ON THE PROPERTIES OF MATRIX BOUND LACTATE DEHYDROGENASE

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1. Introduction

Recent developments in enzyme technology and in the use of matrix bound enzymes as analytical reagents or for clinical potentials have been substantial in recent years.

Several studies [1–3] have indicated increased stability of matrix bound enzyme. This increase in stability could make the matrix bound enzyme a better candidate than the free form of the enzyme for clinical use as well as for its use as an analytical reagent.

The experiments described here were designed to compare some of the catalytic as well as the immunological properties of free and matrix bound lactate dehydrogenase.

2. Materials and methods

Cyanogen bromide, used for the activation of Sepharose, was obtained from Matheson, Coleman and Bell; and Sepharose 4B-200, for the preparation of the matrix bound enzyme, was obtained from Sigma Chemical Company. For the determination of enzyme activity, the reduced form of nicotinamide adenine dinucleotide (NADH) was obtained from P-L Biochemicals, and sodium pyruvate from Nutritional Biochemicals. Enzyme activity was measured by a Beckman Acta III Spectrophotometer as described by Pesce et al. [4]. The inhibition of enzyme activity caused by rabbit antisera, prepared against native lactate dehydrogenase, was determined by the loss of enzyme activity following incubation of the enzyme with increasing amounts of rabbit serum antibody for a period of 90 min at room temperature.

Substrate inhibition of lactate dehydrogenase was determined by comparing the ratio of enzymatic activity at low pyruvate concentration (1×10^{-3} M) to the activity at high pyruvate concentration (1×10^{-2} M).

Protein concentrations were determined by the method of Lowry et al. [5]. Dogfish muscle (M_4) lactate dehydrogenase was prepared as previously described [6] and chicken heart (H_4) lactate dehydrogenase was prepared by the method of Pesce et al. [7]. Quantitive micro complement fixation was carried out according to the method of Levine [8].

Sepharose 4B-200 was activated in 0.1 M phosphate buffer, pH 7.5, according to the method of Axen et al. [9]. However, highest yields of Sepharose-bound enzyme activity were obtained by reducing the amount of cyanogen bromide to 15 mg/ml Sepharose. Enzyme molecules were added in large excess, and the unbound protein was removed by repeated washing of the insoluble matrix.

3. Results and discussion

The data presented in fig.1 show that the matrix bound enzyme is significantly less reactive than the native enzyme when tested by the microcomplement fixation technique. The vertical shift in the extent of

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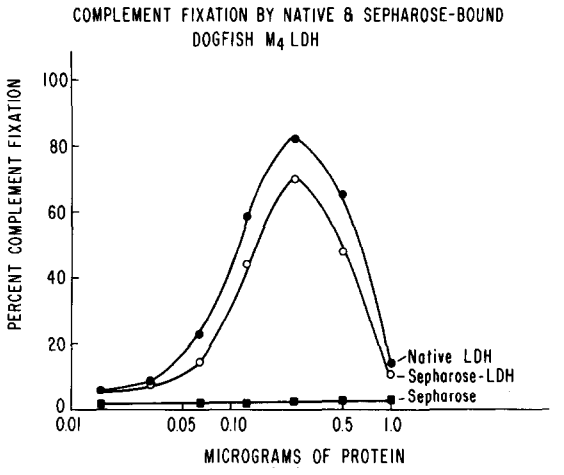

Fig. 1. Immunological comparison of native (free form) and matrix bound dogfish muscle (M_4) lactate dehydrogenase as measured by the quantitative microcomplement fixation technique. (●——●) Native free form lactate dehydrogenase (LDH). (○——○) Sepharose bound lactate dehydrogenase (Sepharose-LDH). (■——■) Sepharose (Control) The presence of Sepharose has no effect on the fixation of complement by the native (free form) lactate dehydrogenase.

complement fixation suggests structural or conformational changes in the protein molecule [8].

Results obtained in our laboratories [6], indicated that structural changes in the lactate dehydrogenase molecule, could be detected by the use of the microcomplement fixation techniques [8,10–12] have also used this immunological technique to detect subtle differences in protein structure. The covalent bonding of the enzyme to the insoluble matrix does not destroy the activity of the enzyme, although it may cause certain conformational changes that alter the complementarity between the antigen and antibody.

Interaction between the antibody and the matrix bound lactate dehydrogenase as compared to the native enzyme was studied by measuring the inhibition of enzymatic activity by rabbit serum antibody. Table 1 shows that the antibodies prepared against native enzyme are much less effective in inhibiting the catalytic activity of the Sepharose-bound enzyme as compared to the free enzyme. This difference in inhibition seems to support the possibility that conformational changes occur upon insolubilization.

Substrate inhibition is a characteristic which is most prominent in the heart type of lactate dehydrogenase [13,14]. The effect of conformational changes in the free form of the enzyme has been investigated [6]. In the previous study, it was observed that the

Table 1
Inhibition of enzymatic activity of free and matrix-bound lactate dehydrogenase by Rabbit serum antibody*

Dilution of Rabbit serum antibody	Per cent activity	
	Sepharose-bound lactate dehydrogenase	Native lactate dehydrogenase
No antibody	100[a, b]	100[a]
1/1	0	0
1/10	0	0
1/50	45	0
1/100	100	0
1/1000	100	7
1/10 000	100	15
1/100 000	100	40

* Antibody used in this experiment was prepared against the free form of the enzyme.

[a] Equal amount of enzyme units of native as well as of matrix bound, were used for the inhibition study.

[b] Specific activity of the matrix bound enzyme was 70–80% of the native (free) enzyme.

Enzyme activity was assayed according to the method of Pesce et al. [4] after incubation of the enzyme sample with rabbit serum antibody for a period of 90 min at room temperature.

Table 2
Effect of insolubilization of Chicken Heart (H_4) lactate dehydrogenase on the substrate inhibition by pyruvate

	Enzymatic activity (Change in A at 340 nm) at pyruvate concentrations of:		Ratio of activities
	1×10^{-3} M	1×10^{-2} M	$1 \times 10^{-3}/1 \times 10^{-2}$
Native	0.185	0.061	3.05
Matrix bound enzyme	0.163	0.130	1.25

Inhibition was determined by the ratio of enzymatic activity at low pyruvate concentration (1×10^{-3} M) to activity at high pyruvate concentration (1×10^{-2} M). Enzymatic activity was determined in 0.05 M Tris–HCl buffer, pH 7.5.

heart type enzyme loses its substrate inhibition characteristics as a result of structural changes. Therefore, the interaction between the insolubilized chicken heart (H_4) type enzyme and its substrate was investigated. Results of this experiment are shown in table 2. Data presented in table 2 indicates that the heart type lactate dehydrogenase loses almost all its characteristic substrate inhibition upon its immobilization by binding to Sepharose. The data presented in table 2 indicate that the native enzyme lose 67% of its maximum activity if incubated at high pyruvate concentration (1×10^{-2} M). On the other hand, the matrix bound enzyme loses only 20% of its activity. Further increase of substrate concentration has little or no effect on the matrix bound enzyme. The results shown in table 2 seem to support the possibility that conformational changes in the protein molecules result from the covalent binding to the insoluble matrix. This conclusion is in agreement with those of Cho et al. [15].

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