J Biochem Tech (2015) 6(1): 883-888
ISSN: 0974-2328

Partial purification and characterization of carboxyl esterase in aged and lithium treated rat brain

Sushma S. Rao, Suneetha P, Nagaveni MB, Prabha M

Received: 19 July 2014 / Received in revised form: 06 February 2015, Accepted: 20 March 2015, Published online: 30 June 2015

Abstract

Specific activity of Carboxyl esterase (CE) was estimated for young, old (2-4 and 16-18 months) and for similar groups of Lithium treated male Albino Sprague dawley rats whole brain cells. Rats of both age groups were orally administered with 37mg of Lithium chloride ($LiCl_2$) per kg body weight daily for 10 days. Old rat brains $LiCl_2$ treated showed significant increased CE specific activity of 0.076 IU/mg protein in crude, 0.1445 IU/mg protein in Ammonium sulphate precipitated and 0.5827 IU/mg protein in ion exchange fraction than their control. So the highly purified samples of carboxyl esterases obtained with $LiCl_2$ treated brain. Enzyme kinetics of substrate concentration showed Km of 0.2 mM and Vmax of 52.631, indicating higher CE activity at pH-6 and at 60oC temperature in crude extracts of control old rats. The crude brain samples of both aged groups $LiCl_2$ treated showed an intense band patterns in Native PAGE for CE expression than their controls. Thus, $LiCl_2$ elevates carboxyl esterase in each step of purification and in future it activates drug metabolism to treat various brain related disorders.

Keywords: Specific activity, Lithium chloride, Ammonium sulphate, Ion exchange chromatography, Native PAGE

Introduction

Hydrolytic enzymes split different groups of biomolecules such as protein, nucleic acids, carbohydrate and fat molecules breakdown into their simpler units (Prabha M et al.2013). Lysosomal hydrolase comprises phosphatases, carboxylesterases, glucuronidases and β-galactosidase, ribonuclease and acid protease are believed to play an important role in brain tumors (Robins E, et al. 1958). Several studies have earlier highlighted the importance of studying hydrolytic enzymes in a variety of pathological conditions such as brain tumors (Ramsey et al. 1980; Wielgat P et al. 2006), neurodegenerative diseases like Alzheimer's disease (Cataldo et al. 1996) and inherited metabolic disorders.

Carboxyl esterase (EC 3.1.1.1) is one of the hydrolytic enzymes which play an important role in the maintenance of the metabolic advantages and essential for neuronal function. Carboxyl esterase (CE) is a drug metabolizing enzyme hydrolyzes molecules containing functional groups such as a carboxylic acid ester, amide, and thioester mainly localized in microsomes (Satoh and Hosokawa 1998). In brain, Carboxyl esterase was reported in endothelial cells and blood brain barrier of human brain (Zhang et al. 2002). In rat central nervous system, CE glycoprotein enzyme may function as a neuroprotective factor against foreign chemicals in glial and neuronal cells.

Carboxyl esterase activity can be influenced by interactions of a variety of compounds including some member of drugs containing metals which acts as modulators involved in the activation of enzymes. Lithium is one of such modulator for enzyme and protein, which is an antidepressant and mood stabilizer (L.Trevor Young et al.2004) either directly or at the level of enzyme regulation. Lithium lightest metal is involved in enhancement of hippocampus neurogenesis (Chen G et al. 2000). The interaction between metals and proteins in the nervous system seems to be a crucial factor for the development or absence of neurodegeneration. Metal accumulation within the nervous system was observed in those diseases could be the result of compensatory mechanisms to improve metal availability for physiological processes. (Susana Rivera-Mancía, *et al,* 2010).

Despite its long-standing clinical use and intensive investigation, there has been no consensus on the molecular mechanisms underlying the therapeutic actions of lithium. Several hypotheses have been presented to explain the mechanism of lithium's actions. One of the most popular models, the inositol depletion hypothesis, is that acute mania in

Sushma S. Rao, Prabha M*

Department of Biotechnology, M. S. Ramaiah Institute of Technology, Bangalore-560054

*Email: prabhamg@gmail.com

Suneetha P, Nagaveni MB,

Department of Biotechnology, Maharani Lakshmi Ammanni College for Women, Bangalore-560012

bipolar illness is caused by hyperactivity of receptor-mediated PI turnover in the brain. Accordingly, lithium, by inhibiting IMPase, would deplete brain inositol levels and dampen PI metabolism (Berridge *et al*.1989).

