Electrophoretic Study of Lactate Dehydrogenase and Alkaline Phosphatase Isoenzymes of the Mongolian Gerbil (*Meriones unguiculatus*)

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ABSTRACT. Lactate dehydrogenase and alkaline phosphatase isoenzymes of the Mongolian gerbil were examined using electrophoretic techniques and were compared with those of the mouse, rat, and guinea pig. Five isoenzymes of lactate dehydrogenase (LDH) were detected in the gerbil with LDH_2 and LDH_5 being equally dominant. Two bands of alkaline phosphatase (ALP) were distinguished in sera treated with neuraminidase in the gerbil and the relative activity of the cathodic fraction was greater than those of the mouse and rat. Genetic polymorphism was not found among the coat color variants of the Mongolian gerbil. A comparative study on LDH and ALP revealed distinct interspecific differences in the rate of the electrophoretic migration of the respective isoenzymes among the mouse, rat, guinea pig, and the Mongolian gerbil. — KEY WORDS: alkaline phosphatase, electrophoresis, isoenzyme, lactate dehydrogenase, Mongolian gerbil.

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The Mongolian gerbil (*Meriones unguiculatus*) is a small rodent frequently used as a model for neurology [21], parasitology [4, 22, 41], virology [1, 39], or metabology [5, 20]. Basic biochemical and physiological studies have been carried out before [2, 27, 36], but to a relatively limited extent compared with those of the mouse or rat. Many colonies of gerbils have been established in laboratories for medical research purpose, but genetic variance among those colonies has not yet been clarified because the essential data of biochemical marker genes or enzymes have not been analyzed. Only a few zymograms [9, 11, 15, 16, 24] or inheritance of coat colors [17, 37] have been reported. During our research into the basic biological features of the Mongolian gerbil colony in our laboratory [3, 29], coat color mutant gerbils appeared in 1985. We performed the selective breeding of those coat color mutants, established their colonies, and conducted comparative studies on their physiological charactericstics, but genetic variance, such as the coat color differences, has not been detected until now [30-32].

In the present study, we used electrophoretic techniques to determine the lactate dehydrogenase and alkaline phosphatase isoenzyme patterns of the Mongolian gerbil in comparison with those of mice, rats, and guinea pigs, and to ascertain the genetic polymorphism of those isoenzymes in the coat color mutant gerbils.

MATERIALS AND METHODS

The Mongolian gerbils used in the present study comprised a total of 50 animals reared at our laboratory, consisting of 5 males and 5 females each in an established line of wild coat color called agouti, as well as the four mutant lines characterized by the respective coat colors; white, called albino; black; agouti color with white spots (white spotted-agouti); and black with white spots (white spotted-black) [31]. The mice, rats, and guinea pigs comprised 5 males and 5 females each of the conventional ddy, Sprague-Dawley (S-D), and Hartley strains, respectively, and were purchased from the Saitama Experimental Animal Supply (Saitama, Japan). After these animals were exsanguinated without fasting under ether anesthesia, their sera were separated and stored at −20°C until analyzed. The activities of serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were measured by Spotchem (Kyoto Daiichi Chemical Co., Ltd.). LDH isoenzyme patterns were determined by agarose gel electrophoresis using a TITAN Gel S-LD set (Helena Laboratories, Saitama, Japan). Cellulose acetate membrane (TITAN LIPO; Helena Laboratories, Saitama, Japan) electrophoresis was done to identify the ALP isoenzyme. To improve the resolution of ALP isoenzymes, sera were treated with neuraminidase (ALP separator; Helena Laboratories, Saitama, Japan) at 1:7 dilution for 30 min at room temperature. The relative activities of each isoenzyme of LDH and ALP were measured by densitometer (Cliniscan; Helena Laboratories, Saitama, Japan).

RESULTS

Figure 1 represents the typical LDH isoenzyme patterns of mice, rats, guinea pigs, and agouti gerbils. Five bands were detected in each species, but the mobility of the respective fractions varied among the species. The 2nd and 5th bands of LDH isoenzymes were stained deeply in the gerbil sera. LDH_1 from rats, mice, and guinea pigs migrated more rapidly than that of gerbils. LDH_5 from rats was the most cathodic while that from the guinea pigs was the most anodic in these 4 species. A 6th band was found in one of the male gerbils as well as one of the female guinea pigs

