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Immunochemical Studies on Lactate Dehydrogenase A Subunit Deficiencies

MASATO MAEKAWA,[1] KAYOKO SUDO,[2] AND TAKASHI KANNO[1]

SUMMARY

In lactate dehydrogenase (LDH) A subunit deficiency, is there not only a lack of activity but also a lack of subunit production? We demonstrated three remarkable points to answer this question: (1) There are no proteins that immunologically react with anti-A subunits. (2) There are no heterotetramers that react with anti-B subunits. (3) B subunits seem to be genetically produced at normal level, and all of them form only one isoenzyme, LDH-B_4. From these points, we concluded that there is a complete lack of A subunit production or production of incomplete A subunits that can neither react with anti-A subunits nor form heterotetramers.

INTRODUCTION

Lactate dehydrogenase (LDH; E.C.1.1.1.27) is separated into five isoenzymes in mammalian tissues [1]. These isoenzymes are formed by the random combination of two different subunits—A (or M) and B (or H)—into tetramers.

A case of complete LDH-B subunit deficiency was first reported by Kitamura et al. [2] in 1971 and that of a complete LDH-A subunit deficiency by Kanno et al. [3] in 1980 and by Maekawa et al. [4] in 1984. In these LDH subunit deficiencies, there seems to be a complete lack of subunit production, although production of an enzymatically inactive subunit in LDH subunit deficiencies has not been excluded.

Here, we tried to demonstrate whether or not there is a production of enzymatically inactive A subunits in the two cases of LDH-A subunit deficiencies

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[1] Department of Laboratory Medicine, Hamamatsu University, School of Medicine. Handa-cho 3600, Hamamatsu City, 431-31 Japan.

[2] Department of Laboratory Medicine, The Jikei University, School of Medicine.

[3, 4]. The following three points were investigated: (1) whether or not there are proteins that react immunologically with anti-A subunits, (2) whether or not there are heterotetramers without LDH activity by observing a reaction against anti-B subunits, and (3) whether or not the absence of LDH-A subunits affects LDH-B subunits.

MATERIALS AND METHODS

Measurement of LDH Activity, Protein Concentration, and Hemoglobin Concentration

We measured LDH activity by the method of Wróblewski and LaDue [5], using an LKB 2086 Mark II reaction rate analyzer (LKB, Bromma, Sweden). Final reagent concentrations (mmol/l) of the assay mixture were as follows: pyruvate, 0.68; NADH, 0.17; and sodium-sodium phosphate, 100; the pH was 7.4. The activity of the enzyme was expressed in U/l at 37°C. The protein concentration was assayed by the method of Lowry et al. [6]. The hemoglobin concentration was measured by the method of Kampen and Zijlstra [7], using samples after conversion to cyanmethemoglobin.

Separation of LDH Isoenzymes

We separated the isoenzymes by electrophoresis on a Cellogel membrane (Chemetron, Milano, Italy) by the method of Shioya et al. [8]. We quantified the bands with a Cliniscan reflectance densitometer at 570 nm. From total LDH activity and each quantified LDH isoenzyme, we calculated the activities of A subunits, B subunits, and B_4.

Preparation of Human LDH-A_4 and B_4

The human LDH-A_4 was prepared from skeletal muscle obtained from autopsied specimens and the B_4 from human erythrocytes for blood transfusion, by the method of Igarashi and Nakayama [9].

Preparation of Antiserum against LDH A Subunit and B Subunit

White male rabbits were immunized with the A_4 and B_4 prepared above, according to Burd et al. [10] for A_4 and to Malvano et al. [11] for B_4. The antisera were used for the following studies.

Immunological Methods

Double diffusion in agarose gel was performed using anti-A subunits according to Ouchterlony [12]. Immunofixation electrophoresis on a Cellogel membrane was performed using anti-B subunits according to Alper and Johnson [13].

