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Low and high affinity receptors mediate cellular uptake of heparanase

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Abstract

Heparanase is an endoglycosidase which cleaves heparan sulfate and hence participates in degradation and remodeling of the extracellular matrix. Importantly, heparanase activity correlated with the metastatic potential of tumor-derived cells, attributed to enhanced cell dissemination as a consequence of heparan sulfate cleavage and remodeling of the extracellular matrix barrier. Heparanase has been characterized as a glycoprotein, yet glycan biochemical analysis was not performed to date. Here, we applied the Qproteome™ GlycoArray kit to perform glycan analysis of heparanase, and compared the kit results with the more commonly used biochemical analyses. We employed fibroblasts isolated from patients with I-cell disease (mucolipidosis II), fibroblasts deficient of low density lipoprotein receptor-related protein and fibroblasts lacking mannose 6-phosphate receptor, to explore the role of mannose 6-phosphate in heparanase uptake. Iodinated heparanase has been utilized to calculate binding affinity. We provide evidence for hierarchy of binding to cellular receptors as a function of heparanase concentration. We report the existence of a high affinity, low abundant (i.e., low density lipoprotein receptor-related protein, mannose 6-phosphate receptor), as well as a low affinity, high abundant (i.e., heparan sulfate proteoglycan) receptors that mediate heparanase binding, and suggest that these receptors co-operate to establish high affinity binding sites for heparanase, thus maintaining extracellular retention of the enzyme tightly regulated.
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Keywords: Heparanase; Glycosylation; Lectin array; Mannose 6-phosphate; Binding; Receptor

1. Introduction

Heparanase is an endo-β-D-glucuronidase capable of cleaving heparan sulfate (HS) side chains at a limited number of sites, yielding HS fragments of still appreciable size (~4–7 kDa). Heparanase activity has long been correlated with the metastatic potential of tumor-derived cells, attributed to enhanced cell dissemination as a consequence of HS cleavage and remodeling of the extracellular matrix (ECM) barrier (Parish, Freeman, & Hulett, 2001; Vlodavsky & Friedmann, 2001). Similarly, heparanase activity was implicated in cell dissemination associated with inflammation, autoimmunity and angiogenesis (Dempsey, Brunn, & Platt, 2000; Vlodavsky

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& Friedmann, 2001). This notion gained further support by employing siRNA and ribozyme methodologies, clearly depicting heparanase-mediated HS cleavage and ECM remodeling as critical requisites for inflammation, angiogenesis, and metastatic spread (Edovitsky, Elkin, Zcharia, Peretz, & Vlodavsky, 2004; Edovitsky et al., 2005). More recently, heparanase up-regulation was documented in an increasing number of human carcinomas and hematological malignancies (Ilan, Elkin, & Vlodavsky, 2006; Vreys & David, 2007). In many cases, heparanase induction correlated with increased tumor metastasis, vascular density, and shorter post-operative survival rate, thus providing a strong clinical support for the pro-metastatic and pro-angiogenic function of the enzyme and positioning heparanase as an attractive target for the development of anti-cancer drugs (Ferro, Hammond, & Fairweather, 2004; Miao, Liu, Navarro, Kussie, & Zhu, 2006; Vlodavsky, Ilan, Naggi, & Casu, 2007). In addition, heparanase activity can liberate multitude HS-bound biological mediators such as growth factors, cytokines and chemokines, thus significantly affecting cell and tissue function. The multitude of polypeptides sequestered and regulated by HS and the ability of heparanase to convert these into bio-available molecules (Elkin et al., 2001; Folkman et al., 1988) require that these activities will be kept tightly regulated. We have shown previously that exogenously added heparanase rapidly interacts with primary human fibroblasts (Nadav et al., 2002) as well as with tumor-derived cells (Gingis-Velitski, Zetser, Flugelman, Vlodavsky, & Ilan, 2004a), followed by internalization and processing into a highly active enzyme (Zetser et al., 2004), collectively defined as heparanase uptake (Gingis-Velitski et al., 2004b). Applying HS-deficient cells, addition of heparin or xylosides, and deletion of HS-binding domains of heparanase, we have provided compelling evidence for the involvement of HS in heparanase uptake and processing (Gingis-Velitski et al., 2004b; Levy-Adam et al., 2005), maintaining extracellular retention of the enzyme tightly regulated. More recently, Vreys et al. (2005) have identified two additional cell-surface receptors that mediate heparanase uptake, namely the low density lipoprotein receptor-related protein (LRP) and the mannose 6-phosphate receptor (MPR) as key elements in this process, although a model that combine the three receptors has not been proposed. Heparanase has long been characterized as a glycoprotein and including Concanavalin A affinity chromatography in the purification scheme brought this feature into practice (Toyoshima & Nakajima, 1999; Vlodavsky et al., 1999; Zcharia et al., 2005). Six glycosylation sites were identified in the 50 kDa heparanase subunit (Hulett et al., 1999), and their role in protein secretion was established (Simizu, Ishida, Wierzba, & Osada, 2004), yet glycan biochemical analysis was not performed to date.

Here, we utilized a new lectin array method for studying glycan structure and composition, the Qproteome^{TM} GlycoArray kit (Qiagen) (Rosenfeld et al., 2007), to study heparanase glycan composition and compared the lectin array results with the more common HPLC analysis. We further employed fibroblasts isolated from patients with I-cell disease (mucolipidosis II) and fibroblasts deficient of LRP and MPR, to explore the role of mannose 6-phosphate in heparanase uptake, and utilized iodinated heparanase to calculate binding affinities. We provide evidence for hierarchy of binding to cellular receptors as a function of heparanase concentration. We report the existence of high affinity, low abundant (i.e., MPR, LRP), as well as low affinity, high abundant (i.e., HSPG) receptors that mediate heparanase binding, and suggest that these receptors co-operate to establish high affinity binding sites for heparanase.

2. Materials and methods

2.1. Glycan analysis by lectin array

Recombinant human heparanase was analyzed according to the manufacturer's (Qiagene, GmbH, Germany) instructions. Briefly, Qiagene Lectin Array (Qproteome GlycoArray Kit) was incubated for 60 min with 1% BSA. Recombinant heparanase (13 μg/ml), purified from medium conditioned by heparanase-transfected CHO cells (Gingis-Velitski et al., 2004b; Zetser, Bashenko, Miao, Vlodavsky, & Ilan, 2003) was then applied to the array for 80 min, followed by incubation with rabbit anti-heparanase 1453 antibody (Zetser et al., 2003) (60 min, diluted 1:1000) and goat anti-rabbit-FITC conjugated antibody (Jackson Immunoresearch Laboratories, West Grove, PA) diluted 1:1000. Data analysis was performed using the automatic analysis software, as described (Rosenfeld et al., 2007).

2.2. Glycan preparation for HPLC analysis

Heparanase was subjected to peptide-N-gycosidase F (PNGase; 0.1 U/μl) cleavage using denaturation protocol, according to the manufacturer (New-England Biolabs, Beverly, MA) instructions. Glycans were fluorescently labeled at their reducing end by 2-aminobenzamide (2AB, Merck), as described (Bigge et al., 1995).

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