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Genetic Variation and Immunochemical Properties of L-Malate : NAD^+ Oxidoreductase Isozymes in the Bandicoot, *Isoodon macrourus*

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Abstract

A genetic polymorphism for the soluble isozyme (MDH-A_2) of malate dehydrogenase (L-malate : NAD^+ oxidoreductase; EC 1.1.1.37) was found in a Townsville–Inkerman population of short-nosed bandicoots, *I. macrourus*. The enzyme seems to be inherited in a normal autosomal codominant manner. The gene frequencies for the *MDH-A* and *MDH-A'* alleles were 0·82 and 0·18 respectively. The genotypic frequencies indicated that the population was in Hardy–Weinberg equilibrium. None of 22 pouch young from seven litters expressed a phenotype inconsistent with its mother's genotype, assuming autosomal codominance. Other populations of *I. macrourus* and of the long-nosed bandicoot (*Perameles nasuta*) exhibited only the *MDH-A* allele. Homologous relationships of bandicoot MDH-A_2 and MDH-B_2 isozymes to those of other vertebrates were established by immunochemical studies.

Introduction

Electrophoretic techniques permit comparisons of net surface charge between multiple nonallelic and allelic isozymes within organisms and are widely used in biochemical genetic studies (see Masters and Holmes 1973). A number of isozyme systems of marsupials have been recently analysed and their comparative electrophoretic properties shown to be useful in supporting species relationships that have been established by classical procedures (Clarke and Poole 1973; Holmes *et al.* 1973, 1974; Richardson *et al.* 1973).

In a search for a genetic marker of different populations of the short-nosed bandicoot (*Isoodon macrourus*), a polymorphism of malate dehydrogenase (L-malate : NAD^+ oxidoreductase; EC 1.1.1.37) was observed. Vertebrate malate dehydrogenase exists as two major isozymes, MDH-A_2 and MDH-B_2, which are localized in the cytoplasm and mitochondria respectively (Siegel and Englard 1961; Thorne *et al.* 1963; Kitto and Kaplan 1966). Genetic and biochemical analyses have shown that these isozymes are coded by distinct nuclear genes (Shows *et al.* 1970; Wheat *et al.* 1971) and have dimeric subunit structures (Devenyi *et al.* 1966; Kitto and Kaplan 1966; Davidson and Courtner 1967*a*, 1967*b*).

Here we report a polymorphism for the MDH-A polypeptide in a Townsville–Inkerman population of short-nosed bandicoots and provide evidence for the homologous relationships of marsupial malate dehydrogenase isozymes to those of other organisms by the use of immunochemical techniques.

Materials and Methods

The animals used in this study were obtained by live-trapping and shooting in three geographical areas. Seventy-four adults and juveniles were collected from within a 125-km radius of Townsville, Qld, 10 adults and juveniles were collected near Iron Range, Qld, and 15 near Gosford, N.S.W.

Frozen heart and liver tissues were thawed and ground in a glass homogenizer with 30 mM tris–citrate buffer (pH 7·0, 30% w/v) and subsequently centrifuged for 15 min at 48 500 *g*. The supernatant solutions were subjected to starch gel electrophoresis at 4°C on 10% gels, using a tris–citrate buffer system (60 mM electrode buffer; 30 mM gel buffer at pH 7·0) with 8 V/cm being applied for 16 h. Following electrophoresis the gels were sliced horizontally, equilibrated at pH 9·0 for 15 min with a 100 mM tris–HCl buffer, then stained for malate dehydrogenase activity at 37°C by a tetrazolium technique previously described (Whitt 1970). Anti-chicken MDH-A_2 and MDH-B_2 antibodies were prepared in sheep against the pure isozymes (Holmes and Scopes, unpublished data) and used in immunochemical titration experiments as previously described (Holmes and Scopes 1974).

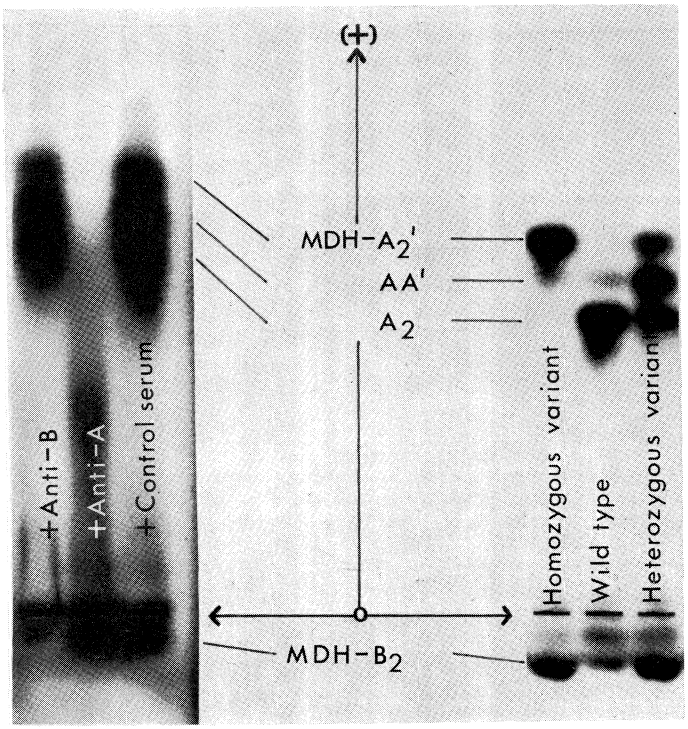

Fig. 1. Genetic variability and immunochemical properties of malate dehydrogenase isozymes from *I. macrourus*. Proposed subunit structures are given. The left starch gel zymogram demonstrates the immunochemical precipitation of malate dehydrogenase isozymes by anti-MDH-A_2 and anti-MDH-B_2 antibodies. The right zymogram illustrates the three phenotypes observed for MDH-A_2 in natural populations of bandicoots.

