BIOLOGICAL AND CLINICAL SIGNIFICANCE OF CATHEPSIN GROUP OF ENZYMES

ESSAY

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بسم الله الرحمن الرحيم

﴿سبحانك لا علم لنا إلا ما علمتنا ،
إنك أنت العليم الحكيم﴾

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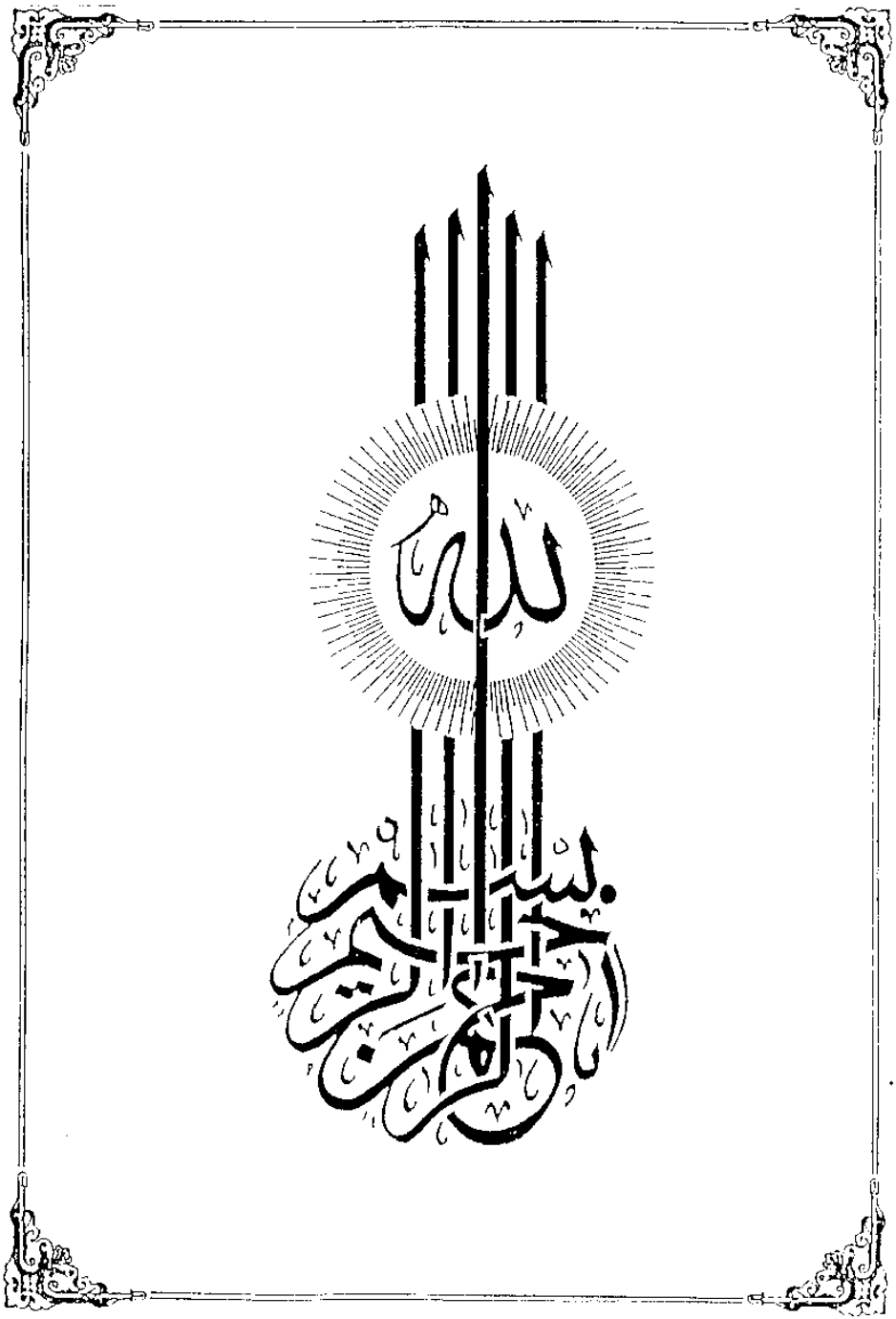

