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028

High-performance liquid chromatographic method for determination of disaccharides of glycosaminoglycans in human plasma

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Hitherto different methods quantifying glycosaminoglycans are reported [1, 2, 4]. For the determination of type and amount of various sulfated and non-sulfated glycosaminoglycans in different tissues a specific and reliable method is used that has been first described by Gurr et al. [3]. The presented method allows the determination of hyaluronate, chondroitin, chondroitin sulfate isomers, dermatan, and dermatan sulfate isomers mainly in plasma. It uses the sequential application of chondroitinases AC and ABC and separation of the resulting disaccharides by high-performance liquid chromatography. This simple and rapid separation is yielding an accurate quantification and an exact distribution pattern.

Materials and methods

Glycosaminoglycans from citrate plasma are isolated according to the method of Staprans and Felts [6]. The sulfated components are separated on Hypersil ODS (125 mm) and Nucleosil 5 SB (125 mm) in a mobile phase of 0.22 mmol/l NaCl (flow rate 0.8 ml/min), the non-sulfated on Hypersil ODS (15 mm) and Hypersil APS (250 mm) in a mobile phase of 2.5 mmol/l Na_2HPO_4, 0.1 mol/l boric acid (pH 3.0) with the same flow rate. Disaccharides are injected in concentrations from 0.07 up to 70 nmol dissolved in 10 µl water. Their absorbance is detected by UV (232 nm). Solutions of commercial available standards are used for the quantification (external standard method).

Results

The determination of glycosaminoglycan disaccharides is linear within concentrations of 7 to 7000 nmol/ml. The precision of the method is determined by standard and plasma samples. The coefficients of variation are between 1.48 and 2.05% (intra), 4.21 and 4.50% (inter) for standards and 4% (intra) and 8% (inter) for plasma. To investigate the recovery of the assay different concentrations of glycosaminoglycan disaccharides are added to the plasma sample. The recovery ranged from 86.1 to 101.8%. The chondroitin and chondroitin-4-sulfate compounds make up to 93% of the whole content of glycosaminoglycans in plasma, merely hyaluronan and dermatan sulfates are detectable in traces (Fig. 1).

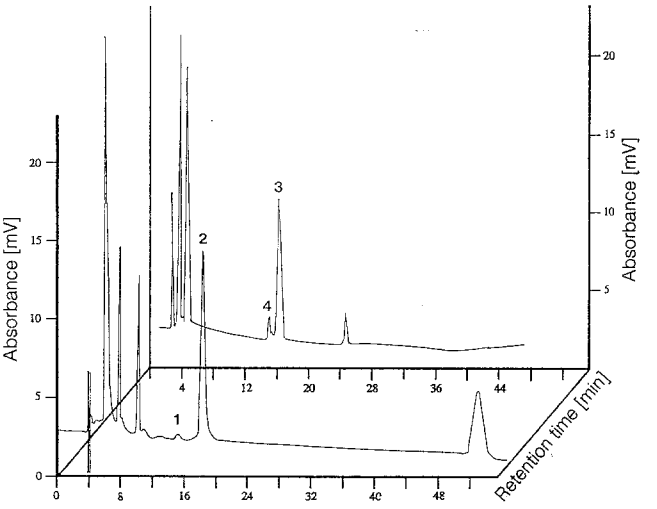

Fig. 1. HPLC chromatogram of sulfate (in front of the picture) and non-sulfate glycosaminoglycan disaccharides from plasma

Discussion

Total glycosaminoglycan concentrations coincide very well with the results of Staprans et al. [6] and Larking [5]. Results of different methods [5, 6] are compatible to the demonstrated distribution pattern of disaccharides. Applying this high specific and sensitive method glycosaminoglycans can be quantified in human plasma with good precision, accuracy and performance. The distribution pattern of glycosaminoglycans are a useful tool for the explanation of biosynthesis and degradation of proteoglycans.

References

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Peak no. Disaccharides of	1 Chondroitin-6-sulfate	2 Chondroitin-4-sulfate	3 Chondroitin	4 Hyaluronan	Dermatan-6-sulfate[a]	Dermatan-4-sulfate[a]	Dermatan[a]
Retention time [min]	15.20	18.12	16.14	14.46	15.20	18.12	16.14
Concentration [nmol/l plasma]	37 ± 11 1.6%	1087 ± 23 47.8%	1030 ± 40 45.3%	85 ± 5 3.7%	11.1 0.5%	26 ± 8 1.1%	not detectable 0%

[a] Chromatograms are not shown