ABSTRACT

Understanding the mechanism of tanning through identification of forces involved in collagen stabilization in the presence of tanning agents is of profound interest. In the present investigation, forces involved in collagen-collagenase interaction and the role of amino acid chirality at the cleavage site of collagenase in collagen has been studied.

The effect of isopeptide bond on collagen structure and its stability against temperature and bacterial collagenase has been studied with the objective of understanding the role played by the charged functional groups present in collagen on collagen-collagenase interaction. Isopeptide bond formation in collagen resulted in an increase in thermal stability and decrease in collagenolytic activity with an increase in concentration of 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC). The percentage inhibition of collagenase activity was found to be 78% for the 98% reduction of free amino group. The formation of isopeptide bond between the side chains reduces the binding affinity of collagenase towards collagen by arresting the electrostatic interaction. The effect of isopeptide bond between two triple helices has been studied using molecular dynamics (MD) method by introducing the iso-peptide bond between Lys-Glu and Lys-Asp, which cross-links the two triple helices. From the results, it can be observed that the
number of inter-helical hydrogen bonds increases in the presence of isopeptide bond.

Interaction of tannic acid (TA) with collagen has been investigated to understand the role of hydrogen bonding and hydrophobic interactions in collagen-collagenase interaction. Interaction of tannic acid with collagen increases the thermal stability of collagen due to formation of hydrogen bonds. Increase in concentration of TA induces significant change in the conformation of triple helix. Interaction of TA with collagen increases the inhibition of collagenase activity, which was found to be 80%. Hydrophobic group of TA molecule combined with the hydrophobic domains of collagen forms tight clusters, which reduces the accessibility of collagenase on collagen.

Role of amino acid chirality at the cleavage site of collagenase on collagenolysis has been addressed using collagen-like peptides (CLPs). The effect of the replacement of L-amino acid (L-AA) in the model collagen-like peptides with D-amino acid (D-AA) on the structural stability has been explored using the MD method. Results reveal that substitution of D-AA produces a large local disruption to the triple-helical structure. Formation of a kink (bulge) at the site of substitution is observed from the detailed analysis of MD trajectory. Collagenolysis has been studied using FALGPA and imino poor region of the type I collagen. At the cleavage site, D-Leu and D-Ala
have been substituted at their L-counterpart of the peptide and the structural, thermal and enzymatic stability investigated. D-AA substitution in CLP induces conformational change in the secondary structure. D-AA substitution at the cleavage site in CLP showed complete inhibition for collagenolysis. The structural stability of native and D-Ala substituted collagen like peptides (imino poor region) has been studied using hydrophilic and hydrophobic solid surfaces. D-Ala substituted peptide showed better orientation compared to native.

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