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SHORT PAPER

Polymorphism of lactate dehydrogenase B subunit in rat erythrocytes

BY V. STOLC

Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

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SUMMARY

Lactate dehydrogenase regulator gene *Ldr-1* controls the amount of the lactate dehydrogenase A subunit in rat serum and the amount of lactate dehydrogenase B subunit in rat erythrocytes because no recombinant rats were found among 295 pups produced in the BDIV × (ACI × BDIV) F1 and (ACI × DI) F1 × DI backcrosses. Two alleles regulate the high (*Ldr-1*^{a}) and low (*Ldr-1*^{b}) activity enzyme. The *Ldr-1* is not linked to hooded coat pattern or glyoxalase-1. Sex and *Ldr-1* assorted independently; hence the gene is autosomal.

1. INTRODUCTION

We have reported recently (Stolc & Gill, 1983) that the lactate dehydrogenase regulatory gene *Ldr-1* controls the amount of lactate dehydrogenase A subunit in rat serum. No polymorphism of lactate dehydrogenase A or B subunits was found in heart, small intestine, kidney, liver, lungs, spleen or testes. Only erythrocytes showed polymorphism of lactate dehydrogenase B subunit. We reported that the *Ldr-1* gene was linked to the haemoglobin (*Hbb*), albino (*c*) and red eye (*r*) genes which are on the linkage group I of the rat. Other loci in the first linkage group of the rat are these for two cell surface alloantigens RT4 and RT6 (Kren *et al.* 1973; DeWitt & McCullough, 1975; Wonigeit, 1979), warfarin resistance (Greaves & Ayres, 1967), fuzzy coat (Palm & Ferguson, 1976), infantile ichthyosis (Knox & Lister-Rosenoer, 1978), waltzing, haematoma and several eye and coat colours (Robinson, 1982).

This article reports that the polymorphism of lactate dehydrogenase B subunit in the rat erythrocytes is caused by the amount of the enzyme produced and two phenotypes LDR-1A and LDR-1B were detected by acrylamide gel electrophoresis. The regulatory locus is probably identical with the *Ldr-1* gene because no recombinant rats were detected among the 295 offspring of two backcrosses.

2. MATERIALS AND METHODS

(i) *Animals*

The BDIV and WF strains were purchased from Harlan, Indianapolis, IN; the DI and ACI were obtained from Small Animal Section, National Institute of Health, Bethesda, MD; the BUF, SHR, WKY, and F344 strains were purchased from Laboratory Supply, Indianapolis, IN, and the BN and LEW strains came from Charles River Breeding Laboratories, Wilmington, MA.

The BDIV and DI strains carry recessive regulatory marker (*Ldr-1^b*) for the amount of lactate dehydrogenase A and B subunits in serum and erythrocytes and the ACI strain carries dominant regulatory marker (*Ldr-1^a*) for the two subunits. Segregation of the allelles was studied in the two backcrosses: BDIV × (ACI × BDIV) F1 and (ACI × DI) F1 × DI.

(ii) *Electrophoresis*

Blood was collected from tail under light ether anesthesia. The serum was harvested after incubation of tubes at room temperature for 1 h and erythrocytes were obtained from EDTA-containing vacutainers. The cells were extensively washed with 0·15 M-NaCl and lysed in 10 mM Hepes buffer, pH 7·4. The lactate dehydrogenase phenotypes in serum were determined by isoelectrofocusing in acrylamide gel (Stolc & Gill, 1983) and the lactate dehydrogenase phenotypes in erythrocytes were determined by electrophoresis in regular polyacrylamide gels (Stolc, 1979). Staining of lactate dehydrogenase in gels was performed by the method described by Harris & Hopkinson (1976).

