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Malate Dehydrogenase: Evidence for Tetrameric Structure in Mus musculus

Abstract. *Two electrophoretically distinct variants of supernatant nicotinamide-adenine dinucleotide phosphate–dependent malate dehydrogenase exist in mice* (Mus musculus). *They are controlled by codominant alleles segregating at an autosomal locus. The two forms exist in a polymorphic condition in wild populations of* Mus musculus *and are fixed in a homozygous condition in inbred lines. These genetic electrophoretic variants are used here to study the subunit structure of this enzyme. Evidence indicating a tetrameric structure for mouse nicotinamide-adenine dinucleotide phosphate–dependent malate dehydrogenase is presented. This interpretation is based on the occurrence in heterozygote tissue extracts of five electrophoretically distinct enzymes. This is the predicted phenotype for tetramers composed of two types of subunits which associate randomly in heterozygotes forming three hybrid enzymes having mobilities intermediate between the parental forms.*

Malate dehydrogenase (decarboxylating; E.C. 1.1.1.40) reversibly catalyzes the oxidative decarboxylation of malate in the presence of NADP (*1*) and manganous ions to pyruvate and carbon dioxide. The enzyme also decarboxylates oxalacetate to pyruvate in the presence of Mn^{++} (*2*, *3*). Henderson has shown that at least two distinct and unrelated forms of NADP-malate dehydrogenase (NADP-MDH) (*1*) exist in *Mus musculus* (*4*). She demonstrated that there were two enzymes which had markedly different electrophoretic mobilities, restricted to either the mitochondrial or supernatant fractions, with allelic variants being in the supernatant, but not in the mitochondrial fraction. These observations provided evidence for two distinct enzymes with similar substrate specificities determined by independent loci. Henderson suggested that the different electrophoretic forms of the supernatant enzyme were determined by two alleles which segregated in a simple Mendelian fashion; the locus specifying the enzyme was possibly situated on linkage unit II; and these alleles coded for subunits which associated randomly, forming a single, though diffuse, hybrid enzyme. Her observations suggested a dimeric association of subunits.

We now report that the supernatant form of mouse NADP-MDH is a tetramer composed of four freely associating subunits, confirm and extend Henderson's genetic interpretation, and tabulate the expression of the two allelic forms of the supernatant enzyme in 30 inbred lines of mice.

The gene for the supernatant enzyme is designated *Mdh-1*, and the alleles *Mdh-1*^{a} and *Mdh-1*^{b} control, respectively, the slow and fast cathodally migrating bands (*4*). These alleles code for the *a* and *b* subunits (slow and fast, respectively), which assort randomly and give rise to the three possible F_2 patterns. These phenotypes are designated A (Fig. 1, channels 2, 6), B (Fig. 1, channel 4) for the two homozygote types, and AB for the heterozygote (Fig. 1, channels 1, 3, 5).

Henderson's data was based on hybrid animals from crosses between inbred strains of mice (C3H/HeJ × AKR/J and C57BL/6J × DBA/2J) homozygous for different *Mdh-1* alleles, and a few F_2 mice showing segregation of the two alleles. We made crosses (F_1, F_2, and BC) between inbred strains SJL/J and C57BL/6J in order to validate the genetic nature of the *Mdh-1* polymorphism. The results of the crosses (Table 1) demonstrate (i) the expression of a hybrid enzyme pattern in all F_1 animals; (ii) an F_2 distribution in agreement with a 1 : 2 : 1 ratio for A, AB, and B phenotypes respectively; (iii) agreement with a 1 : 1 ratio in the backcrosses; and (iv) no sex association of the *Mdh-1* gene. These results indicate that *Mdh-1*^{a} and *Mdh-1*^{b} are codominant alleles controlled by an autosomal locus.

