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RESEARCH ARTICLE

BIOENERGETIC STUDY OF RENAL B-GLUCURONIDASE IN INDIAN MAJOR CARP, *LABEO ROHITA*

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β-Glucuronidase is a lysosomal enzyme. It plays an important role in carbohydrate metabolism. The renal β-glucuronidase at optimum pH-5 was observed to produce ΔG, -2.074 kcal/mole at 453 K. Reduction in ΔG was recorded above and below these pH optima. With the increase in temperature, the ΔG was decreased from -2.3 kcal/mole at 358 K to -10.2 kcal/mole at 834 K at constant pH-4.5. However, with the increase in enzyme and substrate concentration ΔG was seen to increase at constant temperature 435 K and pH-4.5 for 1 h incubation. For all the above experimental parameters, the equilibrium constant (Keq, 0.641) was kept constant. It was concluded that the enzyme is greatly affected by the pH and the temperature in the course of time. These factors might have distorted the physical structure of the enzyme and hence the efficiency to catalyze the reaction hampered and resulted in the decrease in ΔG. However, the increase in enzyme and substrate concentration favors the reaction by enhancing the subsequent increase in ΔG in the renal β-glucuronidase of *Labeo rohita.*

Key words: β-Glucuronidase, pH, Temperature, Enzyme, Substrate, ΔG.

INTRODUCTION

Phenolphthalein-mono-β-D-glucuronide was used as a substrate for evaluating the β-Glucuronidase activity by several workers (Wakabayashi and Fishman, 1961; Kanase, 1978; Herber *et al.*, 1980; Lampe *et al.*, 2002; Heide *et al.*, 2004; Chilke, 2006 and 2009). β-Glucuronidase enzyme is known for many decades to work in the carbohydrate metabolism in bacteria, plants and animals. Himeno *et al.*, (1974) suggested the chemical nature of the enzyme. It consists of amino acids and carbohydtrate and has the molecular weight 29000. It is composed of four identical subunits that play role in the stepwise degradation of glucuronide containing glycosaminoglycans. In human, the deficiency of this enzyme results in the clinical genetic disorder mucopolysaccharides type VII (Sly *et al.*, 1973) which is characterized by an accumulation in lysosomes of glycosaminoglycans containing terminal glucuronic acid residue (Hall *et al.*, 1973 and Sly *et al.*, 1973). Many authors have worked on the kinetics of β-glucuronidase in mammals (Talalay *et al.*, 1946, Fishman, 1947, Bernfeld *et al.*, 1952, Rugenburg and Seligman, 1953, Wakabayashi and Fishman, 1961) and on fish (Chilke, 2009). Though, much work is available on the biochemical and histochemical parameters but is no work has been carried out still on the bioenergetics with respect to this enzyme. Therefore, in the present work, an attempt was made to study the effect of different parameters variable on the energetic of β-glucuronidase in the renal tissues of Indian teleost, *Labeo rohita.*

MATERIALS AND METHODS

Matured and healthy fish *Labeo rohita* was purchased from Amal-Nala Dam, Gadchandur. Kidney was excised after decapitation and brought to the laboratory in an ice cold 0.1 M Phosphate Buffer Saline (PBS) with pH-7.4. Phenolphthalein mono-β-D-glucuronide was used as substrate (Sigma, USA). Sodium Acetate, acetic acid, glycine, sodium hydroxide and sodium chloride were purchased from Himedia, Pvt. Ltd. Mumbai.Tissue extract was prepared in an ice cold PBS and raised to 1% final concentration. Biochemical assay of the β-glucuronidase was carried by using Phenolphthalein mono-β-D-glucuronide as substrate (Talalay *et al.*, 1946 and Fishman *et al.*, 1948). In this experiment, the use of TCA was excluded. Required grades of substrate were prepared in acetate buffer (0.1M, pH-4.5).

For the effect of enzyme concentration, 1% stock tissue extract was used with subsequent increased quantity. At the end of incubation, all the test tubes were immersed in boiling water for one minute and to each tube 1.5 ml double distilled water was added and centrifuged at 2000 rpm for 10 minutes. 2 ml supernatant was mixed with 2.5 ml alkaline glycine buffer (0.1M, pH-10.5) and 1.5 ml distilled water. Optical density was read at 540 nm on visible spectrophotometer (Labtronics) and ΔG was calculated by using Gibbs equation (Becker *et al.*, 2003). Graphs have been produced by using the Origin-50 software and were edited in Adobe Photoshopt-7.

RESULTS

Enzymes are the biocatalysts that enhance the rate of biological reaction under favorable conditions. The enzymatic reactions take place under definite temperature and pH in stipulated time. During this exothermic reaction, the energy released can be calculated as free energy change ΔG. This free energy may not be constant at different variable of pH, temperature, time, and enzyme and substrate concentration. The effect of these parameters has been elucidated as follow:

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Effect of pH on ΔG

The tissue extract was exposed to pH variable at 435 K for 1hr incubation that resulted in ΔG variation at constant equilibrium (Keq) 0.642. Increase in pH subsequently increased the ΔG (Fig.1). This increase was noted from pH-3 to 5. Later, the ΔG was reduced up to pH-6.5. The lowest ΔG (-3.2 kcal/mole) was recorded at pH-3 and highest at pH-5 (ΔG, -2.07 kcal/mole).