3. RESULTS

(i) *B subunit polymorphism*

Polymorphism of lactate dehydrogenase in rat erythrocytes was observed in the leading band of the enzyme (Fig. 1). This band represents the lactate dehydrogenase tetramer consisting of the B subunits (Markery, 1963). The polymorphism was expressed as high or low amount of enzyme and it was caused by the action of a regulatory gene on the lactate dehydrogenase structural gene *Ldh-2* that produces the B subunits in rat erythrocytes. The decreased amount of the enzyme in the LDR-1B phenotype was not caused by a presence of an inhibitory compound because mixing of erythrocyte homogenates prepared from blood of the LDR-1A and LDR-1B phenotypes showed the expected staining of the gels (data not shown). In addition, the erythrocyte homogenates prepared from blood of the F1 hybrids were stained as those as of the LDR-1A parent. The distribution of the LDR-1A and LDR-1B phenotypes in erythrocytes of various rat strains is shown in Table 1.

Both intercrosses (ACI × DI) F1 and (ACI × BDIV) F1 had the LDR-1A phenotype; hence the allele that regulates the high amount of B subunit is dominant to the allele that regulates the low amount of B subunit in the erythrocytes. The segregation of the phenotypes in the intercrosses and backcrosses is shown in Table 2. The (ACI × DI) F1 × DI backcross produced 59 offspring with LDR-1A phenotype and 69 with LDR-1B phenotype; and the BDIV × (ACI × BDIV) F1 backcross had 82 pups with LDR-1A phenotype and 85 with LDR-1B phenotype. The data did not differ significantly ($P > 0·25$) in either backcross from the 1:1 ratio. In addition, the (ACI × DI) F1 × (ACI × DI) F1 intercross produced 33 pups, 23 with the LDR-1A phenotype and 10 with the LDR-1B phenotype. The data did not differ significantly from the 3:1 ratio ($P > 0·25$). Thus the *Ldr-1* genotype was expressed in the backcross and the F2 rats according to the normal Mendelian inheritance.

The sex and the lactate dehydrogenas phenotypes were transmitted independently and the pooled results did not differ from the 1:1 ratio ($P > 0·25$). There were 67 and 80 females with the lactate dehydrogenase phenotype LDR-1A and phenotype LDR-1B and there were 74 and 74 males with the lactate dehydrogenase phenotype LDR-1A and phenotype LDR-1B. Hence the *Ldr-1* gene that regulates the amount of the lactate dehydrogenase B subunit produced in erythrocytes is autosomal.

