Enzyme as a Tool for Establishing the Metabolic Status in Animal Model

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Introduction

All the living organisms are habituated to live in congenial environment. Any change in the environmental conditions will alter the physiological and metabolic activities of the living being. Intensity of exposer to the adverse condition may further lead to the stimulation of stress, which involves primary, secondary and tertiary stress responses, in the meantime, intermediate metabolism gets activated to revamp homeostasis (Wendelaar Bonga, 1997; Iwama *et. al*., 2006). Most of the research carried out on stress and metabolism are mainly based on the metabolite present in the plasma or serum but not on enzyme activity. Though, analysis of metabolites present in the tissue, plasma and serum will provide information about the energy consumption during stress or unfavourable situations, it will not give an idea about the metabolic pathways unless the enzyme activities are analyzed (Montero *et. al*., 1999; Santos *et .al*., 2010).

In the present context, knowing and understanding the mechanisms of organism during the environmental variations, has captured a great attention among the researchers. As an outcome, researchers have shown interest in determining the physiological conditions of living organisms in natural contest (Dahlhoff, 2004). Biochemical indicators of metabolic activity are key components of synthetic or metabolic biochemical pathways that are directly or indirectly linked to the processes important for survival, growth and reproduction. Biochemical indicators of stress are usual process of the cellular stress response, which are elevated when the animal or living organism is exposed to unfavourable conditions. The reciprocal effect will lead to altering protein synthesis or metabolism that impact growth performance and reproduction (Dahlhoff, 2004).

Is it worth and reasonable to manipulate the metabolism of an organism, knowing the fact that cellular process has been constantly modified and refined through evolution and natural selection for adapting, in the most convenient manner to the ongoing environmental situations The answer to this question seems evident, there are three broad areas of research and developments identified, wherein which the manipulation of metabolic pathways is relevant: (a) Drug design to treat diseases, (b) Genetic engineering of organisms of biotechnological interest, and (c) Genetic syndromes therapy (Moreno-Sánchez *et. al.*, 2008). In addition to above, the metabolism of an animal can be manipulated by dietary supplementation.

Enzyme as tool

Use of enzyme in the field of medical science to diagnosis a disease is one of the achievements out of the intensive research in biochemistry since the 1940's. Enzymes have provided the basis for the field of clinical chemistry. Metabolism is a process where in which tremendous biochemical activity exists in living cell. Chemical and physical change goes on continuously in the living organism to build new tissue, repair or replace the old and damaged tissue, conversion of energy, etc. A number of enzyme/ metabolic pathways are existing in the living organism or animal. Many of the molecular transformations that occur within cells require multiple steps to accomplish. The series of chemical reactions is the best example for metabolic pathway in which the product of one reaction becomes the substrate for the next reaction. The biochemical reactions management within the cell by the enzyme is the most crucial part. According to change in the environmental condition, enzymatic activity allows a cell to respond and regulate its metabolic pathways, both of which are essential to cell survival.

Generally, hematological parameters are also used as fish biomarkers. The responses from the fish are non-specific to environmental stressors but provide useful information in the eco-toxicological studies and risk assessment. Enzymes like serum transaminases, alkaline and acid phosphatases, lactate dehydrogenase, and blood glucose are considered very important parameters to evaluate the stress response in fish (Rudneva, 2013).

Transaminases

Alanine transaminase (ALT) and Aspartate transaminase (AST): ALT and AST helps to catalyse the inter-conversion of amino acids and α-keto acids by transfer of amino groups. Alanine transaminase catalyzes the transfer of an amino group from alanine to α-ketoglutarate to form glutamate and pyruvate. Similarly, aspartate

transaminase catalyzes the transfer of the amino group from aspartat to α-ketoglutarate to form glutamate and oxaloacetate (van der Oost *et al.*, 2003). These amino transferases are the bridge between the carbohydrate and protein metabolism. ALT and AST are found in the blood serum, cytoplasm and mitochondria of various tissues. When the living organism or an animal is exposed to unfavourable conditions, the enzyme activity gets disturbed, and any change in the aminotransferase activities cause the disturbances of Kreb's Cycle, decrease the cycle intermediates and AST and ALT compensates through providing α–glutarate (Ugwemorubong *et. al.*, 2009). Elevated enzyme activity in the blood serum, plasma and tissues of an animal exposed to unfavourable conditions was well documented by the researchers across the globe. AST and ALT are being used as clinical diagnostic tool and it is linked with cell necrosis of the liver and skeletal or cardiac muscle starvation. (Coppo *et al.*, 2001;2002).