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CONTENTS

LIST OF ABBREVIATIONS

AFP : alpha - fetoprotein
AFU : alphafucosidase
ALP : alkaline phosphatase
ALT : alanine aminotransferase
AST : aspartate aminotransferase
arginine - 4 - methyl - 7 - coumarylamide.
Bz - DL - Arg - NPH No2 : α - N - benzoyl - D - L - arginine - p - nitrophenylamide.
Bz - DL - Arg - 2 - NNap : α - N - benzoyl - D - L - arginine - 2 - naphthylamide
CA : carbohydrate Antigene.
E64 : trans - epoxy - succinyl - L - leucylamido (4 - guanidiono) butane
EC : Enzyme commission
ECM : Extracellular matrix
EDTA : Ethylenediaminetetra - acetate.
EIA : Enzyme Immunoradiometric Assay.
ELISA : Enzyme Linked Imnunosorbant Assay
ER : Estrogen receptor
GGT : gamma - glutamyl - transferase.
HMWKO : High molecular weight kininogen
IRMA : Immuno radiometric Assay
KDa : kilo Dalton.
LMWKO : low molecular weight kininogen.
MCT : medullary carcinoma of thyroid gland.
MEP : major excreted protein
Mr : molecular mass
OA : osteoarthritis.

PMN : polymorphnuclear cell.

RA : Rheumatoid arthritis.

RIA : Radio Immuno Assay

Z - Arg - Arg - NMec:
benzyloxycarbonyl - Arginyl - Arginine - 4 - methyl - 7 - coumarylamide.

Z - Arg - Arg - NNap:
benzyloxycarbonyl - Arginyl - Arginine - 2 - diazomethane .

Z - Phe - Arg - CHN2:
benzyloxycarbonyl - phenyl - arginine - N - methylcoumdrine.

Z - Phe - Arg - NMec:
benzyloxycarbonyl - phenyl - arginine - 4 - methyl - 7 - coumarylamide.

INTRODUCTION AND AIM OF THE STUDY

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Cathepsin enyzmes are group of lysosomal proteinases. These different enzymes such as B, L, D, G, H, S and N. can degrade native cross - linked collagen at acid pH *(Maciewicz and Etherington, 1988).* Cathepsin B and D were purified from human liver.Cathepsin H and L were purified from human kidney *(Popovic, et al.; 1988).* Cathepsin G was isolated from human granulocytes *(Rouphley and Barrett.; 1977).*

Several authors have demonstrated that these proteolytic enzymes have been emplicated in some way in tumor cell invasion and metastasis such as lysosomal proteinase, cathepsin D and L *(Rozhin, et al.; 1989). Vashist, et al., 1988,* also reported that elevated activities of many proteinases including cathepsin B, H and L in breast cancer tissue .The level of cathepsin B increases also in colon cancer *(Buck, et al., 1994).*

Proteinase released from polymorphnuclear leucocytes in the synovial fluid and the inflamed synovium are important mediators of the degradation of collagen and proteoglycan which largely constitute the matrix of articular cartilage *(Mort et al., 1984).* Cathepsin D and B are enzymes of lysosomal proteinases which when released extracellularly have a high destructive potential .

Aim of the study

In this assay we aim to clarify the nature of cathepsin group of enzymes .We also hope to evaluate thier role in prognosis and diagnosis of various clinical conditions

CHAPTER 1

CLASSIFICATION, NATURE AND BIOCHEMICAL STRUCTURE

CLASSIFICATION , NATURE AND BIOCHEMICAL STRUCTURE

A- CLASSIFICATION :

Cathepsins are the group of acid lysosomal sulfhydryl proteinases *(Roughly and Barrett, 1977).* These enzymes are present in most , if not all mammalian cells including neutrophils , monocytes , macrophages and fibroblasts *(Tryggvason, et al., 1987).* Major classes of proteinases are shown in table 1.

Table (1)

Class	EC number	Examples	pH range of activity	Inhibitors
Aspartic or Acid	3.4.23	" D	2-7	diazo -ketones
Cysteine	3.4.22	" B	3-8	N- ethyl-malei-
or Thiol	3.4.22.15	" L		mide
	3.4.22	" H		
Serine	3.4.21	Cathepsin G	7-9	Flurophosphate

(Tryggvason , et al., 1987).

1- Aspartic Proteinases :

Most intracellular protein digestion in mammalian cells occurs at acid pH in lysosomes. The most prominant lysosomal proteinase acting at acid pH is cathepsin D. Cathepsin D is found in the lysosomes of most cells

including fibroblasts, however, its activity is higher in phagocytic cells such as macrophages and is increased by connective tissue activation. Under inflammatory conditions and during periods of rapid extracellular matrix (ECM) destruction, Cathepsin D is secreted extracellularly by macrophages and connective tissue cells mostly as proenzyme *(Werb and Alexander., 1989).*

2- Cysteine proteinases:

Cathepsin B and cathepsin L are the best known lysosomal cysteine proteinases .These enzymes are related to each other and, evoulutionarily to papain and have catalytic sites that require cysteine and histidine residues *(Barrett and Kirschke, 1981).*

