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Lactate Dehydrogenase Isozymes: Further Kinetic Studies at High Enzyme Concentration

Abstract. *By competition with lactate dehydrogenase (LDH) for nicotinamide adenine dinucleotide (NAD), commonly occurring intracellular proteins, such as glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, and albumin, can protect LDH-1 and LDH-5 from inhibition and ternary complex formation with NAD and pyruvate. The existence of intracellular proteins that compete with LDH for NAD renders unphysiological a model for estimating the extent of intracellular LDH inhibition based on incubations of only LDH, NAD, and pyruvate.*

At low concentrations of enzyme, lactate dehydrogenase LDH-1, but not LDH-5, is inhibited by high concentrations of pyruvate. On the basis of this difference in kinetic behavior a theory was developed to explain tissue specific distributions of LDH-1 and LDH-5. It has been shown that LDH activity assayed under comparable conditions, except for substitution of physiological levels of enzyme, exhibits no substrate inhibition (*1*). Furthermore, Coulson and Rabin (*2*) and Griffin and Criddle (*3*) showed that LDH inhibition results from the enol form of pyruvate, which is present in most commercial preparations of pyruvate; the enol form of pyruvate enhances abortive ternary complex formation (*2, 3*). When enol pyruvate was chromatographically removed, even exceedingly high unphysiologic concentrations of the purified keto form failed to inhibit low concentrations of LDH. Our experiments were designed to determine whether certain commonly occurring intracellular compounds could alter pyruvate inhibition of LDH.

Our experiments reveal that certain intracellular compounds can alter the extent of LDH inhibition by pyruvate. Pyruvate inhibition of LDH has been attributed to the formation of an abortive ternary complex (*1–4*). As a reference, we incubated LDH, nicotinamide adenine dinucleotide (NAD), and pyruvate to form the abortive ternary complex and then investigated changes in LDH inhibition produced by addition of certain common intracellular proteins to the mixture of LDH, NAD, and pyruvate. We selected concentrations of these proteins similar to those of LDH. Such concentrations of these specific proteins may not be physiological, but comparable proteins that could compete with LDH for NAD probably exist intracellularly in at least these concentrations. The NAD concentration in our incubation mixtures (14 m*M*) is approximately 20- and 40-fold greater than physiological levels in rat liver and brain, respectively (*5*). As in our previous experiments (*1*), an Aminco-Bowman spectrophotofluorometer equipped with a stopped-flow apparatus (*6*) was used for measurement of LDH activity. Reactions were initiated in the 0.2-ml stopped-flow cuvette by mixing either NADH or pyruvate with the enzyme solution that had been incubated at room temperature (Tables 1 and 2). The initial linear decrease in reduced NAD (NADH) fluorescence was followed at 460 nm (340 nm excitation) for 0.1 to 5 seconds. Reactions were recorded on a Hewlett-Packard storage oscilloscope.

We recognize that incubation of the constituents of the abortive ternary complex prior to assay of LDH activity, though useful as a reference, is unphysiological for a variety of reasons and cannot therefore serve as a model of the situation in vivo. For example, concentrations of NAD and pyruvate are not stationary, but oscillate intracellularly over the time course of the incubations (*7*). Moreover, the cell contains many proteins that compete with LDH for NAD. Thus incubations solely of LDH, NAD, and pyruvate cannot be accepted as an indication of the extent of ternary complex formation in the cell. Rather they serve as a tool to explore the influence exerted by various commonly encountered intracellular compounds on the extent of formation of the abortive ternary complex. The extent to which a ternary complex forms in vivo with physiologic concentrations of LDH, NAD, pyruvate, and a variety of proteins that compete with LDH for NAD is unresolved and a major consideration of these experiments.

The results indicate that a variety of intracellular proteins compete with LDH for NAD and thereby reduce abortive ternary complex formation from that produced when LDH is incubated only with NAD and pyruvate. Even when the constituents of the

Table 1. Effect of several proteins on LDH inhibition and ternary complex formation. The incubating mixtures contained: $3.5 \times 10^{-6}M$ LDH-1 or LDH-5, 14.0 m*M* NAD, 2.0 or 10.0 m*M* pyruvate, $7 \times 10^{-6}M$ G-3-PD or $3.5 \times 10^{-6}M$ MDH or 2.5 mg of BSA per milliliter, all made up in 0.05*M* tris-HCl buffer, *p*H 7.4. Final concentrations in the cuvette were: $1.75 \times 10^{-6}M$ LDH-1 or LDH-5, 7.0 m*M* NAD, 1.0 or 5.0 m*M* pyruvate, $3.5 \times 10^{-6}M$ G-3-PD or $1.75 \times 10^{-6}M$ MDH or 1.25 mg of BSA per milliliter, and 0.7 m*M* NADH. Reactions were initiated by the addition of reduced coenzyme.

Final pyruvate in cuvette (m*M*)	Incubation (min)	Percent LDH activity* remaining after incubating LDH with:							
		NAD and pyruvate only		NAD, pyruvate, and G-3-PD		NAD, pyruvate, and MDH		NAD, pyruvate, and BSA	
		LDH-1	LDH-5	LDH-1	LDH-5	LDH-1	LDH-5	LDH-1	LDH-5
1.0	10	18	32	47	50	25	33	27	34
	30	6	10	15	17	15	20	10	13
5.0	10	7	13	52	53	20	16	15	17
	30	3	5	18	17	8	11	5	13

* Activity compared to that of control where NAD is deleted from the incubation medium.

