CHAPTER I

INTRODUCTION

1.1 General Introduction

Serine proteases are involved in a number of important physiological processes including blood coagulation, the complement system, fertilization, and protein turnover (1). Many of these enzymes are believed to be involved in diseases such as emphysema, arthritis and tumorogenesis (1). The granule fraction of human polymorphonuclear leukocytes contains almost equal amounts of two serine proteases, human leukocyte cathepsin G (HLC-G) and human leukocyte elastase (HLE). These proteases have given rise to increasing interest in recent years due to their possible involvement in degradation of connective tissues in arthritis and the destruction of lung tissue in chronic emphysema (2). Many research groups have developed specific substrates, in order to establish the preference and substrate selectivity and also to gain information concerning the nature of the active site and its surrounding. Other research groups have synthesized inhibitors for these proteases. This research involves a study of synthetic human leukocyte cathepsin G inhibitors and some related serine proteases.

1.2 Human Polymorphonuclear Leukocyte Enzymes

Several enzymes have been isolated from the granule fraction of human polymorphonuclear leukocytes in Table 1.1 (3), but the most

Table 1.1 Contents of human polymorphonuclear leukocyte granule

Acid phosphatase	N-Acetyl-β-galactosaminidase
Aminodipeptidase	α-Glucosidase
α-Amylase	β-Glucosidase
Cathepsin D	N-Acetyl-b-glucosaminidase
Cathepsin G	β-Glucuronidase
Cationic proteins	Lactoferrin*
Collagenase*	Laminarinase
Dextranase	Lysozyme*
Elastase	α-Mannosidase
α-Fucosidase	Myeloperoxidase
α-Galactosidase	Vitamin B12 binding protein*
β-Galactosidase	

* These have been found in specific granules.

abundant enzymes are collagenases, HLE and HLC-G. There is evidence to show that these enzymes are involved in the degradation of cartilage in both rheumatoid and osteoarthritis (4-5). The destruction of the elastic component of lung connective tissue in chronic obstructive lung disease is currently believed to be caused by HLE and cathepsin G. In arthritic patients, the granule fractions of the polymorphonuclear leukocytes, which have infiltrated into the synovial cavity, have been shown to contain several active proteolytic enzymes (3). These include the serine proteinases elastase and cathepsin G, as well as collagenases (6-7).

1.3 Human Leukocyte Cathepsin G

During 1974, several groups independently reported the existence of a chymotrypsin-like proteinase in human neutrophil leukocytes (8). Subsequently, Starkey and Barrett isolated a chymotrypsin-like enzyme from human spleen (9). Although chymotrypsin-like in most respects, the enzyme also differed from chymotrypsin in some ways, and clearly needed a name. It was suggested to be named " cathepsin G " . The M_r of cathepsin G appears to be close to 30,000 D in SDS-gel electrophoresis, but a value of 23,000-24,000 D has been reported on the basis of compositional data (10). The N-terminal sequence shows distinct homology with that of chymotrypsin (10). The enzyme is classified as chymotrypsin-like due to its ability to act on some synthetic substrates of chymotrypsin (especially of α-chymotrypsin).

Human leukocyte cathepsin G has been implicated in the proteolysis of lung elastin and it strongly stimulates the rate of

solubilization of human lung elastin by HLE. For instance, the elastolytic activity of an equimolar mixture of elastase and cathepsin G is more than 5 times higher than that of elastase alone (11). Cathepsin G has also been implicated in the proteolysis of collagen, cartilage proteoglycan and other connective tissue proteins and has been identified in rheumatoid synovium (12-14). Because of destruction in lung elastin and connective tissue, this enzyme is believed to be probably responsible for the degradative results of diseases such as pulmonary emphysema and rheumatoid arthritis. It has been suggested that cathepsin G may play a role in the development of hypertension, based on its ability to convert both angiotensinogen and angiotensin I directly to angiotensin II without requirement for renin or angiotensin-converting enzyme (15-16). Angiotensin II is an octapeptide which has potent vasoconstrictor and aldosterone secretion activities (17-18). Human cathepsin G has also been reported to cleave myosin and thus may be involved in muscle catabolism (19). Fibronectin either in solution or in matrix form is a rather selective substrate for cathepsin G, thus this enzyme has been implicated in the regulation of fibronectin-mediated processes, such as cell adhesion, cell motility and cell migration (20-22).

1.4 Inhibitors of Human Leukocyte Cathepsin G

There are many classes of compounds reported in the literature, which inhibit human leukocyte cathepsin G. Some of these are listed in Table 1.2.

Table 1.2 Some HLC-G inhibitors.

Class of compound	References
Chloromethyl ketone	23
Heterocyclic transition-state analogues and acylating agents	24-25
Sulfonyl fluorides	26
Azapeptides	27
Diisopropyl fluorophosphate	28-29
Tosylphenylalanyl chloromethane	28-29
Substituted isocoumarins	1

All the compounds in Table 1.2 have one or more reactiv functional groups. They are difficult, if not impossible, to targe onto receptor sites, as they are likely to react with the many components of the body available between the point of administration and the target at the receptor site. In addition, some of these compounds such as diisopropyl fluorophosphate, are rather poor inhibitors of cathepsin G. Tosylphenylalanyl chloromethane also inhibits cathepsin G much more slowly than it does chymotrypsin, so that the inhibition often appears incomplete (28-29). Many protein inhibitors such as lima bean and soybean trypsin inhibitors (28), α_1-chymotrypsin inhibitor, α_1-proteinase inhibitor, α_2-macroglobulin and aprotinin also inhibit HLC-G.

