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Molecular Docking of Short-Chain Glycosaminoglycan Ligands in 3D Model of Bovine Testicular Hyaluronidase

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Abstract

Computational analysis of molecular docking of 3D model of bovine testicular hyaluronidase *in silico* with a trimer (hexasaccharide) chondroitin sulfate and tetramer (octasaccharide) heparin has demonstrated eight significant binding sites (cs1-cs8) for these ligands. Interactions with heparin that inactivates the enzyme and protective effects of chondroitin sulfate were examined. Binding sites have been identified which are critical for stabilization of the protein structure after chondroitin sulfate binding: at positions cs2, cs4, cs7 and cs8 or cs1, cs2, cs4, cs7 and cs8. Occupation of these sites is theoretically sufficient for prevention of irreversible molecular deformations after heparin inclusion into active site. The presence of sensitivity sites on hyaluronidase globule indicates the possibility of regulating the enzyme function via coupling glycosaminoglycan ligands thus initiating delicate/fine formation of effective electrostatic potential. Interactions between glycosaminoglycan ligands and hyaluronidase are based primarily on electrostatic forces.

Keywords: Bovine Testicular Hyaluronidase; Chondroitin Sulfate; Docking; Glycosaminoglycan Ligands; Heparin

Abbrevitiations

BTH	:	Bovine Testicular Hyaluronidase
CHS	:	Chondroitin Sulfate
GAG	:	Glycosaminoglycan

Introduction

Enzyme derivatives used as pharmacological agents constitute a considerable part of enzyme therapy. Glycosidases are prospective regulators of the endothelial glycocalyx, the initial protective layer on luminal surface of blood vessels [1,2]. Hyaluronidases are deeply involved in the regulation of metabolism of hyaluronan, a major constituent of glycocalyx [3]. Bovine Testicular Hyaluronidase (BTH, EC 3.2.1.35) is allowed for intramuscular/subcutaneous injections and local application. Although this enzyme becomes increasingly important in glycobiology research [4], vertebrate hyaluronidases were not studied in sufficient detail due to very low concentration (~ 60 ng/ml in human plasma) and scarce information on their structure [5]. Hyaluronidases display an order of magnitude higher enzyme activity compared with that of other globular biocatalysts; however, their stability upon isolation and purification is very low [4,5].

Production of pharmacological agents based on modified hyaluronidases to achieve stable endoglycosidase activity for correction of glycocalyx function [6] (currently only replacement therapy is used for these purposes) requires deep understanding of molecular structure of the enzyme for optimization of its properties. Elucidation of human hyaluronidase structure [7] facilitated its use as a prototype for *in silico* 3D homologous modeling of BTH [8]. Our further research was based on BTH function in a Glycosaminoglycan (GAG) environment and aimed at understanding of the regulatory mechanisms operating during interaction between the enzyme and GAG of endothelial glycocalyx and extracellular matrix. It was important to define limiting structural factors associated with these mechanisms. GAGs are heterogeneous on their length, sequence, composition. According

to these reasons the experimental studies of GAG 3D conformation and dynamics are difficult performed [9]. It is necessary to use the computer modeling. Computational analysis is the only direction to gain insight of GAG interactions now and then in some cases [10]. Under physiological conditions BTH molecule has a positive charge (pI 8.62) /http://www.uniprot.org/uniprot_/Q7YS45/, while GAG ligands bear a negative charge [11]. Our objective was to examine electrostatic interactions between BTH and GAG by computational analysis of molecular docking in 3D model of BTH and Chondroitin Sulfate (CHS) trimers *in silico*.

Methods

3D models of native BTH, free enzyme and an enzyme-substrate complex (a hyaluronan dodecamer given here), were constructed previously [8] and were used for docking with CHS and heparin ligands.

Molecular docking was performed using UCSF Chimera, DOCK and Swiss-PdbViewer software [12-15]. DOCK was employed to perform docking of a low-molecular-weight molecule into a static macromolecule; UCSF Chimera and Swiss-PdbViewer were used to produce images with further superposition and visualization. Calculation of isosurface for electrostatic potential was done using PDB2PQR, APBS, UCSF Chimera and Delphi software and AMBER, GROMOS and CHARMM [16-21] force field in compliance with Coulomb's law and Poisson-Boltzmann equation. Upon docking, the low-molecular-weight molecule was placed at different positions in relation to the macromolecule at a distance of its half cross-size. The procedure was performed many with real time modeling. Reversibility/irreversibility of conformational changes in BTH molecule was evaluated by its return to the initial state within a given time period (50-100 ps). It should be noted that the absence of changes in the protein structure within this time period indicates that it is stable, i.e., remains unchanged with the accuracy of heat-induced conformational oscillations. Potentials were given in e/a_o natural unit system, where e is the electron charge and a_o is the Bohr radius; in SI unit system potential of 0.4 e/a_o corresponds to approximately 1 V. Molecular dynamic was monitored with NAMD software [22].