Therefore the present study was taken to study further about the role of lithium at the level of CE purification. Hence partial purification of carboxyl esterases was carried out in aged and lithium treated rat brain to understand the activation of lithium as a modulator towards CE relating to the young brain cells. Old rat brains treated with $LiCl_2$, showed significant increased specific activity for each step in crude, Ammonium sulphate precipitated samples and in ion exchange fraction of partial purification of CE when compared with their control old rats. This confirms that the purified fraction obtained were the highly purified samples of carboxyl esterases treated with $LiCl_2$. Hence lithium is a promising modulator for carboxyl esterase activity that helps by hydrolytic function in the modulation of neuronal function. Thus protein/enzyme modulation with metal modulators play an important role in the regulation of biological processes.

Materials

Animals and treatment

The experiments were carried out on locally bred Albino sprague dawley young rats weighing 150-220g and old aged rats weighing 300-350g. These four categories of rats were being selected as shown in (supplementary data). Animals were caged individually in plastic cages and were exposed to a natural day-night cycle with free access to cubes of standard rodent diet and tap water for 3 days before starting the experiment. Body weight and food intake of all rats were monitored in both pre- and post-experimental period. All experiments were performed according to a protocol approved by local animal care ethical committee.

The normal specimens were purchased from Vekateshwara agency, Bangalore after informed consent was available from department of Biotechnology, Maharani Lakshmi Ammanni College for research purposes. The model was first anesthetized using Xylazine and Ketamine in the ratio 1:3, followed by surgical dissection of rat brain. These tissue specimens were collected in a sterile condition by sacrificing the rats. After removal of brain specimens subjected for preservation at -20^{0}C and were used for biochemical studies.

Methods

Lithium chloride dosage

Dose of the drug was calculated by converting adult human therapeutic dose (600-2400mg/day) to animal dose. The average dose of lithium chloride amounted to 37mg/kg of body weight of rats per day. To study the effects of the drug, five young and five old rats were treated with modulator orally for 10 days.

Processing of tissue specimens

The weight of the respective brain tissue sample were noted and used for biochemical studies. Brain specimens were handled aseptically and divided into four categories which were further used for biochemical studies.

Preparation of tissue extraction from specimens for biochemical studies

Specimens were subjected to homogenization with tris buffer saline and protease inhibitors (PBS pH=7.4 containing 1% (v/v) triton-X 100, 0.5mM phenyl methyl sulphonyl fluoride in ethanol) with the tissue to buffer ratio of 1:10 w/v (wet weight). All the steps in extraction procedure were observed and denaturation was prevented by maintaining at low temp (5^o-10^{o}C) for homogenization and centrifugation with extraction buffer. Homogenates were centrifuged at 10,000 rpm for 15 minutes at 4^{o}C. The supernatants were collected for assays of enzymes.

Lowry's method (Lowry et al. 1951; Hartree 1972)

Bovine Serum Albumin as standard protein (1 mg/ml) by Lowry's method (Lowry et al. 1951; Hartree 1972).

Enzyme Activity of Carboxyl esterase(CE)

Enzyme activity of the CE in tissue extracts were assayed by spectrophotometric methods. CE were estimated by monitoring the release of product namely α-naphthol spectrophotometrically wherein a common standard curve derived from α - naphthol were employed to quantify the α-naphthol released. Specific activity will be expressed as micromoles of 1-naphthyl acetate hydrolyzed per minute per mg of protein.

Carboxyl Esterase

Assay was carried out by Gomori (Gomori, 1941) and later modified by Van Asperen (Asperen, 1962). Enzyme reaction was initiated by adding 900 µl of 5 mM a-naphthyl acetate in Phosphate assay buffer (pH 7.0) to pre incubated 100 µl tissue extract and was incubated for 15 min at 27ºC. Subsequently, the reaction was stopped by the addition of 500 µl DBLS reagent and enzyme activity was measured at 600 nm.

