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Mitochondrial Malic Enzyme (E.C. 1.1.1.40) in Human Leukocytes: Formal Genetics and Population Genetics

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Summary. Mitochondrial malic enzyme ME_M (E.C. 1.1.1.40) is present in human leukocytes; the polymorphism of ME_M thus can be easily demonstrated using routine starch gel electrophoresis. Data on formal genetics are given. The gene frequency of ME_M^1 was estimated to be 0.67 ± 0.02.

Introduction

Malic enzyme catalyzes the oxidative decarboxylation of L-malate and the decarboxylation of oxaloacetate (Kun, 1963). It is strictly specific for nicotinamide-adenine dinucleotide phosphate (NADP) (Rutter and Lardy, 1958). The biochemical properties of the human enzyme have been discussed by Burchell et al. (1977).

Two autosomal loci, ME_S and ME_M (Giblett, 1976), respectively, determine the soluble and mitochondrial forms of the enzyme (Cohen and Omenn, 1972; Povey et al., 1975). Povey et al. (1978) have suggested, based on the electrophoretic pattern, that ME_M, like ME_S, is a tetramer.

Gene assignment was performed by Povey et al. (1975) using man-mouse and man-hamster cell hybrids. Their data would be consistent with the ME_M locus being on the 1q chromosome of humans.

Genetic polymorphism of human mitochondrial malic enzyme was first described by Cohen (1971). Cohen described three common patterns, designated MOD-2A, MOD-2AB, and MOD-2B, as the phenotypic expression of two alleles at an autosomal locus. Further data on these phenotypes have been given by Cohen and Omenn (1972) and by Saha et al. (1978).

These investigators used various human tissues, such as brain, heart muscle, liver, kidney, testis, and even foreskin to demonstrate ME_M polymorphism. Gene

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frequencies for ME_M^1 were calculated to be 0.693 (Cohen, 1971) and 0.65 (Saha et al., 1978), respectively.

According to Cohen (1971), no measureable enzyme activity should be present in placenta, hair follicles, leukocytes or phythemagglutinin-stimulated lymphocytes, erythrocytes, and serum.

However, the use of tissue samples as described above seems to be a less than satisfactory approach to the formal genetics or even the linkage relations in this startling polymorphism. Therefore, the intention of our study was to prove that ME_M polymorphism can be demonstrated using routine-methods.

Materials and Methods

Blood samples were obtained from healthy individuals from southwestern Germany. Leukocytes were collected from venous blood using K-EDTA tubes. After storing the whole sample at room temperature for about 24 h, the plasma containing the leukocytes was carefully transferred into glass tubes with a conical bottom. Leukocytes were obtained by centrifugation and stored at −80° C until use.

To prepare samples for electrophoresis the leukocytes were lysed by an equal volume of Triton X-100 (10% solution), followed by ultrasonic disintegration.

Electrophoresis was carried out for 18—20 h using a Tris-histidine × HCL buffer system containing 0.2 M Tris and 0.2 M histidine × HCL (pH 7.6). The gel was a 1 : 7 dilution of bridge buffer (18% Connaught starch, 5 V/cm with cooling at 4°C).

The reaction mixture consisted of 3 g% malic acid, 6 g% Tris, 0.5 g% Mg Cl_2, 50 mg% NADP, 25 mg% MTT, and 100 µl% of a 1% solution of Meldolablau (Boehringer).

Incubation of the gel was done by means of the so-called "sandwich technique" using Whatman no. 3 filter papers at 37° C for about 2 h.

Enzyme patterns of ME_M will then be seen about 3 cm anodally from the starting point.

Results and Discussion

Figure 1 shows the three common phenotypes ME_M 1, ME_M 2-1, and ME_M 2. The homozygous phenotypes have a single band, whereas the heterozygous ones

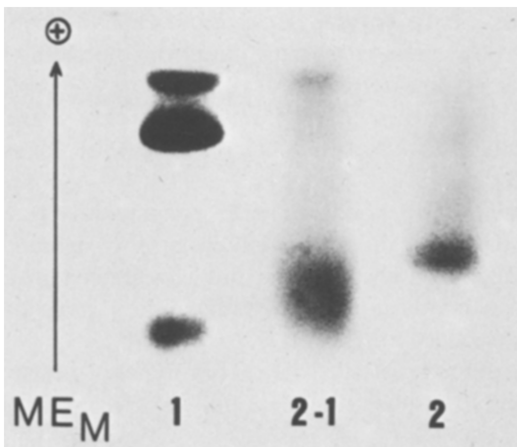

Fig. 1. The common phenotypes of ME_M

Table 1. Distribution of ME_M-phenotypes in 67 mother-child pairs

Phenotype of mother	Phenotype of child			
	1	2-1	2	Σ
1	16 (20.15)	13 (9.92)	—	29
2-1	13 (9.92)	15 (14.81)	4 (4.89)	32
2	—	4 (4.89)	2 (2.41)	6
Σ	29	32	6	67

Table 2. Phenotypic distribution of ME_M in Europeans

Author	*n*	1	2-1	2	ME^1_M
Cohen and Omenn (1972)	132	58	67	7	0.693
Povey et al. (1975)	60	26	30	4	0.69
Burchell et al. (1977)	66	28	27	11	0.63
Saha et al. (1978)	409	164	195	43	0.6505
This investigation (1979)	184	82	82	20	0.67 ± 0.02

have a comparably large area of enzyme activity in an intermediate position. More anodally, the bands of ME_S will be seen.

These zymograms are consistent with the findings of Cohen and Omenn (1972) and Saha et al. (1978).

Data on formal genetics of ME_M are presented in Table 1.
67 mother-child pairs were investigated. Calculation of the expected values were based on Hardy-Weinberg conditions. Observed and expected values are in good agreement ($\chi^2 = 3.164$, $0.4 < P < 0.5$, $df = 4$). These data agree with the results of Cohen and Omenn (1972) and Saha et al. (1978), and prove the formal hypothesis that there are two common alleles, ME^1_M and ME^2_M, at an autosomal locus ME_M.

Table 2 gives the results of testing of 184 individuals from southwestern Germany.

Our ME^1_M frequency is in good agreement with the values given in Table 2. This, along without data on formal genetics, show that ME_M polymorphism can be determined routinely.

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