# Control of ammoniagenesis in the kidney of water- and air-breathing osteoglossids: characterization of glutamate dehydrogenase

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Glutamate dehydrogenases (EC 1.4.1.2) from the kidney of *Osteoglossum bicirrhosum* (called aruana) and *Arapaima gigas* were kinetically characterized. The two enzymes exhibited several common characteristics including $V_{max}$ activity ratio, pH optimum, affinity for cofactors, a marked preference for NAD(H) over NADP(H), and a very low affinity for $NH_4^+$. A variety of regulatory metabolites affected both enzymes. GTP and GDP were inhibitory while ADP, ATP, AMP, and leucine activated the enzymes. Both enzymes displayed potent product inhibition which was partially reversed by low levels of ADP. *Arapaima* kidney glutamate dehydrogenase was tightly regulated by the adenylate and guanylate nucleotides, inhibition by GTP and GDP and deinhibition by ADP and AMP being much stronger for this enzyme than for the aruana enzyme. Aruana glutamate dehydrogenase, however, was more responsive to NAD–NADH control. The enzyme was more sensitive to NAD(H) product inhibition and this inhibition was poorly reversed by ADP. From these data, it was concluded that both fish kidney glutamate dehydrogenases could function in glutamate oxidation in vivo. However, the *Arapaima* enzyme appeared most clearly adapted to a catabolic role, activity being more tightly linked to the energy status of the mitochondrion. Conversely, the aruana enzyme displayed regulatory properties allowing it the potential to function in NADH oxidation during periods of hypoxic stress.

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L'activité des glutamate-déshydrogénases (EC 1.4.1.2) du rein a été déterminée chez *Osteoglossum bicirrhosum* (aruana) et *Arapaima gigas*. Les deux enzymes ont plusieurs caractéristiques communes, notamment le rapport d'activité $V_{max}$, le pH optimal, l'affinité pour les cofacteurs, une préférence marquée pour le NAD(H) plutôt que pour le NADP(H) et une affinité très faible pour l'ion $NH_4^+$. Les deux enzymes sont affectées par une variété de métabolites régulateurs. Le GTP et le GDP sont des inhibiteurs de l'enzyme, alors que l'ADP, l'ATP, l'AMP et la leucine l'activent. Les deux enzymes sont fortement inhibées par les produits terminaux, inhibition en partie renversée par de petites concentrations d'ADP. La glutamate-déshydrogénase rénale d'*Arapaima* subit un contrôle très serré des nucléotides d'adénylate et de guanylate; l'inhibition de cette enzyme par le GTP et le GDP de même que le renversement de l'inhibition par l'ADP et l'AMP sont beaucoup plus fortes que chez l'aruana. La glutamate-déshydrogénase de l'aruana est cependant beaucoup plus susceptible au contrôle NAD–NADH. L'enzyme de ce poisson est plus susceptible à l'inhibition par le produit terminal NAD(H) et cette inhibition est peu réversible en présence d'ADP. On peut conclure de ces données que les glutamate-déshydrogénases rénales des deux poissons peuvent agir au cours de l'oxydation du glutamate in vivo. Cependant, l'enzyme d'*Arapaima* semble mieux adaptée à un rôle catabolique, activité reliée plus directement au statut énergétique de la mitochondrie. Par ailleurs, l'enzyme de l'aruana a des propriétés régulatrices lui permettant de jouer un rôle dans l'oxydation de NADH durant les périodes de stress hypoxique.

[Traduit par le journal]

## Introduction

The transition to obligate air breathing in fishes is often associated with a decrease in the size and perfusion of the gill. In some of the best studied and well known of air-breathing fishes, such as the lungfishes, the gills are reduced to a few small filaments through which blood can be quickly shunted to the lung. Although this arrangement may have respiratory advantages (see Johansen (1970) for review), it also implies a significant reduction in the

