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A High Performance Liquid Chromatography Method for the Determination of Glycosaminoglycans in Human Blood

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Summary: A method is described for the determination of plasma and serum glycosaminoglycans, which can be used in any laboratory equipped with an HPLC system. It is based on the sequential application of chondroitinases AC and ABC and separation of the resulting disaccharides by high-performance liquid chromatography. All reagents are commercially available. This simple and rapid separation yields an accurate quantification and an exact distribution pattern. The determination of glycosaminoglycan disaccharides is linear between 7 and 7000 $\mu\text{mol/l}$ with coefficients of variation between 3.0 and 7.7% for serum and between 2 and 14% for plasma. The recovery of the assay ranged from 93 to 106% for different concentrations of glycosaminoglycan disaccharides. This HPLC method may therefore be considered as a candidate reference method.

Introduction

Glycosaminoglycans, which are widely distributed in human tissues and fluids (1), are intimately related to proteoglycan metabolism. In plasma, glycosaminoglycans have a wide range of molecular masses, but a large proportion have relative molecular masses of less than 5000 (2). Various laboratories have demonstrated the presence of glycosaminoglycans in human plasma or serum (2–5). The results indicate that a great quantitative variability may exist among different methods for determination of blood glycosaminoglycans.

Recently we measured the glycosaminoglycan disaccharides from human blood donors, using two different methods for isolation of glycosaminoglycans in plasma and serum. The chromatographic conditions are identical for both methods of glycosaminoglycan isolation.

Significant sex differences in glycosaminoglycan disaccharide composition were found in the present study.

Materials and Methods

Reagents

Chondroitinase AC (EC 4.2.2.5)¹⁾ of *Arthrobacter aureescens* (lot E88301) and chondroitinase ABC (EC 4.2.2.4) of *Proteus vulgaris* (lot E88301) were purchased from Seikagaku Kogyo (Tokyo, Japan). α -Mannosidase (EC 3.2.1.24) of *Canavalia ensiformis* (lot 48F-9545) was from Sigma (St. Louis, USA) and papain (EC 3.4.22.2) from *Carica papaya* (lot 12385425-75/May 92) from Boehringer Mannheim (Mannheim, Germany).

Calibrator disaccharides

2-acetamido-2-deoxy-3-O-(β -D-gluco-4-ene-pyranosyluronic acid)-D-galactose (Δ Di0S),

2-acetamido-2-deoxy-3-O-(β -D-gluco-4-ene-pyranosyluronic acid)-4-O-sulpho-D-galactose (Δ Di4S),

2-acetamido-2-deoxy-3-O-(β -D-gluco-4-ene-pyranosyluronic acid)-6-O-sulpho-D-galactose (Δ Di6S) and

¹⁾ Enzymes:

Chondroitinase AC (EC 4.2.2.5)
Chondroitinase ABC (EC 4.2.2.4)
 α -Mannosidase (EC 3.2.1.24)
Papain (EC 3.4.22.2)