The NADP-MDH phenotypes in 30 lines of inbred mice are given in Table 2. A number of wild populations of mice from diverse sources have been screened, and all three NADP-MDH phenotypes (A, AB, B) have been observed. No new electrophoretic variants

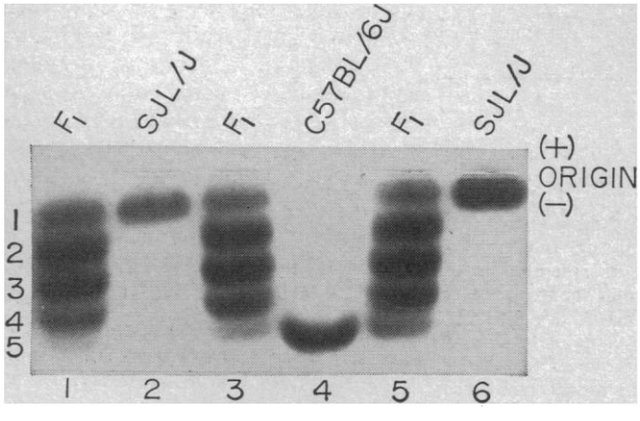

Fig. 1. Kidney NADP-MDH phenotypes after starch-gel electrophoresis, demonstrating the genetic variation and tetrameric nature of the supernatant isozyme. Vertical column of numbers represent enzyme bands, and the horizontal row represents gel channels. (Channels 1, 3, and 5) Hybrid phenotypes showing the five bands characteristic of a protein composed of four subunits; (channels 2 and 6) slow-migrating form, A; and (channel 4) fast-migrating form, B. The same electrophoretic patterns are observed in liver homogenates. Fresh homogenates were prepared for electrophoresis (*5*) from kidneys and livers of adult mice. Starch-gel electrophoresis was carried out vertically at 3°C for 20 hours at 280 volts across the system, the gel buffer consisted of 0.008*M* tris and 0.003*M* citric acid adjusted to *p*H 6.7 with NaOH; the bridge buffer consisted of 0.223*M* tris and 0.086*M* citric acid adjusted to *p*H 6.3 with NaOH. After electrophoresis, the gel was incubated at 37°C for 2 hours in a staining solution consisting of NADP (20 mg), nitro blue tetrazolium (10 mg), and phenozine methosulfate (4 mg) dissolved in a mixture of 90 ml of 0.2*M* tris-HCl, *p*H 8.0; 10 ml of 0.5*M* of L-malic acid (adjusted to *p*H 7.0), and 0.5 ml of 0.25*M* $MnCl_2$.

Table 1. Genetic classification of Mdh-1 phenotypes in *Mus musculus*.

Parental Combinations	Phenotypes	Progeny phenotype: A (slow) ♂	A (slow) ♀	AB (hybrid) ♂	AB (hybrid) ♀	B (fast) ♂	B (fast) ♀
SJL/J × SJL/J	A × A	6	4	0	0	0	0
C57BL/6J × C57BL/6J	B × B	0	0	0	0	6	4
SJL × C57BL/6J (F_1)	A × B	0	0	4	6	0	0
F_1 × F_1 (F_2)	AB × AB	7	20	21	24	3	15
F_1 × SJL/J (BC)	AB × A	6	3	2	5	0	0
F_1 × C57BL/6J (BC)	AB × B	0	0	6	3	3	1

Table 2. Mdh-1 phenotypes in different inbred strains of *Mus musculus*.

A (slow)			B (fast)		
SJL/J	A/HeJ	BALB/cJ	C57BL/6J	C58/J	CBA/J
LP/J	CE/J	SWR/J	C57BL/KsJ	AKR/J	CBA/CaJ
MA/J	C3HeB/FeJ	BuB/BrJ	C57BL/6J-Ob	ST/bJ	NZB
PL/J	129/J	LG/J	C57Br/cdJ	P/J	
DBA/1J	RF/J	DE/J	C57L/J	SEA/GrJ	
DBA/2J	DW/J				

were detected in inbred or wild mice.