Partial purification of Carboxyl esterases for 4 categories of rat brain

Ammonium sulphate precipitation

Ammonium sulphate precipitation was carried out for 80% saturation in all crude fractions of control, lithium treated young and old aged rat brains were subjected to ion exchange chromatography.

Ion exchange chromatography

Partial purification of the protein is carried out by ion exchange chromatography by taking 5ml of the sample (protein) in column with CM Cellulose matrix and 0.05 M-acetate buffer pH-5.5 as Sample buffer. Supernatant of tissue extract are taken for loading of samples. After samples are run through the column, 20-25 fractions are collected for every 5 minutes.

Native electrophoresis (Laemmli system, 1970)

The brain tissue supernatant sample of 20 µl (20 µg to higher protein content) are electrophoresed in a native polyacrylamide gel (10 %) at a constant voltage of 50 Volts for 10 min, followed by 150 Volts for 1 h (approximately). The run is

stopped when the marker dye reached 1–2 mm above the lower edge of the plate and the band patterns are analysed. This is followed by substrate incubation (in gel assay) and the enzyme will be identified by analyzing the band pattern.

Results and Discussions

Estimation of carboxyl esterase activity

Specific activity was calculated to determine the Carboxyl esterases activity in normal young and old aged rats of crude extracts, ammonium sulphate precipitated samples and ion exchange chromatography samples that is presented in table 1. Similarly the same procedure is repeated for Therapeutic Lithium Chloride treated rats of above mentioned groups.

Specific activity of carboxyl esterases was expressed in micromoles of product formed/min/mg Protein. By and large, the activities of esterases in crude and partially purified extracts studied were found to differ in normal young rats and normal old rats. Further, the activity of CE was also found to differ in lithium treated young and old rats. In these four categories of rat brain, the CE enzyme activities of tissue extracts of lithium treated old rats of brain were found to be relatively high as compared to control old rats (normal).

Estimation of protein content

The total protein contents of rat brain extracts for crude and partially purified extracts (ammonium sulphate precipitated extracts and ion exchange pooled samples) in control, lithium treated young and old aged rats were estimated that is presented in Table 1. The significant increase of protein content for crude extracts in young control rats as compared to lithium treated rats was obtained, in contrast, ammonium sulphate fractions showed 1.3 fold increase in lithium treated young rats as compared to control young rats. Whereas incase of old aged rats also there was a significant increase of protein content as compared with lithium treated rats for crude extracts and ammonium sulphate fractions showed 8 fold increase in control old rats as compared with lithium treated old rats. Ion exchange pooled samples of protein increased by 1.2 fold in lithium treated rats as compared to control rats.

Estimation of Total Activity

Total activity of carboxyl esterases was calculated for rat brain crude extracts, partially purified extracts (ammonium sulphate precipitated extracts and ion exchange pooled samples) of control, $LiCl_2$ treated young and old aged rats (figure 1). Crude extracts showed significant increase of CE by 2.73 fold in young control rats as compared to $LiCl_2$ treated rats. Whereas, ammonium sulphate fractions showed more activity by 2.79 fold in control rats than $LiCl_2$ treated rats. There is no significant change in total activity for crude and ammonium sulphate fractions of control young rats.

In old aged $LiCl_2$ treated rats of crude extracts showed 1.2 fold increased CE activity as compared to control rats whereas $LiCl_2$ treated rats of ammonium sulphate fractions also showed increased CE activity by 1.1 fold than control rats. Ion exchange pooled samples of lithium treated rats showed 1.2 fold increased CE activity as compared with control rats. There is no significant change for total activity in crude, ammonium sulphate fractions and Ion exchange pooled samples of control old rats. All 3 categories of $LiCl_2$ treated rats showed higher CE total activity by 1.2 fold as compared to their control. Hence these data showed the positive effect of lithium in elevation of CE activity in old aged rats than young rats. Therefore this study confirms lithium as a modulator activates carboxyl esterase in old aged rat brain and this helps in drug metabolism for treatment of brain diseases like brain tumors and several neurodegenerative diseases.