potential role of the gill in ion regulation. In concert with this trend, we reasoned that the relative importance of the kidney in ion balance should rise in air-breathing fishes. In studies of the obligate air breather, *Arapaima*, our reasoning was encouraged by finding that the kidney is greatly increased in size compared with that of aruana, its obligate water-breathing relative. As well, the *Arapaima* kidney displays a much higher potential for energy-requiring transport functions than does the gill (Hulbert *et al.* 1978; Hochachka *et al.* 1978). Total $Na^+/K^+$-ATPase and $Ca^{2+}$-ATPase activities in *Arapaima* kidney are 15- and 3-fold higher than in the aruana kidney. As ammonia is the major nitrogenous excretory product of most teleosts, it is especially significant that $NH_4^+$ is preferred to $K^+$, and that with $NH_4^+$ as the counterion, the salt-activated ATPase of *Arapaima* kidney is in the order of 20-fold higher than in aruana kidney (Hochachka *et al.* 1978). The source of excretory ammonia in *Arapaima* as in other fishes (Walton and Cowey 1977) appears to be glutamate dehydrogenase (GDH), so it is also relevant that the highest GDH activities in *Arapaima* occur in the kidney, GDH occurring at about eightfold higher levels than in the liver or the gills (Hochachka *et al.* 1978). Given the large mass of the *Arapaima* kidney, the above data taken together raise the possibility that a large fraction of waste ammonia may be formed in, and released by, this organ. To better understand how ammoniagenesis is regulated, we turned our attention to the catalytic and regulatory properties of *Arapaima* kidney GDH, comparing and contrasting the enzyme with its homologue in aruana.

We found that, as with mammalian GDHs (Fisher 1973; Mendes-Maurao *et al.* 1975; McGivan and Chappell 1975; Smith *et al.* 1975), kidney GDHs from aruana and *Arapaima* were subject to regulation by a variety of metabolites and showed kinetic properties consistent with bidirectional function. *Arapaima* GDH was tightly regulated by adenylate and guanylate nucleotides, inhibition by GTP and GDP and deinhibition by ADP and AMP being much more effective than for the aruana enzymes. In comparison, aruana GDH appeared more sensitive to $NAD^+$ (NADH) product inhibition and this inhibition was poorly reversed by ADP. The data suggested that both enzymes could function in glutamate oxidation under normal in vivo conditions. However, in *Arapaima*, in keeping with the increased emphasis on the kidney in overall salt, excretory nitrogen, and acid–base regulation (Hochachka *et al.* 1978), kidney GDH seemed better designed for catabolic function through a closer integration of activity with the energy status of the cell.

## Materials and Methods

Animal collection and holding was as described by Hochachka and Randall (1978). All substrates, cofactors, and reagents were purchased from Sigma Chemical Co., St. Louis, MO.

Kidney tissue was dissected out, blotted, and homogenized in a Sorvall omnimixer in five volumes of 100 m*M* imidazole buffer, pH 7.5. The homogenate was centrifuged at 13 000 × *g* for 20 min and the pellet was discarded. The supernatant solution, containing at least 85% of the extractable activity, could be used directly as a source of enzyme for kinetic studies. No purification of the enzyme was attempted for these studies as conditions on board the *Alpha Helix* were not suitable for the required procedures. Also since it is well established that GDH expresses extremely complicated catalytic and regulatory properties (see for example Goldin and Frieden 1971), we felt that an initial kinetic characterization on crude preparations obtained from fresh material would be most suitable. Our GDH preparation, although contaminated with many other enzyme activities, was free of $NAD^+$ reductase and NADH oxidase activities and none of the regulatory metabolites reported in this study were removed or interconverted by other enzymes in the preparation.

GDH activity was assayed spectrophotometrically by monitoring the change in $A_{340\ nm}$ due to oxidation or reduction of NAD(P)(H). All assays were carried out at 25°C in 50 m*M* imidazole buffer and were started by the addition of enzyme. Reaction conditions are as listed in the appropriate table and figure legends. One unit of GDH activity is defined as 1 μmol of substrate utilized per minute per gram wet weight of kidney. Estimates of apparent $K_m$'s were made from Lineweaver–Burk plots of 1/reaction velocity versus 1/substrate concentration. Apparent $K_i$'s and $K_a$'s were estimated from plots of reaction velocity versus substrate concentration. Kinetic constants for allosteric effectors that did not yield hyperbolic curves were derived from Hill plots. Standard deviations were calculated for kinetic constants and results compared using a *t*-test (Pearson and Hartley 1958; Mendenhall 1971).

