116, 359 – 364), was *N*-deacetylated by hydrazinolysis, *N*-sulfated with $TMA.SO_3$ and *O*-sulfated with $TBA.SO_3$, yielding products with sulfate/carboxyl molar ratios of 1.5 to 2.5. Most of the products potentiated the inactivation of coagulation factor Xa by antithrombin (AT).

Affinity chromatography on AT-Sepharose of one of these products (anti-Xa activity ~20% of that of standard heparin) yielded a number of fractions with apparent affinities intermediate to those of standard heparin with high and low affinity for AT. Structural analysis of these fractions showed major 6-*O*-sulfation of $GlcNSO_3$ units along with some *O*-sulfation of GlcA residues. In addition, small amounts of -GlcA-$GlcNSO_3$(3,6-di-OSO_3)- were present, in proportions increasing with increasing apparent affinity of the parent polysaccharide fraction for AT. These findings suggest that the interaction of modified K5 with AT involves a sequence that mimicks the AT-binding pentasaccharide region of native heparin, with its specific 3-*O*-sulfate marker group (Lindahl *et al.* (1984) *J. Biol. Chem.* **259**, 12368 – 12376).

Analysis by fluorescence titrations of the interaction between AT and the subfraction of modified K5 having the highest affinity for the inhibiter (8% of the total preparation), gave a K_D of $\sim 2 \times 10^{-7}$ M. The fraction thus bound ~50 times more tightly to AT than low-affinity heparin, although ~10 times more weakly than high-affinity heparin. UV difference spectroscopy and circular dichroism both indicated conformational changes of AT, at saturation with the subfraction of modified K5, that were very similar to those caused by native high-affinity heparin. The anti-factor Xa activity of the modified-K5 fraction was about the same as that of unfractionated heparin. Taken together, these findings indicate that the anticoagulant effects of the chemically modified K5 polysaccharide and of heparin depend on similar interactions with AT, involving, in particular, a 3-*O*-sulfated AT-binding sequence.

S5.10

Isolation and Characterization of Heparan Sulfate Proteoglycan from Human Articular Cartilage

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Heparan sulfate proteoglycans (HS-PG) represent a family of macromolecules which are involved in fundamental biological processes like cell-cell interaction and control of cell growth and differentiation. Different types of HS-PG were found on cell surfaces, in basement membranes and in extracellular matrix. Here we report on the isolation and characterization of HS-PG from human articular cartilage.

HS-PG was isolated from human articular cartilage and purified by several chromatographic steps including anion-exchange chromatography, size exclusion chromatography and hydroxylapatite chromatography. Biochemical characterization of the isolated HS-PG was done by electrophoresis and blot onto nylon membranes prior and after digestion with heparinase/heparitinase using immunochemical detection with mAb 1F10/B8. MAb 1F10/B8 has been raised previously against a basement membrane associated HS-PG from human aorta. HS-PG specific mAb were used for immunohistochemical analysis of the localization of HS-PG in human articular cartilage. A strong chondrocyte-associated staining of HS-PG in the extracellular matrix was shown. Whereas a clear staining of the pericellular matrix was observed, no staining of the interterritorial matrix was seen. By the mAb 1F10/B8 basement membrane associated HS-PG were stained in different human tissues, however no chondrocyte-associated basement membranes have been described so far. Therefore the function of this pericellular located HS-PG from human articular carilage has to be clarified. The possible role of this HS-PG in the control of growth and differentiation of chondrocytes by binding of growth factors will now be investigated.

5.11

Decorin Contains a Potential Collagen Binding Site Between Leu-125 and Leu-158

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The small proteoglycan decorin binds to collagen and influences its fibril formation. To investigate collagen binding domains of the core protein, recombinant decorin and decorin peptides were tested for collagen binding. An EcoRI/HpaI fragment encoding the complete translated decorin sequence was cloned into pBEHpac18 and expressed in CHO- and COS-cells. Recombinant decorin was indistinguishable from the normal product of human fibroblasts. It also bound to collagen type I. To further localize binding sites two cDNA-fragments EcoRI-AlwNI (peptide A_E) and EcoRI-DdeI (peptide B_E) were cloned into pBEHpac18. Expression in CHO- and COS-cells generated peptides of the expected sizes of 14 kD (peptide A_E) and 17 kD (peptide B_E) which both reacted with the decorin antibody and carried glycosaminoglycan chains. The molecular weights of these peptides correlate well with the postulated amino acid sequences of Asp-1 — Leu-125 and Asp-1 — Leu-158, respectively. Peptide B_E bound to collagen while peptide A_E showed no binding. To exclude any influence of CHO-proteoglycans and-to test a different expression system, the cDNA fragments BstUI-AlwNI (peptide A_P) and BstUI-DdeI (peptide B_P) were cloned into the prokaryotic expression vector pRSET A. The generated fusion peptides contained the amino acid sequences Asp-15 — Leu-125 and Asp-15 — Leu-158, respectively. Both peptides reacted with the decorin antibody. After renaturation peptide B_P bound specifically to collagen type I and could be competed off with native fibroblast decorin, while peptide A_P showed no binding. These findings suggest that decorin has a collagen binding site between Leu-125 and Leu-158, which is in contrast to a recent publication (Pogány & Vogel (1992) *BBRC* **189**, 165).

5.12

Isolation and Characterization of Proteoglycans from Haematopoetic Stem Cell- and Stromal Cell-Lines

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Proteoglycans represent a family of macromolecules which are