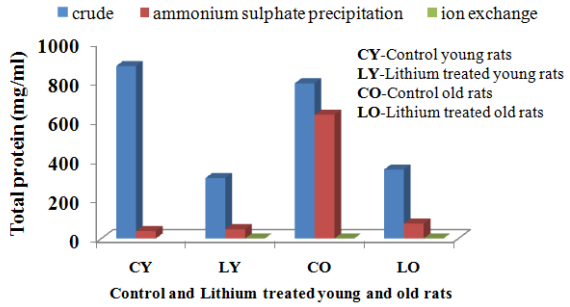


Figure 1: Comparison of total protein in control and lithium treated rats

Estimation of Specific Activity

Specific activity of carboxyl esterases in rat brain crude extracts, partially purified extracts (ammonium sulphate precipitated extracts and ion exchange pooled samples) of control, lithium treated young and old aged rats were estimated presented in Table 1. Young $LiCl_2$ treated rats of crude extract showed 1.2 fold increased CE activity as compared with control rats whereas ammonium sulphate fractions showed higher specific activity by 3.5 fold in control rats as compared to lithium treated rats because of high protein content in ammonium sulphate fractions of lithium treated young rats.
Old aged $LiCl_2$ treated rats of crude extracts showed 3 fold increased CE activity as compared with control whereas ammonium sulphate fractions showed 3.5 fold increase in $LiCl_2$ treated rats than control rats. Ion exchange pooled samples (0.5M NaCl concentration) from $LiCl_2$ treated rats showed 1.2 fold increased activity as compared with control rats, there by the fold purification increases at each stage of purification and the yield of the enzymes decreases subsequently.

High specific activity of carboxyl esterases were shown in $LiCl_2$ treated rats for young crude extracts, crude and partially purified fractions of old rats because of low protein content in this group, thereby the enzyme of our interest is concentrated and hence significant specific activity is observed in lithium chloride treated rats.

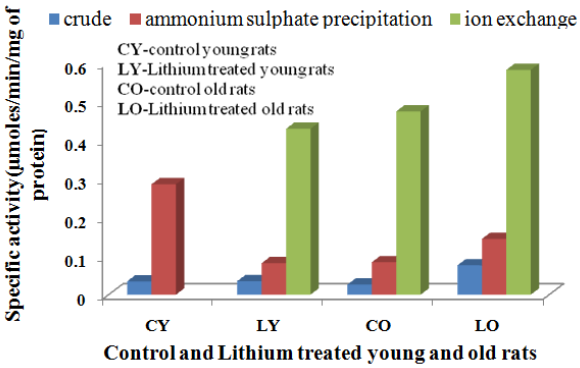


Figure 2: Comparison of specific activity in control and lithium treated rats

Comparison of Fold Purification in Control and Lithium treated rats

Fold purification for carboxyl esterase in partially purified extracts of ammonium sulphate precipitated samples and ion exchange pooled samples control, lithium treated young and old aged rats were estimated presented in Table 1. Control young rats, ammonium sulphate fractions showed 3.5 fold increase of CE than $LiCl_2$ treated rats. Old aged control rats, ammonium sulphate fractions showed 1.7 fold increased fold purification than lithium treated rats. Ion exchange pooled samples showed 2.4 fold increase in control rats as compared to lithium treated rats.

At each stage of purification, total activity of enzyme decreases but specific activity increases as indicated by protein purification table in which each stage of purification CE gets concentrated and concentrates of protein present in the sample where as the fold purification increases at each stage of purification and there by the yield of the enzymes decreases subsequently.

Hence the fold purification of ammonium sulphate fractions and ion exchange pooled samples showed high fold purification for CE in control rats than $LiCl_2$ treated rats for both young and old rats. This may be due to the degradation of matrix step by step purification and the activity decreases in fold purification of old lithium treated rats. Therefore, the efficiency of fold purification decreases in old rats.