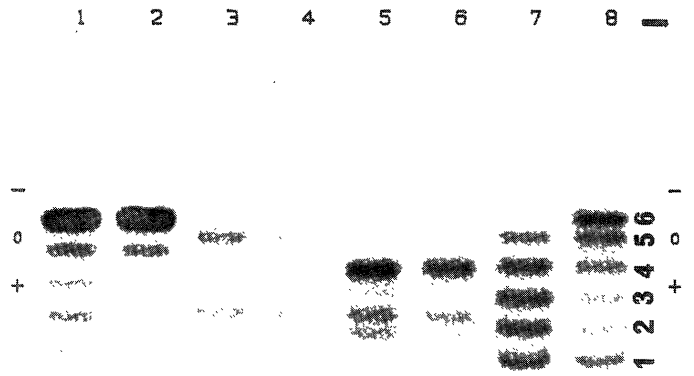

Fig. 1. Electrophoretic patterns of lactate dehydrogenase isoenzymes in normal sera from mice, rats, guinea pigs, and Mongolian gerbils. 1.male S-D rat, 2. female S-D rat, 3. male agouti gerbil, 4. female agouti gerbil, 5. male ddy mouse, 6. female ddy mouse, 7. male Hartley guinea pig, 8. female Hartley guinea pig.

Table 1. Total activity and relative composition of serum lactate dehydrogenase (LDH) isoenzymes of mouse, rat, guinea pig, and Mongolian gerbil

Species	Total activity (IU/*l*)	Relative activities of LDH isoenzymes (%)				
		LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Mongolian gerbil	399 ± 203	13.3 ± 5.4	32.0 ± 9.4	12.6 ± 6.7	5.6 ± 4.7	35.6 ± 12.9
Mouse	754 ± 281	6.4 ± 2.1	8.2 ± 4.5	19.2 ± 0.5	4.4 ± 2.3	61.9 ± 7.2
Rat	951 ± 406	3.3 ± 2.8	6.3 ± 5.4	6.3 ± 5.0	20.9 ± 0.8	63.3 ± 14.4
Guinea pig	429 ± 293	17.2 ± 4.7	14.2 ± 11.5	15.3 ± 11.7	17.6 ± 5.1	18.3 ± 8.1

Each value represents mean ± standard deviation from 10 animals each.

(Fig 1).

Table 1 shows the total activity of serum LDH and the relative composition of each isoenzyme of the agouti gerbil compared with those of the mouse, rat, and guinea pig. LDH_2 and LDH_5 were equally dominant in the gerbil, while the mouse and rat had a marked LDH_5 dominance. The guinea pig specific LDH isoenzyme patterns was not detected because of wide individual variation.

Figure 2 shows some examples of the LDH isoenzyme patterns of the coat color mutant gerbils. Each coat color mutant gerbil had 5 isoenzymes of LDH similar to the agouti gerbil. The relative activities of the fractions were almost the same as those of the agouti gerbil, that is, LDH_2 and LDH_5 were equally dominant, and there were no genetic polymorphisms in the coat color mutant gerbils.

Figure 3 represents the ALP isoenzyme patterns of mice, rats, guinea pigs, and agouti gerbils. Untreated sera from mice, rats, and guinea pigs displayed a single wide band. A distinct delayed band and a slight cathodic one were detected after treatment with neuraminidase in all the animals. The gerbils gave the most distinguished two bands. The effect of neuraminidase treatment on mobility of the isoenzymes varied with the species, being greatest in the guinea pigs and least in the gerbils.

Table 2 shows the total activity of serum ALP and the relative activity of each isoenzyme, fractionated by neuraminidase, of the agouti gerbil compared with those of mice, rats, and guinea pigs. The relative activity of the cathodic ALP isoenzyme of the gerbil was greater than that of the mouse or rat. The guinea pigs did not show a specific densitometoric pattern and they had wide individual differences.

Figure 4 gives some examples of the ALP isoenzyme patterns of the coat color mutant gerbils. Each coat color mutant gerbil had 2 bands of ALP. The dominance of the two fractions varied at random and there were no coat color differences in these patterns.