Electrophoretic patterns of liver and kidney extracts from heterozygotes suggest that the Mdh-1 enzyme is a tetramer. If the enzyme were dimeric and the subunits could be distinguished electrophoretically, heterozygotes would exhibit three enzyme bands: two bands composed of parental-type subunits (*aa* or *bb*) and a single hybrid enzyme of intermediate mobility with the *ab* configuration. The relative activities of the three bands would conform to a ratio of 1 : 2 : 1. If, however, the enzyme were a tetramer, then the expected hybrid phenotype would consist of five bands of activity composed of the following subunit combinations: *4a*; *3a1b*; *2a2b*; *1a3b*; *4b*. The staining intensity of the hybrid phenotype would express a 1 : 4 : 6 : 4 : 1 ratio which would be consistent with expansion of $(a + b)^4$.

Five distinct bands demonstrating the expected activity ratio were reproducibly observed in all hybrid animals (Fig. 1, channels 1, 3, 5); these bands provide evidence for a tetrameric molecule (*6*). In some hybrid animals the most cathodally migrating enzyme band (band 5, channels 1, 3, 5) stained faintly in the *p*H 6.3 bridge and 6.7 gel buffer systems, and they may not be visible on the zymogram reproduction. At *p*H 7.0 and 7.4, band 5 stained more intensely and appeared more stable, but under these electrophoretic conditions the isozymes migrated less cathodally, and bands 1 and 2 were obscured by the gel origin. A five-band isozyme pattern was not observed when crude kidney and liver homogenates from A (slow) homozygotes were mixed with extracts from B homozygotes.

Hsu and Lardy (*3*) reported that the molecular weight of pigeon liver NADP-MDH was 280,000, and from NADPH binding and alkaline dissociation they estimated the number of subunits to be four. This supports the tetrameric structure of mouse Mdh-1. The catalase tetramer (*7*), having a molecular weight of about 240,000, has also been studied with genetic variants and electrophoretic techniques similar to those reported here (*8*). The catalase hybrid phenotype, like the Mdh-1 hybrid, consisted of five enzyme bands typical of a tetramer.

THOMAS B. SHOWS
FRANK H. RUDDLE
Department of Biology, Yale University, New Haven, Connecticut

References and Notes

1. The following abbreviations are used: NADP and NADPH, nicotinamide adenine dinucleotide phosphate and its reduced form; MDH, malate dehydrogenase.
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Hereditary Autoimmune Thyroiditis in the Fowl

Abstract. *In the Cornell C strain of White Leghorn chickens, thyroiditis appeared spontaneously. It was characterized by phenotypic changes due to a decrease of thyroxine, histological damage to the thyroid gland, and the presence in the serum of antibodies to thyroglobulin. Susceptibility to the disease is genetically inherited. Hormonal bursectomy (androgen given to the embryo) suppresses the development of thyroiditis.*

Hereditary hypothyroidism in the domestic fowl (*1*) is characterized by changes in the thyroid glands, which are somewhat similar to those seen in chronic thyroiditis (Hashimoto's disease) of man. In chickens, the phenotypic expression of this genetic variation consists of obesity, a skeletal structure somewhat smaller than normal in size, long silky feathers, and poor laying ability. Such characteristics are similar to those resulting from thyroidectomy and are correlated with a deficiency of thyroxine. Histologically the thyroid glands become infiltrated with lymphoid cells. The latter change begins about 2 weeks after hatching and subsequently becomes more intense. These symptoms (*1*, *2*) occurred spontaneously in the Cornell C strain of White Leghorn chickens (a closed flock since 1935). Selective breeding for this obese-hypothyroid trait demonstrated the hereditary nature of the disease and increased the incidence in females from less than 1 percent to over 90 percent, and in males from zero to over 80 percent. These higher percentages occurred in the latest two generations and represented observations on more than 400 chickens of each sex. Changes in the phenotype and physiology can be reversed by providing iodinated casein, a thyroxine-like substance, in the diet at a level of 0.011 percent.

Chronic thyroiditis in man appears to be associated with the development of antibodies specific for thyroglobulin. Witebsky and Rose (*3*) produced a similar disease in rabbits by autoimmunization. The exact mechanism of tissue damage has not been established. Spontaneously occurring thyroiditis in the fowl may present an interesting opportunity for exploration of many aspects of this disease.

The bursa of Fabricius in the chicken controls the development of humoral