

ISOLATION AND PURIFICATION OF MYELOPEROXIDASE FROM HUMAN POLYMORPHONEUCLEAR-CELLS (PMN)

Essam F.A. Al-Jumaily¹ BSc MSc PhD, Muntaha A.K. Al-Safar² BSc MSc PhD,
Rhagad A.A. AlSuhail³ BSc MSc PhD

Abstract

Background: Myeloperoxidase (MPO) oxidoreductase, EC.1.11.1.7 stored in granules of neutrophils ingest microorganisms by generating of reactive oxidants.

Objective: Isolation and Purification of (MPO) from polymorphonuclear cells.

Methods: The enzyme was purified from polymorphonuclear blood cells by Ion exchange chromatography by CMC and gel filtration Sephacryl S.200 column and SDS electrophoresis.

Results: Polymorphonuclear cell (PMN) were isolated from human blood; cell extract was prepared by homogenization of cell pellets in 0.34 M sucrose. Human (PMN) Myeloperoxidase (MPO) has been purified to homogeneity by two- steps procedure, which included CM-cellulose ion exchange

chromatography and Sephacryl S- 200 column at purification fold and recovery of 1.281 and 43.94% respectively. The final product was homogeneous when examined by SDS -polyacrylamide gel electrophoresis. The molecular weight of the enzyme is 80,000 daltons as determined by SDS- PAGE and 88,000 daltons by Sephacryl S-200.

Conclusion: The purification of MPO with accepted yield may open new approaches for its using in the medical application as preparing of monoclonal antibodies and diagnostic kits for detection of antimyeloperoxidase that are required for some inflammatory diseases

Keywords: Polymorphonuclear cells; Myeloperoxidase

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Introduction

Myeloperoxidase (MPO) (donor, hydrogen peroxide oxidoreductase, E.C. 1.11.1.7 is a heme-containing enzyme stored in immense amounts in azurophilic granules of neutrophils, these granulocytic cells ingest microorganisms into phagosomes where it is killed by generating an array of reactive oxidants^[1,2]. It is assumed that MPO acts by producing hypochlorous acid, HOCl, which is also likely to contribute to the tissue damage caused by neutrophils at sites of inflammation^[3,4]. MPO belongs to the mammalian peroxidase superfamily the enzyme is a disulfide-linked dimer ($\square\square_2$) of 145KDa with each heavy subunit containing

a heme group and this enzymes includes eosinophil peroxidase, lactoperoxidase, thyroid peroxidase^[5,6].

The enzyme represents 5% of neutrophil 1% of monocyte protein but has been believed to be absent from macrophages^[7]. MPO is a major neutrophil protein and may be involved in the nitration of tyrosine residues observed in a wide range of inflammatory diseases that involve neutrophils and macrophage activation, MPO is released into the extracellular medium where its measurement can be used as an index of neutrophil activation^[8]. Most of hydrogen peroxide generated by neutrophils is consumed by MPO^[9].

¹Genetic Engineering and Biotechnology Institute, Baghdad University, ²Health Dept. Medical Tech. Institute, Baghdad, ³Dept. Biology, Science College, University of Baghdad.

Address correspondence to be addressed e-mail samgen992003@yahoo.com

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Methods

Isolation of polymorphonuclear cells (PMN). The method of vasiliauskas was followed by^[10] and the blood was aspirated from 40 healthy donor's.

Purification of human leukocyte Myeloperoxidase:-

First step: Preparation of crude extract

1- (PMN) extracts were freezer and thawed several times^[11].

2-Pellet suspended in 0.34 M sucrose

3-This is considered the crude.

Second step: CM-Cellulose column chromatography

The concentrated crude cell extract which contained all the Myeloperoxidase and then applied to a column of CM-Cellulose (3.5x15cm) was equilibrated with 0.02M sodium acetate and 0.1M NaCl (pH=5.0) overnight at 4°C . The column of CMC washed with 60ml of the same buffer and the enzyme was eluted with a linear gradient in the same buffer from 0-0.5M, NaCl, total gradient was 150ml, fractions of 3ml were collected.

