# Isolation and Purification of Meprin α from the blood Serum of COVID-19 Patient

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**Abstract**

Meprins are zinc metalloproteases that are membrane-bound and secreted from these membranes. Many of these enzymes are secreted from the cell membranes and some of them are attached to the cell membrane and act both extracellularly and intracellularly. They have a role in tissue differentiation and remodeling and the processing of physiologically active peptides and cytokines in adult tissues. The Meprin α enzyme was isolated and purified from the blood serum of COVID-19 patients using a variety of biotechniques, such as sedimentation with 65 percent ammonium sulphate, dialysis, and ion exchange chromatography, where one highly active main peak is separated by the DEAE-cellulose ion exchange method. The number of purifications was 16 times. Then, using Sephadex G-150 for gel filtration, it was found that there was one peak with strong activity for the Meprin α, which had gone through 30 purification cycles with a 55 percent recovery rate of the enzyme. Furthermore, the estimated molecular weight of Meprin α extracted and partially purified from blood serum (140560 Dalton) of one peak was determined using gel filtration chromatography (Sephadex G-150). The results show that the optimal conditions for the activity of the isolated and partially purified enzyme are a potassium phosphate buffer at a pH of (8), a reaction time of (15 minutes), and a temperature of (40°C) using a concentration of (0.48 mmol/L) of tetramethyl benzidine (TMB) substrate, The maximum velocity (Vmax) of Meprin α was 136.9 U/ml with an enzyme concentration of 150 ng/ml and Michaelis-Menten constant (Km) values of 0.133 mmol/L.

Keywords: Meprin α, isolation, purification, gel filtration, ion exchange, maximum velocity (Vmax), Michaelis-Menten constant (Km).

## 1. Introduction

Meprins are zinc metalloproteases that are membrane-bound and secreted from these membranes. Many of these enzymes (metalloproteases) are secreted from the cell membranes and some of them are attached to the cell membrane and act both extracellularly and intracellularly. During embryogenesis, they have a role in tissue differentiation and remodeling, as well as in the processing of physiologically active peptides and cytokines in adult tissues. Metalloproteinases have a role in a variety of disorders, including inflammatory diseases and cancer [1]. Meprins were found in diabetic mice in 1980 because of a quest for proteolytic enzymes [2]. They are highly glycosylated and expressed in epithelial cells of the kidney and small intestine, leukocytes, and cancer cells [1]. extracellular matrix proteins, growth factors, and physiologically active peptides can all be cleaved by Meprins. Meprins are among the most important matrix-degrading metalloproteases in the intestine and kidney [3]. Meprin α and Meprin β were discovered as two separate enzymes in the following years [4]. Proteolytic removal of amino-terminal properties activates Meprins, which are released as zymogens. Depending on the expressing tissue, many activation mechanisms have been discovered. Trypsin converts both human enzymes to their mature versions in the gut [5]. Meprin is selectively activated by plasmin outside the gut [6]. The purification of enzymes is critical for creating a better understanding of how enzymes work. This includes initial protein recovery, concentration, primary purification, and eventually high-end resolution chromatographic purification [7]. The purified enzyme is also needed for property research and a better understanding of the structure-function connection. Protein separation methods, for example, have historically been used to separate and purify certain proteins to assist in investigations of their enzymatic, chemical, physical, and structural characteristics [8]. These sorts of investigations are required to understand the mechanism by which individual enzymes operate and are regulated. Several Meprins have been purified and described from diverse sources. The purification and characterization of the enzyme were deemed factors influencing the characteristics of Meprin to be relevant [9]. In the current investigation, Meprin was isolated from COVID-19 blood serum and purified before being subjected to biochemical characterization and kinetic tests.

## 2. Methods and Materials

### Blood Sample Collection

The patients in this study included (40) COVID-19 individuals that were diagnosed using the Polymerase Chain Reaction (PCR). Patients treated at Al-Shifa Hospital and Ibn-Sina Hospital in Mosul/Iraq, collected samples between January and March 2022. All the patients are between the ages of 20 and 75 years old.

