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Development of an enzyme-linked immunosorbent assay (ELISA)-like fluorescence assay to investigate the interactions of glycosaminoglycans to cells

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ABSTRACT

Sulfated glycosaminoglycans were labeled with biotin to study their interaction with cells in culture. Thus, heparin, heparan sulfate, chondroitin 4-sulfate, chondroitin 6-sulfate and dermatan sulfate were labeled using biotin-hydrazide, under different conditions. The structural characteristics of the biotinylated products were determined by chemical (molar ratios of hexosamine, uronic acid, sulfate and biotin) and enzymatic methods (susceptibility to degradation by chondroitinases and heparitinases). The binding of biotinylated glycosaminoglycans was investigated both in endothelial and smooth muscle cells in culture, using a novel time resolved fluorometric method based on interaction of europium-labeled streptavidin with the biotin covalently linked to the compounds. The interactions of glycosaminoglycans were saturable and number of binding sites could be obtained for each individual compound. The apparent dissociation constant varied among the different glycosaminoglycans and between the two cell lines. The interactions of the biotinylated glycosaminoglycans with the cells were also evaluated using confocal microscopy. We propose a convenient and reliable method for the preparation of biotinylated glycosaminoglycans, as well as a sensitive non-competitive fluorescence-based assay for studies of the interactions and binding of these compounds to cells in culture.

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1. Introduction

Sulfated glycosaminoglycans (GAGs) are linear polysaccharides composed of repeating disaccharides units, containing a hexosamine (D-glucosamine or D-galactosamine) linked by glycosidic linkage to an uronic acid residue (D-glucuronic acid or L-iduronic acid) or a neutral sugar (D-galactose). These polymers are negatively charged due to the presence of sulfate groups in their structure and/or carboxyl groups from the uronic acids, which contribute to the

Abbreviations: BSA, bovine serum albumin; C4S, chondroitin 4-sulfate; C6S, chondroitin 6-sulfate; DAB, 3,3′-diamino benzamidine tetrahydrochloride; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; DS, dermatan sulfate; ECM, extracellular matrix; EDC, (1-ethyl-3-[dimethylaminopropyl] carbodiimide hydrochloride); EC, endothelial cells; FCS, fetal calf serum; HRP, horseradish peroxidase; HS, heparan sulfate; Hep, heparin; GAG, glycosaminoglycan; bGAG, biotinylated GAG; PBS, phosphate buffer saline; SMC, smooth muscle cells.

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highly polyanionic nature of the GAGs. Glucosamine is the hexosamine present in hyaluronan (HA), heparan sulfate (HS), heparin (Hep) and keratan sulfate (KS), whereas galactosamine is characteristic of chondroitin sulfate (CS) and dermatan sulfate (DS). These linear polysaccharides are composed of variable number of repeating disaccharide units [1–3].

With exception of HA, GAGs occur in tissues as proteoglycans covalently attached to core proteins, being present at the cellular surface, extracellular matrix (ECM), basal membrane and cytoplasmatic granules [4–7]. This strategic localization in the plasma membrane and ECM points these compounds as important intermediates between the cells and their environment [5,8–10]. They have been implicated in cell–cell and cell matrix interactions, organization of basement membranes, control of macromolecules diffusion, interactions with a variety of ligands such as growth factors, hormones, and neurotransmitters, among others [1,11–15].

Labeling of GAGs is mandatory for studies of their interactions with cellular surface and ECM components it is of importance to label. Several methods have already been published that use the grafting of radiolabeled elements—namely [^{3}H], [^{35}S], and [^{125}I] [16–21]. However, the use in microscopy of compounds labeled with radioisotopes present limitations, besides the radiobiological risk management. On the other hand, biotin can be used as a label, due to the facility that the labeled compounds can be easily detected and quantified through streptavidin conjugated with diverse markers as peroxidase, alkaline phosphatase and fluorescent markers [22]. The present paper describes a new reproducible methodology for incorporation of biotin into the carboxyl groups of GAGs. Furthermore, in the present work these compounds were then used to study the interaction with cells by a simple, rapid, sensitive non-competitive fluorescence-based assay, which uses europium-labeled streptavidin.

