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

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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 homogenizetion 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 Sephacyl 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 Sephacyl S-200.

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

Keywords: Polymorphonulear 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 macrophags[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].

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:-

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