## Results

The activities of GDH in the kidney of aruana and *Arapaima* are shown in Table 1. GDH activity, 23 U/g, (in the reverse, α-ketoglutarate-utilizing direction) in *Arapaima* kidney is five times that of aruana kidney, possibly reflecting the greater abundance of mitochondria in *Arapaima* kidney (Hochachka *et al.* 1978). These activities can be compared with values of 52 and 11 U/g wet weight (measured at 37°C) for mammalian liver and kidney respectively (Smith *et al.* 1975). Like other GDH's (Goldin and Frieden 1971; Smith *et al.* 1975), the $V_{max}$ activity ratios for these fish GDH's indicate that the enzymes favour the reductive amination reaction.

### *Effect of pH*

The pH optima of both enzymes are similar (Table 1) and closely resemble values reported for other vertebrate GDH's (Goldin and Frieden 1971;

TABLE 1. Comparison of kidney glutamate dehydrogenase from aruana and *Arapaima*

| | Aruana | *Arapaima* |
|---|---|---|
| Activity, U/g wet weight (reverse direction) | 3.6±0.4 | 23.1±1.8 |
| Activity ratio (reverse:forward) | 3.4 | 6.0 |
| pH optimum | | |
| Forward reaction | 9.0 | 9.0 |
| Reverse reaction | 7.5 | 8.0 |
| Kinetic constants, m*M* | | |
| $K_m$ glutamate | 0.20±0.02 | 4.00±0.32 |
| $K_m$ glutamate (+ ADP) | 0.29±0.02* | 4.20±0.34 |
| $K_m$ $NAD^+$ | 1.43±0.11 | 1.00±0.09 |
| $K_m$ $NAD^+$ (+ ADP) | 0.33±0.03* | 0.25±0.02* |
| $K_m$ α-ketoglutarate | 0.20±0.02 | 0.15±0.01 |
| $K_m$ α-ketoglutarate (+ ADP) | 0.29±0.03* | 0.80±0.06* |
| $K_m$ NADH | 0.017±0.001 | 0.010±0.001 |
| $K_m$ NADH (+ ADP) | 0.04±0.003* | 0.09±0.008* |
| $K_m$ $NH_4^+$ | 111.0±8.7 | 50.0±4.1 |
| $K_m$ $NH_4^+$ (+ ADP) | 45.0±4.0* | 50.0±3.8 |

NOTE: Enzymes were assayed under experimentally determined optimal conditions: for the forward reaction 40 m*M* glutamate, 1 m*M* $NAD^+$, 50 m*M* imidazole buffer, pH 7.5; for the reverse reaction 5 m*M* α-ketoglutarate, 0.1 m*M* NADH, 100 m*M* $NH_4^+$, 50 m*M* imidazole buffer, pH 7.1. When added, ADP concentration was 0.1 m*M*. Results are means ± SD of at least six determinations on each parameter.
*Significantly different from $K_m$ in the absence of ADP, $p < 0.01$.

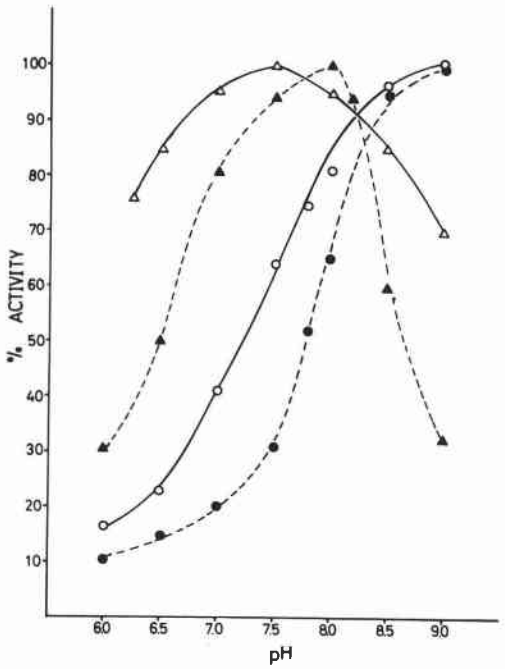


FIG. 1. The pH profiles of kidney glutamate dehydrogenase activity from aruana and *Arapaima*. Enzymes were assayed under optimal substrate conditions (in the presence of 1.0 m*M* ADP) as described in Table 1. Enzyme activity at the pH optimum is arbitrarily set at 100%. Aruana GDH: ○, forward (glutamate-utilizing) reaction; △, reverse reaction. *Arapaima* GDH: ●, forward reaction; ▲, reverse reaction.

Bond and Sang 1968). However, as Fig. 1 shows, the pH profile for the aruana enzyme is much broader in both the forward and reverse directions.

