

CHAPTER 4

SUMMARY AND CONCLUSIONS

Understanding of collagen stabilization against temperature and enzymes irrespective of tanning molecules is of profound interest. Issues related to thermal stability has been addressed in the recent past (Covington et al 1998; Ramasami 2001). In the present study, an attempt has been made to understand the forces involved in collagen-collagenase interaction and its cleavage site specificity. The common forces involved in collagen-collagenase interaction are electrostatic, hydrogen bonding, hydrophobic interactions and weak intermolecular forces such as dipole-dipole, and van der Waals interactions.

To address the role of electrostatic interaction between collagen and collagenase, the charged functional groups have been masked using EDC. This zero length crosslinker facilitated the formation of isopeptide bond. The mass spectral evidence and decrease in amino group indicates the bond formation. Increase in viscosity and enhancement in fibril formation substantiate the formation of inter helical isopeptide bond. From the structural analysis, it is confirmed that, the amide bond formation increases the helicity of the polyproline II conformation. The negative ΔG value indicates that the collagen with isopeptide bond is more stable than the native. The percentage inhibition of collagenase activity has been found to be 78% for the 98% reduction of free amino group. This may be due to the formation of isopeptide bond between the side chains, which reduces the binding affinity of collagenase towards collagen by arresting the electrostatic interaction.

The effect of introduction of iso-peptide bond has been investigated using classical molecular dynamics simulation. From the results, it can be observed that there are no significant changes in the number of inter-chain H-bonds. However, there is an appreciable increase in the inter-helical contacts due to the introduction of isopeptide bond. Thus overall structural stability of triple helix is not significantly affected by the introduction of isopeptide bond.

To address the role of hydrogen bonding and hydrophobic interactions between collagen and collagenase, tannic acid has been used as a crosslinking agent. The interaction of tannic acid with collagen increases the thermal stability by formation of hydrogen bonds. Tannic acid binds to the collagen with high affinity because, the structural flexibility of the collagen is compensated by the structural rigidity of the phenolics. Increase in concentration of tannic acid induces significant change in the conformation of triple helix. Interaction of TA with collagen increases the inhibition of collagenase activity to about 80%. This may be due to the formation of multiple hydrogen bonds and hydrophobic interactions. Hydrophobic group of TA molecule combined with the hydrophobic domains of collagen forms tight clusters, which are inaccessible to collagenase.

Role of chirality of amino acids at the cleavage site of collagenase on collagenolysis has been addressed using collagen mimics. MD simulations have been carried out on the model collagen-like peptides to understand the effect of D-AA substitution on the structure and stability of collagen. D-AA substitution in the triple helix leads to the destabilization. Destabilization occurs only in the local region (substituted site) and it propagates marginally along the length of the entire triple helix. The absence of H-bonds in the D-AA substituted positions and electrostatic repulsions are the predominant factor that destabilizes triple-helical conformation. Cluster analysis reveals the formation of the kink at the site of the D-AA substitution. D-Ala

substituted triple-helical model is more stable when compared to that of D-Pro and D-Asp. The effect of D-amino acids at cleavage site of collagenase on collagen has been addressed using collagen like peptides. Structural analysis reveals that, D-Ala substitution in the imino poor region increases the structural stability through the presence of polyproline II conformation. Enzymatic analysis reveals that, D-AA substitution at the cleavage site in FALGPA and imino poor region leads to complete inhibition of collagenase activity. Present study more precisely shows that collagenase clearly differ in their hydrolyzing abilities on collagen like peptide upon change in chirality of the amino acid at the cleavage site. The conformational stability of native and D-Ala substituted collagen like peptides has been carried out using two different solid surfaces, such as hydrophilic and hydrophobic surfaces. In both the surfaces, D-Ala substituted peptide shows better orientation compared to native.

On the whole, the present investigation has established the possible forces involved in collagen-collagenase interaction and the role of chirality of amino acid at the cleavage site of collagenase on collagen in collagenolysis.

4.1 DIRECTIONS FOR THE FUTURE WORK

Understanding the forces involved in collagen-collagenase interaction will facilitate the development of structure based design in the selection of crosslinkers for collagen stabilization.

Configurational changes in the amino acid at the cleavage site of collagenase on collagen can be used for the development of bio-based tanning system. This will help to overcome the environmental problems related to the present chemical based tanning.