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Aldose-1-Epimerase: Isolation and Purification from Bovine Liver

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Abstract: Aldose-1-epimerase also called as mutarotase is an enzyme that has the key role in the diagnosis of blood glucose level in diabetic patients. Upon reaction, the enzyme converts the α-D-glucose to β-D-glucose, which is a specific anomer of glucose for the enzymatic glucose estimation. Bovine liver was homogenized for the extraction of mutarotase. The extract from bovine liver tissue was subjected to 42-56% ammonium sulfate precipitation, ion exchange and gel filtration chromatographic techniques. The activity and specific activity of the crude enzyme was 4.6 U mL^{-1} and 1.68 U mg^{-1} respectively. After the partial purification by ammonium sulfate precipitation and desalting, the activity and specific activity increased to 5.42 U mL^{-1} and 4.71 U mg^{-1}, respectively. Diethylaminoethyl (DEAE) cellulose column chromatography resulted in the enzyme with a specific activity of 10.62 U mg^{-1}. The enzyme was subjected to sephadex-G-150 and obtained 14.03 U mg^{-1} specific activity. It is concluded that among other sources for the enzyme, bovine liver tissue is another rich source for aldose-1-epimerase.

Key words: Aldose-1-epimerase · Isolation · Purification · Bovine liver

INTRODUCTION

In aqueous form, all the saccharides exist in two different anomeric forms i.e. α and β. The spontaneous inter-conversion of these anomeric forms is acid base catalyzed and is called mutarotation. The inter-conversion occurs in many hours under normal conditions. But in presence of the enzyme aldose-1-epimerase/mutarotase, the process takes place within minutes. The extraction of aldose-1-epimerase has been studied earlier. But as far as the animal/mammalian tissues are concerned, bovine liver tissue has not been studied earlier for this enzyme [1]. Reaction for aldose-1-epimerase is shown as under.

Aldose-1-epimerase

α-D-glucose → β-D-glucose

Miva *et al.* [2] reported that colorimetric determination of blood glucose is improved by the use of mutarotase. Glucose oxidase is the key enzyme in glucose estimation and shows a high degree of specificity for β anomeric form of D-glucose [3], which is produced at rapid rate in presence of aldose-1-epimerase.

The molecular weight of rat liver mutarotase is 41,300 with optimum pH 7.5 and optimum temperature of 35-38°C. This enzyme is heat stable upto 50°C [4] Microbial production of Mutarotase has since been reported from *Penicillium notatun, Escherichia coli* and *Aspergillus niger* [5, 6]. Glucose, galactose and rhamnose mutarotase from plant and other animal sources were investigated by many researchers [8, 9, 10]. Their major concern was to study the exact function of the said enzyme in such tissues. Kidney, intestine from rat and bovine and kidney of human were investigated to find out the function of mutarotase in such tissues [4]. As aldose-1-epimerase has been reported to be a nuclear protein involved in the nuclear sugar transport in cells [11]. This hypothesis also supports the major impact of aldose-1-epimerase on sugars utilization in higher organisms as reported by [12]. While Baba *et al.* [10] reported that aldose-1-epimerase is tremendously soluble enzyme and it leaks from the cellular compartments inside the media. The absence or mutation in mutarotase genes could result in a disorder called galacosemia. The enzyme galactose mutarotase was found to be involved in the conversion of galactose into metabolically useful form of glucose-1-phosphate.

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Recently it has been revealed that in addition to other proteins, maltose utilization in *Enterococcus faecalis* is regulated by mutarotase/aldose-1-epimerase [13].

Aldose-1-epimerase not only plays important function in sugars utilization but also finds its applications in clinical and diagnostic industry. Most of the literature reports the utilization of mutarotase in glucose determination from serum/urine samples. In serum enzymatic glucose determination studies it was reported that α and β-D-glucose exists in a temperature dependent equilibrium. At lower temperatures the equilibrium gets shifted largely towards α-D-glucose. The said enzyme was reported to affect D-glucose α/β anomeric equilibrium [14].

