Fluorophore Assisted Carbohydrate Electrophoresis Applied to Glycosaminoglycan Structural Analysis

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Introduction: carbohydrate analysis plays a key role in many research and industrial segments. In the field of glycoconjugates, evidence shows that chondroitin sulfate and dermalan sulfate proteoglycans have biological roles, and so the fine structure elucidation of their glycosaminoglycan chains is of major importance. In this work we describe the separation of different glycosaminoglycan building blocks and the enzymatic chain depolymerization by Fluorophore Assisted Carbohydrate Electrophoresis (FACE), a polyacrylamide gel electrophoresis (PAGE) technique. Material and Methods: monosaccharides, disaccharides and glycosaminoglycan digestion products were derivatized with 2-aminoacridone (AMAC) and submitted to PAGE in 20% gels. Electrophoresis run was performed in Tris-glycine or Tris-borate-glycine buffer systems, according to the type of sugar and desired separation. Results and Discussion: unsaturated glycosaminoglycan disaccharides (ΔDiHA, ΔDi0S, ΔDi2S, ΔDi4S, ΔDi6S, ΔDi2,6S, ΔDi4,6S, ΔDi2,4,6S) and monosaccharides (GalNAc, GlcNAc, GalNAc-4S and -6S) showed differential migration pattern by FACE using two different buffer systems. This method was applied to disaccharide analysis of chondroitin sulfate extracted from different sources (bovine trachea, chicken kneel, shark, whale, raja and squid cartilage), and our results showed similar sulfation patterns to those described in the literature. Chondroitin sulfate depolymerization by chondroitinase AC from Flavobacterium heparinum was monitored by FACE. In the early stages of the reaction, a predominance of oligosaccharides and tetrasaccharides was observed, followed by an increase in disaccharides during the course of incubation, suggesting an endolytic action pattern of this enzyme. Analysis of dermalan sulfate depolymerization by chondroitinase AC showed several bands in a ladder manner, indicating formation of oligosaccharide chains with different polymerization degrees produced by cleavage of glucuronic regions of this glycosaminoglycan. Conclusions: FACE is a sensitive, reproducible and powerful technique for glycosaminoglycan structural analysis.

Keywords: chondroitin sulfate, chondroitinase, FACE, glycosaminoglycan.