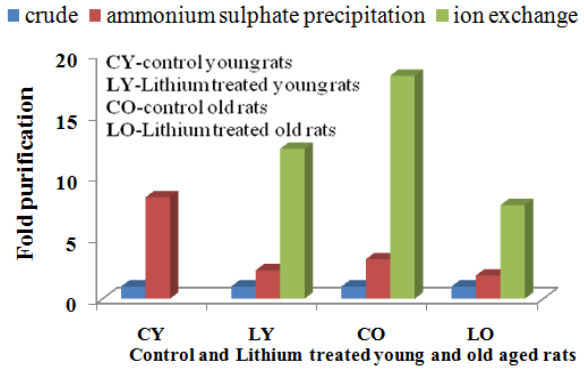


Figure 3: Comparison of fold purification in control and lithium treated rats

Comparison of Yield in Control and Lithium treated rats

Carboxyl esterases yield in partially purified extracts of ammonium sulphate precipitation, ion exchange pooled samples of control and $LiCl_2$ treated old aged rats of similar groups were estimated. Similarly CE yield in partially purified extracts of ammonium sulphate precipitation, $LiCl_2$ treated young rats of similar groups were also estimated (Table 1). Young rats ammonium sulphate fractions showed increased yield by 1.023 fold in control than $LiCl_2$ treated rats (Fig.4). In case of old rats, ammonium sulphate fractions showed high yield by 1.24 fold in control as compared to $LiCl_2$ treated rats. Ion exchange pooled samples showed increased yield by 1.1 fold in $LiCl_2$ treated rats as compared with control rats. Ammonium sulphate fractions and ion exchange pooled samples showed high yield in control rats than $LiCl_2$ treated rats for both young and old aged rats. While young rats did not show any significant difference in the yield of ammonium sulphate fractions among control and $LiCl_2$ treated rats. However, in case of old aged rats, ammonium sulphate fractions showed less yield in lithium chloride treated rats as compared to control, which showed more purity for enzymes. Therefore lesser the yield more is the purity of the fractions.

Table 1: Purification table in Control and Lithium treated rats of both the age groups

Control young rats	Crude	880	0.0344	1	100
	$(NH_4)_2SO_4$ precipitated samples	37.73	0.286	8.31	35.64
Lithium treated young rats	Crude	308.66	0.035	1	100
	$(NH_4)_2SO_4$ precipitated samples	47.41	0.0816	2.33	34.83
	Ion exchange pooled samples	0.088	0.430	12.28	0.3411
Control old rats	Crude	792	**0.026**	1	100
	$(NH_4)_2SO_4$ precipitated samples	631.4	**0.084**	3.23	50.63
	Ion exchange pooled samples	0.176	**0.475**	18.26	0.398
Lithium treated old rats	Crude	351.78	0.076*	1	100
	$(NH_4)_2SO_4$ precipitated samples	75.944	0.1445*	1.901	40.64
	Ion exchange pooled samples	0.197	0.5827*	7.66	0.425

Bold - Indicates low specific activity of control old aged rats as compared to lithium treated old aged rats in crude and partially purified samples.

* - Indicates higher specific activity shown in lithium treated old aged rats in crude and partially purified samples.

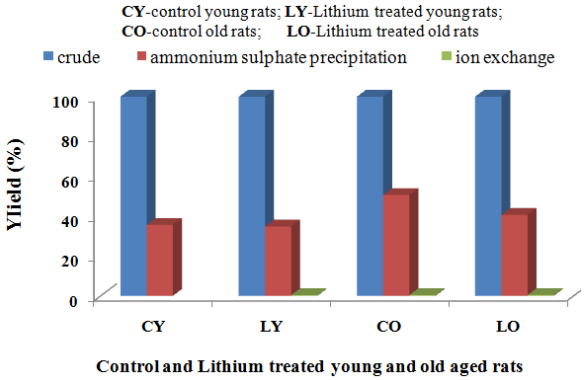


Figure 4: Comparison of yield in control and lithium treated rats

Ion Exchange Elution Profiles in Control and Lithium treated old aged rats

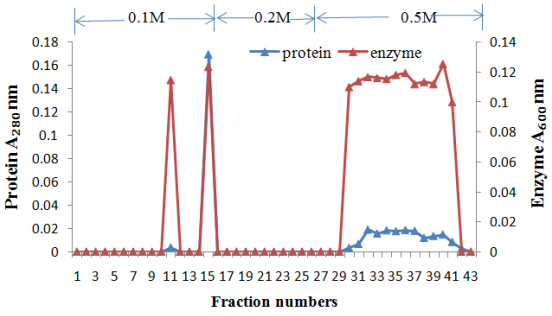


Figure 5: Ion exchange elution profiles in control old aged rat

From the elution profiles it was found that major isoforms were eluted in 0.5M NaCl concentration and some were eluted in 0.1M and 0.2M NaCl concentration in control old aged rats at 40th fraction and 0.5M NaCl fractions showed greater esterase activity (Fig 5).