Lactate and malate dehydrogenase

Lactate dehydrogenase: LDH is the pivotal enzyme of the Embden–Meyerhof–Parnas (EMP) Pathway. LDH converts lactate to pyruvate in the presence of coenzyme NADH which is converted to NAD^+. LDH helps in upholding the glycolysis cycle by supplying NAD^+. In the presence of sufficient oxygen, pyruvate enters the Kreb's Cycle, on the contrary, pyruvate is converted to lactate (Murray *et al.*, 2000). In the EMP pathway, this reaction is reversible (Kaneko *et al.*, 2008 and Panteghini *et al.*, 2008). Increased level of serum LDH has been well recorded during the renal infarction (Winzelberg *et. al.*, 1979). Drent *et. al.* (1996) has reported that LDH acts as an indicator of disturbed cellular integrity induced by pathological conditions. Any damage in the tissue level can significantly elevate LDH activity; therefore, LDH enzyme assay can be used to identify cell injury or cell death (Panteghini *et. al.*, 2008). Exposing fish to endosulfan resulted in the increase in LDH activity in kidney and found to influence by the concentration and period of exposure (Rani and Ambili, 2006). Recently, Tejpal *et. al.*, (2017) has reported that dietary supplementation of thiamine and pyridoxine-loaded vanillic acid-grafted chitosan microparticles could modulate the expression of energy metabolic enzymes, lactate dehydrogenase and uphold the metabolic homeostasis at tissue level in albino rats.

Malate dehydrogenase: Malate dehydrogenase is the critical enzyme in the mitochondrial TCA cycle. It is a homodimeric (α) sulfhydryl enzyme which oxidizes malate to oxaloacetate, using NAD^+ as the electron acceptor. MDH activity is expected to increase during gluconeogenesis. The oxaloacetate formed from the deamination of aspartate and the pyruvate formed from deamination of various amino acids *viz.* alanine,

serine, tryptophan are converted to malate or phosphoenol pyruvate (PEP) in the mitochondria. The malate and PEP are transferred to cytosol where they are converted to glucose. The conversion of oxaloacetate to malate in the mitochondria and the reverse in cytoplasm is catalyzed by MDH (Das *et. al.*, 2006). Published reports in the field of medical sciences have shown that the patients died with Alzheimer's disease are found to have higher MDH activity in brains (Bubber *et .al.*, 2005). Akhtar *et. al.* (2010) has recorded higher MDH activity in *Labeo rohita* fingerlings exposed to endosulfan-induced stress, and it indicates greater activity of TCA cycle mainly because of its energy demand.

Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP): Alkaline phosphatase (ALP) is membrane-associated glycoprotein which is produced in the liver and playing a role in transport of ions and absorption of water across cell membranes. It is used as a diagnostic tool for evaluation of liver function and its pathologies and its activity depends on age of fish, maturation stage, water pollution, food composition, temperature and peculiarities of fish biology and ecology (Mehdi *et. al.*, 2011). Acid phosphatase (ACP) is a phosphatase enzyme, usually freely attached to phosphoryl groups from other molecules during assimilation. ACP are found in different forms in various organs. ALP and ACP enzymes are considered as indicators of health status of the animals or living beings. Elevated activity of ALP and ACP are reported to be related with various diseased conditions. Dietary supplementation of thiamine and pyridoxine loaded vanillic acid-grafted chitosan are reported to have lower ALP and ACP activity and maintain metabolic homeostasis in rats (Tejpal *et. al.*, 2017).

Acetylcholine esterase (AChE): Acetylcholine esterase is the primary cholinesterase present in the human body. The enzyme helps in break down of acetylcholine into choline and acetic acid. This splitting process helps to avert the accumulation of acetylcholine in the synaptic buds and maintain the normal function in the body. On the contrary, accumulation of acetylcholine disturbs the energy metabolism of nervous system and stops the communication of nerve impulses; further this may cause the behavioral changes in the animal (Sarma *et. al.*, 2010; Colovic *et. al.*, 2013). Lower level of AChE activity in control group indicates higher levels of accumulation of acetylcholine in the brain tissue. The increase in concentration of TPVGC in the diet increases the AChE activity. In the neurotransmission process of central and peripheral nervous system, AChE plays a critical role.

Antioxidant enzymes: Antioxidants are of different types. They are natural, scavenging or chain breaking, pharmacologic antioxidants and others. Antioxidants helps in the

process of counteracting free radicals. Therefore, one must continually produce more of the antioxidants in the body or ingest them either in diet or by supplementation. Many enzyme systems help to catalyze the reactions to counteract free radicals and reactive oxygen species, for example superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione *S*-transferases (GST).

Superoxide dismutase (SOD): In the year 1967, Irwin Fridovitch and Joe McCord of Duke University have discovered the antioxidant enzyme SOD, which provides an important means of cellular defence against free radical damage. Superoxide dismutase helps in the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in blood plasma, serum and different tissues. In addition, SODs contain metal ion cofactors which are isozyme specific (Krishnamurthy and Wadhwani, 2012).

Catalase (CAT): The presence of catalase was first noticed by Louis Jacques Thénard in the year 1811, when he discovered H_2O_2 and suggested that breakdown of hydrogen peroxide is by unknown substance. In the year 1900, Oscar Loew has coined it as catalase and reported the presence of CAT in many plants and animals. Catalase is an antioxidant enzyme which helps in conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese as a cofactor. CAT is present in the blood plasma, serum and different tissues (Krishnamurthy and Wadhwani, 2012).

Conclusion: Enzymes are proteinaceous in nature and are organic catalysts which assist in facilitating physical and chemical reactions in the living being. Enzymes are needed to carry out the physiological and metabolic activities in the body and will play vital role in respiration, digestion and other important life processes. When enzymes function properly, homeostasis is maintained. Generally, during unfavourable conditions, enzyme activity gets altered and it can be maintained by taking suitable dietary supplementation.

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