Role of dehydrogenase competition in metabolic regulation. The case of lactate and alpha-glycerophosphate dehydrogenases.

M Guppy and P W Hochachka


Access the most updated version of this article at [http://www.jbc.org/content/253/23/8465](http://www.jbc.org/content/253/23/8465).

Find articles, minireviews, Reflections and Classics on similar topics on the [JBC Affinity Sites](http://www.jbc.org/affinity-sites).

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/253/23/8465.full.html#ref-list-1) to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/253/23/8465.full.html#ref-list-1](http://www.jbc.org/content/253/23/8465.full.html#ref-list-1)
Role of Dehydrogenase Competition in Metabolic Regulation

THE CASE OF LACTATE AND α-GLYCEROPHOSPHATE DEHYDROGENASES*

Michael Guppy and P. W. Hochachka‡

From the Department of Zoology, University of British Columbia, Vancouver, B. C., Canada V6T 1W5

Many tissues expressing capacities for both anaerobic and aerobic glycolysis contain significant amounts of both lactate dehydrogenase and α-glycerophosphate dehydrogenase. Since the first serves in oxidation-reduction balance during anaerobic metabolism, while the second serves in the α-glycerophosphate cycle during aerobic metabolism, a provision seemed to be made (through competition for coenzyme) to encourage relatively exclusive function of either one or the other dehydrogenase. Competition for coenzyme was found to depend upon the isozyme form of each dehydrogenase (which determines the sensitivity of each reaction to modulators) and the concentration of two key metabolites, α-glycerophosphate and creatine phosphate, which differentially influence α-glycerophosphate dehydrogenases and lactate dehydrogenases. The sensitivities of various dehydrogenase isozymes to these modulators correlated well with their expected roles in the tissue of origin.

Lactate dehydrogenase (EC 1.1.1.27) and α-glycerophosphate dehydrogenase (EC 1.1.1.8) often occur simultaneously and apparently in the same cell compartment, the cytosol (1). When they both occur at relatively high activities, such as in tuna white muscle (2), the two enzymes clearly compete for the same cytoplasmic supply of NADH. The consequences of such competition are potentially detrimental, since under aerobic conditions, significant lactate dehydrogenase activity would reduce the flow of glucose-derived carbon and hydrogen to oxidative metabolic pathways, while under anaerobic conditions, significant lactate dehydrogenase activity would reduce the flow of glucose-derived carbon and hydrogen to α-glycerophosphate (3). Thus, when both enzymes occur in the same cell, minimizing simultaneous function seems essential.

Such control is evidently widely achieved, since under hypoxic or anoxic stress lactate always accumulates to much higher levels than does α-glycerophosphate, even though both lactate dehydrogenase and α-glycerophosphate dehydrogenase are present in the cell. In the ischemic perfused rat heart, for example, lactate rises to about 20 μmol/g wet weight, while α-glycerophosphate increases to only about 2 μmol/g wet weight (4). In isolated rat skeletal muscle, lactate and α-glycerophosphate increase to about 30 and 3 μmol/g wet weight, respectively, during anaerobic work (5). During burst swimming in tuna, lactate and α-glycerophosphate in white muscle increase to 100 and 4 μmol/g wet weight, respectively (2). In some of these cases, such as tuna white muscle, the level of α-glycerophosphate dehydrogenase (over 100 μmol of substrate converted/g wet weight/min at 25°C) is easily high enough to channel a large fraction (up to one-half) of glucose-derived carbon and hydrogen to α-glycerophosphate. Yet this does not occur. Similarly, under aerobic conditions, when the α-glycerophosphate cycle is active, the activity of lactate dehydrogenase is potentially far in excess of any other step in the overall metabolism of glucose, and lactate could in theory accumulate. Yet this too does not occur. Why not?