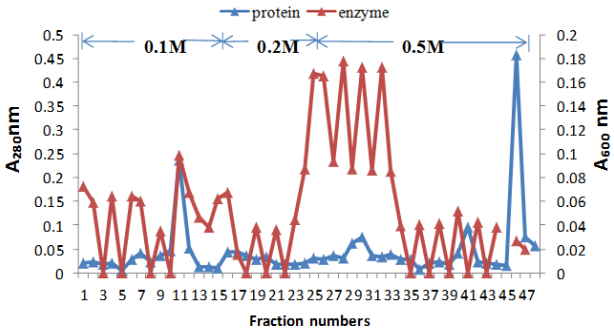


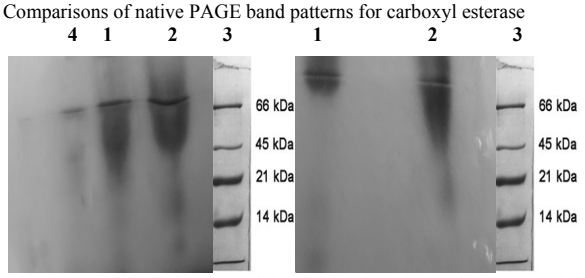
Figure 6: Ion exchange elution profiles in lithium treated old rats

Elution profiles showed major isoforms bound protein which was eluted in 0.5M NaCl concentration and some were also eluted in 0.1M and 0.2M NaCl concentration for lithium treated old aged rats at 46th fraction and 0.5M NaCl fractions showed higher carboxyl esterase activity (Fig.6).

Native PAGE of carboxyl esterase

Native PAGE of CE was performed for young and old aged rats to retain CE activity in its native form in the steps of purification. Crude and purified enzymes for control and $LiCl_2$ treated of old aged rats were subjected to Native PAGE and stained for CE activity to detect and separate the pure carboxyl esterase from other

Comparisons of native PAGE band patterns for carboxyl esterase



Part A Lane 1-Ammonium sulphate precipitated sample of control young, Lane 2-Crude homogenate of control young, Lane 3-Crude homogenate of therapeutic young
Part B Young Brain: Lane 1- crude homogenate of control old rats, Lane 2-crude homogenate of therapeutic old rats

Figure 7: Native PAGE band pattern

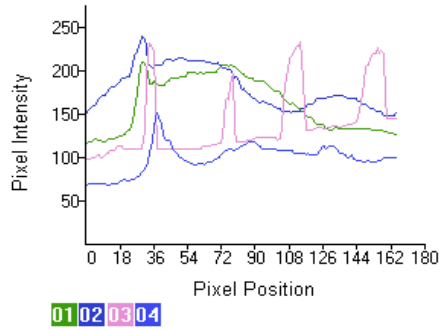


Figure 8: Densitometric graph for young rats

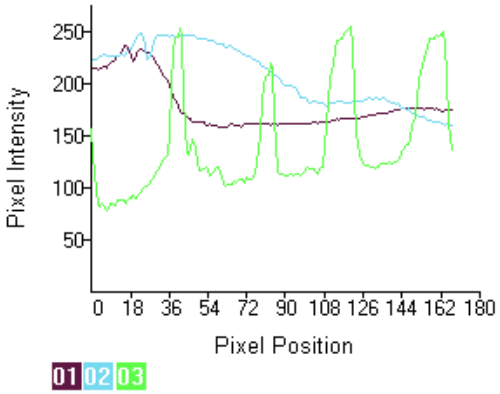


Figure 9: Densitometric graph for old rats

enzymes in the mixture. The gel was stained for CE activity by incubating the gel for 15 minutes at 37°C with suitable substrate (α-napthyl acetate) and chromogen (DBLS) that showed thick intense bands only for crude extracts of control and lithium treated old and young rats, however pale band was also observed in ammonium sulphate precipitated extracts of control young rats but no bands were observed in ion exchange fractions of control and therapeutic $LiCl_2$ treated old rats. Therefore the band patterns of gel confirm the expression of carboxyl esterase in lithium treated rat crude samples.

