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# ISOLATION AND STUDY OF MYELOPEROXIDASE AND GLUTAMATE DEHYDROGENASE ENZYMES FROM TUMOR PULMONARY TISSUE

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***ABSTRACT**--The research included the isolation of Myeloperoxidase and Glutamate Dehydrogenase from tumor lung tissue using different biological techniques. These techniques included: ammonium sulfate precipitation, dialysis, gel filtration chromatography on sephadex G–200. For partially purified Myeloperoxidase, The results showed that specific activity and the number of fold of purification were (42.59 U/ml) and (36.3), respectively. On the other hand, the results showed that specific activity and the number of fold of purification for partially purified Glutamate Dehydrogenase were (22.69 U/ml) and (44.1), respectively. Furthermore, using gel filtration chromatography, the comparative molecular weights of the partially isolated Myeloperoxidase and Glutamate Dehydrogenase was (150.3 kDa) and (332.3 kDa), respectively. The study showed that the optimum conditions of Myeloperoxidase were obtained at the first minute using sodium citrate (0.1 M), as buffer at pH (5.5), at a temperature (45 ºC) and (14 mM) of o-dianisidin as substrate. It was found that Vmax and Km have the values of (18.86 U/ml) and (2,69 mM), respectively. Finally, The optimum conditions of Glutamate Dehydrogenase were obtained using Tris-HCl (100 mM) as a buffer, at pH (8.6), at a temperature (40 C°) and (35 mM) of glutamate as a substrate. It was found that Vmax and Km have the values of (14.1 U/ml) and (16.56 mM) respectively.*

***Key words**--Myeloperoxidase, Glutamate dehydrogenase, Purification enzyme, Tumor pulmonary, Tumor*

## I. INTRODUCTION

The tumor is a result of many process induce multi gene changes cause unbalance of cell division and cell death [1]. It causes an increase in growth signals with on response to the antigrowth signals, reduction of gene replication in DNA, also a tissues invasion by tumor, finally metastasis of tumor to neighboring tissues. Cancer is the more diseases can be leads to death in the world [2]. Tumors can be divided into benign tumor and malignant tumor [3].

Lung cancer occurs when cells grow abnormally in one or both lungs, and prevents lungs from functioning normally [4]. It can kill over a million person yearly. Lung cancer caused by smoking kill (75%-80%) of people annually in the United States of America [5]. This research includes isolation and study of Myeloperoxidase and Glutamate Dehydrogenase enzyme from lung cancer.

Myeloperoxidase enzyme (EC1.11.1.7: MPO) is one of the peroxidases that belongs to oxidation and reduction enzymes and contains heme as prosthetic group[6], It is an important component of the oxidation in

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white blood cells. It can acts against different types of bacteria, viruses and parasites. Myeloperoxidase is also responsible for the green colour in the infected area [7].

Myeloperoxidase enzyme was isolated for the first time in 1941 [8]. White blood cells are important source of the enzyme, which present in relatively-large quantities in pigmented granules [7]. This enzyme exist in various body fluids, and also found in infected tissues such as heart, artery and kidney [9]. Pure MPO enzyme isolate from neutrophils, contains two chains of polypeptide. Each chain is consist of light and heavy chain. There is controversy about the actual molecular weight of the enzyme as different researches showed different molecular weight 140,000 -150,000 Da [10,11].

Recent studies showed that MPO enzyme is responsible of many pathway in the body such as hydrogen peroxide reaction with chloride to produce hypochlorose, which is considered an oxidant agent of many biomolecules, In addition it can kill bacteria and other pathogens [12, 13], and activate production of tyrosyl radical which has role in multiple oxidation processes like lipids and proteins oxidation [14].

High plasma level of MPO enzyme is consider as an early risk factor of angina [15], acute coronary syndrome [16], and myocardial infarction [17]. In addition, MPO enzyme can be considered as risk indicator for a future development of artery disease [18]. Furthermore studies mentioned that MPO enzyme has a role in kidney and lungs diseases [19]. Buss and other found that increased level of 3-chlorotyrosine in trachea to newborns suffering from breath disease which develop to chronic lung disease [20]. Moreover, a high activity of enzyme was noticed in hepatitis [21], non-alcoholic hepatitis [22] and Alzheimer disease [23].