Known substrates of mutarotase have a reducing pyranose ring in C-1 confirmation with equatorial hydroxyls at C-2 and probably C-3. Crystal structure of the mutarotase has been reported from *Lactococcus lactis* [15]. The enzyme is widely used in quality control laboratories of biochemical, pharmaceuticals and clinical diagnostics. The present research was designed for the extraction and purification of aldose-1-epimerase using bovine liver tissue as source.

MATERIALS AND MEHTODS

Preparation of Crude Extract for the Enzyme: A volume of 250mL sodium EDTA buffer 7.4 pH containing 50g of freshly procured Bovine liver tissue (BLT) was homogenized in homogenizer for 2 minutes. To prepare an extract, the mixture was filtered and centrifuged at 10,000 revolutions minute^{-1} for 15 minutes at 4°C [16].

Salt Extraction of Proteins: BLT extract proteins were subjected to ammonium sulfate precipitation. The ionic strength resulting after 42-56% ammonium sulfate inside the solution resulted in the precipitation of aldose-1-epimerase. The precipitated enzyme was dialyzed against buffer to remove ammonium sulfate contents. Using sodium EDTA buffer 7.4 pH, salt extraction of proteins was performed strictly at 4°C to avoid enzyme denaturation or instability [5].

Purification of Aldose-1-Epimerase Through Column Chromatography: Salt extracted protein (aldose-1-epimerase) having some other impurities/proteins were further subjected to purification through column chromatography. A column of DEAE-cellulose was prepared by heating DEAE cellulose in distilled water at 95°C for 5 hours. A total of 50 fractions, each having a volume of 2 mL were collected and subjected to enzyme and proteins assay. The fractions with significant enzyme activity were pooled and subjected to sephadex G-150 for gel filtration chromatography [16].

Determination of Enzyme Activity and Protein Contents: The enzyme assay was performed by using polarimeter. Mutarotation of glucose between alpha and beta forms was recorded for calculation of enzyme activity U mL^{-1} using 5 mM sodium EDTA buffer 7.4 pH [16]. Proteins concentrations were determined in recovered fractions by Biuret protein assay method [17, 18].

RESULTS AND DISCUSSION

Specific rotation of the sugar was determined for different time intervals. As the time passed, the specific rotation decreased and anomeric form i.e. α-D-glucose concentration also decreased. A 5 minutes reaction of the crude enzyme resulted in enzyme activity of 4.6 U mL^{-1}, with protein contents of 2.75 mg mL^{-1} as shown in Table 1. Aldose-1-epimerase was then separated from impurities through salt treatment. The enzyme material was dialyzed against sodium EDTA buffer 7.4 pH. The enzyme could lose its catalytic properties after the dialysis against water, as reported by Bailey *et al.* [8]. While they reported 50% renovation of aldose-1-epimerase activity after using buffer. The recovering enzyme specific activity after salting out was 4.71 U mg^{-1}. Toyoda *et al.* [4] reported the enzyme specific activity as 1.02 U mg^{-1} from rat liver. The increase in specific activity of bovine liver aldose-1-epimerase after the salt extraction was 2.80 fold. However, the protein contents were decreased from 2.75 mg mL^{-1} to 1.15 mg mL^{-1}, which indicates the purification of the specific protein (Table 1).

It's been investigated by Bouffard *et al.* (1994) [19] that aldose-1-epimerase in bacteria is involved in the metabolism of carbohydrates. Aldose-1-epimerase function in higher organisms was not discovered as reported by Bailey *et al.* (1967) [8]; however they argued against metabolic breakdown of carbohydrates in higher organisms due to its uncontinuous distribution with glycolytic activity. Later on Hees-Peck and Raikhal (1998) [20] reported that aldose-1-epimerase functions for sugars utilization not in metabolism in higher organisms.