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Blood samples of five millilitres were collected from the veins of each patient and healthy subject group. It was divided into two parts, the first being in glass tubes containing the gel. The serum was separated by centrifugation at 6000 rpm for 10 minutes.

## 2.1. Purification and Isolation of Meprin α

The following processes were used to purify the Meprin α from the COVID-19 patient's blood serum using customary purification techniques.

### A. Precipitation of enzymes with ammonium sulphate

The ammonium sulphate precipitation procedure is one of the earliest phases of purification, during which the proteins in the blood serum are precipitated. The crude enzyme was precipitated by gradually adding salt 65% to the serum and stirring continuously for 1 hour with a magnetic stirrer. After that, leave the solution at 4°C for 24 hours to finish the sedimentation process. The process of sedimentation is carried out using a cooling centrifuge at 10,000 rpm for 15 minutes [10].

### B. Purification by dialysis

The dialysis was carried out by injecting the protein solution obtained from the previous stage into cellophane tubes, which were then washed with distilled water and dipped in a volumetric container containing 2 L of ammonium bicarbonate ($NH_4HCO_3$) at a concentration of 0.1 M to prevent the Donnan effect. After the dialysis procedure is completed, the final volume and concentration of the total protein are calculated, and the enzyme activity in the dialysis solution is determined [11].

### C. Purification by Diethylaminoethyl cellulose ion-exchange chromatography

The enzyme solution from the preceding stage was put on a DEAE-cellulose column (2.5 x 30cm) that had previously been equilibrated with 0.05M phosphate buffer pH 7. The protein solution from the previous stage was silently injected at the top of the column. The column was then washed with an equivalent amount of the same buffer, and the bound proteins were eluted in steps with increasing sodium chloride concentrations (0.1–1 M). Each fraction collected was measured for absorbance at 280 nm using a UV-VIS spectrophotometer at a flow rate of 60 ml/hr throughout the column. Each fraction's enzyme activity was measured [12].

### D. Purification by Gel Filtration Chromatography using Sephadex G-150

After DEAE-cellulose ion exchange, the lyophilized protein was dissolved in buffer and loaded onto an equilibrated Sephadex G-150 column, where fractions were collected at a flow rate of 3 ml/minute while the absorbance of protein at 280 nm and enzyme activity was measured using the standard kit assay method.

## 2.2. Determination of the molecular weight of the separated enzyme by gel filtration technique.

Gel filtration chromatography is used to determine molecular weight. Gel filtration chromatography was used to determine the protein's molecular weight. The Sephadex G-150 column (2.5x30cm) was equilibrated with 0.05M phosphate buffer (pH 7) and eluted with the same buffer. Alcohol-dehydrogenase (150,000 Dalton), albumin (66,000 Dalton), carbonic anhydrase (29,000 Dalton), and lysozyme were used as molecular weight indicators (14300 Dalton). The Blue Dextran was used to determine the void volume at 600 nm, and the elution volume for each standard protein was quantified at 280 nm [13].

Dissolve 2 mg/ml blue dextran in an equilibration solution containing 5% glycerol. The peak percentage of this concentration of blue dextran will have an A280 of 1.0. The sample volume recommended is 2 mL. To calculate Vo, carefully apply the blue dextran sample to the column.

Measure the elution Volume (Ve) of effluent collected from the point of a sample application to the center of the effluent peak to calculate the Ve for the protein standards. An A280 of 1 in the peak fraction is obtained for a 2.5 ×30 cm column when 2.0 ml of individual samples are applied at the recommended concentration. The same sample volume and flow rate as for the blue dextran sample should be used to apply protein standards to the column [14].

### 2.2.1. Protein estimation using the Bradford technique

A spectrophotometer set to 595 nm was used to assess the total protein content of the Meprin α enzyme. The standard protein was bovine serum albumin (BSA) [14].