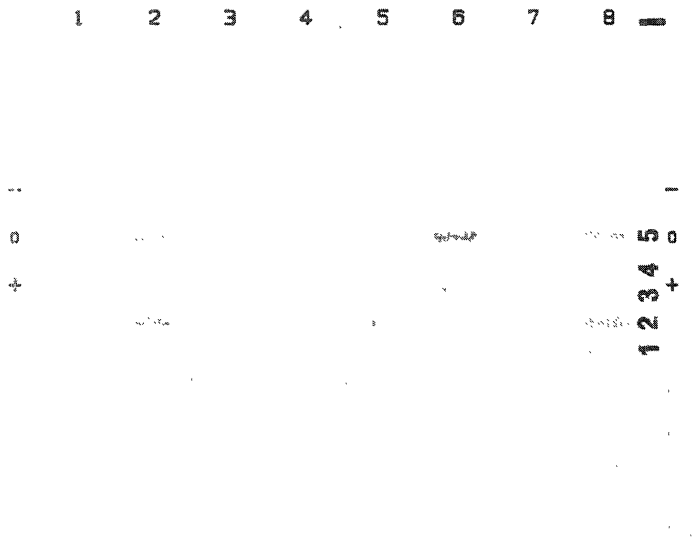

Fig. 2. Electrophoretic patterns of lacate dehydrogenase isoenzymes of the coat color mutant Mongolian gerbils. 1. male albino gerbil, 2. female albino gerbil, 3. male black gerbil, 4. female black gerbil, 5. male white spotted-agouti gerbil, 6. female white spotted-agouti gerbil, 7. male white spotted-black gerbil, 8. female white spotted-black gerbil.

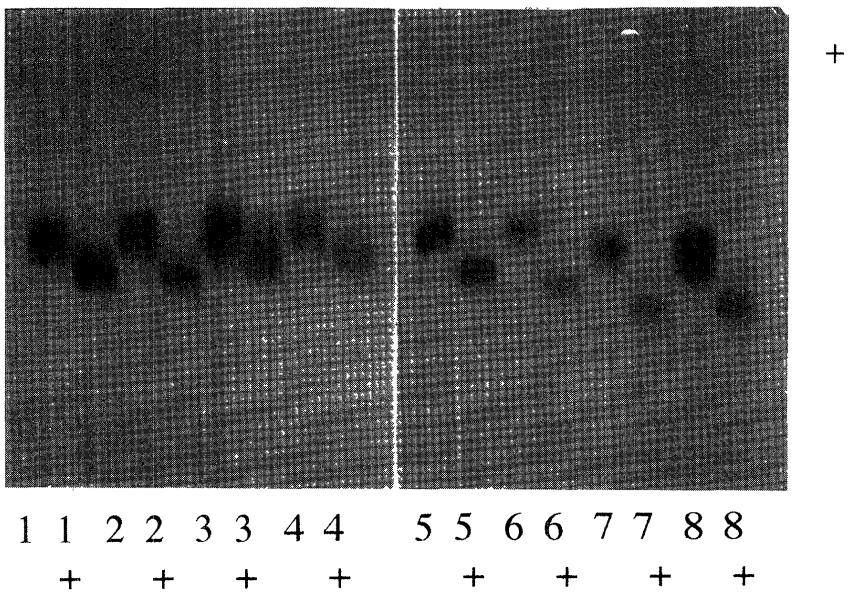

Fig. 3. Electrophoretic patterns of alkaline phosphatase isoenzymes in normal sera from mice, rats, guinea pigs, and Mongolian gerbils. 1. male S-D rat, 2. female S-D rat, 3. male Mongolian gerbil, 4. female Mongolian gerbil, 5. male ddy mouse, 6. female ddy mouse, 7. male Hartley guinea pig, 8. female Hartley guinea pig. + indicates each sample treated with neuraminidase.

DISCUSSION

Normal levels of ALP and LDH activity in experimental animals have been reported in many articles and books. These data do, however, have a wide range because of the influence of rearing conditions, as well as differences among strains or individuals even in the same species [35]. The essential requisite for evaluating experimental results is the establishment of reference levels for a particular colony, determined by several repeated measurements under specific rearing conditions.

LDH is an enzyme involved in the final stage of the glycolytic system, and is ordinarily known to possess five isoenzymes because it is a tetrameter composed of two types

Table 2. Total activity and relative composition of serum alkaline phosphatase (ALP) isoenzymes of mouse, rat, guinea pig, and Mongolian gerbil

Species	Total activity (IU/l)	Relative activities of ALP isoenzymes (%)	
		Cathodic	Anodic
Mongolian gerbil	320 ± 71	46.0 ± 20.5	54.1 ± 20.5
Mouse	186 ± 37	6.2 ± 2.0	93.8 ± 31.5
Rat	345 ± 62	10.1 ± 3.5	89.9 ± 29.5
Guinea pig	211 ± 59	12.0 ± 11.2	88.0 ± 47.3

Each value represents mean ± standard deviation from 10 animals each.

