Cross-linking and Three Dimensional Stereospecific Structure of Fibrils in Mineralized and Soft Tissue Type I Collagens

Gerald L. Mechanic, Elton P. Katz, Kayo Teraoka and Mitsuo Yamauchi
University of North Carolina at Chapel Hill, 27514 USA

Type I collagen is the most ubiquitous connective tissue matrix protein in soft and hard tissues. Covalent intermolecular cross-links are essential for collagen matrices to maintain their cohesiveness, tensile strengths, mechanical properties, and stability. Recently we have determined the structure and fibrillar molecular locus of the stable non-reducible trifunctional intermolecular cross-link histidinohydroxylsinonorleucine (HHL) in skin fibrils. This cross-link was only found on skin and cornea and it is absent from all the major soft and hard skeletal tissue collagens. Its molecular fibrillar locus was found to be, $\alpha_1(I)[\text{Lys}_{16}]\times \alpha_1[\text{Hy1-87}] \times \alpha_2(I)[\text{His}_{92}]$.

We also quantified the molecular fibril locations of the bifunctional intermolecular cross-links in skeletal tissues. The ratios of cross-linked $\alpha_1(I)$ chains to cross-linked $\alpha_2(I)$ chains in all cases were 3 to 1. A random staggered molecular packing of collagen molecules should have yielded a cross-linked chain ratio of 2 to 1. Our findings suggest that the angular orientation of packing of collagen molecules on skeletal tissues is stereospecific and the chemical reactions that form the cross-links are stereochemical in nature. The results of the quantitative molecular locations of the bifunctional cross-links in skeletal tissue and locus and quantity in skin of HHL indicate that the staggered azimuthal molecular packing of these two tissue type I collagens are fundamentally different.

The histidine-based cross-link dehydro-histidinohydroxymerodesmosine (deH-HHMD) is one of the major reducible tetrafunctional cross-links in soft tissue type I collagen such as skin, tendon, ligament, cornea, etc. These tissues contain from 0.3-1.0 mole deH-HHMD/mole of collagen depending on the organism's chronological age. However, little if any of this tetrafunctional cross-link is found in bone and dentin. We have recently purified, from NaB$_4$H$_4$-reduced bovine skin, the tryptic cross-linked peptides that contain deH-HHMD in its reduced form histidinohydroxymerodesmosine (HHMD). Our initial data strongly indicate that it is formed at the NH$_2$-terminal nonhelical peptide regions of $\alpha_1(I)$ chains and Hyl and His of staggered juxtaposed molecules. Chromatographic patterns of tryptic peptides from NaB$_4$H$_4$-reduced corneal collagen as well as achilles tendon suggested that the major molecular fibril locus to be the same as just described.

It is felt that deH-HHMD constrains the NH$_2$-terminal nonhelical portions in the collagen molecules in soft tissue collagens while the latter portions of the molecules in mineralized tissues such as bone and dentin are much less constrained. In addition there is little if any of the trifunctional cross-link pyridinoline (PYR) and the iminium bifunctional cross-links in the NH$_2$-terminal peptide region. This contrasts to their relatively larger abundance in the COOH-terminal nonhelical peptide.
portions of mineralized tissues as we have found.

It has been proposed the NH$_2$-terminus of molecules in fibrils are the nucleation and epitaxial site of mineralization. The relative paucity of aldehyde derived cross-links in this region observed in mineralized tissues may be favored for nucleation and crystal growth.

Supported in part by NASA grant NAG 2-181, NIH grants AR-19969, AR-30587, DE-08522, DE-00233, DE-08611 and AR-37604.