## 2.3. Kinetic analysis of purified Meprinα enzyme

#### A: Effect of the enzyme. concentration on activity Meprin α

The activity of the Meprin α was determined by the presence of various concentrations (50–250) ng/mL of the partly purified enzyme {Formatting Citation}.

#### B: The effect of the type of buffer on Meprin α activity:

The effects of the following buffer solutions were studied: ($Na_2HPO_4$ – $NaH_2PO_4$), ($C_6H_8O_7$ – $C_6H_5O_7Na_3$), ($K_2HPO_4$ – $KH_2PO_4$), ($C_6H_8O_7$ – $C_6H_5O_7Na_3$) ($C_4H_{11}NO_3$– HCl ) on the activity of Meprin α at (0.05M. pH 7).

#### C: Effect of. pH. on Meprin α Activity

The enzyme activity of Meprin α was measured in a buffer solution with different values of pH and in

the range of (5 - 9 pH) to find the highest activity of the enzyme.

D: Effect of Incubation time on Meprin α Activity

The reaction time was examined to determine the best period for maximum enzyme activity. The enzyme activity was monitored at 5–25-minute intervals.

E: Effect of Temperature on activity Meprin α

The enzyme activity was measured at different temperatures (37-40 °C) to determine the optimum temperature that gives the highest enzyme activity.

F: Effect of Substrate Concentrations on Meprin α Activity

To study the effect of the substrate on the activity of the Meprin α partial purified, different concentrations of 3,3',5,5'-Tetramethylbenzidine (TMB) were prepared to determine the optimal concentration that results in the highest speed maximum velocity (Vmax) and Michaelis -Menten Constant (Km) [16].

## 3. Results and Discussion

The Meprin α enzyme was isolated and purified from the blood serum of COVID- 19 patients using a variety of biotechniques, such as sedimentation with 65 percent ammonium sulphate, dialysis, and ion exchange chromatography, Results in Figure (1), showed one highly active main peak is separated by the DEAE-cellulose ion exchange method. The results shown in Table (1) indicated that the number of purifications was 16 times, and the percentage of enzyme recovery is 61%. Then, using Sephadex G-150 for gel filtration, it was found that there was one peak with strong activity for the Meprin α as shown in Figure (2), which had gone through 30 purification cycles with a 55 percent recovery rate of the enzyme.

Table (1): Purification steps for Meprin α from blood serum of covid-19 patients.

| Purification step | Volume (ml) | Protein concentration (mg/ml) | Total Protein (mg) | Enzyme activity* (U/ml) | Total activity (U) | Specific activity (U/mg) | Purification (folds) | Yield (%) |
|---|---|---|---|---|---|---|---|---|
| Crude enzyme | 43 | 56.2 | 2416.6 | 101.2 | 4351.6 | 1.8007 | 1 | 100 |
| $(NH_4)_2SO_4$ precipitation 65% | 13 | 49.41 | 642.33 | 157.3 | 2044.9 | 3.1835 | 1.7677 | 47 |
| Supernatant | 37 | 5.6 | 207.2 | 0.21 | 7.77 | 0.037 | 0.02 | 0.178 |
| Dialysis | 14 | 17.5 | 245 | 132.7 | 1857.8 | 7.58 | 4.20 | 43 |
| DEAE-Cellulose | 21 | 4.3 | 90.3 | 126.4 | 2654.4 | 29.39 | 16.32 | 61 |
| Sephadex G150 | 21 | 2.1 | 44.1 | 113.9 | 2391.9 | 54.23 | 30.11 | 55 |

