CHAPTER IV

CONCLUSION

The study by Ashe and Zimmerman(31) appears to show that HLE possesses a hydrophobic region, which differs from that of other serine proteases. This is shown by the observation that a series of cis-unsaturated fatty acid are able to specifically inhibit HLE, but not other enzymes. In the course of this research, a series of compounds were tested against HLE, PPE, trypsin and chymotrypsin, to determine the structural requirements necessary for specific inhibition of HLE and of other serine proteases.

The inhibition of HLE, PPE, trypsin and chymotrypsin by N-alkyl peptide esters and N-protected alkyl amino acids are shown in Table 3.1 and 3.2. The N-alkyl peptide esters (compounds 3 and 5) inhibit HLE, PPE and chymotrypsin to approximately the same degree. On the other hand, N-protected alkyl amino acids (compounds 1, 9, 14 and 15) are better inhibitors of HLE. These results suggest that not only does HLE posses a hydrophobic binding region for long hydrocarbon chains, but that such a binding region also exists in PPE, trypsin and chymotrypsin, although its effective size might be different. The hydrophobic binding regions of HLE and other serine proteases must have different orientation with respect to the actual active site, as the specificity of the inhibitors of these enzymes depends on the orientation of the hydrocarbon chain. N-alkyl peptides will generally inhibit HLE, PPE, trypsin and chymotrypsin while specificity

is achieved with N-protected amino acids with an alkyl chain substitued at the carboxamide end. It was found that a chain length of at least 8 to 12 carbon units long on the amide or carboxyl terminal is required to achieve resonable inhibition of PPE.

It is possible that the orientation of these hydrophobic pockets may influence the structural requirements for achieving specific inhibition of the respective enzyme. In order to establish the amino acid specificity of these enzymes, a series of alkylated and N-protected amino acids were synthesised. The best inhibitor was found to be the valine compounds.The alanine compounds showed only a slight decrease in inhibition when compared to valine. The enzyme binding subsite seems to be of such size as to be able to accommodate a hydrophobic side chain such as a bulky valine residue.

The influence of the amino protective groups were also investigated in order to determine their effect on enzyme specificity and inhibition. Table 3.2 lists the inhibition results of dodecyl amino acids and peptides protected at the N-terminal by carbobenzoxy (Z),t-butyloxy carbonyl (t-BOC) and succinyl (Suc) groups. The structural changes at the P_2 position of the inhibitor were recognised by the four enzymes, thus revealing further differences between HLE, PPE, trypsin and chymotrypsin near their active site. HLE preferred the t-BOC group at P_2 while PPE preferred the Z group, and the presence of a succinyl group or no protecting group at all, at P_2, abolished the inhibitory activity of these compounds towards HLE and PPE.
It is clear therefore, that there is a hydrophobic area at or near where the N-terminal of the substrate (or inhibitor) interacts with the active site of the enzyme.

The N-BOC alkyl amino acids and peptides were also tested as inhibitors of other serine proteases. No inhibitory activity was found against trypsin and chymotrypsin and all compounds showed less than 30% inhibition of HLE. These experiments clearly show the specificity of these compounds towards HLE.

To develop specific inhibitors of HLE certain structural requirements are necessary. Specific inhibition of HLE requires that the inhibitor has a t-BOC group protecting the N-terminal of the amino acid, at the P_2 site. The amino acid of the inhibitor should be valine. Increasing the number of valine residues to two slightly increases the inhibitory activity of the compound. The protecting group and the amino acid or peptide residues may well be acting as recognition sites for the binding of the inhibitor to the enzyme. An important requirement for specificity is the presence of a hydrocarbon chain of between 8 to 12 carbons in length attached to the C-terminal of the amino acid or peptide. The long alkyl chain may act by blocking some of the small subsites in the hydrophobic pocket of the enzyme.

The alkyl peptides have a great advantage over other inhibitors, for not only being specific, we have at least the same or even better inhibitory capacity than the currently used drugs. (To determine whether these type of inhibitors are toxic or not, the toxicological testing was performed by Pharmaceutical Consulting Services, NSW.2158, Australia). These specific inhibitors were administrated orally to rats and mice as 10%(w/w) aqueous samples. The LD_{50} values were found to be more than 3 g./Kg. Therefore these specific inhibitors have potential for application as drugs as they have been shown to be non-toxic.