Glutamate dehydrogenase enzyme (EC1.4.1.2-4: GDH ) stimulates addition of reductive amines to α-Ketoglutarate producing glutamate by NAD(P)H, or removing of oxidative amines from glutamate to produce ammonia and α-Ketoglutarate by $NAD(P)^+$ [24].

All Glutamate dehydrogenase enzymes is consist of six units Homo hexametric except of fungi GDH is consist of four units Homo tetramer. Molecular weight of each units is 47000-55000 Da [25, 26]. Structure of GDH enzyme depends on the enzyme source [27]. The big difference between the enzyme in mammals and fungi is the presence of antenna domain in subunits of mammals. This antenna consist of 48 amino acids [28]. It is believed that GDH enzyme has a catabolic role in eukaryotes [29], and it is able to absorb nitrogen in yeast and fungi in prokaryotes [30]. Glutamate dehydrogenase enzyme in mammals is consist of six units with molecular weight is 56,000 Da for each unit [31].

The enzyme has a high activity in brain, liver, pancreas and kidney [32].The study of purification and properties of GDH enzyme in mammals from different tissues was investigated in liver [33], kidney [34] and heart [35]. The molecular weight of GDH enzyme in Bovine liver is 320,000 Da [36], while another study of the pure enzyme from human liver was 332,694 Da [28].

Allosteric regulation of GDH enzyme in mammals by positive agent such as ADP and leucine amino acid, and some negative factors or agents such as GTP [37].

## II. MATERIAL AND METHODS

The Myeloperoxidase and Glutamate dehydrogenase were isolated from the pulmonary tissue infected with cancer, Immediately after the obtaining of the tissues from the operation hall of hospital, the tissues were washed

thoroughly with distilled water and transformed to the laboratory the tissues were dried using filter paper, then the dry weight of the tissues were calculated then sliced to small parts followed by the addition of distilled water with a ratio of 3:1. Three steps of freezing and thawing were carried out, then homogenized with homogenizer followed by sonication with ultrasonic for 30 minutes, The suspension was stirred with magnetic stirring in ice bath for two hours. The homogenate solution was filtered through gauze to obtain the crude homogenate, then centrifuged at 2000 xg for 20 minutes to get rid of insoluble particulate, and obtain the pure extract in the supernatant represents . All the processes mentioned above were carried out at cold conditions, then the activity of MPO and GDH were measured.

The activity of MPO enzyme was measured by using Kumar method[38], in which the oxidation of *o*-Dianisidin in presence of hydrogen peroxide to provide colored, solution can be measured at 450 nm using spectrophotometer. The activity of GDH enzyme was measured the spectral by determining the increase of absorbance at 340nm after 1 minute of reaction time [39]. Protein was measured using lowery method [40] modified by Schactele and Pollack [41] to determine the protein concentration.

The protein was isolated by precipitation using Ammonium sulphate at 75% saturation by gradual addition of sold salt with continuous stirring of homogenate at 4 $^0$C for 60 min, the homogenate was left over night at 4 $^0$C in the fridge to complete the precipitation, followed by centrifugation at 15000 xg for 20 min. The precipitate was separated and dissolved in small amount of distilled water, then the protein concentration, MPO and GDH activity were measured.

Dialysis was carried out using the method explained by Plummer [42], in this method tightly closed dialysis tubes were used and sunk in a beaker containing 2 L of ammonium bicarbonate 0.1M at 4 $^0$C. The tubes were stirring using magnetic stirrer for 24 h with replacement of ammonium bicarbonate solution. Protein concentration, MPO and GDH activity were measured, then the lyophilized sample was obtained by dialysis using lyophilizer. Protein concentration, MPO and GDH activity were measured.

This method was used to separate MPO and GDH enzymes and to determine molecular weight as explained by Andrews [43].