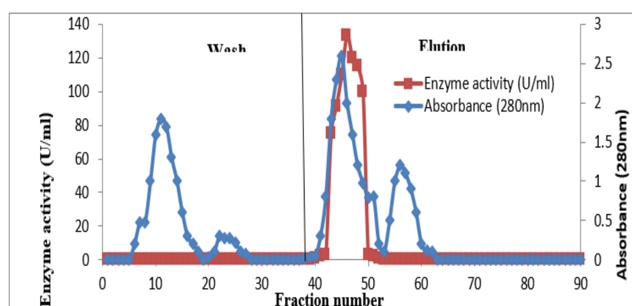



*Figure (1): The elution profile of the Meprin α proteinous peak from the DEAE-cellulose ion exchange column.*

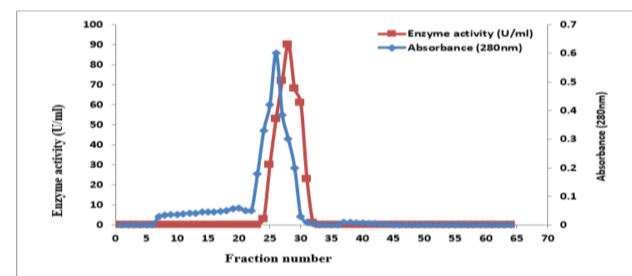



*Figure (2): Profile of the Meprin α proteinous peak from the serum of COVID-19 patients using a gel filtration technique using a gel column type of Sephadex G-150*

### 3.1. Calculation of molecular weight

The estimated molecular weight of the protein band focused on one peak arising from the separation column containing Sephadex G-150 has been determined, As a result, it has been used to estimate the molecular weight of the enzyme by passing several compounds with recognized molecular weights ranging from (14300 - 2000000 Dalton) through the separation column to determine the column's properties. Table (2) displays the standard chemicals that pass through the Sephadex G-150-containing separation column, together with their molecular weights and elution volumes. The approximate molecular weight of Meprin α partially purified from serum was (140560 Daltons) by making a standard curve between the logarithm of the molecular weight and elution volume Figure (3).

Table (2): Ve/Vo and molecular weights of the standard substances that were passed through the separator column containing the gel Sephadex G-150.

| Ve/Vo | Molecular weight (Dalton) | Standard substances |
|---|---|---|
| 1.4 | 150000 | Alcohol dehydrogenase |
| 2.3 | 66000 | Egg albumin |
| 2.7 | 29000 | Carbonic anhydrase |
| 2.9 | 14300 | lysozyme |
| 1.5 | 140560 | The unknown (Meprin )* |

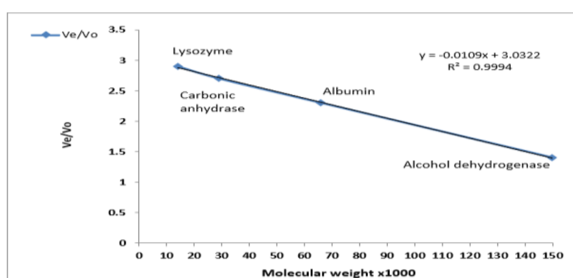



*Figure (3): Standard curve for approximate molecular weight*

### 3.2. Characterization of isolated and purified Meprin α from COVID-19 Patients' Serum.

The results show that the optimal conditions for the activity of the isolated and partially purified enzyme are at an enzyme concentration of 150 ng/ml (Figure 4) using a concentration of (0.48 mmol/L) of tetramethylbenzidine (TMB) substrate(Figure 5), The best buffer was a potassium phosphate at pH (8) (Figure 6,7), a reaction time of (15 minutes) (Figure 8), and a temperature of (40°C) (Figure 9), Also, the kinetic studies of Meprin α showed that the Vmax of Meprin α was 136.9 U/ml and the Km value was 0.133 mmol/L (Figure 10).