### *Cofactor Specificity*

In common with GDH's from other fish sources (Corman and Kaplan 1967; Corman *et al.* 1967) and from non-mammalian sources in general (Goldin and Frieden 1971; Storey *et al.* 1978), GDH from the kidneys of these two osteoglossiform fishes is essentially specific for NAD(H). NADP(H) is a poor cofactor for the reaction; the aruana enzyme is essentially inactive (<1% of activity with NAD(H)) in the presence of NADP(H) while maximal activity with NADP(H) is only 12% of that with NAD(H) for the *Arapaima* enzyme.

### *Kinetic Constants*

Substrate $K_m$'s in the presence and absence of ADP are given in Table 1. The kinetic constants are similar for both enzymes with the exception of the $K_m$ for glutamate which is 20-fold higher for the *Arapaima* enzyme. However, in the absence of measurements of intramitochondrial glutamate concentrations in these two kidneys, little can be said about this difference. In common with GDH from other non-mammalian sources (Corman and Kaplan 1967; Gilles 1974; Storey *et al.* 1978), the affinity of both enzymes for $NH_4^+$ is low. These high $K_m$'s for ammonium ions, taken together with the modest concentrations of $NH_4^+$ thought to occur in vivo (McBean *et al.* 1966; Walton and Cowey 1977), argue against a role for this enzyme in the reductive amination of α-ketoglutarate under normal circumstances. The $K_m$ for $NAD^+$ is approximately 1 m*M* for both enzymes, and is much higher than that recorded for other GDH's (Goldin and Frieden 1971; Storey *et al.* 1978; Gilles 1974).

The effects of ADP on reaction rate and substrate affinities of aruana and *Arapaima* GDH (Table 1) are similar to the effects of ADP on other GDH's (Goldin and Frieden 1971; Chen and Engel 1975; Fisher 1973). ADP decreases the affinity of the *Arapaima* enzyme for NADH and α-ketoglutarate (about a 10- and 5-fold increase in $K_m$ respectively) while increasing the affinity for $NAD^+$. If these in vitro effects of ADP are valid in vivo the net effect of an increase in intracellular ADP concentrations (a signal indicating the need for increased energy production) would be an increase in the net flux through the forward reaction, i.e., an increase in amino acid oxidation. The effects of ADP on the aruana enzyme are less likely to dramatically alter net flux. ADP lowers the enzyme affinity for both

TABLE 2. Kinetic effectors of kidney glutamate dehydrogenase

| Kinetic constants, m*M* | Aruana | *Arapaima* |
|---|---|---|
| Activators | | |
| $K_a$ ADP | 0.060±0.006 | 0.040±0.004 |
| $K_a$ ATP | 0.30±0.03 | 0.30±0.03 |
| $K_a$ leucine | 0.40±0.04 | 0.30±0.04 |
| Inhibitors | | |
| $K_i$ GTP | 0.0030±0.0003 | 0.0010±0.0001 |
| $K_i$ GTP (+ ADP) | 0.0090±0.0009* | 0.030±0.003* |
| $K_i$ GDP | 0.035±0.003 | 0.010±0.001 |
| $K_i$ GDP (+ ADP) | 0.100±0.010* | 0.080±0.008* |

NOTE: Glutamate dehydrogenase was assayed in the direction of glutamate synthesis using the optimal conditions described in Table 1. $K_i$'s in the presence of ADP were determined at 0.1 m*M* ADP. Results are means ± SD of four determinations on each parameter.
*Significantly different from $K_i$ in the absence of ADP, $p < 0.01$.

α-ketoglutarate and glutamate as well as for NADH. ADP increases the affinity for $NAD^+$ and unlike its effect on the *Arapaima* enzyme, also increases the affinity for $NH_4^+$.

*Metabolite Effects on Enzyme Activity*

Osteoglossiform kidney GDH is activated by ADP and to a lesser extent by leucine, ATP, and AMP (Table 2, Fig. 2). The enzyme is inhibited by GTP and also by GDP (Tables 2 and 3). NADH, and to a lesser extent glutamate, α-ketoglutarate, and $NAD^+$ are product inhibitors of the reaction (Table 3).