Effect of temperature on ΔG

The enzyme exposed to temperature variable showed changes in ΔG at constant pH and equilibrium constant (Keq, 0.642) for 1hr incubation. Rise in temperature from 358 to 834 K reduced the value of ΔG (Fig.2). And at low temperature it was -2.3 kcal/mole; however, at highest temperature it was noted to be -10.2 kcal/mole.

Effect of Time incubation on ΔG

The enzyme was exposed at constant temperature 453 K and pH-4.5 at different time period ranging from 60 to 420 minutes. The rate of ΔG was increased with the increased time period (Fig.3). However, this increase was observed up to 300 minutes; later, it was remained constant. At 60 minutes incubation, the Δ**G** was recorded to be -2.2 kcal/mole and at 300 minutes and later it remained -1.4 kcal/mole.

Effect of Enzyme on ΔG

Renal extract prepared in PBS was exposed at constant temperature 453 K and pH-4.5 for 1 hr incubation gave interesting results. Increase in enzyme concentration from 5% to 100% resulted in increase of ΔG at Keq 0.541 (Fig.4). At 5% enzyme concentration ΔG was -4.7 kcal/mole; however, at 100% it was -1.9 kcal/mole.

Effect of Substrate on ΔG

Increase in substrate concentration from 0.1 mM to 4 mM increased the ΔG as -3.3 kcal/mole to -1.4 kcal/mole at temperature 453 K, pH-4.5, Keq-0.641 for 1 hr incubation (Fig.5).

DISCUSSION

The β-glucuronidase has been studied in detail in mammals in relation to xenobiotics including various aspect of physiology. Literature is available on the histochemical distribution, localization and biochemical aspect of the enzyme. Little work in this regards was carried out in fish with reference to enzyme kinetics (Chilke, 2009). But still, no work has been carried out in the direction of bioenergetic approach of β-glucuronidase anywhere in any of the organism. Hence, the present work was carried out to fill the lacunae. It was observed that the β-glucuronidase is present in almost all the organs of the fish and might be involved in the deactivation of drug and toxic material. It was observed to be present in large amount in the liver and therefore it can be called as metabolic center of the enzyme β-glucuronidase. However, the kidney was also observed to contain quite appreciable amount of the enzyme which is distributed both in granular and diffused form in *Labeo rohita* (Chilke, 2006).

In the present study the effect of different parameters like pH, temperature, time, and enzyme and substrate concentration on free energy change ΔG was carried out. The enzyme showed response to all the parameters whenever either ΔG reduced or increased. Increase in pH subsequently increased the ΔG. This increase was noted from pH-3 to 5. Later the ΔG was reduced up to pH-6.5. The lowest (-3.2 kcal/mole) ΔG was recorded at pH-3 and highest at optimum pH-5 (ΔG, -2.07). The enzyme work reliable at optimum pH might be due structural integrity. However at lower and higher pH, the structural deformities in the enzyme might have inducted, that resulted in the reduction in ΔG. Temperature affects the enzyme activity at both higher and lower degree. Rise in temperature from 358 to 834K reduced the value of ΔG at constant pH-4.5 and equilibrium constant (Keq, 0.642) for 1hr incubation. At low temperature as 358 K and high as 834 K, the ΔG was recorded to be -2.3 kcal/mole and -10.2 kcal/mole respectively. This continuous decrease in ΔG corresponding to increase in temperature indicated that the higher temperature induces the structural change and hence it loses successive ability to react with the substrate result in reduction in ΔG. The rate of ΔG was increased with increased time period from 60 to 420 minutes of incubation at constant temperature 453 K and pH-4.5. However, this increase was noticed up to certain time period which later remained constant. It indicates that product saturation prevails after specific time of incubation and then no change in ΔG can be allowed. Both the enzyme and substrate concentration exhibited the same result in relation to ΔG. Increase in enzyme concentration from 5% to 100% resulted in the increase of ΔG at Keq 0.541 and temperature 453 K. Similarly, increase in substrate concentration from 0.1 mM to 4 mM simultaneously increased the ΔG from -3.3 kcal/mole to -1.4 kcal/mole at temperature 453 K, pH-4.5, Keq-0.641 for 1 hr incubation.

It was concluded that the optimum pH is required for maintaining the ΔG at peak. However, the increase in temperature affects the enzyme activity and also the ΔG. Time is the limiting factor and hence beyond certain time, ΔG can not be changed. The ΔG depends upon both the enzyme and substrate concentration. The increase in the quantity of enzyme and substrate resulted in increase in the rate of reaction and also the ΔG.

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