ASIAN JOURNAL
ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 4, Issue, 11, pp. 058-059, November, 2012

RESEARCH ARTICLE

HISTOCHEMICAL LOCALIZATION OF ß-GLUCURONIDASE IN SOME VERTEBRATE TISSUES

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Received: 28th*, August, *2011; Received in Revised from: 29th* , September, *2011; Accepted:* 04th, October, *2011; Published online: 27th*, November, *2012

ABSTRACT

ß-Glucuronidase is the novel enzyme known to present in the tissues of several vertebrates. It can be detected by biochemical, histochemical and immunocytochemical techniques in bacteria, plant and animal. The traditional method used for histochemical demonstration of ß-glucuronidase is quite tedious and substrate utilized in a given concentration is not affordable to researchers of the developing country. In the present study the attempt is made to improve the localization of ß-glucuronidase by employing the same method with change in substrate concentration and by avoiding the use of some chemicals. It was observed that under the light microscope the enzyme can be localize clearly both in the granular and diffuse form without any artifact. This modified method could be beneficial for the localization of ß-glucuronidase in other animals beside mammals and fishes.

Key words: histochemistry, localization, ß-glucuronidase, tissues, vertebrate.

INTRODUCTION

β-Glucuronidase enzyme is known for many decades to work in the carbohydrate metabolism in bacteria, plants and animals. It is an acid hydrolase expressed at variable levels by virtually every cell in the vertebrate body (Paigen 1989). 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). In its active form it participates in the degradation of glycaminoglycans by cleaving ß-glucuronosyl residues at the non-reducing end of polysaccharides (Birkenmeier *et al*. 1989). Absence of this enzyme in humans results in the fatal lysosomal storage disease mucopolysaccharidosis type VII (MPS VII), or Sly syndrome (Sly *et al*. 1973, 1974). The traditional method used in the demonstration of β-glucuronidase by employing the Naphthol-AS-BI-b- D-glucuronic acid as a substrate gives poor resolution under light microscopy. In the present study, the traditional method is slightly changed with respect to concentration of substrate and skipping the use of some chemicals. This technique may prove beneficial for the demonstration of β-glucuronidase in the tissues of all vertebrates under light microscope.

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MATERIALS AND METHODS

The histochemical localization of ß-glucuruonidase was carried out from in the lower and higher vertebrate to find out the intensity of localization and differentiation of staining. From the higher vertebrate adult Albino rat was selected and from the lower vertebrate fish, adult *Labeo rohita* was selected. The Albino rat was obtained from animal house of Department of Biochemistry, Nagpur University, Nagpur. Live fish was purchased from the Ambazari tank in Nagpur located very close to University Campus. Live fish was carried to the laboratory in polythin bag containing water. Albino rat and fish was perfused with ice-cold 0.01M phosphate buffer saline (PBS), pH-7.45 after providing anesthesia with intramuscular injection. Gonad, kidney and liver was and fixed in Bouins fixative for histology and in 10% Neutral buffer formalin (NBF) for histochemistry of ß-glucuruonidase. The tissues in the fixative were kept for more than 72 hrs at 4-8 °C in the refrigerator. 10 µm thick sections of testes were cut on cryostat (Leica) at -20°C and the sections were spread on slide treated with adhesive glycerol jelly. 11 mg Naphthol-AS-BI-β-D-glucuronic acid (Sigma) was dissolved in 0.05 M Sodium carbonate and then diluted to 100 ml with 0.1 M acetate buffer (pH-4.5). This stock solution was preserved in the refrigerator at 4°C. It was then diluted with 0.1 M acetate buffer (pH-4.5) to get solutions of variable molarities. It was observed that $1x10^{-4}$M substrate solution gives the best result. Immediately, prior to incubation, 0.5 ml solution of sodium nitrate was added to 0.5ml acidic pararosaniline solution, mixed gently by inversion and allowed to stand for 5 minutes. Later, 38 ml prewarmed deionized water, 5 ml acetate buffer (0.1M; pH-4.5) and 5 ml Naphthol-AS-BI-β-D-glucuronic acid