Table 1: Purification summary of aldose-1-epimerase from bovine liver tissue

Purification steps	Activity (U mL^{-1})	Protein contents (mg mL^{-1})	Specific activity (U mg^{-1})	Fold Purification
Crude	4.60	2.75	1.68	1.00
$(NH_4)_2$ SO_4 desalted	5.42	1.15	4.71	2.80
After ion exchange chromatography	9.03	0.85	10.62	6.15
After gel filtration chromatography	10.66	0.76	14.03	8.35

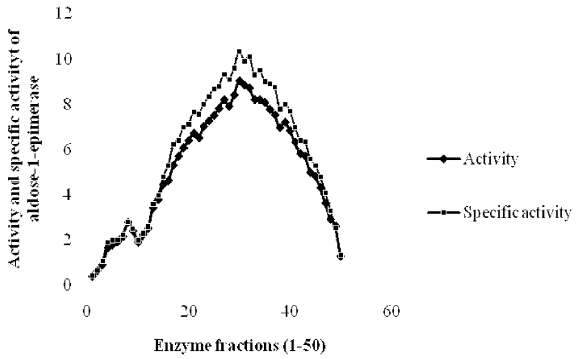

Fig. 1: Activity (U mL^{-1}) and specific activity (U mg^{-1}) of aldose-1-epimerase after ion exchange chromatography

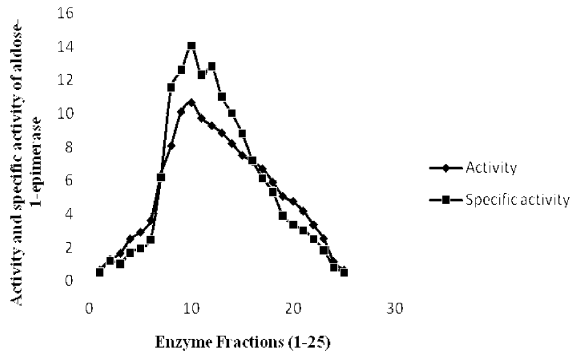

Fig. 2: Activity (U mL^{-1})and specific activity (U mg^{-1}) of aldose-1-epimerase after gel filtration chromatography

Specific activity from bovine liver after DEAE cellulose and Sephadex G-150 treatment was 10.62 and 14.03 U mg^{-1} respectively (Figs. 1 & 2). While Toyoda *et al.* 1983 [4] reported the specific activity of 9.22 and 5.13 U mg^{-1} respectively. According to Bailey *et al.* 1967 [8], aldose-1-epimerase from plant source indicated 16% increase in activity from ion exchange after gel filtration, here we report 118% increased enzyme activity (U mL^{-1}).

Aldose-1-epimerase specific activity increased upto 6.15 and 8.35 folds after purification through DEAE-cellulose and sephadex-G-150; while proteins after ion exchange and gel filtration chromatography were 0.85 and 0.76 mg mL^{-1} respectively. This indicates the rich presence of aldose-1-epimerase and its importance in sugars utilization in bovine liver. In addition to its major role in exploitation of carbohydrate, aldose-1-epimerase has been used in association with glucose-peroxidase system to determine sugars level. The accretion of aldose-1-epimerase in the reaction mixture resulted in shortening of time for the assay [6, 16]. However, in present study, the high yield of the enzyme indicates the presence of well established correlation between enzyme and active transport process of sugars in such tissues. Aldose-1-epimerase contents in bovine liver tissue are sufficient to be studied further. On the other hand, small amount of tissue is available to study from rats. Human kidney/liver are also hard to study for; whereas, bovine liver tissues are easily available source.

