Heparanase: Structural Investigation of Binding Affinities

Molecular Dynamics Investigations of Binding Affinities between Heparan Sulphate and Heparanase Isoforms 1 and 2 and Selected Active Site Mutants

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In Short

- Heparanase isoform HPSE-1 is an endo-β-d-glucuronidase involved in the detaching of heparan sulphate from the cell surface and from the proteoglycan of the extracellular matrix
- Heparanase isoform HPSE-2 protects heparan sulphate from detachment by HPSE-1
- Both isoforms are attractive targets for anticancer drugs
- HPSE-1 has been intensively studied, but not much is known about the functional properties and cellular role of HPSE-2
- Molecular Dynamics simulations are used to scan the conformational space of the heparan sulphate binding sites of both isoforms and their mutants

At this point in time not much is known about Heparanase-2 (HPSE-2). HPSE-2 is a protein found throughout the body with the most prominent concentrations in smooth muscle containing tissues. Cellular studies have shown, that HPSE-2 reduces lipopolysaccharides mediated toll-like-receptor 4 activation, subsequent cell signalling, and cytokine production 1. Production of HPSE-2 can contribute to a feedback loop by which HPSE-2 enhances Endoplasmic reticulum (ER) stress 2. A follow-up study has shown that increased expression of the gene encoding HPSE-2 is triggered by activating-transcription-factor 3 (ATF3) 3. In the case of pancreatic and some other forms of cancer, tumour growth is attenuated by the resulting increase in apoptosis 4. HPSE-2 is thought to be involved in several other cellular pathways, including tumour suppression through activation of the p53/p21 signalling cascade 5. In addition, HPSE-2, with Heparan Sulphate (HS) probably acting as cofactor, can induce the production of Sox2, suggesting that HPSE-2 plays a role in determining and maintaining the differentiation and identity of normal endothelial cells 2,4. A structurally closely related isoform is the enzyme Heparanase-1 (HPSE-1). HPSE-1 is an endo-β-d-glucuronidase involved in the degradation of both cell-surface HS and extracellular matrix (ECM) HS. HPSE-1 is the only enzyme that can shed HS, which is part of the glycocalyx and is essential to maintain integrity and function of the endothelial layer 6,7,8. Endothelial glycocalyx shedding occurs during many pathological conditions 9, specifically it plays a critical role in tumour metastasis 10. The sequence of HPSE-1 splicing form 1 and HPSE-2 splicing form c are 40% identical and share 59% similarity 11. HPSE-2 does not possess heparan sulphate-degrading activity, notwithstanding a predicted close structural similarity with HPSE-1 and the presence of two conserved residues (Glu_{225} and Glu_{343}) that are required for the enzymatic properties of HPSE-1 10,11. It has been suggested that HPSE-2 acts as a tumour suppressor by inhibiting the activity of HPSE-1 in a competitive manner binding more strongly to HS 10.

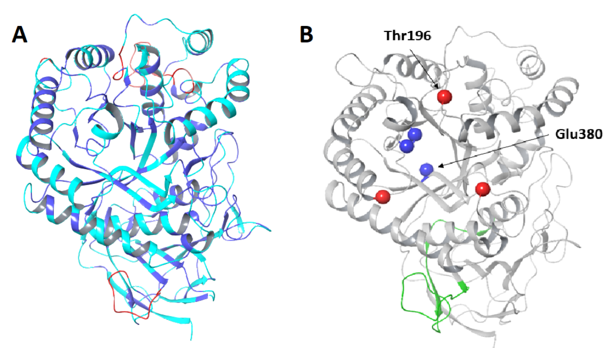

***Figure 1:** Homology Model of HPSE-2 (Uniprot: Q8WWQ2) based on HPSE-1 (PDB: 5LA7) with a resolution of 1.94Å. A: Regions with high confidence score are shown in blue and those with low confidence scores in orange. The blue sections of the chain correspond for the most part to the regions that have a large sequence match with HPSE. B: HPSE-2 with the positions of the positively charged HS binding amino acids from HPSE in red. In blue the catalytic residues and in green the additional HS binding site.*

This project aims to complement direct experimental studies of the purified enzymes and selected mutant forms with *in silico* structural analyses to obtain a better understanding of the impact of changes in sequence on overall molecular dynamic behaviour and how structural differences affect HS binding and ECM degradation. By understanding this process, new ways of treatment of HPSE-1 conditions caused by abnormally increased expression of HPSE-1 can be investigated. For this purpose, we created a homology model for HPSE-2, based on HPSE-1 (Figure 1A). With long-time MD-simulations we want to study the conformation of the catalytic residues of HPSE-1 in comparison to HPSE-2 (Figure 1B).

We want to determine the reason for the observed missing catalytic activity of HPSE-2, although it contains the necessary residues. Therefore, we aim to explore in detail the active site and the effects of changes at specific residues on the conformation and binding capabilities of HS to HPSE-1 and HPSE-2.

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https://www.mhh.de/bpc

More Information

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Project Partners

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