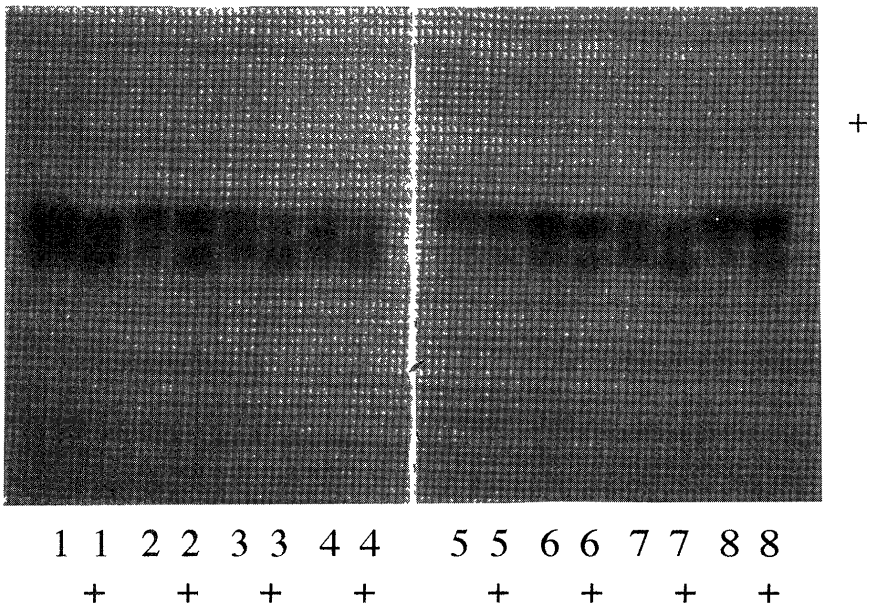

Fig. 4. Electrophoretic patterns of alkaline phosphatase isoenzymes of the coat color mutant Mongolian gerbils. 1. male albino gerbil, 2. female albino gerbil, 3. male black gerbil, 4. female black gerbil, 5. male white spotted-agouti gerbil, 6. female white spotted-agouti gerbil, 7. male white spotted-black gerbil, 8. female white spotted-black gerbil. + indicates each sample treated with neuraminidase.

of subunits. Five LDH bands were detected in the gerbil sera and LDH_2 and LDH_5 were equally dominant in the gerbil. The relative activities of the LDH isoenzymes of the gerbils were different from past reports, and this might have been caused by a colony difference, because the dominance of LDH_5 in the sera of mice and rats agreed with the results of previous reports [6, 11]. The electrophoretic mobility of each fraction of LDH varied among the species. This study confirmed the result of a previous report [11] that there was a species specificity in the electrophoretic mobilities of the LDH isoenzymes.

Serum ALP gave two distinguished bands in the gerbil after neuraminidase treatment, whereas the cathodic fraction was very slight in the mouse and rat. Treatment with neuraminidase makes sialic acid residues split off from the molecule, and slows the electrophoretic migrations of bone, liver, and serum ALP, whereas it does not affect that of the intestine [26, 28]. Consequently it improves the resolution of some overlapping isoenzymes. There have been some reports that the bones are a major source of ALP in sera of mice and rats in a fasted condition, while the intestinal isoenzyme played a greater role in fed rats than in mice [18], and furthermore, that sera from fed rats contained a significant amount of the intestinal ALP, which had a migration rate which was not affected by neuraminidase unlike that of the fasted rats [26]. In the present study, the relative activity of the cathodic ALP was greater in the rat than in the mouse after neuraminidase treatment. This results confirmed the results of previous reports and the cathodic fraction in treated sera is suggested to be of intestinal origin. If this hypothesis is correct, the gerbil has a greater portion of intestinal ALP than the rat in a fed condition. Further study will be necessary to determine the major source of the serum ALP of the gerbil.

Genetic polymorphism of some isoenzymes including LDH and ALP has been demonstrated in many kinds of animals [14, 19, 33, 34] and the genes that code those enzymatic variants are used as biochemical markers [10,

12, 13, 38], however, we could not find genetic polymorphism of LDH and ALP isoenzyme patterns in gerbils when comparing coat color variants. A 6th band was detected on the cathode side of the LDH_5 in sera from one gerbil and one guinea pig. A similar band which looked like an additional LDH fraction was found in human sera, but it was determined to be alcohol dehydrogenase (ADH) [25]. This suggests that ADH of the gerbil is detected by electrophoresis in the same manner as that of the human.

The present experiments clarified the isoenzyme patterns of LDH and ALP in the Mongolian gerbil. Five LDH isoenzymes were detected, and two ALP bands were clearly stained. No genetic polymorphism was observed in LDH and ALP isoenzyme patterns among coat color variants of the gerbils. Comparison with the corresponding results for mice, rats, and guinea pigs revealed interspecific differences in the mobility of each isoenzyme. The importance of the isoenzyme patterns of animals has increased recently in studies on the genetic backgrounds of the animals [8, 23, 40] and in addition to the veterinary clinical applications [7]. The present study will be a useful basic data of isoenzyme patterns of the Mongolian gerbil and further analysis on DNA, which codes every isoenzyme, will help our understanding of the characteristics of the Mongolian gerbil.

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