REGULATORY CHANGES IN ENZYMES OF MALATE-ASPARTATE SHUTTLE DURING DEVELOPMENT AND AGING OF MICE

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ABSTRACT

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ABSTRACT

Enzymes are specific proteins that catalyze chemical reactions in biological systems. Living cells have evolved a complex regulatory mechanism to control the concentrations of their enzymes, particularly those catalyzing critical metabolic reactions. In an organism, several physiological and biochemical changes occur during development, growth, adulthood and senescence. The developmental phase includes an increase in the number and size of cells, and their differentiation to perform specialized functions. The metabolic events that occur during development might influence the later part of lifespan. Aging is the characteristic of all multicellular organisms. The functional abilities of most organs and the organisms decrease during senescence. The decline becomes perceptible towards the later part of the reproductive phase. Thus, the reproductive phase smoothly merges into the senescence phase, unlike the transition from the developmental to the reproductive phase in which specific genes are expressed and specific structures and functions appear that confer reproductive ability to the organism. During senescence, adaptability to external and internal stresses decreases and the homeostatic mechanisms deteriorate and that increase the susceptibility in old age.

During development and aging, different metabolic adjustments take place as an adaptation to the changing demand made upon them. Study of all the enzymes of a particular metabolic pathway provides a complete profile of their biological functions. Keeping in view the importance of studying all the enzymes of a particular metabolic cycle, the work embodies in this thesis was planned to study the regulatory changes in enzymes of malate-aspartate shuttle to elucidate the mechanism of regulation of this shuttle during development and aging.

The malate-aspartate shuttle appears to be the primary mechanism for the transfer of reducing equivalent from the cytosolic NADH to the mitochondria in many animal tissues. It has been seen that inner mitochondrial membrane is impermeable to NADH. The NADH formed during glycolysis in the cytoplasm by the oxidation of glyceraldehyde-3-phosphate must be regenerated to NAD^+ for glycolysis to operate. The shuttle involves an influx of malate and glutamate and efflux of aspartate and ketoglutarate from the mitochondria. The main enzymes of the shuttle are malate dehydrogenase and aspartate aminotransferase. Both these enzyme have two homologous and genetically independent isoenzymes. One in the cytosolic and the other in the

mitochondrial fraction. The present study aims :

(a) to assess the endogenous activity level of shuttle enzymes in a postnatal age - and tissue - specific manner

b) regulation of enzymes of shuttle by various hormones such as glucocorticoid and thyroid hormone during development of mice

c) lastly, to purify one of the shuttle enzymes that is cytosolic aspartate aminotransferase and to study its chemical and kinetic properties in order to find out changes, if any, in properties as a function of age.

Endogenous level of shuttle enzymes :

The endogenous activities of isoenzymes of malate dehydrogenase and aspartate aminotransferase show a significant change during postnatal development of mice. The activities of both the isoenzymes (cytosolic and mitochondrial) of malate dehydrogenase (MDH) and aspartate aminotransferase (AsAT) were significantly higher in the liver of mice at day 15, declined at day 30 and remained unchanged thereafter until day 60. In contrast, the activities of these isoenzymes showed a lower value at day 15, increased to a peak value at day 30 in the kidney of mice. It indicates an early developmental expression of shuttle enzymes in the liver than in the kidney of mice which may in turn exhibit an early involvement of malate-aspartate shuttle in the transfer of reducing equivalents to compensate the metabolic demands of this tissue in growing mice. Reconstitution studies confirmed the observation of malate- aspartate shuttle enzymes in liver and kidney during postnatal development of mice.

Hormonal regulation of shuttle enzymes :

It was observed that adrenalectomy decreases and administration of hydrocortisone to adrenalectomized mice increases the activity of cytosolic and mitochondrial malate dehydrogenase and cytosolic aspartate aminotransferase in the liver of 15-, 30-, and 60 -day old mice. Per cent decrease following adrenalectomy is almost similar in all the postnatal ages studied. This indicates that adrenal steroid do play a role in the regulation of the shuttle enzymes.

Adrenalectomy also decreases and administration of hydrocortisone to adrenalectomized

mice increases the activity of kidney cytosolic and mitochondrial malate dehydrogenase and cytosolic aspartate aminotransferase only in 30- and 60- day old mice. It has not shown any effect on the activity of these enzymes in preweaned mice (15-day old). It may be due to the differential level of glucocorticoid receptors and other trans-acting factors in the liver and kidney of mice during this phase of postnatal development. These findings corroborate the observations that the same enzyme in different tissues of the developing animals might be regulated differentially by the same physiological stimuli. The hormonal signals important in the developmental formation of the enzymes may or may not regulate the level of the same enzyme in adult tissues. Differential hormonal regulation of AsAT isoenzymes indicate that they are subject to different physiological controls in different tissues.