Three step: Sephacryl S-200 column chromatography

The active concentrated Myeloperoxidase was placed on column (2 x 70 cm) which had been equilibrated with 0.1 potassium phosphate (pH=7.3) and washed with the same solvent. Fractions of 3ml were collected.

Estimation of Myeloperoxidase activity:

Myeloperoxidase activity was determined by the method of Chance and Maehly^[12]. The reaction mixture (3ml) contained 1ml of 50mM Sodium phosphate (pH=7.3). 2ml of 20mM guaiacol, 20ml of

40mM H₂O₂ and then enzyme. The reaction was started by adding H₂O₂ and increase in absorbance at 470nm was followed in Spectrophotometer. One unit Myeloperoxidase was defined as the amount of enzyme causing increase of 1 unit in the absorbance at 470nm in 1min. at 20°C under these Conditions.

Protein Determination:

The protein content of the cell free extract was determined by the method of Barford *et al.*,^[13]. With Bovine Serum Albumin (BSA) as the standard.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

This was performed as described in Garfine^[14] for the Lamemml system using Bio-Red vertical slab gel cell. Electrophoresis utilized a 0.1M Tris-glycine buffer, pH=8.3 and 1% SDS and 2-mercaptoethanol. A 7.5% gel was employed.

Results

The purification of human (MPO) from (PMN) cells (Table I) included two steps, firstly, CM-cellulose ion exchange chromatography, in this step one peak was obtained (Figure1) which had fold and recovery of 2.96 and 92.12% respectively.

Table 1: Purification of Myeloperoxidase form human neutrophil

Sample	Volume	Enzyme activity u/ml	Total unit	Protein mg/ml	Specific activity u/mg	Fold	Yield
Crude	20	117.3	2346	1.35	86.9	1	100
CM-Cellulose	12	180.09	2161.08	0.7	257.27	2.96	92.12
Sephacryl S-200	9	105.5	949.5	0.32	329.69	1.281	43.94

The active product of the CMC step which was loaded on Sephacryl S-200 column gave one peak (Figure 2) and with

fold and yield 1.281; 43.94% respectively. The specific activity of the purified human MPO was 234.44 unit/ mg.

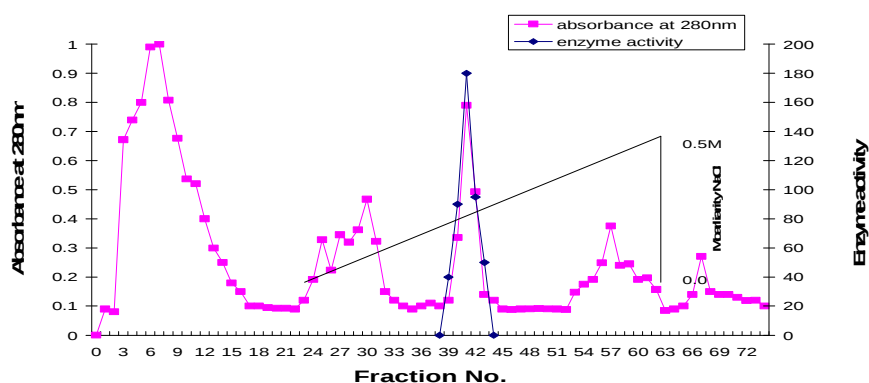


Figure 1: CM-Cellulose chromatography of human polymorphonuclear myeloperoxidase fractions obtained from crude sample. The column (3.5x15 cm) was equilibrated with 0.02M sodium acetate and 0.1M NaCl, pH 5.0 and eluted with a linear gradient in the same buffer from 0-0.5M NaCl. Total gradient was 250 ml.

