GLYOXYLATE REDUCTASE ACTIVITY OF LACTATE DEHYDROGENASE

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It is now well known that some single enzymatic proteins are capable of catalyzing reactions with more than one substrate (1—5). In particular, Yielding and Tomkins (4) showed that crystalline glutamate dehydrogenase (EC 1.4.1.3) from beef liver active toward alanine oxidation. Moreover, it has been found that steroids have the capacity to modify the enzymatic structure. Some authors (6) suggested that the small molecules such as GTP, NADH and ADP change the conformation of the enzyme. In a preliminary report from this laboratory (7), it was found that the crystalline lactate dehydrogenase (EC 1.1.1.27, Boehringer rabbit muscle enzyme) showed reductase activity toward glyoxylate.

The purpose of this communication is to show that the crystalline lactate dehydrogenase (Boehringer pig heart enzyme) catalyzes the reduction of glyoxylate with the similar results to those obtained with the rabbit muscle lactate dehydrogenase.

MATERIAL AND METHOD

Two commercial preparations of crystalline lactate dehydrogenase were used, obtained from C. F. Boehringer Company, Germany. These enzymes are prepared from rabbit muscle and pig heart. Quantitative determination of dehydrogenase activities was carried out by measurement of NADH diminution (at 23° and at 340 m μ). The assay solution contained 1.5 ml of 0.05 *M* phosphate buffer, pH 7.6, 0.2 ml of 0.0017 *M* NADH, 0.5 or 0.25 ml of 0.005 *M* lactate (or glyoxylate) and the enzyme (10 μ g) to be assayed, in a final volume of 2.5 ml. Specific activities of the enzyme were expressed as moles of NADH reduced per hour under the above conditions. On the other hand, as to the qualitative study cellogel electrophoresis was performed at 4° and the enzyme activity was visualized using phenazine methosulfate, nitroblue tetrazolium and glycollate to stain the NADH production by the enzyme reaction. The procedures have been reported in a previous publication (8).

RESULTS

The results are shown in Table 1, 2 and Fig. 1.

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TABLE 1

Activity of the Crystalline Lactate Dehydrogenase (Crystalline Boehringer Pig Heart Enzyme) with two Substrates

Specific activities are expressed as μ moles of NADH reduced/10 μ g of protein/hr.

Substrate	Concentration	Specific activity
	<i>M</i>	
Pyruvate	0.001	36.6
Glyoxylate	0.001	34.8
Pyruvate	0.0005	27.6
Glyoxylate	0.0005	21.6

TABLE 2

Activity of the Crystalline Lactate Dehydrogenase (Crystalline Boehringer Rabbit Muscle Enzyme) with two Substrates

Specific activities are expressed as μ moles of NADH reduced/10 μ g of protein/hr.

Substrate	Concentration	Specific activity
	<i>M</i>	
Pyruvate	0.001	39.6
Glyoxylate	0.001	37.2
Pyruvate	0.0005	27.2
Glyoxylate	0.0005	25.6

FIG. 1 *Photograph of Cellogel, Upper Two Developed with Lactate, the Remainders with Glyoxylate*

A, crystalline Boehringer rabbit muscle lactate dehydrogenase; B, crystalline Boehringer heart lactate dehydrogenase.

Our observations demonstrate that the lactate dehydrogenase catalyses the oxidation of NADH in the presence of either pyruvate or glyoxylate with almost equal efficiency.

Furthermore, similar results were observed between the muscle lactate dehydrogenase and heart lactate dehydrogenase. Fig. 1 is a photograph of a cellogel exhibiting the crystalline lactate dehydrogenase and its glyoxylate reductase.

On the cellogel preparations, the enzymic patterns obtained from the two different substrates were identical except for a difference in intensity of the bands. These crystalline enzymes from two sources were found to possess glycollate oxidase activity. These results apparently provide that some single enzymatic proteins are capable of catalyzing reactions with more than one substrate. In this case, it seems likely that there appear to be some conformational changes, but the structure of the enzymic protein is not quite clear.

DISCUSSION

Many proteins such as lactate dehydrogenase and glutamate dehydrogenase exhibit reversible association-dissociation equilibria without loss in enzymic activity. Especially, the work of Yielding and Tomkins (4) has been noteworthy in demonstrating the conformation of enzymatic structure.

The above results show that two types of crystalline lactate dehydrogenase may have glyoxylate reductase activity. Further study is required to rule out completely the possibility of enzymatic contamination.

SUMMARY

It has been established that glycollate is oxydized to glyoxylate by the crystalline lactate dehydrogenase. These findings suggest the possibility that some single enzymatic proteins may catalyze multiple reaction.

REFERENCES

1. Selim, A. S. M., and Greenberg, D. M., *Biochim. Biophys. Acta* **42**, 211 (1960).
2. Waksman, A., and Roberts, E., *Arch. Biochem. Biophys.* **109**, 522 (1965).
3. Shaw, C. R., and Koen, A. L., *Biochem. Biophys. Acta* **92**, 397 (1964).
4. Yielding, K. L., and Tomkins, G. M., *Amer. J. Med.* **33**, 1 (1962).
5. Naoi, M., *Seikagaku* **37**, 173 (1965).
6. Frieden, C., *J. Biol. Chem.* **283**, 3286 (1963).
7. Sawaki, S., and Yamada, K., *Nature* (in press.)
8. Sawaki, S., and Morikawa, N., *J. Vitaminol.* **10**, 179 (1964).