

**MITOCHONDRIAL LACTATE DEHYDROGENASE:
A SOLUTION TO SUBSTRATE DEPLETION AND
REDOX IMBALANCE CAUSED BY
GLUTAMATE/GLUTAMINE FORMATION**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Ammonia is an important toxicant produced and detoxified internally in animals. Ammonia is normally excreted by teleosts directly to the water by diffusion across the gills. However, under certain conditions, such as lack of water and high ambient pH or ammonia, when ammonia excretion is impeded, ammonia concentration inside the fish body increases. If fish are unable to detoxify ammonia accumulating inside the body, they will enter into convulsions, and die.

Many strategies for dealing with increased ammonia levels in animals have been described (Ip et al., 2001). One of them is the conversion of ammonia to less toxic compounds such as urea and glutamine. Glutamine is produced from glutamate and NH_4^+ , catalyzed by glutamine synthetase (GS). Glutamate may in turn be produced from α -ketoglutarate (α -KG), catalyzed by glutamate dehydrogenase (GDH), with NADH acting as co-enzyme. In other words,

formation of one glutamine molecule allows detoxification of two ammonia molecules.

There are, however, potential problems associated with glutamine formation. Glutamine formation requires glutamate as a substrate. Glutamate is formed in the mitochondrial matrix. When a large amount of glutamate is turned into glutamine for ammonia detoxification, equally large amounts of α -KG and NADH are needed. Consumption of α -KG would pull it away from the Krebs cycle, and oxidation of NADH in the mitochondrial matrix would disrupt the redox balance of this compartment. It has been suggested that this could be the mechanism behind ammonia toxicity (Bessman and Bessman, 1955).

Yet, it has been observed that certain fish species choose this strategy of ammonia detoxification. The sleeper, *Bostrichthys sinensis*, has been reported to produce and store glutamine during aerial exposure (Ip et al, 2001). Therefore, this fish has been chosen for investigation of the mechanisms involved in dealing with problems associated with glutamine formation.

The GS activities in sleeper exposed to 15 mM NH_4Cl for 48 h were 4-fold higher than those in control. Subcellular localization of liver enzymes reveals that GS in this fish is found in the cytoplasm. This suggests that glutamine formation takes place in the cytoplasm, while its substrate, glutamate, comes from the mitochondria. In addition, it was found that glutamine was not a good oxidative substrate for the mitochondria, unlike the situation in most other fish. This could prevent a futile cycle of glutamine formation in the cytoplasm and degradation in the mitochondria.

Lactate was found to be a good oxidative substrate for the mitochondria. High specific activities of lactate dehydrogenase (LDH) were found to associate with the mitochondrial fraction. We propose a model explaining the role of this mitochondrial LDH in solving the problems caused by glutamate/glutamine formation (Figure 1). Lactate enters the mitochondrial matrix and is turned into pyruvate by LDH. NADH thus formed can be used for the GDH reaction, balancing the mitochondrial redox. Pyruvate formed from lactate can feed into the Krebs cycle, producing α -KG, and replenishing the substrate used for glutamate formation. The following experiment was carried out to support this model: incubation of mitochondria with ^{14}C -lactate and ammonia resulted in ^{14}C -glutamate formation.

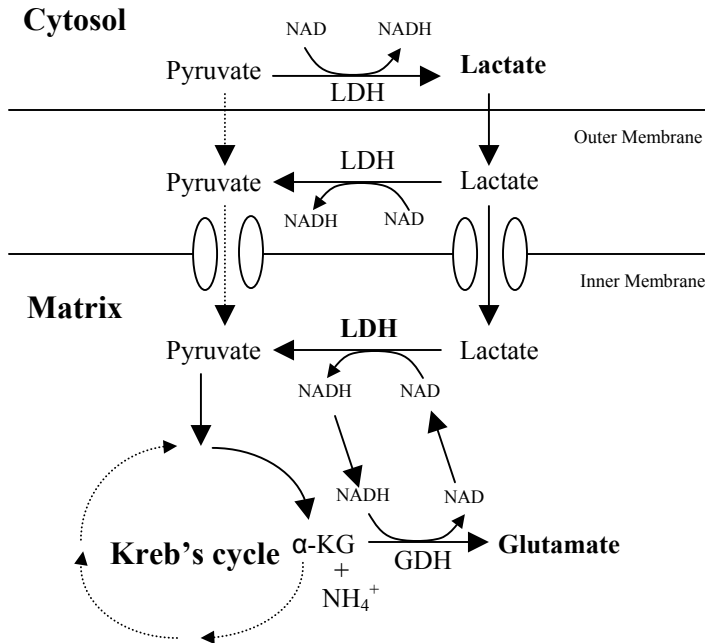


Figure 1. Proposed model for the role of mitochondrial lactate dehydrogenase (LDH) in glutamate formation. α -KG: α -Ketoglutarate; GDH: Glutamate dehydrogenase.

The presence of mitochondrial LDH has been demonstrated recently in rat mitochondria (Brooks et al, 1999). The present paper, however, represents the first report on the role of mitochondrial LDH in glutamate/glutamine formation. It is well known that animal brains detoxify ammonia to glutamine (Cooper and Plum, 1987). If mitochondrial LDH is present in the brains of different animals, then the “problems” with glutamate/glutamine formation may actually not exist. Together with other data obtained on brain metabolites during hyperammonemia (see Cooper and Plum, 1987 for review), the proposed model dispels the “Krebs cycle depletion theory” put forward by Bessman and Bessman (1955). Ammonia toxicity is therefore mediated via some other mechanism(s).

References

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