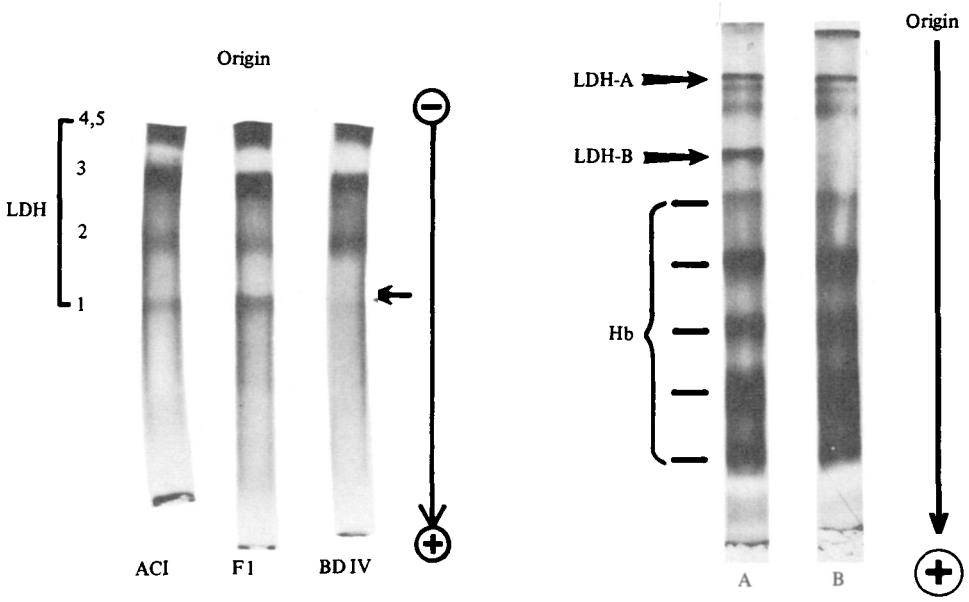

Fig. 1. Polyacrylamide gel electrophoresis of lactate dehydrogenase isozymes in rat erythrocytes. The gel patterns in the ACI, the (ACI × BDIV) F1 hybrid and the BDIV strain are shown. The position of the leading band that contains the B_4 tetramer (the LDH 1 isozyme) is shown by the arrow. The haemoglobins in the lysate were eluted out during the electrophoresis (panel on the left). The lactate dehydrogenase isozymes before elution of haemoglobins from the gels. The A gel represents the ACI and the B gel represents the BDIV strains. The A_4 and B_4 lactate dehydrogenase tetramers (LDH-A and LDH-B) are marked by the arrows (panel on the right).

Table 1. *Representative strains for the lactate dehydrogenase B subunit regulator gene in rat erythrocytes*

Phenotype	Genotype	Strain
LDR-1A	*Ldr-1^a/Ldr-1^a*	ACI, F344, WKY
LDR-1B	*Ldr-1^b/Ldr-1^b*	BDIV, DI, BN, BUF, LEW, SHR, WF

Table 2. *Genotypes and number of phenotypes of the lactate dehydrogenase B subunit in erythrocytes of the various crosses of the rat*

Mating	Parental alleles	No. of offspring	No. of phenotypes	
			LDR-1A	LDR-1B
ACI × ACI	*Ldr-1$^{a/a}$ × Ldr-1$^{a/a}$*	12	12	0
BDIV × BDIV	*Ldr-1$^{b/b}$ × Ldr-1$^{b/b}$*	10	0	10
DI × DI	*Ldr-1$^{b/b}$ × Ldr-1$^{b/b}$*	6	0	6
ACI × BDIV	*Ldr-1$^{a/a}$ × Ldr-1$^{b/b}$*	15	15	0
ACI × DI	*Ldr-1$^{a/a}$ × Ldr-1$^{b/b}$*	8	8	0
BDIV × (ACI × BDIV) F1	*Ldr-1$^{b/b}$ × Ldr-1$^{a/b}$*	167	82	85
(ACI × DI) F1 × DI	*Ldr-1$^{a/b}$ × Ldr-1$^{b/b}$*	128	59	69
(ACI × DI) F1 × F1	*Ldr-1$^{a/b}$ × Ldr-1$^{a/b}$*	33	23	10

The females are shown first.

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(ii) *Linkage study*

Table 3 shows that the lactate dehydrogenase regulator gene *Ldr-1* controls the amount of lactate dehydrogenase A subunit in serum and the amount of the lactate dehydrogenase B subunit in erythrocytes. There were no recombinant rats among the 295 pups produced in two backcrosses. No linkage was found between the *Ldr-1* gene and hooded coat pattern or glyoxalase-1 (Table 4).

Table 3. *Segregation of the lactate dehydrogenase A subunit and of the lactate dehydrogenase B subunit genotypes in the BDIV × (ACI × BDIV) F1 and (ACI × DI) F1 × DI backcrosses*

		BDIV × (ACI × BDIV) F1: erythrocytes B subunit		(ACI × DI) F1 × DI: erythrocytes B subunit	
		Ldr-1^a	*Ldr-1*^{b}	*Ldr-1*^{a}	*Ldr-1*^{b}
Serum A subunit	*Ldr-1*^{a}	82	0	59	0
	Ldr-1^b	0	85	0	69
		$\chi^2 = 163{\cdot}0$ $P < 0{\cdot}001$		$\chi^2 = 124{\cdot}0$ $P < 0{\cdot}001$	

Table 4. *Parental : recombinant ratio of* Ldr-1, h *or* Glo-1 *genes*

	BDIV × (ACI × BDIV) F1		(ACI × DI) F1 × DI	
	h	*Glo-1*	*h*	*Glo-1*
Ldr-1	92:75	89:78	63:54	56:61
h	—	94:73	—	66:51