Subjects

Two patients with LDH-A subunit deficiency were tested. Femoral muscle was obtained by needle biopsy for the propositus of the first case [3], and rectal abdominal muscle was surgically obtained during a Caesarean section for the propositus of the second case [4]. They were homogenized and centrifuged at 4°C, for 60 min at 100,000 *g*. The supernatants were used as tested samples. The human liver and rectal abdominal muscle obtained from autopsied specimens and the femoral muscle obtained by needle biopsy were treated similarly and used as control samples. Erythrocytes prepared from 5 ml of heparinized venous blood from the two patients with LDH-A subunit deficiency and normal control were washed with isotonic saline three times, then hemolyzed by

TABLE 1

THE RELEVANT DATA OF THE SAMPLES FOR IMMUNOLOGICAL METHODS

SUBJECTS	LDH (U/l)	LDH ISOENZYMES (%)					B/A RATIO	PROTEIN (g/l)	LDH (U/g OF PROTEIN)	HEMOGLOBIN (g/l)	LDH (U/g OF HEMOGLOBIN)
		I	II	III	IV	V					
Normal control:											
Femoral muscle	21,925	2.8	13.3	27.1	26.4	30.5	0.49	10.00	2,193	...	...
Rectal abdominal muscle	17,449	0.2	2.6	13.9	20.6	62.8	0.17	6.51	2,680	...	...
Liver	23,760	0	1.5	18.4	30.4	49.7	0.22	40.70	584	...	...
LDH-A_4	204,000	0	0	0	0	100	...	0.62	329,032	...	...
LDH-B_4	32,219	100	0	0	0	0	...	0.39	83,686	...	...
Hemolysate	28,980	32.6	31.4	34.6	1.4	0	2.82	...	...	179	162
LDH-A subunit deficiency:											
The first case (femoral muscle)	790	100	0	0	0	0	...	8.80	90	...	...
The second case (rectal abdominal muscle)	1,918	100	0	0	0	0	...	6.67	288	...	...
The first case (hemolysate)	25,812	100	0	0	0	0	...	...	...	156	165
The second case (hemolysate)	10,689	100	0	0	0	0	...	...	...	61	176

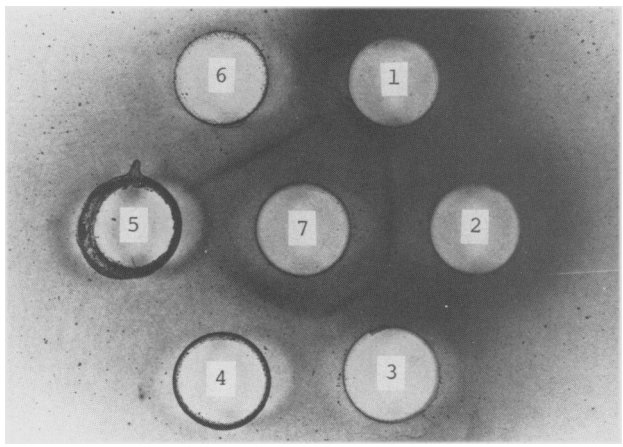

FIG. 1.—Immunodiffusion of LDH. The central well (*7*) contained 20 μl of anti-LDH-A subunits, and the peripheral wells contained various samples: *1*, rectal abdominal muscle of the second case with LDH-A subunit deficiency; *2*, control rectal abdominal muscle; *3*, control liver; *4*, control femoral muscle; *5*, femoral muscle of the first case with LDH-A subunit deficiency; *6*, the prepared LDH-A_4.

adding an equal volume of distilled water. The hemolysates were used as the samples of immunofixation electrophoresis. LDH activity, protein concentration, hemoglobin concentration, and LDH isoenzyme (%) of these supernatants and hemolysates were measured by the method described above. Table 1 shows the relevant data of both LDH-A subunit deficiency and control samples for immunological methods.