*Nucleotide Effectors*

The apparent inhibition constants for GTP and GDP, shown in Table 2, indicate that the guanylates are somewhat more effective inhibitors of *Arapaima* than of aruana GDH. Apparent $K_i$'s for GTP and GDP are fully threefold lower (significantly lower, $p < 0.01$) for *Arapaima* versus aruana GDH. ADP acts not only as an activator of the enzymes (Fig. 2) but also releases the inhibition caused by GTP (and GDP) as evidenced by the large increase in apparent $K_i$'s for GTP and GDP upon addition of 0.1 m*M* ADP. This effect is most pronounced for the *Arapaima* enzyme; ADP increases the apparent $K_i$ for GTP fully 30-fold compared with 3-fold for the aruana enzyme. The effects of nucleotides at saturating substrate concentrations are illustrated in Table 3. GTP at a concentration of 0.01 m*M* reduces the relative activity of GDH to less than 10% of the control activity. The subsequent addition of ADP (at 0.6 m*M*) to the GTP-inhibited aruana enzyme increases GDH activity 10-fold. ADP almost completely overrides GTP inhibition of the *Arapaima* enzyme, increasing enzyme activity by over 100-fold.

ADP, the most effective of the activators of GDH, is also the most effective at reversal of the inhibitory effects of GTP. Thus while ADP can almost completely reverse the effects of 0.01 m*M* GTP on *Arapaima* GDH (90% of the activity in the absence of GTP is restored), leucine, AMP, and ATP cause only 50, 45, and 40% reversal of GTP inhibition respectively.

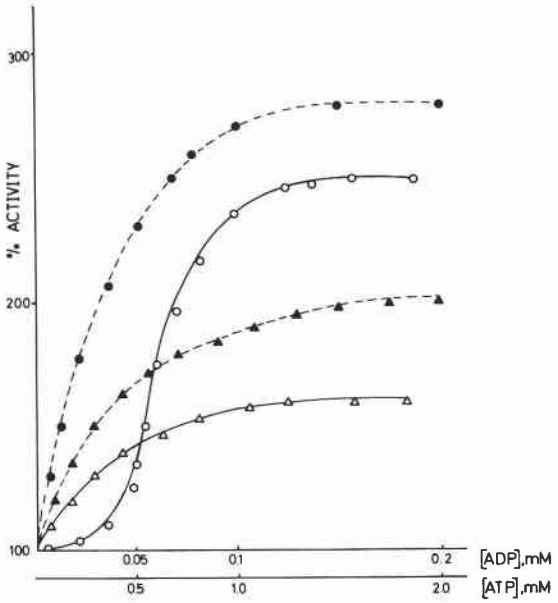


FIG. 2. Effect of activators (ADP or ATP) on kidney glutamate dehydrogenase from aruana and *Arapaima*. Enzymes were assayed under optimal conditions for the reverse (glutamate-producing) reaction as described in Table 1. Activity in the absence of an activator is set at 100%. ATP levels (at 0 ADP) were varied from 0 to 2.0 m*M* while ADP levels (at 0 ATP) were varied from 0 to 0.2 m*M*. In no instances were activators covaried. Aruana GDH: ○, ADP activation; △, ATP activation. *Arapaima* GDH: ●, ADP activation; ▲, ATP activation.

The effect of activators on GDH activity is shown in Fig. 2. Under saturating conditions of activator, both ATP and ADP activate the *Arapaima* enzyme more than the aruana enzyme. Although the activity of both GDH's is potentiated by ADP, the curve relating enzyme activity to ADP concentration (Fig. 2) is hyperbolic for the *Arapaima* enzyme but distinctly sigmoidal for the aruana enzyme. The sigmoidal curve suggests an allosteric interaction between aruana GDH and ADP (Monod *et al.* 1965). The absence of such an effect for the *Arapaima* enzyme suggests some qualitative difference between the two enzymes with respect to nucleotide interactions. Both enzymes are also activated by AMP. The activation curves are hyperbolic and maximal activation occurs only at high AMP concentrations (in the range of 2 m*M*).