($1x10^{-4}$M) were added to get the final incubation solution. The formation of precipitation was rejected. Sections were rinsed with cold 0.01M phosphate buffer saline (PBS), pH-7.45. Later, sections were slightly allowed to dry at room temperature. The sections were incubated for 90 minutes at 37°C and during this process the light contact was avoided. The sections were rinsed for 2-3 minutes in running water and then air dried for 15 minutes before counterstaining in methylene green. The sections were rinsed in deionised water and then mounted in Glycerol jelly. Lastly, the microphotographs were taken by Nikon Camera and adjusted with Photoshop-7.

RESULTS

Ice-cold, preserved transverse section of Kidney, liver, testis and ovary of the lower vertebrate (fish-*Labeo rohita*) and higher vertebrate (Albino rat) were used for comparative study of clarity in staining intensity for the enzyme ß-glucuronidase. The method employed with skipping the use of some chemicals and decreasing the concentration of substrate Naphthol-AS-BI-ß-D-glucuronide show good staining for 90 minutes incubation at 37°C. The enzyme activity appears in the form of red deposits either in diffuse or granular and or both.

Fish tissues

Through out the hepatic cells, staining for the enzyme beta-glucuronidase was clear with no ambiguity. In the kidney-glomerulus, renal tubules, haematopoetic tissues exhibit enzyme activity in granular and diffuse form. In the testes, the primary and secondary spermatogonia showing granular and diffused staining, but in the secondary spermatogonia staining intensities is more as compared to primary spermatogonia. The wall of semeniferous lobule is slightly stained. Enzyme activity is more clearly visible in the secondary oocytes, the nucleoli, nuclear membrane, cytoplasm and cell membrane exhibits enzyme activity (Figure 1, 2, 3 & 4).

Rat tissue

The enzyme activity in the liver of Albino rat is more pronounced. The staining was both in diffused and granular form. Kidney also exhibits both the granular and diffused reaction in the wall of blood vessel, glomerulus, Bowman's capsule and haematopoetic tissues. Testes show clear enzyme activity both in granular and diffused form in the seminiferous tubule, spermatogonia and spermatocytes. In the sperm, enzyme activity is restricted to the head region only. Sertoli and interstitial cells also shows the enzyme activity in their cytoplasm. Ovarian tissues shows dispersed, diffused and granular staining in the ovary and towards the peripheral area of ovary (Figure 5, 6, 7 & 8).

DISCUSSION

Enzymes are very mportant protein that inevitable involve in the metabolic process in the living organism. Most of the enzymes are heat labile and destroyed when exposed to more than optimum temperature. It is reported that the enzyme β-glucuronidase in fish *Labeo rohita* shows enzyme activity above 50°C at pH-5 (Chilke, 2009 and 2010). Enzyme histochemistry is an invaluable tool for the demonstration of endogenous enzyme activity in virtually any type, as well as exogenous reporter genes expressed by ex vivo-manipulated somatic cells. Histochemical demonstration of enzyme activity typically requires tissues to be processed with mild fixatives and cryosectioned to prevent enzyme denaturation. The unfortunate consequences, however, is often poor preservation of cytological architecture (Gene *et al.,* 1999). The present investigation suggests that for proper demonstration of enzyme activity the proper fixation of the tissue is necessary. The mild fixative could not have infiltrating the tissues in short duration and therefore, the tissues should be fixed for more than 72 hours at 4-8°C in the refrigerator. It was observed that the tissues of lover and higher vertebrate exhibit good staining for enzyme β-glucuronidase without any cellular damage. It has been concluded that this slight modified method may prove useful for the demonstration of β-glucuronidase in the tissues of all the vertebrates under light microscopy without change in the cytoarchitect.

Acknowledgement

Author is thankful to Dr. Milind Shinkhede for providing rats for the experiment and also to the Head, Department of Zoology, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur for providing the laboratory facilities.

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