# DIFFERENTIAL ISOENZYME PATTERNS OF SOLUBLE AND PARTICLE-BOUND LACTATE DEHYDROGENASE OF RAT BRAIN

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## 1. Introduction

Differential electrophoretic patterns of multiple forms of an oligomeric enzyme such as lactate dehydrogenase (LDH, EC 1.1.1.27) are a potential means to identify distinct metabolical compartments at the cellular level. As summarized [1] only those forms of LDH containing subunits of type A tend to bind to particulate matter of skeletal muscle. In brain tissue most of the LDH activity occurs in a soluble form, be it cytoplasmic in origin, be it entrapped within vesicular structures like synaptosomes [2]. Still, a small percentage of total LDH activity was found to be associated with synaptosomal membranes too [3]. In view of these results it was felt that the subcellular compartmentation of LDH of nervous cells may be more complex than hitherto recognized. Therefore, the electrophoretic patterns of LDH isoenzymes of rat brain, derived from cytoplasm, the fluid space of subcellular particles, and particle fragments, respectively, were compared to each other.

## 2. Materials and methods

Cerebral hemispheres of male Wistar rats (250–300 g) were used throughout to prepare 10% (w/v) homogenates by means of a motor-driven glass–Teflon homogenizer. After nuclei and cell debris had been removed at 1000 × *g* for 10 min the supernatants were recentrifuged at high speed (100 000 × *g*) for 60 min yielding a cytoplasmic supernatant $S_I$ and a pellet $P_I$ of microsomes and crude mitochondria. $P_I$ was then thoroughly lysed by resuspension in 1 mM EDTA in 2.5 mM Tris buffer, pH 7.5, using a Dounce homogenizer fitted sequentially with a loose and tight pestle. This was followed by freezing and thawing, and high speed centrifugation resulting in the particulate cytoplasm $S_{II}$ and particulate fragments $P_{II}$, respectively.

Samples were assayed for total lactate dehydrogenase activity spectrophotometrically at 25°C [4], and for protein according to the method in [5], using a modification to account for Triton X-100 if present [6].

LDH isoenzymes were separated by standard electrophoretic techniques on 1% agar gel slides using a barbital buffer of pH 8.2 and 0.5 $\mu$M ionic strength. The slides were run at a constant 200 V for 100 min and a constant temp. 8°C. The staining solution contained 0.7 mM $NAD^+$, 0.6 mM nitro-blue tetrazolium-chloride, 27 mM sodium lactate and 0.2 mM phenazine methosulfate/litre, 0.1 M Tris–HCl buffer, pH 9.0. The fixed and dried pherograms were scanned at 578 nM.

## 3. Results and discussion

It is apparent from table 1 that the relative distribution of LDH isoenzymes of $S_I$ and $S_{II}$ distinctly differ from each other. The homotetramer $B_4$ assumes by far the largest percentage of LDH activity of $S_I$, i.e., the main cytoplasmic compartment, whereas it is the heterotetramer $A_3B$ which prevails in the particulate fluid space ($S_{II}$). The proportions of the other three isoenzymes including $A_4$ which sometimes even escapes detection [2,7] were much lower.

Soluble LDH activities listed in table 1 displayed a marked non-binomial distribution pattern similar to those reported for rat brain [7–9], but unlike that in human brain when tissue samples were extracted with

Table 1
Total activities and isoenzyme distributions of soluble lactate dehydrogenase of cytoplasm ($S_I$) and particle fluid spaces ($S_{II}$), respectively

