

Disruption of collagen triple helix hydrogen bonding in ochronotic human cartilage in alkaptonuria observed by dynamic nuclear polarisation-enhanced solid-state nuclear magnetic resonance spectroscopy

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Alkaptonuria (AKU) is an ultra-rare autosomal recessive disease caused by lack of the enzyme homogentisate 1,2-dioxygenase (HGD). The primary biochemical consequence of HGD deficiency is elevated plasma homogentisic acid (HGA) which accumulates over time in collagenous tissues, particularly cartilage. In a process called 'ochronosis', accumulated HGA is thought to undergo oxidation and subsequent polymerisation, causing pigmentation of cartilage. Presence of ochronotic pigment in cartilage alters its physico-mechanical properties, giving rise to inevitable severe, early-onset osteoarthritis particularly affecting weight bearing joints. However, the binding mechanism of ochronotic pigment to cartilage collagen matrix remains unknown, as does the chemical structure of the pigment itself. To investigate these 'unknowns', DNP-NMR experiments were carried out on pigmented and non-pigmented cartilage samples obtained from a patient with AKU. Comparison of fixed versus non-fixed mouse femoral articular cartilage showed that 1D and 2D NMR spectra can be obtained from formalin-fixed cartilage with DNP-enhancement. 1D ¹³C spectra showed unexpected and striking similarity between the biochemical composition of ochronotic and non-ochronotic tissue. Differences became apparent with high-resolution 2D NMR experiments. A common signal was obtained from pigment derived from synthetic HGA and ochronotic cartilage corresponding to aromatic carbons, indicating a structure related to HGA. Disruption to the glycine signal was observed in ochronotic cartilage in 2D experiments. The glycine C α -NH signal showed a lower chemical shift on the ¹H dimension, indicating disruption to the interchain hydrogen bond participated in by the glycine amide within the triple helical type II collagen structure. This study showed for the first time a) DNP-enhanced ssNMR spectra from formalin-fixed tissue and b) a clear NMR signal attributed to pigment in degraded cartilage from an AKU patient. The altered 2D spectra indicates global disruption to the type-II collagen triple helix in ochronotic cartilage likely to underlie its mechanical failure leading to osteoarthropathy in AKU.