In summary the data indicate that *Arapaima*

TABLE 3. Inhibition of kidney glutamate dehydrogenase and the reversal of inhibition by ADP (relative activities)

| Additions | Aruana | | *Arapaima* | |
|---|---|---|---|---|
| | Control | + 0.6 m*M* ADP | Control | + 0.6 m*M* ADP |
| No addition | 100 | 250 | 100 | 280 |
| Nucleotide inhibitors | | | | |
| 0.01 m*M* GTP | 8 | 75 | 2 | 252 |
| 0.05 m*M* GDP | 40 | 180 | 5 | 283 |
| Product inhibitors | | | | |
| 5 m*M* glutamate | 80 | 233 | 72 | 221 |
| 1 m*M* $NAD^+$ | 63 | 135 | 72 | 280 |
| 0.05 m*M* NADH | 0 | 40 | 60 | 196 |
| 1.0 m*M* α-ketoglutarate | 25 | | 27 | |

NOTE: Enzyme activities were measured in the reverse (glutamate-producing) direction except for product inhibition by NADH and α-ketoglutarate. Conditions for the forward and reverse reactions are as in Table 1. $V_{max}$ activity under conditions of no additions is arbitrarily set at 100. Results are the means of four determinations under each condition and are reproducible to within ± 10%.

TABLE 4. Effects of activators and their interaction with ADP (relative activities)

| Additions | Aruana | | *Arapaima* | |
|---|---|---|---|---|
| | Control | + 0.6 m*M* ADP | Control | + 0.6 m*M* ADP |
| No additions | 100 | 250 | 100 | 280 |
| 1 m*M* leucine | 150 | 250 | 250 | 290 |
| 1.5 m*M* ATP | 160 | 250 | 200 | 270 |
| 1 m*M* AMP | 200 | 250 | 230 | 260 |

NOTE: Enzymes were assayed in the forward (glutamate-utilizing) direction under the conditions listed in Table 1. Similar results were found for the reverse reaction. Enzyme activity under conditions of no additions was arbitrarily set at 100. Results are the means of four determinations under each condition and are reproducible to within ± 10%.



GDH is much more tightly regulated by nucleotide interactions than is aruana GDH; GTP (or GDP) is a more potent inhibitor of this enzyme and ADP is more effective in deinhibition of enzyme activity. Of the two GDH's, therefore, *Arapaima* GDH appears to be more tightly regulated by the energy charge of the cell.

*Product Inhibitors*

The products of the GDH reaction, especially those of oxidative deamination, are inhibitors of enzyme activity (Table 3) (Goldin and Frieden 1971; Smith *et al.* 1975). Inhibition in the reverse direction (by glutamate and $NAD^+$) is rather weak at levels of these products thought to be physiological (McBean *et al.* 1966) and the inhibition is reversed by low levels of ADP. The effect of ADP reversal of $NAD^+$ inhibition is greater for the *Arapaima* enzyme. The forward direction of both GDH's is inhibited by NADH concentrations well within the physiological range (McBean *et al.* 1966; Edington *et al.* 1973). The inhibition is especially strong for the aruana enzyme, converting the inhibited enzyme into an essentially unidirectional catalyst in vitro (Table 3). ADP partially reverses NADH inhibition of *Arapaima* GDH but is less effective on the aruana enzyme, restoring only 17% of the total activity in the absence of NADH.

While the *Arapaima* enzyme appears to be tightly controlled by GTP and ADP concentrations, aruana GDH appears to be modified to respond to NAD(H) control. A sudden increase in intracellular NADH concentrations (such as would occur during anoxia) would tend to accelerate the reverse direction of the GDH reaction (which has NADH as a substrate) while inhibiting the forward direction. The net effect of this would be to greatly potentiate reductive amination of α-ketoglutarate. This effect would be much less marked for the *Arapaima* enzyme; NADH is a less effective product inhibitor of this enzyme and the inhibition is readily reversed by moderate levels of ADP.

*Activator Interactions*

The effects of multiple activators on GDH activity are outlined in Table 4. Although mammalian GDH's have specific and distinct binding sites for ADP, ATP, leucine, GTP, and AMP, both activation and inhibition of the enzyme has been shown to be "mutually exclusive" (Fisher 1973; Goldin and Frieden 1971; Frieden 1971). Once the enzyme is fully activated it cannot be further stimulated by

another metabolite and once inhibited, enzyme activity cannot be further dampened by addition of another inhibitor. In this respect the fish enzymes resemble the enzyme from other sources. Thus, GDH fully activated by ADP cannot be further stimulated by the addition of other activators whereas the enzyme partially activated by leucine, AMP, or ATP can be further activated, to a maximum activity, by the addition of another activator, ADP. Similarly, the inhibitors GTP and GDP (data not shown) are 'mutually exclusive' in their effects on the fish GDH's.