Column of 80 x1.0 cm was loaded with Sephadex G-200 gel with high of 77 cm, 1.0 ml of protein liquid obtained by lyophilization and added to the column followed by adding 1.0 ml distilled water. Protein was washed out with flow rate of 12 ml\h and 10 min for each fraction using fraction collector. The protein elution continuously scanned at 280 nm, also MPO and GDH activity were measured in each fraction, the fractions of same enzyme pooled and lyophilized. After the purification of both enzymes, MPO and GDH enzymes were standard with known molecular weight between 2000-0.204 kDa (Blue dextrin, Glucose oxidase, Bovine serum albumin, α-amylase, Egg albumin, Papain, Tryptophan) to detect their molecular weight. Optimum conditions of MPO and GDH enzymes were studied and compared with the published literatures.

## III. RESULTS

The result of partial purification of MPO and GDH enzymes are shown in table (1).The elution volume of the protein solution on gel filtration demonstrate two bands of MPO and GDH as shown in separation curve in fig.(1).The purification fold of MPO and GDH enzymes were 36.3 and 44.1 respectively as shown in table (1).

**Table 1:** Summary of results for purification of MPO and GDH enzyme from tumor pulmonary tissues

| Stage | | Protein conc. mg/ml | Vol. ml | GDH enzyme | | | | MPO enzyme | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Purif. fold | % recovery | Specific activity U/mg | Activity U/ml | Purif. fold | % recovery | Specific activity U/mg | Activity U/ml |
| Crude solution | | 9.63 | 61 | 1 | 100.0 | 0.5148 | 4.958 | 1 | 100.0 | 1.1734 | 11.30 |
| Supernatant solution | | 5.77 | 53 | 1.8 | 92.9 | 0.9191 | 5.303 | 1.8 | 95.4 | 2.1507 | 12.41 |
| Protein ppt. Solution | | 9.75 | 13 | 3.9 | 84.5 | 2.016 | 19.656 | 4.2 | 89.7 | 4.8810 | 47.59 |
| Dialysis | | 4.621 | 15.5 | 6.6 | 80.3 | 3.3885 | 15.658 | 7.2 | 87.5 | 8.4181 | 38.90 |
| Gel Chrom. Sephadex G-200 | MPO | 0.3105 | 40 | - | - | - | - | 36.3 | 76.8 | 42.5990 | 13.227 |
| | GDH | 0.2765 | 33.5 | 44.1 | 69.5 | 22.6908 | 6.274 | - | - | - | - |

Activity: numbers of enzyme unites in 1 ml of solution. , U: quantity of enzyme which convert 1µl of substrate to product in 1min.

specific activity: numbers of enzyme unites in 1 mg of protein.

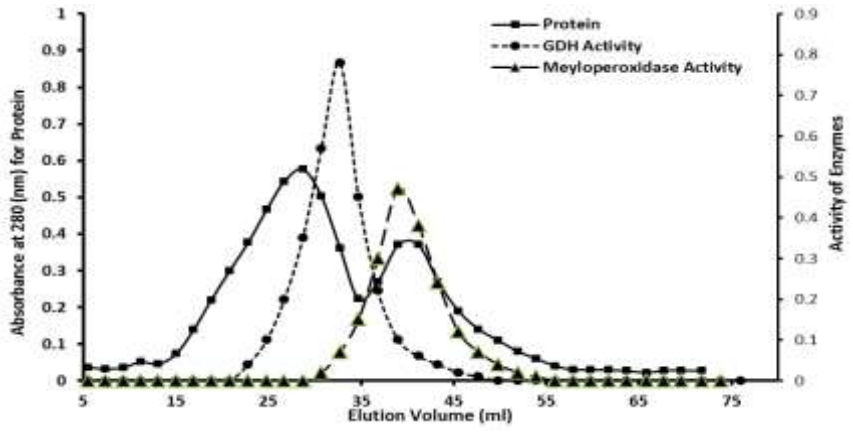



**Figure 1:** Elution curve for protein precipitate solution by gel filtration chromatography using Sephadex G-200

The molecular weight of both enzymes estimated by gel filtration (Sephadex G-200) using standard curve of molecular weight fig. (2), the molecular weights of MPO was 150.3 KDa obtained from elution volume 39.9 ml. This result agreed with the molecular weight of MPO in W.B.C.[11], while other researchers pointed the molecular weight were 146 KDa [10] and 140 KDa [44].

