Developmental stage-dependent regulation of prolyl 3-hydroxylation in tendon type I collagen

3-Hydroxyproline (3-Hyp) is a post-translational modification (PTM) specific to collagen, and Pro residues at the X position of Gly-X-Y repeating triplets are rarely hydroxylated to 3-Hyp. Although 3-Hyp was found in collagen more than 50 years ago, the physiological role of 3-Hyp is still unclear. Recently, some studies have suggested a function of 3-Hyp in fibril assembly and its relationships with certain disorders including osteogenesis imperfecta and high myopia. In this study, we surveyed the relationship between aging and prolyl 3-hydroxylation in skin, bone, and tendon to find a clue to elucidate the function of 3-Hyp.

We first estimated the overall alterations in collagen PTMs in collagens purified from skin, bone, and tail tendon of 0.5-18-month-old rats by liquid chromatography-mass spectrometry (LC-MS) following acid and alkaline hydrolysis. 3-Hyp was found to increase by 3-fold in tendon collagen from 0.5 to 3 months of age and then remain constant. In addition, site-specific analysis of prolyl 3-hydroxylation was performed by LC-MS following trypsin digestion. While 3-Hyp did not increase at a previously known modification site, Pro986 in type I collagen, significant increases in 3-Hyp were observed at a specific sequence region, including newly identified sites at α1(1)/α2(I) Pro716 and Pro719, at the early ages.

We postulate that the tendon- and modification site-specific increases in 3-Hyp are correlated with tissue development, rather than with aging. In addition, we suggest that prolyl 3-hydroxylation has a function in regulation of collagen fibril diameter in tendon.

Key words: collagen, 3-hydroxyproline, tendon, mass spectrometry