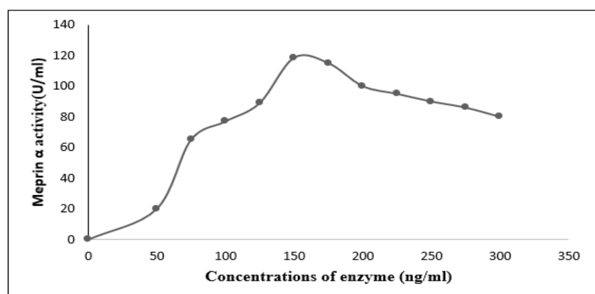



*Figure (4): Effect of different concentrations of the substrate on the activity of the Meprin **α** enzyme*

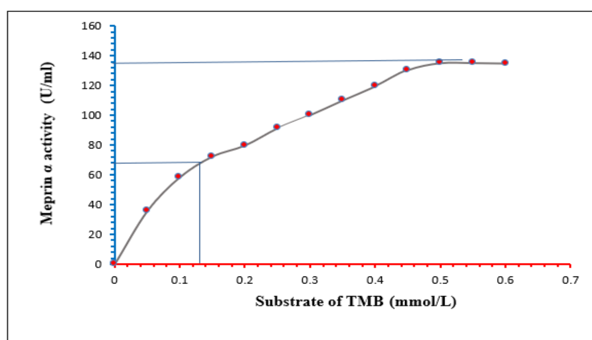



*Figure (5): Effect of different concentrations of the substrate on the activity of the Meprin **α** enzyme.*

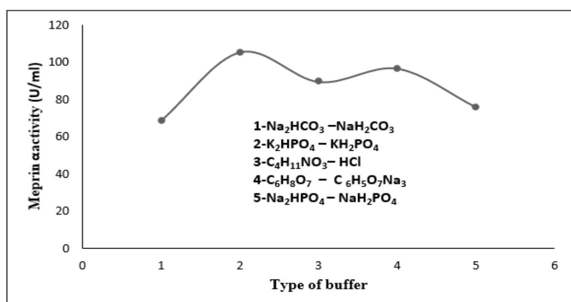



*Figure (6): The effect of the type of buffer on Meprin **α** activity*

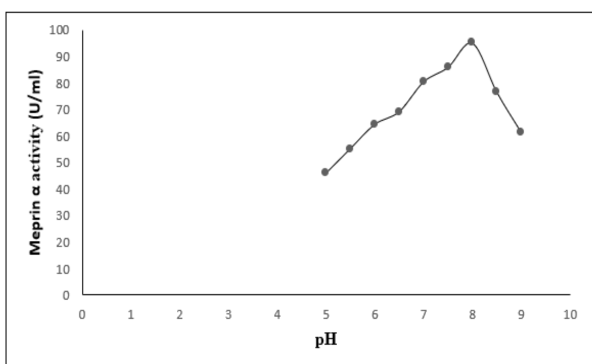
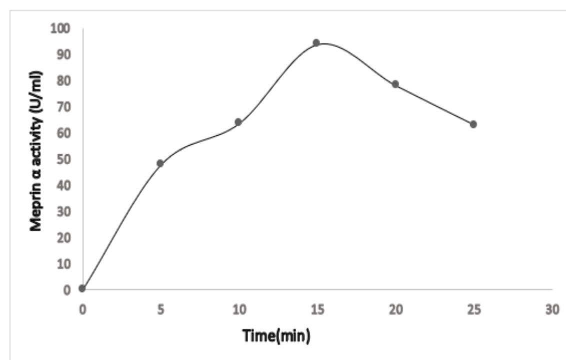