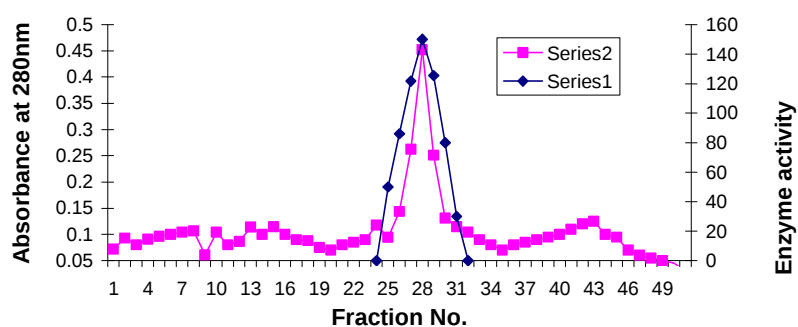


Figure 2: Sephacryl S-200 gel filtration of myeloperoxidase. Concentrated solution from CM-Cellulose (3 ml) was loaded on a Sephacryl S-200 column (2x70 cm) which was equilibrated Myeloperoxidase was eluted with the same solution and fractions of 3 ml were collected

The molecular mass estimated by two methods; the gel filtration by using Sephacryl S-200 column and with the standard molecular weight protein (Figure 3). The molecular weight was estimated to

be 88,000 Daltons .The second method was SDS-polyacrylamide gel electrophoresis, the purified (MPO) appeared as one band at position corresponding to molecular weight of 80,000 daltons (Figures 4 and 5).

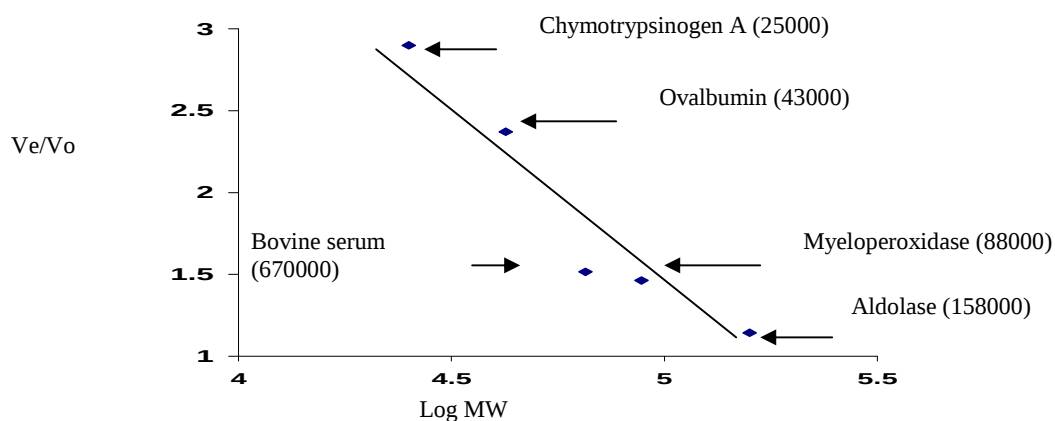


Figure 3: Determination of molecular weight for human MPO by gel filtration by using Sephacryl S-200 Column (70x2 cm)