In normal individuals, proteolytic enzymes such as HLE and HLC-G are primarily inhibited by alpha-1-proteinase inhibitor (α_1-PI

or α_1-antitrypsin) which is the major serum protease inhibitor and also a normal constituent in the fluid of the bronchioalveolar system. However, this inhibitor is unstable. It is readily inactivated by oxidizing agents such as present in cigarette smoke. Janoff and Carp (29) had demonstrated that smoke condensate inactivates α_1-PI and they showed that α_1-PI is inactivated by oxidation of methionine residues. It is also inactivated by oxidases such as myeloperoxidase. This particular oxidative enzyme is normally active in the phagocytic cells during inflammatory states. In addition, the presence of α_1-PI in humans is genetically dependent. Some persons are found to have the inhibitor levels as low as 25 % of normal due to genetic reasons. Thus, in persons with lower concentration of α_1-PI, the enzyme/inhibitor balance is upset, so the onset of chronic emphysema is facilitated. Since α_1-PI is a rather large peptide, replacement or supplementation by using cloned α_1-PI is not feasible as it does not cross the cell wall. Therefore, a drug with a low molecular weight, which is active as an elastase or cathepsin G inhibitor to supplement α_1-PI would be a good candidate to prevent such degradative processes.

1.5 Synthetic Inhibitors of HLC-G in This Study

Since human leukocyte cathepsin G has a significant preference for substrates with aromatic amino acids such as phenylalanine or tyrosine at active site (2), we have sought to utilize these preferences for the design and synthesis of specific, non-toxic inhibitors of HLC-G. However, the enzymes in both the pancreas and the human polymorphonuclear leukocytes are mainly

serine proteases with catalytic mechanism and sequence homologies related to trypsin and chymotrypsin. Thus, in this research it was necessary to investigate the effect of synthetic inhibitors on other proteolytic enzymes such as trypsin and chymotrypsin, in order to develop specific inhibitors of the selected enzymes only.

The general formulae are I or II

X-B-NH-R (I) X-A-B-NH-R (II)

where X is benzyloxycarbonyl or benzoyl

A is alanine or glycine

B is tyrosine or phenylalanine

R is $-(CH_2)_9CH_3$, $-(CH_2)_{11}CH_3$ or $-(CH_2)_{13}CH_3$

The individual compounds synthesized and tested are shown in Table 1.3

Table 1.3 Synthesized and tested compounds.

No	Name	Short formulae (a)
1	Benzoyl-L-phenylalanylamido dodecane	Bz-Phe-NH-C_{12}
2	Benzoyl-L-phenylalanylamido tetradecane	Bz-Phe-NH-C_{14}
3	Benzyloxycarbonyl-L-alanyl-L-phenylalanylamido decane	Z-Ala-Phe-NH-C_{10}
4	Benzyloxycarbonyl-L-alanyl-L-phenylalanylamido dodecane	Z-Ala-Phe-NH-C_{12}
5	Benzyloxycarbonyl-L-alanyl-L-phenylalanylamido tetradecane	Z-Ala-Phe-NH-C_{14}

Table 1.3 Continued.

No	Name	Short formulae (a)
6	Benzyloxycarbonylglycyl-L-phenylalanylamido dodecane	Z-Gly-Phe-NH-C_{12}
7	Benzoylglycyl-L-phenylalanylamido dodecane	Bz-Gly-Phe-NH-C_{12}
8	Benzoylglycyl-L-phenylalanylamido tetradecane	Bz-Gly-Phe-NH-C_{14}
9	Benzyloxycarbonyl-L-alanyl-L-tyrosylamido dodecane	Z-Ala-Tyr-NH-C_{12}
10	Benzoylglycyl-L-tyrosylamido dodecane	Bz-Gly-Tyr-NH-C_{12}

(a) The abbreviations in the short formulae are represented as follows :

Bz = benzoyl

Z = benzyloxycarbonyl

Gly = glycine

Ala = L-alanine

Phe = L-phenylalanine

Tyr = L-tyrosine

C_{10} = $-(CH_2)_9CH_3$

C_{12} = $-(CH_2)_{11}CH_3$

C_{14} = $-(CH_2)_{13}CH_3$

1.6 Objectives of This Study

In summary, the aims of this investigation are :

1. To synthesize and test the compounds, with respect to percentage inhibition against the enzymes HLC-G, trypsin and chymotrypsin in order to ascertain their mode of actions.

2. To synthesize and test compounds with various hydrocarbon chains in order to study the effect of chain lengths.

3. To study the effect of the protecting groups.

4. To determine the type of inhibition of these compounds with respect to specific enzymes.