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Relation Between Purification Gamma Glutamyl Transferase Activity And Some Electroparameters

Jamal.A.AL.Jabbar.Attawi and Raya Majid Alsharfa

*Middle Technical University, Baghdad, **IRAQ**

Email: rusul.aljabbar@yahoo.com

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ABSTRACT

Gamma glutamyl transpeptidase (GGT) is a transferase, which is of great importance in sustaining intra cellular cysteine and glutathione levels. The abnormal expression of GGT is significantly associated with features of many metabolic syndromes (e.g.,hepatocellular carcinoma). So study the relationship between purification Gamma-glutamyl transferase (GGT) activity, kinetic constants and some electro parameters. We studied on 80 patients with heart failure who attended for treatment in Baghdad Medical City, the patients characterized in Electromyo Cardio Gram (ECG) for treatment in hospital. The patient samples were divided into 40 Females and 40 Males with ages from 29 to 85 years and 80 normal healthy controls, 40 Males, 40 Females with ages from 27 to77 years.

Keywords: Gamma-glutamyl transferase (GGT), maximal velocity (Vmax),Michaelis-Menten kinetic parameter(Km).

INTRODUCTION

Gamma glutamyl transpeptidase (GGT) is a transferase, which is of great importance in sustaining intracellular cysteine and glutathione levels. The abnormal expression of GGT is significantly associated with features of many metabolic syndromes (e.g., hepatocellular carcinoma) [1]. Elevated serum GGT levels may be a reflection of high degree of oxidative stress and oxidative stress is known to be associated with central obesity [2]. High levels of GGT have also been found to be associated with various atherosclerotic risk factors such as Diabetes mellitus, hyperlipidemia, and hypertension, increased GGT activity and occurrence or progression of atherosclerosis [3]. Gamma-glutamyltransferase (GGT) has been found to be involved in the pathogenesis of cardiovascular diseases, especially coronary artery disease, and the prognosis of cardiovascular disease may be predicted by increasing GGT levels. GGT levels are related to cardiovascular emergencies of chronic heart failure [4].

MATERIALS AND METHODS

Optimum Conditions GGT Activity

Optimum pH: The effect of pH on the steady state kinetics of gamma-glutamyl transferase (GGT) from *Bacillus subtilis* was examined using glutamyl-(3-carboxyl)-4-nitroanilide as the chromogenic substrate. The enzyme was active in the pH range 7.0–11.0 with the optimum activity at pH 11.0. We noticed a pH dependent transformation in the nature of substrate consumption kinetics [5].

Optimum Temperature: GGT activity has been studied over the temperature ranged between 20-50˚c The optimum temperature for the enzyme 37˚c [6], and when studying the effect of thermal stability, the enzyme is more stable in ranging between 20 and 40˚c, the enzyme activity remained at 50˚c and for one hour while the activity of the enzyme strongly lower if the temperature more 50˚c [7].

Kinetic Constants: In previous studies we developed a mathematical model and kinetic constants for GGT prepared from normal human liver and kidney and for GGT in pathological human sera [8]. The kinetic constants for GGT prepared from human pancreas for GGT in human serum. (Km of GGT of 5.9 ± 1.00, mM) in present (glycylglycine) as substrate, and (Km = 0.70 ± 0.04, mM) in present, (Gamma-glutamyl-3-carboxy-4-nitroanilide), as substrate [9].

Voltage and current in electrophoresis: The reason why macromolecules migrate in the gel is the difference of potentials (generally referred to as 'voltage'). The higher is the voltage the faster is the migration. The voltage is the controlling parameter here, the actual "driving force" of the whole process(10). It is better to run SDS-PAGE in lower voltage, especially to separate close molecular weight bands. The higher voltage generates more heat. Too much heat can cause diffuse or smile bands [11]. The current depends on both the voltage and the resistance and the resistance increases gradually during electrophoresis [12]. Heating the gel causing resistance to increase and hence decrease of current. The application of constant current gets fluctuated and migration is affected. Thus, by controlling the current you do not really control the proteins migration [13].

We studied on 80 patients with heart failure who attended for treatment in Baghdad Medical City, the patients characterized in Electromyo Cardio Gram (ECG) for treatment in hospital. The patient samples were divided into 40 Females and 40 Males with ages from 28 to 80 years and 80 normal healthy controls, 40 Males, 40 Females with ages from 30 to70 years. Blood samples were collected from October 2015 to February2016. The blood was allowed to coagulate at room temperature and was centrifuged at 3000 r.p.m. for 15min, and the resulting sera were placed into test tubes and were used for purification of GGT, purification of the enzyme GGT serum of patients with heart failure and healthy [7].

1-Precipitation by ammonium sulfate: Precipitation by Ammonium Sulphate deposition was blood proteins using ammonium sulphate then placed in a centrifuge for (15min) and then separates and the buffer solution with a pH 8.3.
2-Separation membrane (dialysis): Dialysis for the purpose of removing the remainder of the ammonium sulfate to precipitate proteins placed the protein dissolved in the above step in the separation of membrane dialysis.
3-Concentration of dissolved protein, it was dissolved in membranous bag and dipped in sucrose crystals for between 30-45 min at a temperature 4 ˚c.
4-Gel filtration: This purification depends on the basis of the difference in molecular weight, and proteins associated with the enzyme, as was the use of gel filtration Sephadex G-150 column.
5-Ion Exchange Chromatography used chromatography technique of ion exchange resin column using DEAE-Cellulose A50 to purify the protein.

