TETRAZOLIUM OXIDASE POLYMORPHISM IN RAINBOW TROUT

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ABSTRACT

Tetrazolium oxidase from the blood and liver of rainbow trout was found to be genetically polymorphic. The inheritance pattern of the liver enzyme was compatible only with a one locus-two allele hypothesis. The enzymes in the blood while having an electrophoretically identical polymorphism could differ genotypically from that of the liver in a given fish. The significance of these findings to the understanding of the evolution of the salmonid genome is discussed.

TETRAZOLIUM oxidase is an empirical designation for an enzyme first described by BREWER (1967) which is demonstrated in most animal tissues when tetrazolium dyes are used to detect the electrophoretic isozyme pattern of a variety of dehydrogenases. It catalyzes the transfer of electrons from reduced tetrazolium dyes to oxygen in the absence of added cofactors and is detected as bleached or achromatic areas against the colored background of the reduced dye. Its physiological role is obscure.

OHNO (1970) has assembled convincing evidence to suggest that some members of the salmonid family of fish such as the rainbow trout (*Salmo irideus* or *S. gairdneri*) and the various species of Pacific salmon have arisen after a duplication of the entire genome of their ancestors and thus represent an evolving tetraploid species. Cytogenetic evidence (OHNO *et al.* 1965) suggests that the rainbow trout has partially completed the process of diploidization and biochemical evidence in this and other similar fish support the presence of disomic, tetrasomic, and duplicated loci as part of this process (HOLMES and MARKERT 1969; WOLF, ENGEL and FAUST 1970).

In this paper, we will demonstrate polymorphism for tetrazolium oxidase in the blood and liver of the rainbow and steelhead trout and present evidence that the liver enzymes are specified by a pair of alleles at a single locus. In addition, we will present preliminary evidence to suggest that a second locus for electrophoretically identical bands is independently inherited and specifies the same enzyme in the blood.

MATERIALS AND METHODS

Rainbow and steelhead trout were obtained from the Department of Fish and Game, State of

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Washington, and the Department of Fisheries, University of Washington. The fish came from separate, partially inbred, populations. The different populations were combined for statistical presentation where analysis of each group separately clearly confirmed the final conclusion. Crude enzyme preparations were used for electrophoresis without further purification. They were prepared by homogenization of blood and liver in 5×10^{-3} M phosphate buffer, pH 7.4.

Horizontal starch gel electrophoresis was usually carried out using a Tris-EDTA-borate buffer at pH 8.6 (TEB) (PORTER *et al.* 1964). When blood was analyzed, phosphate buffer at pH 7.4 was used (MATHAI, OHNO and BEUTLER 1966). In this system, the bands of tetrazolium oxidase separated completely from hemoglobin which interferes with the interpretation.

Staining was carried out by the method of BAUR and SCHORR (1969), or with an agar overlay containing the same reagents plus NAD and glucose 6-phosphate. Both were allowed to develop in the light at room temperature for 2–3 hr.

The phenotype frequencies expected from a two or four-allele hypothesis were calculated from gene frequencies estimated by the method of least chi squares on a time sharing computer system using a program developed by Dr. LEONARD ROBBINS.

RESULTS

Examination of the electrophoretic pattern in the liver of these fish revealed a polymorphism for tetrazolium oxidase consisting of a rapidly migrating species, a slowly migrating species, and a putative hybrid dimer equidistant between them. The fast and heterozygote patterns are seen in slots 1 and 4 of Figure 1 which also demonstrates the approximate 1:2:1 ratio between the strength of the three bands in heterozygote fish.

A population survey of 63 fish drawn at random from the two population groups outlined in METHODS was performed for the phenotype of tetrazolium

FIGURE 1.—Tetrazolium oxidase electrophoretic patterns in rainbow trout blood and liver run in phosphate buffer for 16 hr at 3 v/cm. (1) liver f/f, (2) blood 4 f/f, (3) blood s/f, (4) liver 4 s/f, (5) blood f/f, and (6) blood f/f.

TABLE 1

Proportions of the three phenotypes of tetrazolium oxidase in a population of rainbow and steelhead trout

	Homozygous fast	Heterozygous	Homozygous slow	Chi square
Observed	11.	25.	27.	...
Expected 2 allele hypothesis	8.9	29.5	24.6	1.44
Expected 4 allele hypothesis	1.8	53.7	7.5	112.11

oxidase in the liver. The data were then analyzed to determine if the results were most compatible with an hypothesis of a one locus-two allele system or a two locus-four allele system. The calculations assumed that all the criteria necessary for the appropriate application of the standard Hardy-Weinberg equilibrium equation were met. These best gene frequencies calculated by the method of least chi squares were then used to compute the expected frequencies of the several phenotypes and were compared with the observed. The results of these calculations are presented in Table 1. It is clear that the chi square for the two-allele system is far lower than that for the four-allele system. Reference to tables of chi square reveals that the probability of getting the observed results from the two-allele system by chance alone is less than 0.25 but greater than 0.1. The probability for the four-allele system is less than one in a million. This conclusion may be drawn intuitively from simple inspection of the results. Since in a four-allele system the frequency of homozygotes is a function of the fourth power of the gene frequencies, the high number of homozygotes of both types virtually excludes such an hypothesis. Similar reasoning excludes the possibility for four alleles at a single locus, a tetrasomic condition.