Table 2. Effect of incubating several proteins with LDH-1, NAD, and NADH. The incubating mixtures contained: $3.5 \times 10^{-6}M$ LDH-1, 14.0 mM NAD, 1.4 mM NADH, and $7.0 \times 10^{-6}M$ G-3-PD or $3.5 \times 10^{-6}M$ MDH or 2.5 mg of BSA per milliliter, all made up in 0.05M tris-HCl buffer, pH 7.4. Final concentrations in the cuvette were: $1.75 \times 10^{-6}M$ LDH-1, 7.0 mM NAD, 0.7 mM NADH, $3.5 \times 10^{-6}M$ G-3-PD or $1.75 \times 10^{-6}M$ MDH or 1.25 mg of BSA per milliliter, and 0.5, 1.0, 5.0, and 10.0 mM pyruvate. Reactions were initiated by the addition of pyruvate, and, in the case of NAD alone (last column), by pyruvate and NADH.

Final pyruvate in cuvette (mM)	Incubation (min)	Percent initial LDH-1 activity remaining after incubating LDH-1 with:				
		NAD, NADH, and G-3-PD	NAD, NADH, and MDH	NAD, NADH, and BSA	NAD and NADH	NAD
0.5	10	100	100	100	75	51
	30	100	100	60	25	51
1.0	10	100	100	100	63	45
	30	100	100	57	20	47
5.0	10	100	100	85	25	44
	30	100	100	57	6	44
10.0	10	100	100	57	13	46
	30	100	85	43	3	45

ternary complex are incubated alone, inhibition is never complete (Table 1) because, when NADH is added to start the reaction, NADH begins to displace NAD from the complex. Hence, the ternary complex cannot stop the reaction completely. Table 1 shows that at physiologic enzyme concentrations LDH-1 (pig heart) is inhibited more than LDH-5 (rabbit muscle) when LDH, NAD, and pyruvate are incubated together without exposure to commonly occurring protective intracellular compounds. In previous experiments on ternary complex formation, we demonstrated that LDH-1 and LDH-5 from these species reacted similarly to homologous isozymes obtained from a single tissue (*1*). Table 1 also shows the considerable reduction in LDH-1 inhibition produced by glyceraldehyde 3-phosphate dehydrogenase (G-3-PD) (rabbit muscle) when G-3-PD is incubated in a mixture of LDH-1, pyruvate, and NAD. If another common intracellular enzyme, malate dehydrogenase (MDH) (pig heart), is substituted for G-3-PD, LDH inhibition is also decreased (Table 1). Reduced LDH inhibition presumably results from binding of NAD to MDH or G-3-PD. This binding decreases the extent of abortive ternary complex formation. Table 1 shows that bovine serum albumin (BSA) protected LDH-1 activity slightly.

Another important intracellular substance that can affect abortive ternary complex formation but ignored by the model that considers only incubation of LDH, NAD, and pyruvate is NADH. Table 2 shows that, when NAD and NADH are incubated with LDH, alteration occurs in the extent of LDH inhibition. Furthermore, incubation of either MDH or G-3-PD with NAD, NADH, and LDH almost completely eliminates LDH inhibition (Table 2). Under these conditions even BSA exerts a protective effect (Table 2).

These experiments show that such commonly occurring intracellular compounds as G-3-PD, MDH, or BSA reduce the extent of LDH inhibition and abortive ternary complex formation. Therefore, the relative availability of NAD to LDH as compared to other proteins appears to be an important factor determining the extent of abortive ternary complex formation and LDH inhibition. Since NAD and NADH concentrations change continuously within cells (*7*), their concentrations at actual sites of the LDH isozymes and other NAD-linked dehydrogenases are difficult to determine as are the extent of abortive ternary complex formation and LDH inhibition. However, as Coulson and Rabin suggest, LDH inhibition by pyruvate is attributable to the enol form present in commercial preparations (*2*); thus the actual extent of intracellular LDH inhibition would be restricted by the enol–keto tautomerization rate of pyruvate.

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Sexual Reproduction in Geotrichum candidum

Abstract. *A perfect state of* Geotrichum candidum *was isolated from soil in Puerto Rico. Wild-type cultures are self-fertile but give rise to self-sterile, cross-fertile mating types morphologically different in some respects from the wild type. This discovery of the perfect state of* G. candidum *and its unique pattern of sexuality may contribute to knowledge of its ecology, the origin of pathogenic races, and speciation.*

Geotrichum candidum Lk. *ex* Pers. is a versatile ubiquitous asexual fungus of considerable importance to man. It causes plant disease (*1*), contributes to slime accumulations in polluted streams (*2*) and in pulp and paper mills (*3*), and causes sludge bulking in disposal systems for human waste (*4*). It is associated with chronic bronchitis and intestinal disorders of man (*5*) and mycoses in animals (*6*). Few fungi are adapted to as many different habitats.

In an ecological study of *G. candidum* in soils of Puerto Rico, two self-fertile ascosporogenous cultures were isolated, each from soil supporting the growth of unidentified grasses. They are Pr-47 (30 July 1967) from the Sierra de Luquillo, at about 1500-foot (457-m) elevation on a north slope; and Pr-11 (26 June 1967) from near Maricao in West Central Puerto Rico from a south slope at about 2500-foot (761-m) elevation. The collection sites were in areas of high rainfall (100 inches or more per year) and probably