## Discussion

To date, although an enormous amount of information is available on the control properties of glutamate dehydrogenase (Goldin and Frieden 1971; Smith *et al.* 1975), it is still not possible to clearly define the metabolic role of this enzyme in animal tissues. We focused on kidney GDH because all evidence indicated that this enzyme has a well-defined biological role, that of the deamination of amino acids. While GDH is capable of function in either direction depending on the substrate being utilized (McGivan and Chappell 1975; Tischler *et al.* 1977; Goldin and Frieden 1971), the fish kidney enzyme is most probably involved in $NH_4^+$ synthesis in vivo. The regulatory properties displayed by the enzyme in vitro may indicate the controls important to a GDH which functions as a deaminase.

The information obtained on the control properties of these two fish GDH's can be classified into two broad categories: (1) the general set of GDH control properties, common to all animal GDH's which serve to define the general role of GDH in metabolism, and (2) the set of special or selected properties of these enzymes which appear to be unique to the organism and seem to fit each enzyme to function in its particular cellular environment. Osteoglossiform kidney GDH's resemble the enzymes from other non-mammalian sources in several general aspects including Michaelis constants (Goldin and Frieden 1971; Storey *et al.* 1978; Di-Prisco 1967), activation by ADP, ATP, AMP, and leucine and inhibition by GTP, GDP, and reaction products (Storey *et al.* 1978; Goldin and Frieden 1971). Using the adenylate control hypothesis of Atkinson (1968), the key regulatory roles of GTP and ADP (i.e. of high versus low energy status) suggest that a primary function of GDH in both aruana and *Arapaima* kidney is in the supply of energy via the oxidation of glutamate.

Of the two enzymes, *Arapaima* GDH seems to be much more tightly linked to the energy status of the cell. The effects of nucleotide regulators on the enzyme are much greater than the corresponding effects on the aruana enzyme and all of the effects appear to function to enhance the enzyme's capacity for the oxidation of glutamate. The *Arapaima* enzyme is controlled in an essentially on–off manner by GTP–ADP interactions. Low levels of ADP severely limit the reductive amination of α-ketoglutarate while potently activating glutamate oxidation (Table 1). Although product inhibition by NADH is evident, low levels of ADP almost completely reverse this effect. From these data we conclude that the enzyme in *Arapaima* kidney is well suited for glutamate oxidation, in keeping with a need for increased ammonia production and excretion by the kidney in this species (Hochachka *et al.* 1978).

Aruana GDH, while clearly capable of a glutamate oxidizing function, seems less tightly linked to a catabolic role. The enzyme is less strongly affected by GTP–ADP interactions than is *Arapaima* GDH (Table 3). This decreased dependence on nucleotide control is further evidenced by the reduced effectiveness of ADP in dampening the reductive amination reaction (Table 1) and in reversing the potent NADH inhibition of the enzyme. In an animal experiencing frequent hypoxic excursions, any increase in NADH concentrations in the kidney as a result of hypoxia could be partially handled by utilizing the GDH reaction for intramitochondrial NADH reoxidation. Such a rise in NADH concentrations would strongly inhibit glutamate oxidation (Table 3) while potentiating the reductive amination of α-ketoglutarate. A rise in ADP levels during hypoxic stress would have little effect in overriding this NADH effect on the aruana GDH. Thus the control properties of this enzyme ensure function in the reverse direction as long as NADH levels remain high. This role in redox regulation is unlikely for the *Arapaima* enzyme as NADH inhibition of the forward reaction (glutamate oxidation) is much less potent than that shown by the aruana enzyme and is completely reversed by low levels of ADP.

Thus, in conclusion, *Arapaima* GDH seems to have been modified to perform a primarily catabolic role in keeping with the increased emphasis on $NH_4^+$ secretion by the kidney in air-breathing fish. The aruana enzyme retains the more generalized regulatory pattern that would allow an alternating role for the enzyme in glutamate catabolism and redox regulation.

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ATKINSON, D. E. 1968. The energy charge of the adenylate pool as a regulatory parameter. Interactions with feedback modifiers. Biochemistry, **7**: 4030–4034.

BOND, P., and J. SANG. 1968. Glutamate dehydrogenase from *Drosophila* larvae. J. Insect Physiol. **14**: 341–359.

CHEN, S., and P. ENGEL. 1975. Equilibrium protection studies of the interaction of bovine glutamate dehydrogenase with purine nucleotide effectors. FEBS Lett. **58**: 202–205.