| Medium Fraction | Lactate dehydrogenase (U/g) | Relative distribution of LDH isoenzyme activity | | | | |
|---|---|---|---|---|---|---|
| | | $B_4$ | $A_2B_3$ | $A_2B_2$ | $A_3B_1$ | $A_4$ |
| EDTA 1 mM/sucrose 0.32 M/ 2.5 mM Tris, pH 7.5 [3] | | | | | | |
| $S_I$ | 57.5 ± 0.8 | 44.8 ± 2.7 | 14.6 ± 3.5 | 6.4 ± 1.6 | 31.0 ± 2.2 | 3.0 ± 1.7 |
| $S_{II}$ | 39.8 ± 4.4 | 25.8 ± 2.5 | 11.8 ± 0.3 | 15.8 ± 0.3 | 42.0 ± 3.1 | 4.6 ± 0.1 |
| Sodium chloride 0.15 M [2] | | | | | | |
| $S_I$ | 76.8 ± 6.8 | 37.3 ± 0.9 | 14.6 ± 2.1 | 3.5 ± 1.0 | 29.8 ± 2.0 | 14.9 ± 6.1 |
| $S_{II}$ | 39.5 ± 2.9 | 28.5 ± 0.4 | 16.3 ± 0.1 | 9.2 ± 1.3 | 35.5 ± 0.4 | 10.5 ± 0.2 |

Values are means ± SD of triplicate determinations or means ± range of duplicate determinations

isotonic sodium chloride [10]. Although the possibility of an experimental artifarct therefore existed it was ruled out for the following reasons. The relative distributions of LDH isoenzymes of $S_I$ and $S_{II}$ were the same no matter whether Tris-buffered isotonic sucrose or sodium chloride was used. Neither could the distinct non-binomial distribution of LDH of rat brain be due to catalytically unstable assymmetric tetramers as described for LDH of alewife brain [11], if the high percentage of $A_3B_1$ of rat brain is considered. Besides, it was only after dissociation and reaggregation of the enzyme according to [12] that a marked binomial distribution was obtained. The disparity in the proportions of LDH isoenzymes between human and rat brain therefore reflects an interspecies difference rather than the consequence of experimental factors.

Total LDH activities of $S_I$ extracted with saline exceeded those of $S_I$ in Tris-buffered sucrose by as much as one third (table 1). Still, the relative isoenzyme distribution remained essentially unaffected as were total LDH activities of $S_{II}$. The increased yield of LDH of $S_I$ could therefore be due to a specific interaction of ions with dissociable groups of cytoplasmic enzyme protein, or else with the screening near particle surfaces affecting their binding properties [13]. Significantly, additional 'latent' LDH activity of particle membranes derived from sucrose homogenates was rendered both detectable and soluble using a non-ionic detergent (table 2) as in [14]. A portion of cytoplasmic LDH is therefore likely to be or to become adsorbed onto particles, and saline as a solvent of higher ionic strength compared to Tris-buffered sucrose either prevents the adsorption or mediates the desorption of LDH. There are reports of similar effects of salts on the reversible binding of LDH [1], aldolase [15], and hexokinase [16] onto brain particulate matter, or mitochondria, respectively.

To characterize the membrane-bound enzyme complex particle fragments were isolated from lysed whole particles by centrifugation and analysed before and after treatment with the non-ionic detergent Triton X-100 (table 2, fig.1). LDH activity of this fraction comprised as much as 12% of the sum total of LDH ($S_I$, $S_{II}$ and $P_{II}$) of ~111 U/g wet wt. Triton X-100 almost doubled the measurable LDH activity of $P_{II}$ while solubilizing close to 60% of the total membrane-bound enzyme activity, similarly to its effect on particle-bound creatine phosphokinase of rat brain [17]. Protein assays disclosed a preferential release of the enzyme protein as the specific activity increased from 217 mU/mg and 348 mU/mg, respectively, to 657 mU/mg protein in the supernatant.

On electrophoresis particle fragments were found to contain a high proportion of $A_4$ which may be highly typical of this fraction as shown by the effect