RESULTS

Ouchterlony double diffusion experiments were carried out using anti-A subunits. Immunodiffusion was performed with different ratios of antibody to the samples. Figure 1 shows the typical results of double diffusion. All control

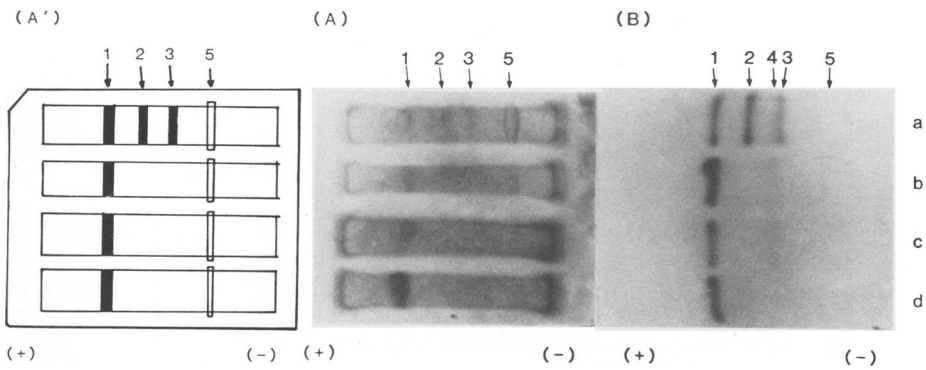

FIG. 2.—Immunofixation electrophoresis by anti-LDH-B subunits. After electrophoresis on a Cellogel membrane, LDH activity was visualized (*B*), and LDH isoenzymes containing B subunits were fixed against anti-B subunits and stained by Nigrosine (*A*). Schema of (*A*) was showed as (*A'*). *a*, Hemolysates of normal control; *b*, hemolysates of LDH-A subunit deficiency (the first case); *c*, hemolysates of LDH-A subunit deficiency (the second case); *d*, the prepared LDH-B_4; *1*, B_4; *2*, B_3A_1; *3*, B_2A_2; *4*, hemoglobin; *5*, origin.