There are two potential explanations, one involving thermodynamic, the other involving kinetic considerations. The first would assume that the free energy change (ΔG') for the lactate dehydrogenase reaction is larger than for the α-glycerophosphate dehydrogenase reaction; lactate, therefore, would be a thermodynamically more favorable carbon and hydrogen sink. Since lactate accumulates only under anoxic conditions and since the ΔG' values for the two reactions are quite similar, this explanation is, at best, incomplete.

An alternative explanation of the data assumes reciprocal kinetic control of the two enzyme activities. Previously, we showed that creatine phosphate inhibits tuna white muscle lactate dehydrogenase but not α-glycerophosphate dehydrogenase. We also found that α-glycerophosphate is a product inhibitor of α-glycerophosphate dehydrogenase but has no effect on lactate dehydrogenase (2). In normoxic conditions in vivo, we reasoned that creatine phosphate levels remained high and lactate dehydrogenase activity is not favored, while α-glycerophosphate levels are low and α-glycerophosphate dehydrogenase activity (thus α-glycerophosphate cycling) is favored. In hypoxic or anoxic conditions, α-glycerophosphate levels rise, and α-glycerophosphate dehydrogenase activity is dampened, while dropping creatine phosphate concentrations lead to lactate dehydrogenase deinhibition, favoring lactate formation.

In this study, we demonstrate that the creatine phosphate and α-glycerophosphate effects on lactate dehydrogenase and α-glycerophosphate dehydrogenase depend on the origin of each enzyme (isozyme) type. Creatine phosphate inhibition is most pronounced in aerobic tissues or in tissues with predominantly H type lactate dehydrogenase subunits, while α-glycerophosphate inhibition of α-glycerophosphate dehydrogenase is least in the most O2-dependent muscles. In two-enzyme experiments, the outcome of competition for limiting NADH depends upon both the kind of isozyme utilized and the concentration of the two key regulatory metabolites, creatine phosphate and α-glycerophosphate.

MATERIALS AND METHODS

Experimental Animals—Frozen skipjack tuna (Euthynnus pelamis) were obtained from the International Tropical Tuna Commission at the La Jolla Laboratories, California. Turtles (Pseudemys scripta) were purchased from NASCO Co., Ltd., Fort Atkinson, Wisconsin, and were kept at 10°C. Honey bees (Apis mellifera) were

* This work was supported by a National Research Council (Canada) operating grant (to P. W. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ During part of this work, P. W. H. held a Guggenheim Fellowship.

(Received for publication, June 6, 1978)
collected from hives around Vancouver. White laboratory rats and rainbow trout (Salmo gairdneri) were obtained from holding facilities at the University of British Columbia. Amazon fishes (Osteoglossum bicirrhosum, Arapaima gigas, Hoplias malabaricus, and Hoploerythrus unitaeniatus) were obtained on an R/V "Alpha Helix" expedition (September, 1976) to the Amazon River (6) and transferred to the laboratory at dry ice temperatures.

Enzyme Preparation—The preparation of α-glycerophosphate dehydrogenase from skipjack tuna, turtle, and trout and lactate dehydrogenase from skipjack tuna was as already described (7, 8). α-Glycerophosphate dehydrogenase from the honey bee thorax was prepared using only the G-100 step described in the previous paper and contained no lactate dehydrogenase. Lactate dehydrogenase from turtle white muscle, rat brain, and Amazon fish muscle was prepared by homogenizing the tissues in approximately 5 volumes of 50 mM imidazole buffer, pH 7.0, containing 2 mM EDTA. The homogenate was spun at 12,000 g for 20 min and the supernatant used for the assays of lactate dehydrogenase. H₁ and M₁ lactate dehydrogenase were prepared from tissues intentionally chosen to represent a wide range of lactate dehydrogenase present in such tissues usually correlates with its O₂ dependence. As already mentioned, in mammals lactate dehydrogenase occurs as a tetramer formed from random combinations of H and M type subunits, with the two homotetramers (H₄ and M₄) showing distinct kinetic characteristics (9). Usually, M₄ type lactate dehydrogenase predominates in highly glycolytic tissues, while H type subunits are more abundant in aerobic tissues. However, kinetic features also can vary without corresponding alterations in electrophoretic properties (10). Moreover, in fishes, at least eight subunit types are now known (11), but kinetic specializations, although probable, have not yet been fully clarified.