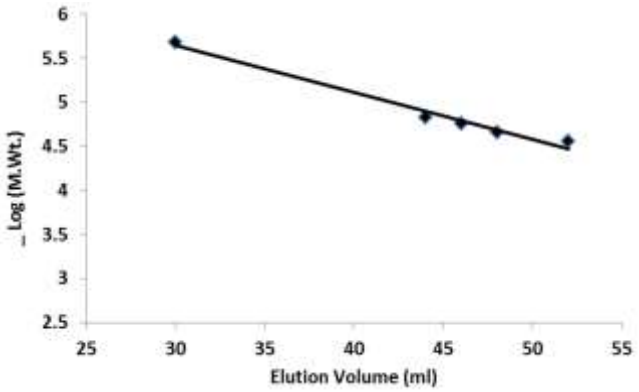



**Figure 2:** Standard curve for determining the molecular weight by gel filtration chromatography using Sephadex G-200

The molecular weight of GDH was 332.3 KDa that obtained from the elution volume of 32.5 ml, the result was similar to molecular weight of GDH in human liver [45], while the molecular weight of GDH in Bovine liver was 320 KDa [36].

The research also included the study of the factors affecting on activity of purified enzymes. The results showed that the activity of MPO and GDH enzyme were increased with the increasing of the enzyme concentration as shown in fig(3), the common concentration was 0.3 mg/ml for each enzyme and was used in all the following experiments.

The result reface that the optimum activity of MPO and GDH enzyme was at 1 min from reaction time of both enzymes, as shown in fig.(4).

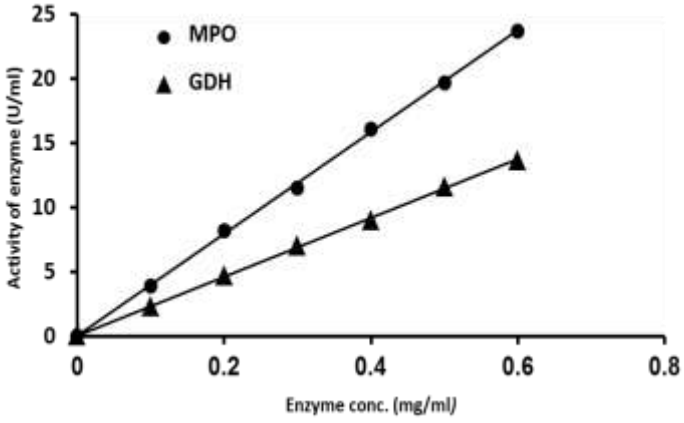



**Figure 3 :** Effect of enzyme concentration on activity of MPO and GDH enzymes

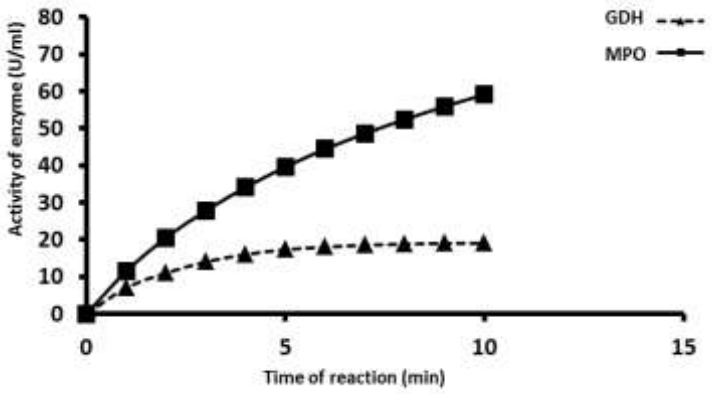



**Figure 4:** Effect of the reaction time on the activity of MPO and GDH enzymes

The results showed that the optimum pH of MPO enzyme was 5.5 using 0.1 M citrate buffer, this result agreed with Mathy-Harter[45], and the optimum pH of GDH enzyme was 8.6 using 0.1 M Tris-HCl buffer, as shown in figure (5).