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REFERENCES

1. Poolman, B., T.J. Royer, S.E. Mainzer and B.F. Schmidt, 1990. Carbohydrate utilization in *streptococcus thermophilus*: characterization of the genes for Aldose-1-Epimerase (mutarotase) and UDP-glucose-4-epimerase. J. Bacteriol, 172(7): 4037-4047.
2. Miva, I., J. Okuda, K. Maeda and G. Okuda, 1972. Clin Chim Acta, 37: 538-540.
3. Hamid, H.M., K. Rehman, M.A. Zia and M. Asghar, 2003. Optimization of various parameters for the glucose oxidase from rice polishing using *Aspergillus niger*. Biotechnol., 2: 1-7.
4. Toyoda, Y., I. Miwa and J. Okuda, 1983. Multiple forms of mutarotase from the kidney, liver and small intestine of rats: Purification, properties, subcellular localization and development changes. J. Biochem., 94(2): 421-431.

5. Bailey, J.M., P.H. Fishman and P.G. Penchev, 1969. Isolation and characterization of mutartoase from bovine kidney cortex. J. Biol. Chem., 244(4): 781-788.
6. Kinoshita, S., K. Kadota and H. Tahuch, 1981. Purification and properties of aldose-1-epimerase from *Aspergillus niger*. Biochem Biophys Acta, 662(2): 285-290.
7. Okuda, J.I., I. Miwa, K. Maeda and K. Taqke, 1977. Rapid and sensitive, cosaimetric determination of the anomers of D-glucose mutase, peroxidase and mutarotase. Carbohydrate, 58(2): 267.
8. Bailey, J.M., P.H. Fishman and P.G. Penchev, 1967. Purification and properties of mutarotase from higher plants. J. Biol. Chem., 242(18): 2463-2469.
9. Richardson, J.S., X. Carpena, J. Switala, R.P. Luque, L.J. Donald, P.C. Loewen and I.J. Oresnik, 2008. RhaU of *Rhizobium leguminosarum* is a rhamnose mutarotase. J. Biotechnol., 190(8): 2903-2910.
10. Baba, S., Y. Arimoto, D. Yoshikawa, Y. Toyoda, I. Miwa and J. Okuda, 1979. Localization of mutarotase in rat kidney. Experimentia, 8(35): 1094-1097.
11. Duverger, E., C. Pellerin-Mendes, R. Mayer, A.C. Roche and M. Monsigny, 1995. Nuclear import of glycoconjugates is distinct from the classical NLS pathway. J. Cell Sci., 108: 1325-1332.
12. Thoden, J.B. and H.M. Hazel, 2005. The molecular architecture of galactose mutarotase/UDP-galactose 4-epimerase from *Saccharomyces cerevisiae*. J. Biol. Chem., 280(23): 21900-21907.
13. Berton, Y.L., V. Pichereau, N. Sauvageot, Y. Auffray and A. Rince, 2005. Maltose utilization in *Enterococcus faecalis*. J. Appl. Microbiol., 98: 806-813.
14. Evert, L.J. and N.S.E. Lennart, 1991. Method for determination of glucose in whole blood and cuvette and photometer for carrying out said method. US Patent, 5: 866-349.
15. Thoden, J.B. and H.M. Holden, 2002. High resolution X-ray structure of galactose mutarotase from *Lactococcus lactis*. J. Biol. Chem., 277(23): 20854-20861.
16. Zia, M.A., 2002. Standardization of conditions for glucose estimation using indigenously purified enzymes. M.Phil thesis. Dept. of Chem. and Biochem. University of Agriculture, Faisalabad.
17. Calzyme Laboratories Manual, 1998. http://www.calzyme.com.
18. Gornall, A.G., C.S. Bradwil and M.M. David, 1949. Determination of serum proteins by means of the biuret reactions. J. Biol. Chem, 177: 751-766.
19. Bouffard, G.G., K.E. Rudd and S.L. Adhya, 1994. Dependence of lactose metabolism upon mutarotase encoded in the gal operon in *Escherichia coli*. J. Mol. Biol., 244: 269-278.
20. Heese-Peck, A. and N.V. Raikhel, 1998. A glycoprotein modified with terminal N-Acetylglucosamine and localized at nuclear rim shows sequence similarity to aldose-1-epimerases. Plant Cell Online, 10(4): 599-612.