Despite these complexities, creatine phosphate was found to inhibit all the lactate dehydrogenases examined at least to some extent. Dixon plots of 1/velocity versus creatine phosphate concentration for M type lactate dehydrogenase of tuna white muscle are consistent with creatine phosphate inhibition, being mixed competitive with respect to either pyruvate or NADH (see Fig. 1, A and B).

The creatine phosphate sensitivity of the 12 preparations we studied appears to roughly correlate with the oxidative capacity of each tissue. The sources of lactate dehydrogenase listed in Table I, for example, are arranged approximately in order of increasing oxidative capacity (2, 6). This correlation is probably secondary and derives from the fact that the lactate dehydrogenase isozyme function (Table I) and content vary in these tissues (12). That is, creatine phosphate sensitivity appears to depend upon the relative abundance of lactate dehydrogenase subunits displaying M type versus H type properties. Not surprisingly, pure M₄ lactate dehydrogenase is one of the least creatine phosphate-sensitive preparations.
studied, while pure H4 lactate dehydrogenase is one of the most sensitive (Table I).

H4 and M4, Lactate Dehydrogenase versus a-Glycerophosphate Dehydrogenase—The above experiments establish that large differences occur in lactate dehydrogenase sensitivity to creatine phosphate. Therefore, in the presence of creatine phosphate, different lactate dehydrogenases should show differing capacities to compete with α-glycerophosphate dehydrogenase for a common source of NADH. Accordingly, appropriate competition experiments were set up to directly test this hypothesis.

Table II summarizes results of two-enzyme competition experiments between rabbit muscle α-glycerophosphate dehydrogenase and either of two (H4 and M4) kinds of lactate dehydrogenase enzymes in the presence and absence of creatine phosphate. Equal initial activities of both dehydrogenases led to approximately equal contributions to total NADH oxidation in both cases. In the case of the M4 lactate dehydrogenase, a creatine phosphate-resistant enzyme, creatine phosphate had no measurable effect on the fraction of NADH oxidized by lactate dehydrogenase. In contrast, fully 3 times more NADH was oxidized by α-glycerophosphate dehydrogenase compared to H4 lactate dehydrogenase when 20 mM creatine phosphate was included in the medium. Thus, under conditions of limited NADH, creatine phosphate is an important modulator of lactate dehydrogenase contribution to oxidation-reduction regulation.

α-Glycerophosphate Dehydrogenase Product Inhibition—Our original interest in this problem arose from the finding that tuna white muscle contains α-glycerophosphate dehydrogenase at activity levels high enough to drain up to one-half the glucose-derived carbon into α-glycerophosphate and thus to significantly reduce the already low energy yield of glycolysis (2, 3). During glycolytic activation in this muscle, when lactate dehydrogenase activity is favored, α-glycerophosphate dehydrogenase activity needs to be, and apparently is, dampened by α-glycerophosphate product inhibition. Thus, in tuna white muscle, α-glycerophosphate dehydrogenase is unusually sensitive to product inhibition by α-glycerophosphate, the K, determined from Dixon plots being about 0.25 mM (3). Table III indicates that a similar mechanism may operate in skeletal muscles of other vertebrates as well, since all of the α-glycerophosphate dehydrogenases examined showed relatively low K, values for α-glycerophosphate. In tuna muscle (2) and mammalian muscle (4, 5), α-glycerophosphate accumulates to values above the K, range of α-glycerophosphate dehydrogenase. Thus, there is good correlation between in vitro enzyme data and tissue metabolite measurements, implying that α-glycerophosphate sensitivity may be of physiological significance.