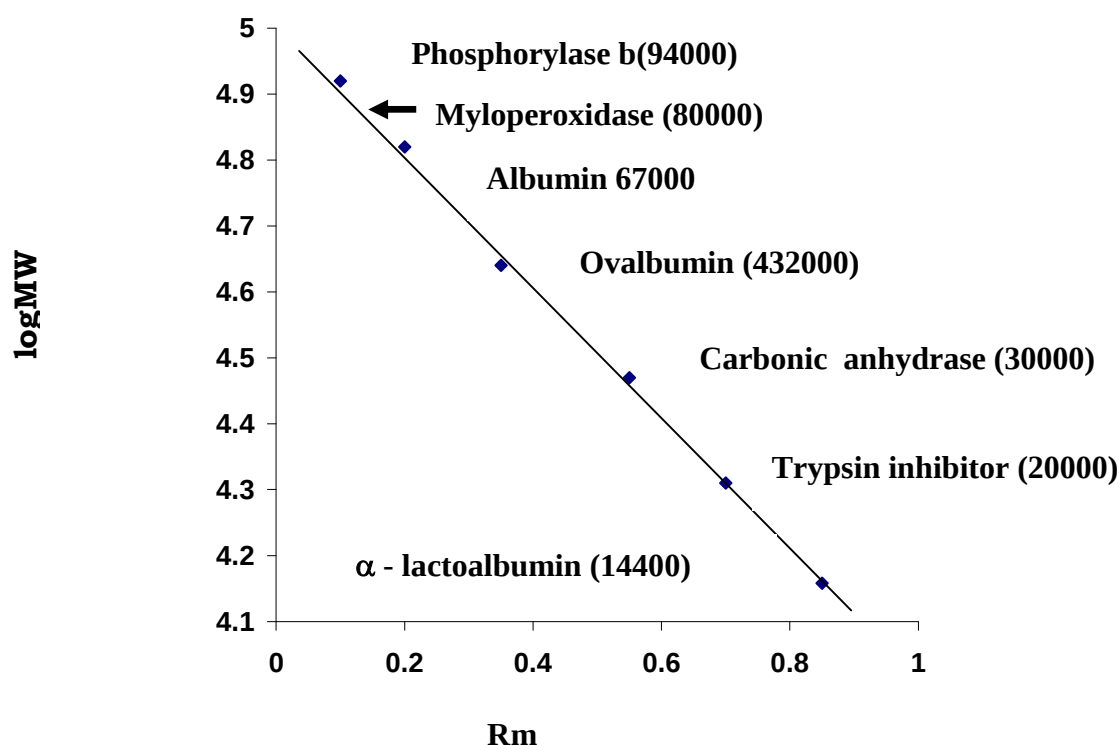


Figure 4: Determination of molecular weight for human MPO by SDS polyacrylamide gel electrophoresis.

In previous studies human (MPO) was purified from Leukocytes of pool peripheral blood from several donors, heterogeneity has been observed in the purified (MPO) obtained in this way possibly due to the heterogeneity of its source and several form of human (MPO) have been separated by polyacrylamide gel electrophoresis^[15].

Discussion

Matheson^[16] purified human leukocyte (MPO) to homogeneity by three steps namely, dialysis of agranule extract against low salt buffer, Sephadex G-75 chromatography and Carboxy methyl cellulose chromatography. The final yield of activity was excellent and represented 79% of the original activity in leukocyte homogenate, the final homogeneous when examined by acid polyacrylamide gel electrophoresis and Sedimentation equilibrium ultracentrifugation, while^[17] purified human (MPO) from Leukemia HL-

60 by Carboxymethyl Sepharose CL-6B column chromatography, and Sephacryl S-200 gel Filtration with 31.2% yield and 737.1 unit/mg specific activity. They found that MPO consisted of a small size; Mr 79,000 daltons in addition to large size Mr, 153,000 daltons the small MPO differed in immunological properties from large MPO.

Brown^[18] purified human myeloperoxidase to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and a minor band with apparent molecular masses of 60000 Daltons and 15000 Daltons respectively, were recognized by both antibodies- under reducing and denaturing conditions on polyacrylamide gel electrophoresis, human myeloperoxidase gave rise to bands of Mr 57,000;39,000;500^[5].

Kettle^[19] purified (MPO) from neutrophils, azurophilic granules released by sonication of cells are extracted using cetyltrimethyl-aminonium bromide (CTAB), purification of the enzyme was

done by column chromatography using concanavalin A bound Sepharose followed by CM-Sepharose, final purification achieved by Chromatography on a phenyl - Sepharose column.

The purification of MPO with accepted yield may open new approaches for its using in the medical application as preparing of monoclonal antibodies and diagnostic kits for detection of antimyeloperoxidase that are required for some inflammatory diseases^[19-21].

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