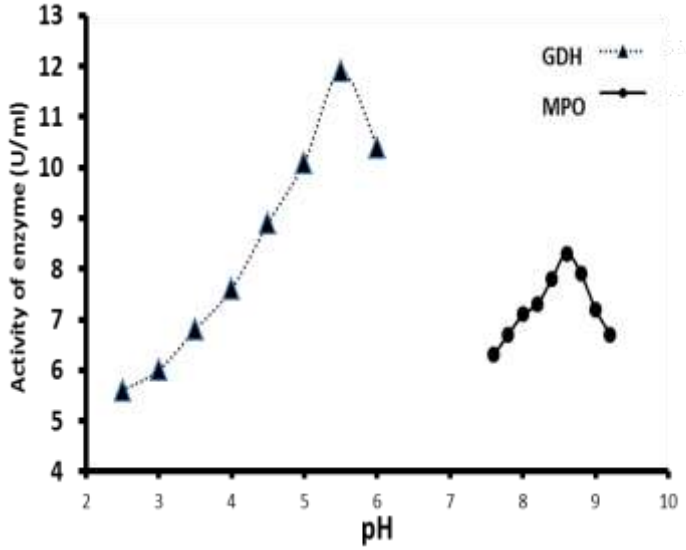



**Figure 5:** Effect of pH on the activity of MPO and GDH enzymes

The effect of the temperatures studied for MPO and GDH enzymes were ranged from 10 $^0$C to 55 $^0$C. The optimum temperature for MPO was 45 $^0$C , while the optimum temperature for GDH was 40 $^0$C, the results showed that the Optimum temperature of GDH was lower than the optimum temperature of MPO because the stability of the reaction solution of GDH was decreased [46, 47], as shown in fig.(6).