4. DISCUSSION

Lactate dehydrogenase (EC 1.1.1.27) is a tetrameric molecule composed of A and B subunits. The structural gene *Ldh-1* for the A subunit is located on chromosome 11 in man and chromosome 7 in the mouse (Boone, Chen & Ruddle, 1972; Britton-Davidian *et al.* 1978). The structural gene *Ldh-2* for the B subunit is located on chromosome 12 in man and chromosome 6 in the mouse (Chen *et al.* 1973; Minna *et al.* 1978). Two regulatory genes *Ldr-1* and *Ldr-2* that regulate the amount of the B subunit are also on the mouse chromosome 6 (*Mouse News Letter*, 1984). In spite of an extensive search no polymorphism for the lactate dehydrogenase A and B subunits has yet been found in the rat (Eriksson *et al.* 1976; Bender & Gunther, 1978; van Zutphen *et al.* 1981).

We reported recently that the amount of the A subunit in rat serum is regulated by *Ldr-1* locus that has two alleles: *Ldr-1*^{a} that regulates the high amount of the A subunit and *Ldr-1*^{b} that regulates the low amount of the A subunit. However, no polymorphism of A or B subunit was observed in the homogenates prepared from various tissues of different strains of the rat (Stolc & Gill, 1983). Only the lactate dehydrogenase B subunit in erythrocytes showed polymorphism. We have found that the staining of the lactate dehydrogenase isozymes in the rat erythrocytes did not follow the classical pattern. The leading band that represents the B_4 tetramer had extremely low enzyme activity in the LDR-1B phenotype. However, the next B_3A and B_2A_2 tetramers had similar intensity of staining in both LDR-1A and LDR-1B phenotypes. Such data suggest that the B_4 tetramer is either unstable or is catabolized at high rate in the rat erythrocytes. The better explanation probably is that an inhibitory factor binds to the B subunits and prevents

their association. Glass and Doyle (1972) reported similar phenomena for the A subunit. They suggested that a factor (probably reduced nicotinamide adenine dinucleotide) was bound to the A subunit and prevented its association with the B subunit. Thus the regulatory gene *Ldr-1* controls either the synthesis or the rate of degradation of the B subunit or it may regulate the level of a factor that affects the association of the lactate dehydrogenase B subunits.

The rates of synthesis and degradation of the A and B subunits are extremely variable in rat tissues and organs. The same isozyme in different tissues and different isozyme in the same tissue are metabolized differently (Fritz *et al.* 1969, 1975). The presence of the A subunit or of the B subunit in given cells depends on the developmental stage of the cells, and in addition, on the presence or absence of a malignant process. The B subunit predominates in lymphocytic leukemia whereas the A subunit appears abundant in myeloid leukemia (Dioguardi, Agostini & Fiorelli, 1965). The data are extremely diverse during the embryological development. The A subunit in the rabbit embryo and the B subunit in the mouse embryo are the dominant species in the preimplantation stage (Brinster, 1973). In contrast, the human embryo has both subunits present at the same level (Pfleiderer & Wachsmuth, 1961).

It seems that the amount of the B subunit in the rodent erythrocytes is controlled by a regulatory locus (*Ldr-1*) because similar results were found by Shows & Ruddle (1968) in the mouse red blood cells. Recently, another B subunit controlling locus (*Ldr-2*) was found in the early postnatal mouse liver (Khlebodarova & Serov, 1980). There may be a difference between the mouse and the rat concerning the location of the regulatory gene. The rat *Ldr-1* is on the linkage group I linked to the *Hbb* and *c* genes. In the mouse, however, the *Ldr-1* is not on the same chromosome as the *Hbb* and *c* genes. The mouse *Ldr-1* is linked to the *Ldh-2* (chromosome 6) and the mouse *Hbb* and *c* are linked to the *Ldh-1* (chromosome 7). Thus it may be possible that the rat *Ldr-1* will be found to be linked to the *Ldh-1* and not to the *Ldh-2*.

The finding of lactic dehydrogenase A and B subunit polymorphisms will be useful for the genetic mapping of the rat.

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