Cysteine proteinases are inactivated by thiol-blocking reagents in general, but more selective inhibitors in biologic system include leupeptin, **L-trans -epoxysuccinyl - leucylamido (4-guanidino) butane** (E 64) and certain chloromethanes *(Werb and Alexander., 1989).*

3- Serine proteinases :

These proteinases with a catalytically essential serine residue at their active site, are most active at about neutral pH. Cathepsin G, a chymotrypsin enzyme of polymorphnuclear cells (PMN), is structurally related to the chymases of mast cell granules. Physiological inhibitors of cathepsin G include, the plasma protein α_1 - antichymotrypsin , α_1 - proteinase inhibitor and α_2 -

macroglobulin. Cathepsin G is an activator of metaloproteinases *(Werb and Alexander., 1989).*

B- CATHEPSIN B

1- STRUCTURE :

Cathepsin B is a thiol - dependent proteolytic enzyme of molecular weight 25 kilo Dalton(KD_a) *(Barrett, 1973).* It exists in two forms, a single - chain form and a double-chain form. Single - chain form consists of 254 amino acid with a molecular weight about 30 KD_a and the double-chain form consist of a heavy chain approximately 25 KD_a and light chain nearly 5 KD_a*(Moin et al., 1992).*

The light chain containing the catalytic cysteine that is formed by cleavage of single - chain form of the protein near the N - terminus *(Mason et al., 1985)* The double - chain form is derived from the single - chain form as a result of cleavages between residues 47 and 50 with the loss of dipeptide *(Moin, et al., 1992).*

Cathepsin B purified from normal human kidney exists as two forms of similar molecular mass .The single - chain form from human fibroblast is more active than the double - chain form *(Moin, et al., 1992).*

2- PRECURSOR FORM OF CATHEPSIN B :

Precursor form are identified immunochemically in ascitic fluid but have not yet been purified to enable direct

demonstration that these forms are proteolitically active *(Mort and Recklies, 1986).*

Many lysosomal enzymes are synthesized as higher - molecular mass (Mr) proenzyme forms. The cathepsin B precursor would be expected to be stable under normal physiological condition to allow it to pass from its site of synthesis to acid enviroment of the lysosome *(Mort and Rechlies, 1986).*

3- Effect of pH on Cathepsin B :

Cathepsin B is enzymatically active over a wide pH range *(Mort, et al., 1984)* which is greater at pH 5.0 but extended into the neutral range *(Morrison et al., 1973).* On the neutral range active enzyme is stable but losses this property on treatment with Hg^{++} ions *(Mort, and Recklies, 1986).*

On the other hand when pH increases it is increasingly unstable but when released into an extracellularly milieu maintained between pH 7- 7.5, activity can persist for a limited period and could cause proteolysis before it is denatured, since denaturation occur at alkaline pH *(Mort, et al., 1984), (Starkey and Barrett, 1973).* So cathepsin B activity is isolated at or above neutral pH due to denaturation of the enzyme *(Buck et al., 1992).*

Activity of cathepsin B against small synthetic substrate is decreased by incubation at pH $\geq$ 7.0 , a loss of activity against a small synthetic substrate occured concomitantly with an apparent auto - degradation of cathepsin B. This autodegradation at neutral pH may be

responsible for the decrease in the activity at this pH .It is possible that denaturation of a portion of the cathepsin molecules may lead to autodegradation of it *(Buck, et al., 1992)*. The half lives of cathepsin B at pH 7.5 found to be 7 minutes and at pH 8 found to be 1.7 minutes respectivly *(Mort, et al., 1984)*.

4- Autodegradation of Cathepsin B:

Cathepsin B from normal and tumor tissues at neutral pH, undergoes an apparent autodegradation, which is not observed at pH 5.0 for incubation period up to 12 hours. In contrast, degradation of extracellular matrix proteins by cathepsin B occurs most rapidly at acid pH. This autodegradation process is time - dependent and occurs more slowly if the incubation at neutral pH is carried out in the prescence of an alternative protein substrate such as laminin, fibronectin or type IV collagen. This auto degradation is inhibited by the cysteine - proteinase inhibitor E-64 *(Buck, et al., 1992)*.

5- Site of Cathepsin B :

Cathepsin B is localized in lysosomes of normal pancreatic B - cells as observed by electron microscopy *(Pitras and Roberts, 1981)* and in cytoplasmic granules of normal fibroblasts viewed by flurescence and bright field microscopy. In contrast, a one report provided evidence for a substantially different distribution of cathepsin B in neoplastic cells. Using fluorescein-labelled antisera directed against a partially purified preparation of cathepsin B, it was found that antigen with immunochemical similarity to