Although sufficient information is not available on various vertebrate tissues to attempt to closely correlate α-glycerophosphate dehydrogenase sensitivity to α-glycerophosphate product inhibition, two forms of the enzyme are known whose K, values are far out of line with the typical vertebrate situation. These are the α-glycerophosphate dehydrogenases from bee flight muscle and squid mantle muscle (Table III). These two enzymes show the lowest sensitivity to product inhibition by α-glycerophosphate of any α-glycerophosphate dehydrogenases thus far known. Interestingly, they function in tissues that are extremely O2-dependent and may never go anaerobic under normal physiological conditions; if made anaerobic experimentally, these muscles sustain an accumulation of α-glycerophosphate to much higher concentrations (up to 20 mM) than ever seen in vertebrate tissues (13, 14). Taken together, these data suggest that product-resistant α-glycerophosphate dehydrogenases should be more competitive with lactate dehydrogenase under conditions of high α-glycerophosphate levels than would be the typical vertebrate, product-sensitive α-glycerophosphate dehydrogenase. Moreover, any major differences between these two kinds of α-glycerophosphate dehydrogenases would indicate the physiological relevance of the low K, for α-glycerophosphate seen in vertebrate tissues.

Product-sensitive and Insensitive α-Glycerophosphate Dehydrogenases versus II Type Lactate Dehydrogenase—The above hypothesis was directly tested by two-enzyme competition experiments using one lactate dehydrogenase form (H4) and two types of α-glycerophosphate dehydrogenases (Table IV). Rabbit muscle α-glycerophosphate dehydrogenase displays a high sensitivity to α-glycerophosphate inhibition, while the honey bee enzyme is strongly resistant to the reaction product (Table III). When α-glycerophosphate was absent

### Table I

<table>
<thead>
<tr>
<th>Source of lactate dehydrogenase</th>
<th>Type of lactate dehydrogenase function</th>
<th>Percentage of inhibition by 20 mM creatine phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoplias white muscle</td>
<td>Pyruvate reductase</td>
<td>25</td>
</tr>
<tr>
<td>Turtle white muscle</td>
<td>Pyruvate reductase</td>
<td>26</td>
</tr>
<tr>
<td>M. from rabbit muscle</td>
<td>Pyruvate reductase</td>
<td>29</td>
</tr>
<tr>
<td>Hoploerythrinus white muscle</td>
<td>Pyruvate reductase</td>
<td>39</td>
</tr>
<tr>
<td>Skipjack white muscle</td>
<td>Pyruvate reductase</td>
<td>38</td>
</tr>
<tr>
<td>H4 heart</td>
<td>Bifunctional</td>
<td>39</td>
</tr>
<tr>
<td>Arapaima heart</td>
<td>Bifunctional</td>
<td>41</td>
</tr>
<tr>
<td>Hoploerythrinus heart</td>
<td>Bifunctional</td>
<td>47</td>
</tr>
<tr>
<td>H4 from beef heart</td>
<td>Lactate oxidase</td>
<td>71</td>
</tr>
<tr>
<td>Rat brain</td>
<td>Lactate oxidase</td>
<td>71</td>
</tr>
<tr>
<td>Weddell seal heart</td>
<td>Lactate oxidase</td>
<td>77</td>
</tr>
</tbody>
</table>

* 0.3 mM pyruvate in the case of the Amazon fishes.