6-Electrophoresis Method for the separation of biochemical compounds such as nucleic acids and nucleotides, amino acids and proteins. The separation of molecules depend on several factors including: the size, the number of molecules, in addition to the electric field [14].

Sodium Dodecyl Sulfate- Polyacryl Amide Gel Electrophoresis (SDS-PAGE): The preparation method of Laemmli gel electrophoresis and deportation with some modulations [15].

Statistical analysis: Data expressed as means ± SD. Students't-test used to evaluate differences between the groups .For all tests $P \leq 0.05$ and 0.01 considered statistically significant. All calculations were made using Excel 2007 program for Windows.

RESULTS AND DISCUSSION

The separation and purification of GGT from the serum of patients with heart failure the purification of the enzyme and to dispose of a large proportion of the water and get a degree of purity and is often used for this purpose salts such as ammonium sulfate concentration 60% because of the good solubility in water, where reducing the solubility protein deposition and this is called Salting out [16].The enzyme get a degree of purity, and the separation membrane dialysis process by (0.125 M) Tris-HCl with pH 8.3, where the degree of purification reached enzyme this stage 3.334 and the outcome of the enzymatic 37.33 % and then was used method of chromatography gel filtration using gel Sephadex G-150 ,degree of purification reached 3.40 times and the outcome of the enzymatic 46.30%, as shown in table1. For the purpose of completing purification by chromatography ion exchange using resin DEAE-Cellulose A50 as a mobilization for the column scalable and solutions of sodium chloride which is chromatography ion exchange one of the methods used for the separation and purification of enzymes.

Table 1: separation and purification enzyme GGT of patients with heart failure

Step	*Elute (ml)*		*Total activity (I U)*	*Protein conc. (mg/L)*	*Total protein (mg)*	*Specific activity (I U/mg)*	*Purification (Fold)*	*Yield %*
Crude	7.5		0.57	53.4	0.358	1.3189	-	100
Ammonium sulphate	5.5		0.36	32.2	0.161	1.833	1.40	60.60
Dialysis	5		0.260	8.9	0.0354	5.43	3.334	37.33
Concetrated	4.5		0.3 39	15	0.036	5.735	3.390	54.63
Gel filtationSephadex G-150	5		0.359	12	0.05	5.6	3.40	46.30
Ion exchanange DEAE-Cellulose A50	3		0.113	4.2	0.014	8.26	6.396	21.8

When using a separation by electrophoresis method on SDS-PAGE gel concentration of 10% and the use of Kumasi CBB R250 the enzyme GGT way exchange it appeared the enzyme carries a negative charge toward the anode as protein movement in the gel depends on the size and protein shape [7], [17].

It was the migration of the enzyme protein in pH 8.3 within the electric field between the anode and cathode. The structure of enzyme GGT was stable at pH 8.0-9.4 with an optimum pH of 8.3, and was stable at 20-50°C with an optimum activity at 37°C. The table 2 shows Km values was 1.77 mM and was (Vmax 26 mM min^{-1}) in the sera of patients with heart failure, but in the healthy (Km 0.6mM) (The Vmax 7.4 mM min^{-1}). The Km of the enzyme GGT in the pancreas of human found 0. 70(mM) [9].

Table 2: Km and Vmax for GGT purified from the serum in patients with heart failure and healthy

	(Km)mM	Vmax(mM/min)
patients	1.77	26
Control	0.6	7.4

Low voltage gives more time for protein to separate well on the gel; therefore we get better resolution at low voltage. This leads to increased run times, which allow the proteins more time to diffuse. At high voltage apart from heating proteins run very fast and do not get much time get separated very well [10]. If the voltage is held constant throughout a separation, the current and power (heat) decrease as the resistance increases. Separations using constant voltage are often preferred because a single voltage is specified for each gel type that is independent of the number of gels being run [11]. If the current is held constant during a run, the voltage, power, and consequently the heat of the gel chamber increase during the run. Constant current conditions, as a rule, result in shorter but hotter runs than do constant voltage runs [13].

It is better to run SDS-PAGE in lower voltage, 100V in mini-gel, and 200V maxi-gel. The higher voltage generates more heat. Too much heat can cause diffuse or smile bands. Lower voltage it will give time to the proteins to compact in a very thin front before starting the separation phase. The constant current (20 - 25 mA) for separating and time (40-45 min) .So stable current may indicate stable protein flow. With fresh running buffer during electrophoresis and the current didn't change at all. Stable current would lead to better protein separation since smaller proteins would not have to pass through the stuffed by large proteins gel.

APPLICATIONS

The present study is useful to know some of the reasons for Heart failure patients, the separation and purification of GGT from the serum of patients and Optimum Conditions GGT Activity.

CONCLUSIONS

GGT levels are related to cardiovascular emergencies of chronic heart failure, the better voltage, current and Optimum Conditions GGT Activity for the separation and purification of GGT.

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AUTHOR ADDRESS

1. **AL. Jabbar**
Middle Technical University, Baghdad, IRAQ
Email: rusul.aljabbar@yahoo.com