Examination of the relative strength of the three bands in the heterozygotes yields similar conclusions. Since fewer than half of all heterozygotes in a four-allele system at either one or two loci would be expected to have equal proportions of both polypeptide chains, at least some heterozygotes would be expected to have a proportion of the three bands compatible with the presence of three of one allele and a single copy of the second, a 9:6:1 ratio. Since all 25 of the homozygotes were found to have the three bands present in a roughly 1:2:1 ratio and none in the 9:6:1 ratio, this lends further support to the one locus-two allele hypothesis.

Figure 1 also shows a fast and a heterozygote pattern from the blood of the same species and demonstrates migration rates indistinguishable from those in accompanying liver extracts. The 1:2:1 band ratio is also seen here suggesting an interaction not unlike that which occurs in liver. Perhaps the most dramatic fact illustrated here is the contrast of the pattern for the liver in the fourth slot with that of the blood in the second, both taken from the same fish. This discrepancy which occurred twice in four fish studied suggests that although the blood and liver of this species contains electrophoretically identical isozymes, they are coded for by the same alleles at independent loci.

DISCUSSION

Tetrazolium oxidase is an empirical name for a catalytic activity that behaves like a protein in its heat lability, migration in electrophoretic systems, and its definability in genetic terms. Variants or polymorphisms have been described in humans (BREWER 1967), dogs (BAUR and SCHORR 1969), and bluefin tuna (EDMUNDS and SAMMONS 1971). These have proven useful in genetic analysis despite the lack of knowledge of the natural function of the enzymes.

OHNO (1970) has marshalled a large amount of evidence to suggest that the rainbow trout and Pacific salmon among other fish species have evolved after a precipitous increase in the DNA content per cell resulting from duplication of the entire genome of an ancestral species. The initial result of such an event, a tetraploid species, might be expected to have considerable difficulty in distributing its chromosomes correctly during meiosis and, therefore, the cytogenetically observed trend toward diploidization (OHNO *et al.* 1965) is not unexpected. The fate of the individual loci that were duplicated in this hypothesized event is less predictable. For the loci on the chromosomes still behaving in a tetraploid manner, a tetrasomic condition would be predicted and such has been observed in the case of the soluble form of NADP dependent isocitrate dehydrogenase in trout (WOLF *et al.* 1970). On the other hand, the fate of the loci on chromosomes that have assumed a diploid state can be more varied. The second locus could be found superfluous and have been eliminated. It may be retained and function along with the first as exemplified by the B locus of brook trout lactate dehydrogenase (LDH) (HOLMES and MARKERT 1969). The duplicated locus could also serve as the starting point for evolutionary change specifying a new enzyme with the same or different substrate specificity and functioning under different circumstances. The A and B loci of lactate dehydrogenase provides an example of this (HOLMES and MARKERT 1969, and others). The latter is a widely accepted hypothesis for evolutionary development of new enzymatic potentialities.

The data that we have presented in this paper are most compatible with the idea that on electrophoresis, rainbow trout liver tetrazolium oxidase behaves as a dimer, the molecules of which are made up of the random association of the monomeric gene products of two alleles at a single locus. A study of liver glucose 6-phosphate dehydrogenase of salmonid fish is also compatible with the one locus-two allele hypothesis (CEDERBAUM and YOSHIDA, in preparation).

The data comparing the isozyme patterns in the liver and blood of four fish may provide an important clue as to the fate of the second locus which should exist as a result of the genome duplication. The disparate patterns in two of the four fish suggest that the second locus has diverged from the first in tissue specificity while retaining enzymes with apparently identical electrophoretic mobility. The potential for independent tissue control may be viewed as of great advantage to an organism adapting to a variety of evolutionary pressures. The fate of the two loci here bears certain resemblance to 6-phosphogluconate dehydrogenase in fish (BENDER and OHNO 1968) and lactate dehydrogenase in many organisms as

well as fish (HOLMES and MARKERT 1969, and others), but differs in the apparent failure to develop new alleles at the second locus.

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