### Table II

<table>
<thead>
<tr>
<th>Percentage of total oxidation by lactate dehydrogenase</th>
<th>Percentage of total oxidation by α-glycerophosphate dehydrogenase</th>
<th>Percentage of total oxidation by lactate dehydrogenase</th>
<th>Percentage of total oxidation by α-glycerophosphate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 mM Creatine phosphate</td>
<td>52.4</td>
<td>47.7</td>
<td>25.6</td>
</tr>
<tr>
<td>20 mM Creatine phosphate</td>
<td>49.5</td>
<td>50.6</td>
<td>48.3</td>
</tr>
</tbody>
</table>

### Table III

The affinities for α-glycerophosphate of α-glycerophosphate dehydrogenases from various vertebrate and invertebrate muscles

<table>
<thead>
<tr>
<th>Source of α-glycerophosphate dehydrogenase</th>
<th>K&lt;sub&gt;a&lt;/sub&gt; (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna white muscle</td>
<td>0.25</td>
</tr>
<tr>
<td>Trout white muscle</td>
<td>1.1</td>
</tr>
<tr>
<td>Turtle white muscle</td>
<td>0.93</td>
</tr>
<tr>
<td>Rabbit mixed muscle</td>
<td>0.5</td>
</tr>
<tr>
<td>Honey bee flight muscle</td>
<td>5.0</td>
</tr>
<tr>
<td>Squid mantle muscle</td>
<td>15.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> α-GP, α-glycerophosphate.

<sup>b</sup> Storey and Hochachka (15).


**Lactate versus α-Glycerophosphate Dehydrogenase**

**TABLE IV**

<table>
<thead>
<tr>
<th></th>
<th>Percentage of total oxidation by lactate dehydrogenase</th>
<th>Percentage of total oxidation by α-glycerophosphate dehydrogenase</th>
<th>Percentage of total oxidation by lactate dehydrogenase</th>
<th>Percentage of total oxidation by α-glycerophosphate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit muscle α-glycerophosphate dehydrogenase</td>
<td>58.6</td>
<td>41.5</td>
<td>89.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Honey bee α-glycerophosphate dehydrogenase</td>
<td>50.0</td>
<td>50.9</td>
<td>65.7</td>
<td>36.4</td>
</tr>
</tbody>
</table>

From the incubation medium, the two forms of α-glycerophosphate dehydrogenase competed with similar effectiveness for NADH. Their contributions to total NADH oxidation were not exactly equal (about 42% versus 50% of total NADH oxidation by the rabbit and bee α-glycerophosphate dehydrogenases, respectively), possibly because of differences in their respective $K_v$ values for substrates and NADH (15). In sharp contrast, large differences appeared in the behavior of the two enzymes in the presence of 2 mM α-glycerophosphate. Under these conditions, the fractional lactate dehydrogenase-dependent NADH oxidation exceeded the oxidation due to rabbit muscle α-glycerophosphate dehydrogenase by nearly 10-fold while it exceeded the oxidation due to bee muscle α-glycerophosphate dehydrogenase by less than 2-fold (Table IV). It is worth re-emphasizing that during anaerobic work in mammalian muscle, α-glycerophosphate levels rise to about 3 μmol/g wet weight (4, 5), i.e. somewhat higher than the concentrations used in the above competition experiments. Thus, with both lactate dehydrogenase and α-glycerophosphate dehydrogenase competing for the same limiting pool of NADH, a high α-glycerophosphate dehydrogenase sensitivity to reaction product strongly diminishes the amount of carbon and hydrogen that can be wastefully channeled from "mainline" glycolysis into α-glycerophosphate.