Results and Discussion

Fig. 1 presents a starch gel zymogram illustrating the electrophoretic resolution of bandicoot malate dehydrogenase isozymes. Treatment of the tissue extract prior to electrophoresis with antisera prepared against pure isozymes isolated from chicken hearts selectively removed the appropriate isozyme. For example, anti-chicken MDH-A_2 precipitated or interacted with the three allelic isozymes migrating anodally while anti-chicken MDH-B_2 treatment selectively removed the cathodal isozyme. This demonstrates the homologous relationships of these isozymes to those of the chicken, which have been fully characterized with respect to their subcellular location, subunit composition and genetic control (Kitto and Kaplan 1966). Similar immuno-

chemical results have been previously reported by Grimm and Doherty (1961), who established the immunochemical specificities of these isozymes.

The subunit structures of the malate dehydrogenase isozymes resolved in Fig. 1 are inferred from previous biochemical and genetic studies (Davidson and Courtner 1967*a*, 1967*b*; Whitt 1970; Shows *et al.* 1970). The *MDH-A* locus exhibits a two-allele codominant polymorphism which results in the synthesis of three isozymes in heterozygotes: MDH-A_2, MDH-AA' and MDH-A'_2. MDH-B_2 is invariant among the individuals studied and is resolved into two main forms of activity. Recent studies on MDH-B_2 from other organisms have shown such multiplicity to be the result of epigenetic modification rather than genetic multiplicity (Meizel and Markert 1967; Shows *et al.* 1970).

Table 1. Population data for the MDH-A polymorphism in *I. macrourus* (pouch young excluded)

Locality	Latitude (°S.)	Longitude (°E.)	Genotype: A_2	AA'	A'_2	Total	Gene frequency A	A'
Iron Range, Qld	12·39	143·13	10	0	0	10	1·00	0·00
Townsville, Qld	19·12	146·48	50	22	2	74	0·82	0·18
Gosford, N.S.W.	33·25	151·18	15	0	0	15	1·00	0·00

Table 1 is an analysis of the phenotypic frequencies of the *MDH-A* alleles in bandicoot populations from three separate locations. No significant differences between frequencies in males and females were observed, implying autosomal codominant inheritance. The gene frequencies for the *MDH-A* and *MDH-A'* alleles in the Townsville–Inkerman population were 0·82 and 0·18 respectively. No significant differences in gene frequencies were detected from subsamplings within this geographical area, although the four individuals from north-west of Townsville were monotypically A_2. The analysis of observed versus expected genotypes showed the Townsville–Inkerman population to be in Hardy–Weinberg equilibrium ($P=1$). The 10 animals from Iron Range and the 15 from Gosford were all A_2. The sample sizes were sufficiently large to establish that both populations have significantly different frequencies from the Townsville–Inkerman population using 2×2 contingency table tests ($P=0{\cdot}028$ for the Iron Range sample of 10). In addition to the short-nosed bandicoots, 20 tissue samples from the long-nosed bandicoot and a single specimen of *Perameles gunnii* all expressed the MDH-A_2/MDH-B_2 phenotype. Table 2 gives incomplete family data for the MDH-A polymorphism in wild-caught mother–pouch young combinations of *I. macrourus* from Townsville. Seven mothers homozygous for the *A* allele were carrying 15 offspring like themselves and seven heterozygotes, but no offspring of the homozygous *A'* type. These data are consistent with autosomal codominance.

We have recently reported a comparative study of the electrophoretic variability of marsupial MDH-A_2 (Holmes *et al.* 1974). Our investigation confirmed the conservative nature of this enzyme since all marsupials studied, with the exception of the grey kangaroos (*Macropus giganteus* and *M. fuliginosus*), brush-tailed and mountain possums (*Trichosurus vulpecula* and *T. caninus*) and the bandicoots (*I. macrourus* and *P. nasuta*), exhibited identical electrophoretic mobilities for the isozyme. Grey kangaroos were monotypic for a faster migrating form of MDH-A_2 of anodal mobility 116

relative to the normal mobility for marsupials of 100. The usual mobility in *Trichosurus* species is 85, although a rare variant was observed in *T. vulpecula* for which the three allelic isozymes had mobilities of 85, 78 and 71. The more common allele in the short-nosed bandicoot as well as the single allele observed in the long-nosed bandicoot have a mobility of 82; the heterodimer (AA′) in heterozygous *I. macrourus* individuals has a mobility of 91, while the homodimer (A'_2) in both heterozygotes and homozygotes has the normal marsupial mobility for MDH-A_2 of 100. It appears likely then that a gene mutation has occurred for the *MDH-A* locus in the immediate ancestor to the two groups of bandicoots. Whether the Townsville–Inkerman population of *I. macrourus* has maintained both alleles in its gene pool or whether a back mutation has occurred since has not been determined.

Table 2. Incomplete family data for MDH-A polymorphism in wild-caught mother–pouch young combinations of *I. macrourus* from Townsville

Phenotype of mother	Phenotype of pouch young: A_2	AA′	A'_2	No. of litters
A_2	15	7	0	7
AA′	0	2	0	1
A'_2	0	0	0	0

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