*Figure (7): The Influence of pH on Enzyme Activity*




*Figur (8): Effect of Incubation Period on Meprin **α** Activity*

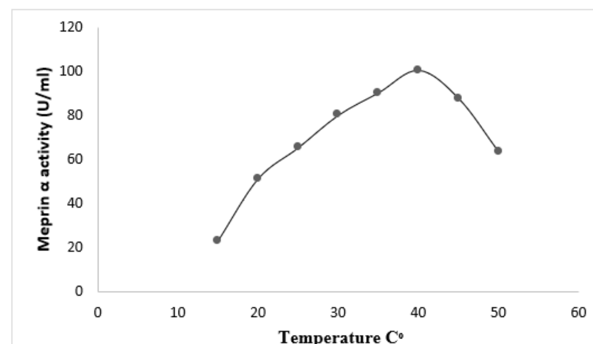



*Figure (9): Effect of Temperature on activity Meprin **α***

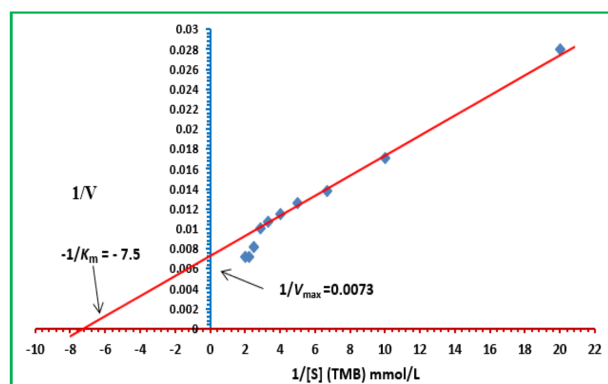



*Figure (10): Lineweaver–Burk plot to show the values of the Michaels constant and the maximum velocity.*

The summary of the optimal conditions for measuring the effectiveness of Meprin α purified from the serum of COVID-19 patients is shown in Table (3).

| Table (3): Optimal conditions for Meprin α purified from the serum of COVID-19 patients. | | | | | |
|---|---|---|---|---|---|
| Substrate concentration (mmol/L) | Temperature C° | Reaction time (min) | pH | Buffer type | Enzyme concentration (ng/ml) |
| 0.48 | 40 | 15 | 8 | potassium phosphate | 150 |

Purification of the enzyme is critical for getting a better understanding of how the enzyme works. It's also necessary for property research and gaining a better grasp of structure-function links. In our investigation, Meprin α was isolated from COVID-19 blood serum 14 and 23 times using DEAE-cellulose ion exchange and gel filtration chromatographic methods, respectively. Meprin α has been

demonstrated to be active throughout a broad pH range of 5–9 depending on the substrate and has special properties including thermostability and pH stability [17]. According to a different study, an enzyme from rat kidneys was most active at 45 °C [18]. At 40 °C and pH 9.0, [19] calculated the kinetic parameters (Km and Vmax) for Meprin α from mice kidneys for casein concentrations ranging from (0.5-7.5) mg/ml. The purified Meprin α has a Km and Vmax of 3.3 mg/ml and 15 U/mg protein, respectively [19]. Based on the results for Meprin α [20], it was determined that the enzyme had a typical Michaelis-Menten kinetic, with an apparent Km for the substrate of 3.2 mg/ml. Iqbal et al [18] used casein as a substrate and produced pure Meprin α with Km and Vmax values of 0.03564 M and 69.76 U/mL. The current study's findings reveal that ammonium sulphate precipitation, ion exchange, and gel filtration chromatography all result in a considerable increase in Meprins α specific activity. The refined Meprin α has a molecular weight of 140560 Daltons. Studies of the purified Meprin's characterization show that key characteristics, such as high activity at neutral pH and temperature and low Km, which are particularly present in this Meprin (compared to other Meprin from literature), can be very useful to design a future study on the competitive inhibitory effects for different chemical compounds or ions to reduce or inhibit Meprin's activity, which is highly desired in the medical field as an attempt to protect Meprin may work in vivo to break down big proteins, which calls for several neighboring active protomers [21]. The presence of many subunits may improve proteolysis efficiency. Linking these subunits together in a certain geometric shape might aid in protein digestion [22]. Furthermore, Meprin α resembles other proteases such as aminopeptidase I, a large multimeric complex formed of homo-oligomers that operate as polypeptide digestors and is involved in protein turnover inside the cell [23],[24]. The Meprin homo-oligomer, on the other hand, is released from the cell and destroys extracellular peptides and proteins [25].

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