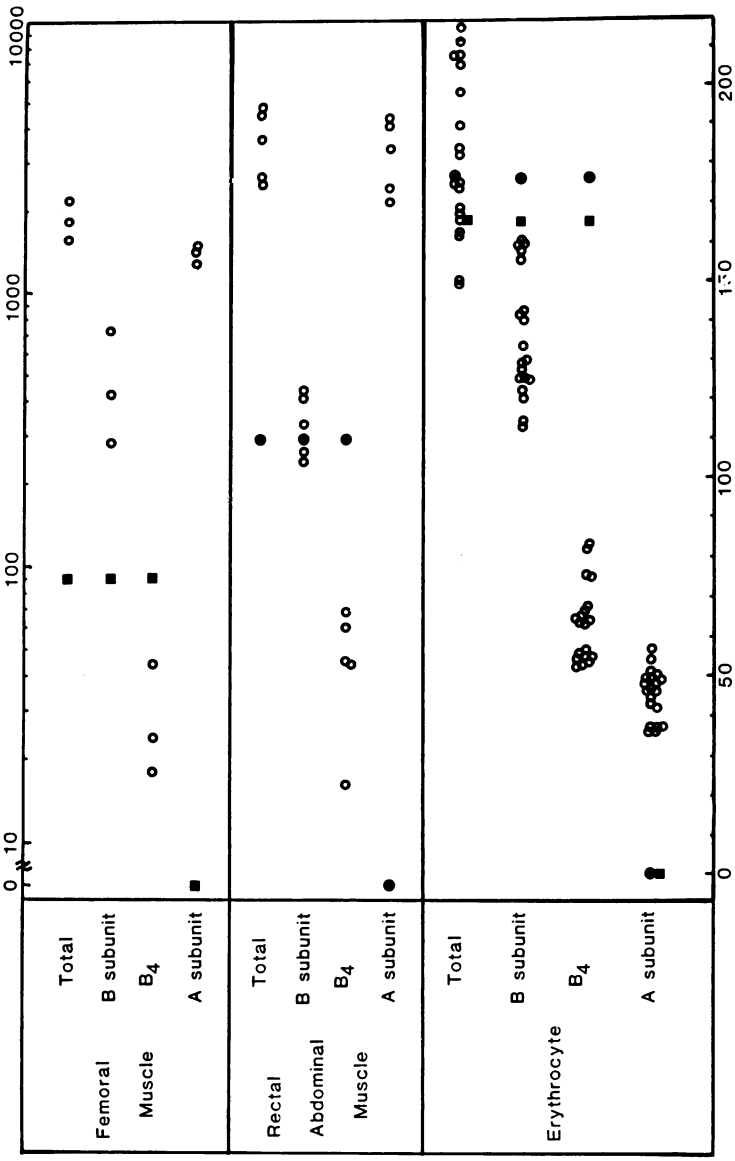

FIG. 3.—LDH activities in various tissues of LDH-A subunit deficiency and normal control. LDH activities were expressed as unit per gram of protein (muscle) or hemoglobin (erythrocyte). Not only total activity but also calculated activities as A subunit, B subunit, and B_4 of the samples were plotted on the logarithmic scale (muscle) or the normal scale (erythrocyte): normal control (*open circle*); LDH-A subunit deficiency, the first case (*closed square*), the second case (*closed circle*).

samples—prepared LDH-A_4, supernatants of muscle or liver homogenates—formed a single precipitin line that showed complete identity, while no precipitin line was formed by anti-A subunits vs. tested samples of LDH-A subunit deficiency.

Immunofixation electrophoresis was carried out using hemolysates and anti-B subunits. Figure 2 shows the electrogram. The hemolysates of the normal controls had nearly equal levels of activity of the three LDH isoenzymes (B_4, B_3A_1, B_2A_2), while prepared LDH-B_4 and hemolysates of LDH-A subunit deficiency had only LDH-B_4 activity (fig. 2*B*). Protein staining after immunofixation showed that the hemolysates of the normal controls had three bands of LDH isoenzymes (that was B_4, B_3A_1, B_2A_2); however, the hemolysates of LDH-A subunit deficiency had only one band of LDH-B_4, which was the same as that of prepared LDH-B_4 (fig. 2*A*).

Figure 3 shows LDH activities as B subunit, A subunit, and B_4 in tissues of LDH-A subunit deficiencies, compared with those of normal control. LDH activities as B subunit in LDH-A subunit deficiency were not significantly different from those in normal controls; however, LDH-B_4 activities in the former were higher than in the latter.

DISCUSSION

The two propositus with LDH-A subunit deficiency had no proteins that could react with anti-LDH-A subunits by double diffusion. If inactive A subunits that could form heterotetramers with normal active B subunits were present, the heterotetramers (B_3A_1, B_2A_2) in hemolysates could be fixed by anti-B subunits. However, the heterotetramers were not present in LDH-A subunit deficiency. Therefore, we might conclude from these results that there was a complete lack of A subunit production or production of incomplete A subunits that could neither react with anti-A subunits nor form heterotetramers.

In hemolysates of LDH-A subunit deficiency, LDH activities as B_4 were higher and as B subunits were relatively close to the activities of hemolysates of normal controls. We could not find an increase of B subunits by a feedback regulation due to the absence of A subunits. The results indicate that LDH-B subunit production is normal and there are no other subunits that can form with B subunits to tetramer; therefore, the produced B subunits form homotetramer, B_4, only. This speculation seems reasonable to explain the present data.

As for LDH-B subunit deficiency, both homozygous individuals and heterozygous individuals are reported to have production of enzymatically inactive B subunits ([14] and K. Sudo, M. Maekawa, and T. Kanno, two manuscripts in preparation). These reports are interesting in contrast with the present data.

The production and regulation among LDH subunits at the gene level remains an area of interest. Structural analysis of the RNA and DNA of LDH-A subunits is clearly necessary.

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