Densitometry Tracing is used to find the content percentage of the Bands which showed maximum pixel intensity for control and therapeutic young and old rats.

Characterization of enzymes with different parameters

Enzyme Kinetics

Enzyme kinetics deals with the several factors affecting the rates of enzyme catalyzed reactions. The most important factors are: Substrate concentration, pH and temperature.

Effect of substrate concentration (Km and Vmax)

Effect of Km and Vmax of carboxyl esterases for different substrate concentration was carried out for crude extracts of old rats which showed Km of 0.2 mM and Vmax of 52.631. Lower the Km value, higher is the affinity for the substrate and has higher enzyme activity.

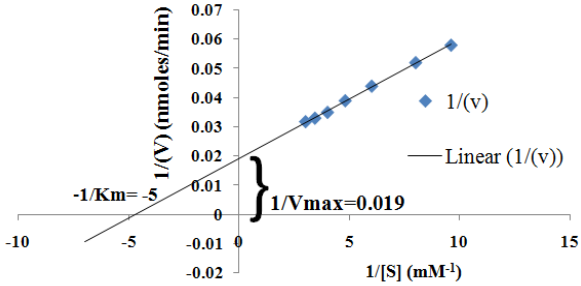


Figure 10: LB plot for determination of Km and Vmax of esterase enzyme

Effect of temperature

Temperature plays a vital role in the activity of any enzyme. It is observed that every enzyme has maximum activity at a particular temperature which is characteristic of that enzyme,

called the optimum temperature. CE activity was obtained from 31°C-40°C and elevated from 45°C to 60°C and at 70° C and 80° C CE activity was declined. Effect of different temperatures on carboxyl esterase activity was carried out from 31°C-80° C in which optimum temperature for CE activity obtained from 45°C to 60°C with maximum activity of CE at 60°C that indicates the thermostability of CE for such a high temperatures (Fig.11).

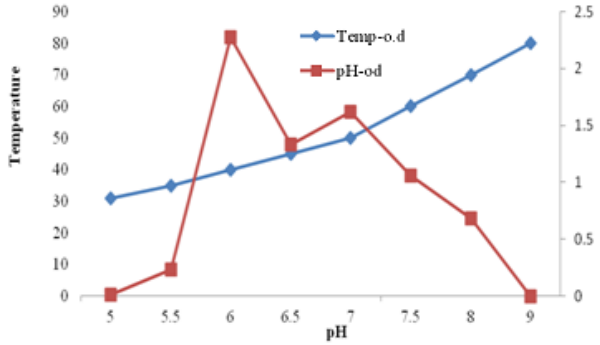


Figure 11: Determination of optimum temperature and pH of esterase activity

Effect of pH

Effect of different buffers for various pH on carboxyl esterases was carried out from pH 5 to 9, which showed maximum CE activity at pH6 indicating optimum pH for CE activity. Here the enzyme is exposed to a wide range of hydrogen ion concentration. During the assay, the pH is brought back to optimum to detect the maximum activity. A plot of reaction rate against pH could suggest the information about the rate and hydrogen ion concentration at which the enzyme is stable (Fig 11).

The present study showed significant carboxyl esterase activity in crude and partially purified fractions of lithium treated old age rats and also significant specific activity with untreated young rats. Ion exchange elution profiles showed that all major isoforms of CE were eluted in 0.5M NaCl concentration in lithium treated old rats which showed greater CE activity. Native PAGE was performed to retain enzyme activity in native state in crude samples which concludes that lithium treated rats of both aged groups showed higher expression for carboxyl esterase in crude samples as compared to young rat models. Enzyme kinetics of CE with substrate concentration, pH, and temperature were carried out indicating higher CE activity in crude extracts of control old rats.

