Nuclear Magnetic Resonance and Mass Spectrometric Studies on the Action of Proteases on Pig Articular Cartilage

Jürgen Schiller, Jürgen Arnhold and Klaus Arnold

Institut für Medizinische Physik und Biophysik, Medizinische Fakultät, Universität Leipzig, Liebigstr. 27, 04103 Leipzig, Germany

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Rheumatic diseases are accompanied by a progradient diminution of the cartilage layer. Unfortunately, degradation mechanisms (role of different enzymes and reactive oxygen species) are not yet understood. Since nuclear magnetic resonance (NMR) spectroscopy was often used for the investigation of synovial fluids, the aim of the present work was to detect cartilage degradation products upon proteolytic digest of cartilage.

Cartilage samples were incubated at 37 °C with phosphate buffer in the absence or presence of different proteases (collagenase, trypsin and papain). Supernatants were subsequently assayed towards their content of carbohydrate and protein degradation products by NMR (\(^{1}H\)- and \(^{13}C\)-) and MALDI-TOF mass spectrometry.

Intense resonances of relatively mobile N-acetyl side chains (ca. 2.01 ppm) of polysaccharides of cartilage were only detectable on digestion with papain. The appearance of these resonances indicates intense degradation of the core protein of proteoglycan aggregate of cartilage, whereby polysaccharides are released. Additionally, broad resonances at 0.85 ppm arising from collagen degradation products were observed upon the action of all applied proteases. However, glycine as a marker of exhaustive collagen degradation was only observed, if cartilage was digested by collagenase. Using more vigorous incubation conditions, additionally high-abundant amino acids of collagen (proline, hydroxyproline) could be detected in the \(^{13}C\)-NMR- and the MALDI spectra. The observed differences are correlated with the different selectivities of the applied enzymes.

It is concluded that NMR allows the detection of characteristic protein and polysaccharide degradation products. The observed differences may be of some relevance for the diagnosis of rheumatic diseases.

Reprint requests to Dr. Jürgen Schiller. Fax: 0049-341-9715709. E-mail: schij@server3.medizin.uni-leipzig.de