Pentosan polysulfate increases affinity between aggrecanases (ADAMTS-4 and -5) and TIMP-3 by a conformational change.

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ADAMTS-4 and -5, adamalysin metalloproteinases with disintegrin and thrombospondin motifs, are thought to be the primary enzymes responsible for degrading the cartilage glycosaminoglycan aggrecan. As such, they are important contributors to the development of osteoarthritis and are attractive drug targets. We recently showed that calcium pentosan polysulfate (PPS), a chemically sulfated xylanopyranose from beechwood, protects cartilage against aggrecan breakdown, partly by increasing affinity between ADAMTS-4 and -5 and their endogenous inhibitor, tissue inhibitor of metalloproteinases 3 (TIMP-3). Here we investigate the structural requirements and mechanism underlying this increased affinity.

Wild-type and mutant forms of TIMP-3 and ADAMTS-4 and -5 were recombinantly expressed in mammalian cells and purified by anti-FLAG affinity chromatography. The effect of PPS on TIMP-3-ADAMTS affinity was investigated using a fluorescent quenched peptide substrate and standard enzyme kinetic approaches.

The improvement of ADAMTS-TIMP-3 affinity by PPS appeared to require binding of both the enzyme and the inhibitor to PPS. Forms of ADAMTS-4 and -5 lacking the non-catalytic spacer domain exhibited only weak PPS binding and required 10-fold more PPS to achieve the same increase in affinity for TIMP-3. Similarly, a non-ECM binding mutant of TIMP-3 that was unable to bind to PPS showed little increase in affinity for ADAMTS-5 in the presence of PPS. Longer forms of PPS were more effective at increasing affinity than short forms, with PPS of 9.4 kDa (36-mer) being 100-fold more effective than 1.8 kDa (7-mer) PPS. The PPS effect was blocked by NaCl concentrations above 200 mM, showing the effect is largely electrostatic. The increased affinity was still observed in a large molar excess of PPS, indicating that PPS does not act by a template mechanism, but more likely causes a conformational change in the enzyme and/or the inhibitor.

The results indicate that sulfated compounds with binding activities similar to PPS may be developed to inhibit osteoarthritis-associated degradation of the cartilage extracellular matrix.