**DISCUSSION**

According to most current concepts, control of glycolysis in muscle and heart is achieved through metabolite regulation of key regulatory enzymes, such as glycogen phosphorylase (16), hexokinase (17), and phosphofructokinase (18). In lower vertebrates, muscle pyruvate kinase also displays characteristics consistent with a regulatory role in glycolysis (19, 20). However, in cases such as tuna white muscle, which displays an exceptional capacity for anaerobic as well as aerobic glycolysis, control of the above enzyme steps cannot account for the relatively exclusive function of either lactate dehydrogenase (in anaerobic glycolysis) or α-glycerophosphate dehydrogenase (in aerobic glycolysis). The enzymes α-glycerophosphate dehydrogenase and lactate dehydrogenase catalyze what are termed near-equilibrium reactions (21, 22). In *in vivo* measurements of substrates and products always show that the mass action ratio is approximately equal to the thermodynamic equilibrium constant. It is usually assumed that since they always tend toward equilibrium, and usually display high activities, their function simply transmits carbon flow along the pathway, flux changes per se being generated elsewhere. As these enzymes are by definition always near or at equilibrium they are usually unsuitable for regulatory positions, and thus it has become dogma that the "equilibrium" enzymes play secondary roles, if any, in metabolic regulation (23). However, if there is either competition for the substrate of an equilibrium enzyme, or if the substrate has an alternate way of being metabolized, alterations in the rate at which the reaction comes to equilibrium could effectively re-route carbon flow. Control of lactate dehydrogenase and α-glycerophosphate dehydrogenase appears to be an example of this type of role for "equilibrium" enzymes of particular importance in transition from aerobic to anaerobic glycolysis.

Under aerobic conditions creatine phosphate levels are high, and α-glycerophosphate levels are low; lactate dehydrogenase cannot compete for NADH under these conditions, nor for pyruvate. The majority of carbohydrate carbon thus is oxidized in the mitochondrion, and α-glycerophosphate dehydrogenase produces NAD$^+$ for continued glycolysis using catalytic amounts of dihydroxyacetone phosphate. When oxygen becomes limiting the creatine phosphate pool is depleted, and α-glycerophosphate levels rise. The activity of α-glycerophosphate dehydrogenase consequently drops, and competition for NADH now favors the deinhibited lactate dehydrogenase. Pyruvate dehydrogenase can no longer compete with the much higher activities of lactate dehydrogenase, and thus pyruvate is channeled into lactate with no wastage of carbon at the triose phosphate level. Competition is the key element here. Even if lactate dehydrogenase and α-glycerophosphate dehydrogenase always tend toward thermodynamic equilibrium, in slowing down this tendency, the coenzyme of the reaction becomes unavailable as it is used by the competing dehydrogenase. The mass action ratio does not deviate from the equilibrium constant when the reduction reaction is slowed, since excess substrate can be channeled off into glyceraldehyde 3-phosphate or acetyl-CoA, respectively.

Central to our interpretation is the assumption that NADH, at least under some conditions, occurs at limiting concentrations. Until recently, reliable estimates of cytosolic NADH were not available. However, most previous measurements of NADH range between 0.03 and 0.15 μM/g wet weight; these values are for the whole cell, and the NADH concentration in the cytosol can only be less (24-26). NAD$^+$/NADH ratios in the cytosol of liver, brain, and fibroblasts vary from 7 to 2000 (27-29). Assuming about a 1 mM pool size, NADH levels would be estimated at about 0.01 to 0.0005 μM. Such earlier estimates of cytosolic NADH concentration ranges have been closely checked using the technique of turbulent flow to rapidly lyse isolated hepatocytes (30). From these studies, the concentration of free NADH in the cytosol appears to be in the 0.06 to 1.5 μM range under differing metabolic conditions (fasted versus fed nutritional states with and without exogenous ammonia). Perhaps because the NADH-binding site of dehydrogenases is conservative (9), the affinity constants for NADH also are fairly constant, usually in the 0.01 to 0.02 μM range (3, 9, 15, 31). Thus it appears that the affinity constants are substantially higher than the lower limits of current estimates of NADH concentration *in vivo*. So it seems reasonable to assume, at least tentatively, that NADH would often, if not always, be limiting in the cytoplasm. At such times, creatine phosphate and α-glycerophosphate effects on lactate dehydrogenase and α-glycerophosphate dehydrogenase, respectively, would profoundly influence transitions from anaerobic to aerobic glycolysis, and vice versa.

**Acknowledgments**—Special thanks are due to National Marine Fisheries of Honolulu for making available tuna for our experiments.
REFERENCES