The current research on these aspects may help in the elevated carboxyl esterase activity with $LiCl_2$ which contributes for neuronal cell function by stimulating new neural growth and protect neurons from death. Hence, it can be concluded that lithium can be complexed with enzyme or drug for their activation in neuronal function for the applications of fundamental biology, proteomics and biomedicine.

Acknowledgement

Authors would like to acknowledge Dr. Channarayappa, Head of the Department of Biotechnology, MSRIT for his valuable guidance and assistance in project. We are extremely grateful to Dr. Harikishore and Mr. Sushil kumar, Department of Biotechnology, MLACW for providing support and guidance in handling animals. We sincerely thank Mr. Lokesh K.N., Asst Professor and Mr. Shantha Kumar, Department of Biotechnology, MSRIT for their invaluable technical inputs in this project.

References

Asperen VK (1962) A study of housefly esterases by means of a sensitive colorimetric method. J Insect Physiol 8:401–16

Berridge MJ, Downes CP, Hanley MR *(*1989*)* Neural and developmental actions of lithium: A unifying hypothesis. Cell 59:411–419

Cataldo AM, Hamilton DJ, Barnett JL, Paskevich PA, and Nixon RA (1996) Properties of the endosomal-lysosomal system in the human central nervous system: disturbances maik most neurons in populations at risk to degenerate in Alzheimer's disease. J Neurosci 16(1):186–l99

Chen G, Rajkowska G, Du F, Seraji-Bozorgzad N, Manji HK (2000) Enhancement of hippocampal neurogenesis by lithium. J Neurochem 75(4),1729-34

Gomori G (1941) The distribution of phosphatase in normal organs and tissues. J Cell Comp Physiol 17:71–83

Hartree EF (1972) Determination of proteins: a modification of the Lowry method that gives a linear photometric response. Anal Biochem 48:422–7

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 (5259): 680–685

L. Trevor Young (2004) What exactly is a mood stabilizer? J Psychiatry Neurosci 29(2): 87–88

Lowry OH, Rosebrough NJ, Farr AR, Randoll RJ (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem 193: 265–75

Prabha M (2005) A Comparative biochemical study on hydrolytic enzymes in normal postmortem human brain, brain tumors and in their derived cell lines. Thesis, Bangalore University

Prabha.M, Ravi V, Shetty KT, Swamy NR (2006) Pineal gland of elevated hydrolytic enzyme activities and similar carboxyl esterase activity to the rest of the brain regions. Proceedings of the National conference in Chemistry. Sept 27–29, Bangalore

Prabha M, Ravi V, N. Ramachandra Swamy (2013) Activity of hydrolytic enzymes in various regions of normal human brain tissue. Ind J Clin Biochem 28(3):283-291

Ramsey RB, Smith KR Jr., Crafts DC, Chung HD, Fredericks M (1980) Hydrolytic enzyme activities of the nervous system. Arch Neurol 37(6):356–9

Robins E, Smith DE, Daesch GE, Payne KE (1958) The validation of the quantitative histochemical method for use on post mortem material—II: the effects of fever and uremia. J Neurochem 3:19–27

Susana Rivera-Mancía, Iván Pérez-Neri, Camilo Ríos, Luis Tristán-López, Liliana Rivera-Espinosa, Sergio Montes (2010) The transition metals copper and iron in neurodegenerative diseases. Chemico - Biological Interactions. Volume 186, Issue 2, 30, Pages 184–199

Tetsuo Satoh., Masakiyo Hosokawa (1998) The mammalian carboxylesterases: From Molecules to Functions. Annual Review of Pharmacology and Toxicology 38: 257-288

Wielgat P, Walczuk U, Szajda S, Bien M, Zimnoch L, Mariak Z, Zwierz K (2006) Activity of lysosomal exoglycosidases in human gliomas. J Neurooncol 80(3):243–9

Zhang W, Xu G, McLeod HL (2002) Comprehensive evaluation of carboxylesterase-2 expression in normal human tissues using tissue array analysis. Appl Immunohistochem Mol Morphol 10(4): 374–80