2. Experimental

2.1. Cell culture

An endothelial (EC) and a smooth muscle cell (SMC) lines derived from rabbit thoracic aorta [10,23] were used in this study. Cells were grown in Ham's Nutrient Mixture F-12 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf

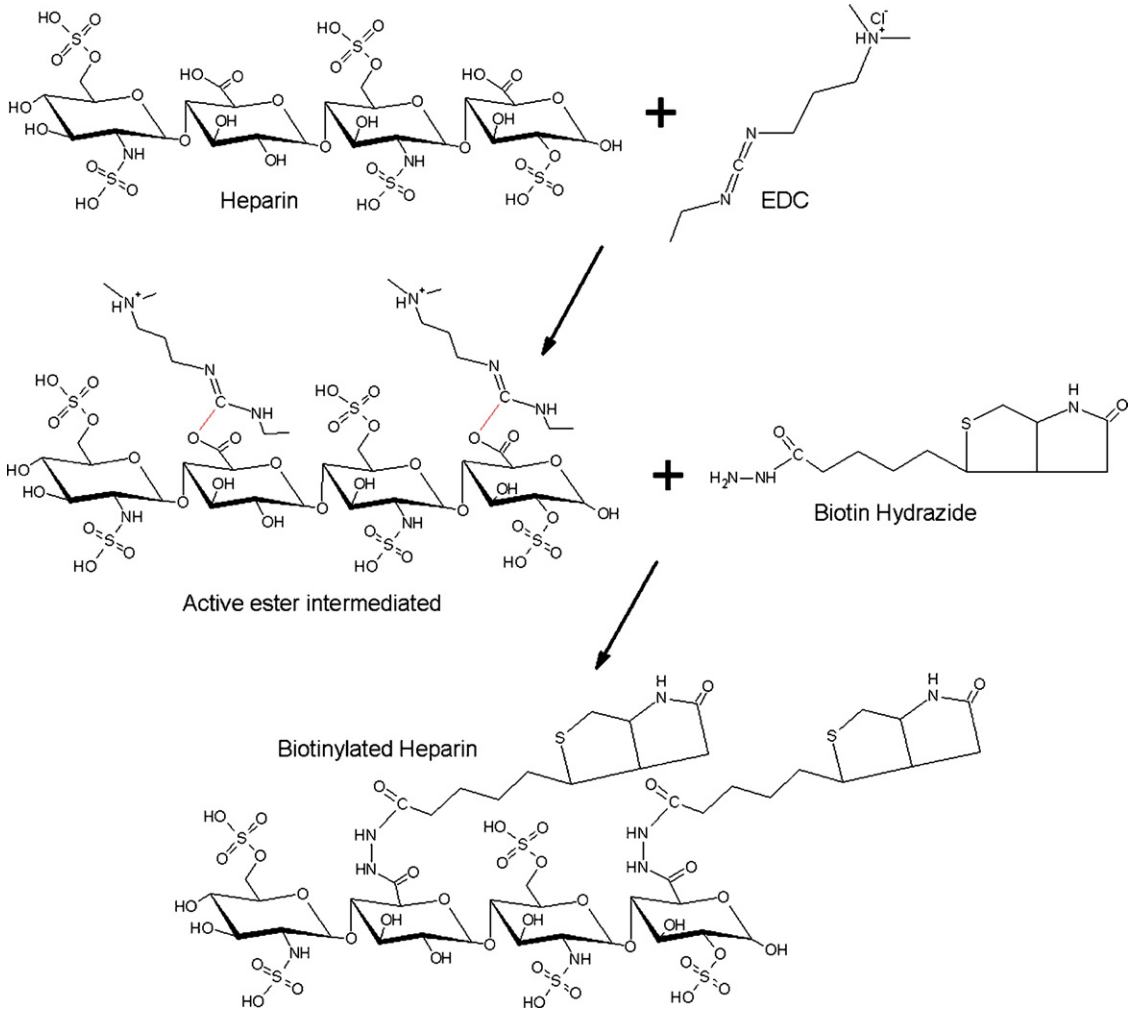

Fig. 1 – Scheme of the reactions involved in GAGs biotinylation. The figure depicts the reaction using Heparin, and the same type of intermediates and reactions are found for the other GAGs.

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