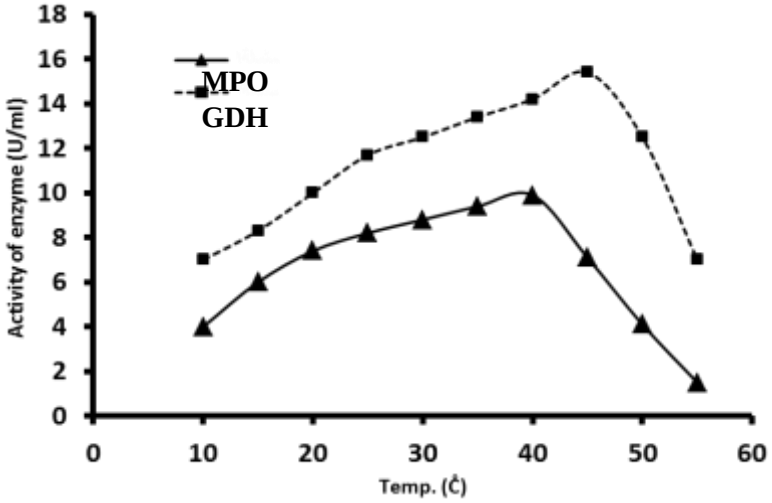


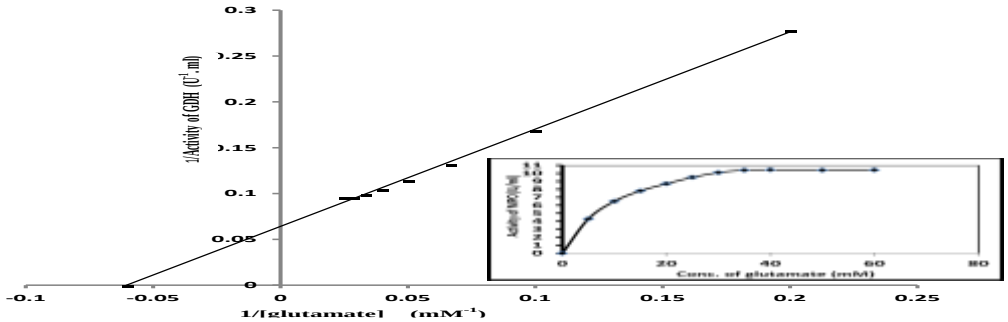

**Figure 6:** Effect of temperature on the activity of MPO and GDH enzymes

The results showed the optimum velocity (Vmax) for MPO and GDH enzyme occur when using 14 mM (*o*-dianisidin) and 35mM (glutamate) respectively using Michaelis-Menten curve, and the Michaelis-Menten constant (Km) and Vmax for each enzyme were 18.86 U/ml , 2.69 mM for MPO enzyme and 14.1 U/ml , 16.56 mM for GDH enzyme using Lineweaver-Burk plot, as shown in fig.(7),(8).

The research provided the optimum condition for MPO and GDH enzymes that purified from tumor pulmonary tissue, as shown in table (2).

**Table 2:** The optimum conditions for MPO and GDH enzymes that purified from tumor tissues

| Enzyme | Reaction time min | Enzyme concentration mg/ml | pH of reaction solution | Temp. of reaction $^0$C | Substrate concentration mM |
|---|---|---|---|---|---|
| MPO | 1.0 | 0.3 | Citrate, 100 mM, pH =5.5 | 45 | 14 |
| GDH | 1.0 | 0.3 | Tris-HCl, 100mM , pH =8.6 | 40 | 35 |




**plot for GDH**

**Figure 7:** Effect of substrate (glutamate) concentrations on the activity of GDH and Lineweaver-Burk

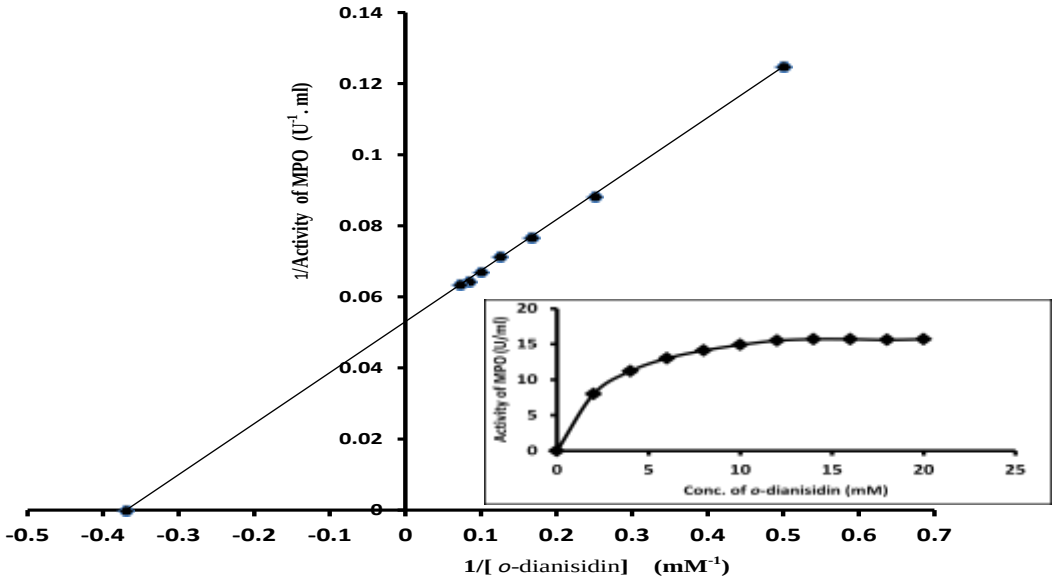



**Figure 8:** Effect of substrate (o-dianisidin) concentration on activity of MPO and Lineweaver-Burk plot for MPO

## IV. CONCLUSION

In this study, molecular weight of Myeloperoxidase and Glutamate dehydrogenase was identified after isolated and purified from the pulmonary tissue by using Gel filtration. Moreover, the study demonstrated the optimum conditions for purified Myeloperoxidase and Glutamate dehydrogenase, then determined the Km ,Vmax values for both enzyme.

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