Table 2
Total activity and isoenzyme distribution of membrane-bound lactate dehydrogenase

| Fraction | Lactate dehydrogenase (U/g) | Protein (mg/g) | Relative distribution of LDH isoenzyme activity | | | | |
|---|---|---|---|---|---|---|---|
| | | | $B_4$ | $A_1B_3$ | $A_2B_2$ | $A_3B_1$ | $A_4$ |
| $P_{II}$ total[a] | 13.04 ± 0.16 | 61.5 ± 4.9 | 37.9 ± 1.2 | 12.5 ± 3.0 | 7.5 ± 1.8 | 11.1 ± 3.4 | 31.0 ± 0.3 |
| $P_{II}$ Triton-treated | 24.17 ± 0.9 | 69.4 ± 4.5 | 37.3 ± 4.5 | 11.9 ± 0.8 | 9.0 ± 0.2 | 13.1 ± 2.0 | 28.6 ± 1.5 |
| Supernatant of triton-treated $P_{II}$ | 14.32 ± 0.19 | 21.8 ± 1.9 | 51.3 ± 9.2 | 13.4 ± 0.8 | 7.4 ± 0.3 | 19.5 ± 5.3 | 8.5 ± 3.4 |
| Residual pellet of triton-treated $P_{II}$ | 9.81 ± 1.20 | 47.2[b] – | 20.8 ± 4.5 | 3.1 ± 0.1 | –[c] | 8.5 ± 2.6 | 67.7 ± 1.8 |

[a] A suspension of particle fragments $P_{II}$ was obtained from fraction $P_I$ as in section 2. Aliquots thereof were made 0.2% with respect to Triton X-100 (v/v) and incubated at 25°C for 45 min. This preparation was further separated into a particle-free supernatant and a residual pellet by immediate centrifugation at 100 000 × *g* for 60 min. The pellets were resuspended in hypotonic Tris-buffer (see section 2)

[b] Calculated as difference

[c] Not detectable on the zymogram

Values are means of duplicate determinations ± range

of the detergent. The isoenzyme complex solubilized by triton is almost identical to that of the cytoplasmic enzyme (table 1) in its relative distribution of activities. In sharp contrast, the residual membrane-bound LDH is made up of $A_4$ by as much as 2/3 total activity.

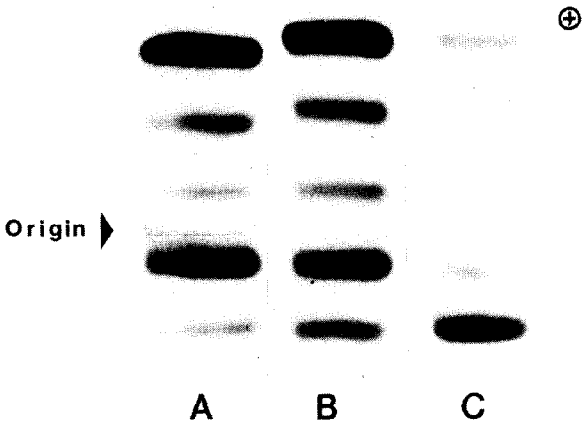


Fig.1. Zymograms of LDH of rat brain. (A) Cytoplasmic fraction $S_I$. (B) LDH of particle fragments $P_{II}$, solubilized by 0.2% (v/v) Triton X-100. (C) Residual pellet-bound LDH of detergent-treated particle fragments.

The similarity between the zymograms of the Triton-supernatant and $S_I$ further supports the opinion expressed above, namely that particle surfaces exposed to the cytoplasm may adsorb soluble LDH. On the other hand a preferential adsorption of $A_4$ would adequately explain the differential zymograms of cytoplasmic and membrane-bound LDH. In this respect LDH-$A_4$ behaves very similarly to $A_4$ of brain aldolase [15], the isoenzyme with the highest positive charge which was also observed to be the most tightly bound form of aldolase.

It should be pointed out that even particulate samples afforded a satisfactory electrophoretic migration of enzyme proteins (fig.1). There was no specific staining with material that had remained at the origin despite some opacity of this area. It appears that the combined action of the electric potential and the buffer environment completely mobilized membrane-bound enzyme molecules during electrophoresis. The results indicate that in rat brain LDH may occupy at least three different cellular compartments, namely the main cytoplasm, the fluid space of subcellular particles, and particle fragments, including membranes. The latter compartment was found to comprise 12%

total LDH activity only. This percentage may be higher since additional catalytically masked LDH was observed to be associated with structural elements. Whether bound LDH of brain is of any metabolic significance in terms of a postulated mechanism of intracellular enzyme regulation through reversible adsorption [18] remains to be determined.

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