Various doses of Bt_2-cAMP, a membrane permeable analog of cyclic AMP, were administered in different postnatal ages of normal male mice. None of these single doses of Bt_2-cAMP were effective on both isoenzymes of MDH and AsAT in the liver and kidney of mice. The finding indicated that none of the shuttle enzyme of mice liver and kidney are regulated by cAMP at those postnatal ages studied. Last couple of years, group of workers visualized the cross-talk between steroid and protein/ peptide hormone action. Hence, in order to find out the synergistic or antagonistic role of cyclic AMP on hydrocortisone action, a combination of Bt_2-cAMP with hydrocortisone was injected in the 15-, 30- and 60- day old mice. It has been observed that only liver mitochondrial malate dehydrogenase and cytosolic aspartate aminotransferase show an increase in the activity. In case of mitochondrial malate dehydrogenase, the increase in activity has been seen only at 30- and 60- day of postnatal age. The activity of both the shuttle enzymes show no effect of this combination in the kidney of mice at either of the age groups. This indicates that only liver and not the kidney is possibly equipped with the cross-talk mechanism in regulating the enzyme activities.

It is seen that administration of T_3 which is a potent thyroid hormone on normal mice of three different postnatal ages (i.e. 15-, 30- and 60- day) showed no significant change in the activities of cytosolic and mitochondrial malate dehydrogenase as well as aspartate aminotransferase in liver and kidney. Most likely this might be due to the tonic regulation of the enzymes by the endogenously circulating level of the thyroid hormones.

Chemical and kinetic properties of c-AsAT :

To find out the change, if any as a function of age, in the chemical and kinetic properties, one of the shuttle enzyme i.e. c-AsAT was isolated and purified from the mice liver of two selected ages (i.e. 15- and 180- day) using similar experimental conditions. The enzyme preparations from both the ages were passed through the CM-cellulose column and the elution profile of the specific activities of this isoenzyme from the liver of two ages of mice exhibited the requirement of two different ionic strength. This indicates that there might be an overall charge difference on the isoenzyme from two different age groups. It was further confirmed by running enzyme preparations onto polyacrylamide gel electrophoresis and staining the gels with general and specific stains. The isoenzyme from immature and mature ages migrated at two different levels, confirming the change difference onto C-AsAT from two ages. Changes in the isoenzyme patterns and their electrophoretic mobilities have earlier been reported and reviewed. They can arise due to genetic variability or sometimes due to epigenic events (such as acetylation, phosphorylation and proteolysis) depending on the metabolic demand to commensurate the requirement at specific stage of development.

Kinetic analysis of data indicates no significant difference between the K_m values of this enzyme for both the substrates in immature and mature mice. However, the enzyme from the mature mice showed higher V_{max} and K_{cat}, indicating higher turnover compared to the immature one. This indicates that the c-AsAT from mature mice catalyses the reaction at a faster rate than that of c-AsAT from immature mice, albeit the binding affinities for substrates remained the same. It may reflect that the substrate binding site of the enzyme is not affected for by the charge difference between the enzymes from the two ages. However, the charge difference in the c-AsAT of two ages might contribute to the catalytic turnover of the enzyme at respective ages. The higher catalytic rate of mature enzyme might extend an adaptation to control the metabolic demands of the mature mice since malate-aspartate shuttle is one of the major control point for glycolysis, Krebs cycle and gluconeogenesis. Inactivation studies of the enzymes from both the ages depict differential folded structure as envisaged by the different requirement of urea for their 50% inactivation. It further corroborates our earlier assumption that there is a difference in the overall charge of the enzyme at two ages.

It may be concluded that the enzymes of malate-aspartate shuttle as well as the shuttle activity expressed differentially in different tissues of mice as a function of postnatal development.

And the shuttle enzymes are also regulated differentially by glucocorticoid where as they do not exhibit any change in intact mice with the exogenously added cAMP as well as thyroid hormones. However, a combination of cAMP and glucocorticoid regulates the shuttle enzymes differentially ia tissue- and age- specific manner. Purification and kinetic analyses show a definite charge difference in C-AsAT isoenzyme at two different ages i.e. immature and mature. The K_m remains the same while catalytic efficiency is higher in mature as compared to immature owing to greater adaptation in mature animals.

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