CORMAN, L., and N. O. KAPLAN. 1967. Kinetic studies of dogfish liver glutamate dehydrogenase. J. Biol. Chem. **242**: 2840–2846.

CORMAN, L., L. PRESCOTT, and N. O. KAPLAN. 1967. Purification and kinetic characterization of dogfish liver glutamate dehydrogenase. J. Biol. Chem. **242**: 1383–1390.

DIPRISCO, G. 1967. Desensitization of the allosteric sites of glutamate dehydrogenase by fluorodinitrobenzene. Biochem. Biophys. Res. Commun. **26**: 148–152.

EDINGTON, D., G. WARD., and W. SAVILLE. 1973. Energy metabolism in working muscle: concentration profiles of selected metabolites. Am. J. Physiol. **224**: 1375–1380.

FISHER, H. 1973. Glutamate dehydrogenase-linked complexes and their relationship to the mechanism of the reaction. Adv. Enzymol. **39**: 369–417.

FRIEDEN, C. 1971. Glutamate dehydrogenase. Annu. Rev. Biochem. **40**: 666–696.

GILLES, R. 1974. Effect of NaCl on the activity of *Eriocheir sinensis* glutamate dehydrogenase. Int. J. Biochem. **5**: 623–628.

GOLDIN, B., and C. FRIEDEN. 1971. L-Glutamate dehydrogenases. Curr. Topics Cell. Regul. **4**: 77–117.

HOCHACHKA, P. W., T. W. MOON, J. BAILEY, and W. C. HULBERT. 1978. The osteoglossid kidney: correlations of structure, function, and metabolism with transition to air breathing. Can. J. Zool. **56**. This issue.

HOCHACHKA, P. W., and D. J. RANDALL. 1978. *Alpha Helix* Amazon Expedition, September–October 1976. Can. J. Zool. **56**. This issue.

HULBERT, W. C., T. W. MOON, and P. W. HOCHACHKA. 1978. The osteoglossid gill: correlations of structure, function, and metabolism with transition to air breathing. Can. J. Zool. **56**. This issue.

JOHANSEN, K. 1970. Air breathing in fishes. *In* Fish physiology. Vol. 4. *Edited by* W. S. Hoar and D. J. Randall. Academic Press, New York and London. pp. 361–411.

MCBEAN, R., M. NEPPEL, and L. GOLDSTEIN. 1966. Glutamate dehydrogenase and ammonia production in the eel. Comp. Biochem. Physiol. **18**: 909–920.

MCGIVAN, J., and J. CHAPPELL. 1975. On the metabolic function of glutamate dehydrogenase in rat liver. FEBS Lett. **52**: 1–7.

MENDENHALL, W. 1971. Introduction to probability and statistics. 3rd ed. Duxbury Press, Belmont. pp. 230–232.

MENDES-MAURAO, L., J. MCGIVAN, and J. CHAPPELL. 1975. Effects of L-leucine on the synthesis of glutamate and urea by isolated rat liver cells. Biochem. J. **146**: 457–464.

MONOD, J., J. CHANGEUX, and M. JACOB. 1965. On the nature of allosteric transition: a plausible model. J. Mol. Biol. **12**: 88–118.

PEARSON, E., and H. HARTLEY. 1958. Biometrika tables for statisticians. Vol. 1. Cambridge University Press, Cambridge. p. 164.

SMITH, E., B. AUSTEN, K. BLUMENTHAL, and J. NYC. 1975. Glutamate dehydrogenases. *In* The enzymes. Vol. 11. *Edited by* P. Boyer. Academic Press, New York. pp. 293–368.

STOREY, K. B., J. H. A. FIELDS, and P. W. HOCHACHKA. 1978. Purification and properties of glutamate dehydrogenase from the mantle muscle of the squid, *Loligo pealeii*. Role of the enzyme in energy production from amino acids. J. Exp. Zool. In press.

TISCHLER, M., P. HECHT, and J. R. WILLIAMSON. 1977. Effect of ammonia on mitochondrial and cytosolic NADH and NADPH systems in isolated rat liver cells. FEBS Lett. **76**: 99–104.

WALTON, M., and C. COWEY. 1977. Aspects of ammoniagenesis in rainbow trout. Comp. Biochem. Physiol. **57B**: 143–149.