC-7 buffer, the gel was heated at 72°C for 10 minutes, and then it was cooled at 4°C and stained.

The parental genotype combinations at the *Ldh-B1* locus were examined in five pair matings of goldfish as shown in Table 2. All offspring examined were obtained from the crosses $A/A \times A/A$, $A/A \times B/B$ and $A/B \times A/B$, respectively. The segregation of offspring from the cross $A/A \times A/B$ was observed, and their genotypes, A/A and A/B, were found with the expected ratio of 1 : 1. Offspring from the cross $A/B \times A/B$ exhibited A/A, A/B and B/B genotypes with the expected ratio of 1 : 2 : 1. The pairmating confirmed our presumed genotypes based on two alleles, A and B, at the *Ldh-B1* locus.

The genotypic frequencies indicate that analyzed populations were under Hardy-Weinberg equilibrium and that eight races of the goldfish were different from each other in the allele frequencies (Table 3).

Discussion

In comparison with members of the Cyprinid family, not only twice the chromosome number but also numerous duplicated gene loci have been reported in carp (*Cyprinus carpio*), goldfish (*Carassius auratus*) and berbel (*Burbus berbus*) (9, 10). In the present work, the existence of a duplicated gene was demonstrated in s-MDH-A, s-MDH-B and m-MDH-C in the form of a fixed multibanded phenotype found in all individuals of a population in the goldfish. The gene duplication was also demonstrated in LDH-B by a polymorphism in a fixed single-banded phenotype. However, the gene duplication could not be demonstrated in LDH-A, liver-specific LDH-F, and brain-specific LDH-E, because a fixed single-banded phenotype was in all individuals of a population. Duplicated gene loci in the goldfish have been found in 6-phosphogluconate dehydrogenase (6PGD) (8), glucosephosphate isomerase (GPI) (11), m-aspartate aminotransferase (m-AAT), α-glycerophosphate dehydrogenase (α-GPD), s-isocitrate dehy-

TABLE 1 *Distribution of LDH Isozyme Patterns of the Skeletal Muscle in Eight Races of the Goldfish*

Race	No. of fish examined	Type of LDH isozyme		
		I	II	III
Wakin	70	22	34	14
Comet	44	7	23	14
Ryukin	44	8	26	10
Kuro-demekin	30	0	8	22
Sansyoku-demekin	30	1	12	17
Aka-demekin	13	0	6	7
Oranda-shishigashira	28	0	7	21
Ranchu	30	0	12	18

TABLE 2 *Presumed Genotypes of the Parents and Genotypic Segregation in Their Offsprings at the* Ldh-B1 *locus in the Goldfish*

Cross	Genotypes of parents	Genotypes of offsprings			Total ratio	χ^2
		A/A	*A/B*	*B/B*		
Type I×Type I	*A/A*×*A/A*	30	0	0	—	—
Type I×Type III	*A/A*×*B/B*	0	44	0	—	—
Type I×Type II	*A/A*×*A/B*	21	22	0	1 : 1	0.091
Type II×Type I	*A/B*×*A/A*	5	8	0	1 : 1	0.692
Type II×Type II	*A/B*×*A/B*	15	23	14	1 : 2 : 1	0.660

TABLE 3 *Genotype and Allele Frequencies at Ldh-B1 Locus in Eight Races of the Gold fish*

Race	No. of fish examined	Genotype frequency			Allele frequency		χ^2
		A/A	*A/B*	*B/B*	q*A*	q*B*	
Wakin	70	22 (21.7)	34 (34.5)	14 (13.8)	0.557	0.443	0.014
Comet	44	7 (7.8)	23 (21.4)	14 (14.8)	0.420	0.580	0.245
Ryukin	44	8 (10.0)	26 (22.0)	10 (12.0)	0.477	0.523	1.460
Kuro-demekin	30	0 (0.5)	8 (6.9)	22 (22.6)	0.133	0.867	0.691
Sansyoku-demekin	30	1 (1.7)	12 (10.7)	17 (17.6)	0.233	0.767	0.878
Aka-demekin	13	0 (0.7)	6 (4.6)	7 (7.7)	0.231	0.769	1.190
Oranda-Shishigashira	28	0 (0.4)	7 (6.1)	21 (21.5)	0.125	0.875	0.645
Ranchu	30	0 (1.2)	12 (9.6)	18 (19.2)	0.200	0.800	1.875

The expected number of genotype under Hardy-Weinberg equilibrium are presented in parentheses.

drogenase (s-IDH), m-isocitrate dehydrogenase (m-IDH) and phosphoglucomutase (PGM) (unpublished data).

Ferris and Whitt (12) reported that loss or retention of expression by one of duplicated gene was observed with about 50% in common carp. The isozymes in which the existence of a duplicated gene have not been demonstrated for a fixed single-banded phenotype may be interpreted by the following alternative explanations : the duplicated gene was lost in the expression, or not be detected due to the

lack of polymorphism. Polymorphism of LDH isozyme in Cyprinid fish has been found in carp, tench, crusian carp (13) and various species of *Notropis* (14). Valenta (15) reported that polymorphism of the LDH isozyme was found at the *B* locus in bitterling, at the *A* locus in white bream, and at the liver-specific *C* locus in bream, rudd, silver carp and barbel. The polymorphism at the *Ldh-B1* locus is utilized for genetic characteristics of several races in the goldfish.

Acknowledgements

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