The choice of GAG ligands /Pubchem.ncbi.nlm.nih.gov/ for molecular docking with 3D model of BTH was arbitrary since it is unknown which GAG fragment selectively inhibits the enzyme. Since glycosaminoglycans demonstrate high conformational flexibility, docking of only their short fragments (up to tetramers) can be reliably calculated. Therefore, were used a [GlcA - GalNAc]$_3$ trimers, where GlcA is glucuronic acid 2-sulfate bound to N-acetylgalactosamine 4-sulfate via β1-3 glycosidic bond, and an [IdoA – GlcNAc (2, 6) - IdoA –GlcNAc (2, 6) - IdoA – GlcNAc (2, 3, 6) - IdoA - GlcNAc (2, 6)]) heparin tetramer, where IdoA is iduronate 2-sulfate bound to glucosamine 2,6 sulfate / GlcNAc (2, 6) / of glucosamine 2,3,6 sulfate / GlcNAc (2, 3, 6) / via α1-4 glycosidic bond. The lengths of straightened CHS and heparin fragments correspond to cross-size of BTH molecule.

Results and Discussion

Binding Sites on Molecular Surface of Hyaluronidase

Construction of 3D model of BTH (Figure 1)

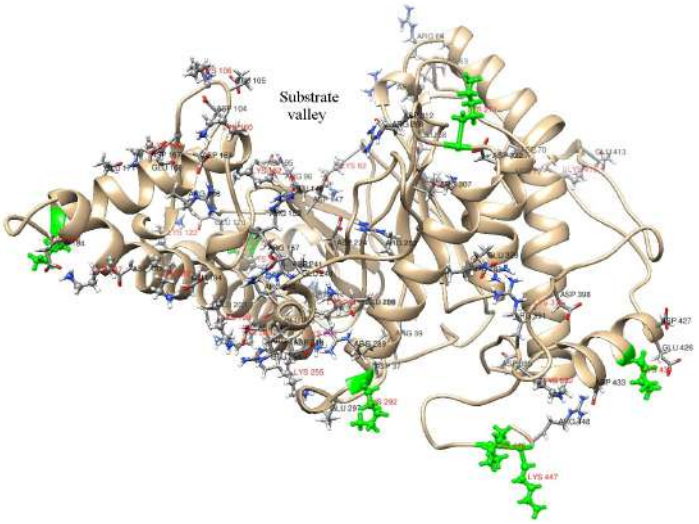

Figure 1: 3D thread-ribbon model of bovine testicular hyaluronidase. Six sites with lysine residues of the first-level availability for covalent modification [8] are highlighted in dark green. Noted lysine residues are located in the zones absolutely accessible for solvent and hydrophilic reagent with Stokes radius 12 Å. They are not involved in close intramolecular Coulombic or van der Waals interactions also. According to such collocation these lysine residues are most appropriate for chemical modification of enzyme (first-level availability).

facilitated computation of surface electrostatic potential of native enzyme (Figure 2). The potential is characterized by definite predominance of positively charged areas. It is noteworthy that electrostatic interactions have an effect on folding stability of human $FKBP_{12}$ protein [23], play an important role in sirtuin 2 inhibition [24] and contribute to protein-ligand interactions depending on dielectric constant of the medium [25]. Computational analysis of electrostatic potential allows prediction of GAG binding sites in individual systems and is useful for proposing protein surface regions as putative glycosaminoglycan binding sites [26]. The motives of these binding sites on a protein molecule surface are represented by parts of protein chain with xBBBxxBx or xBBxBx amino acid sequence where B is a basic amino acid (arginine, lysine or histidine) [11]. Effective binding in 3D structural models of proteins is achieved by juxtaposition of two binding sites. Computationally, we have identified 12 GAG binding sites on the surface of BTH molecule. Calculations were reduced to 8 sites with free binding energy at least 2-fold higher than energy of free ligands (Figure 3).

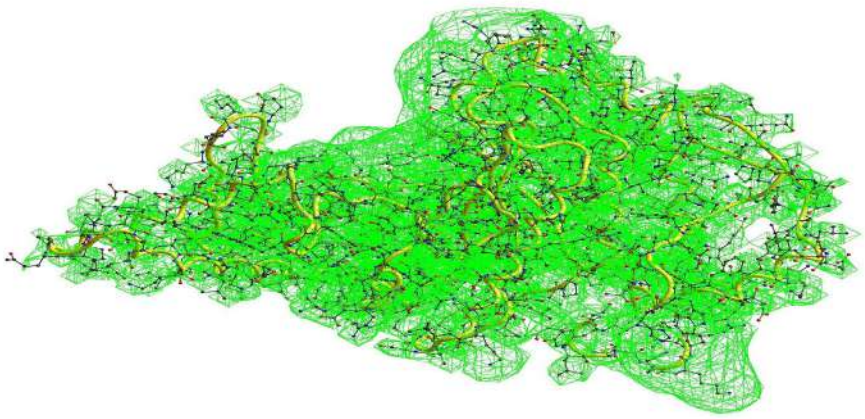

Figure 2: Surface electrostatic potential of free bovine testicular hyaluronidase (here and further at 0.4 e/a_o level). Negative and positive charges are indicated (here and further in Figures 4, 7 and 8) with red and green color, respectively.

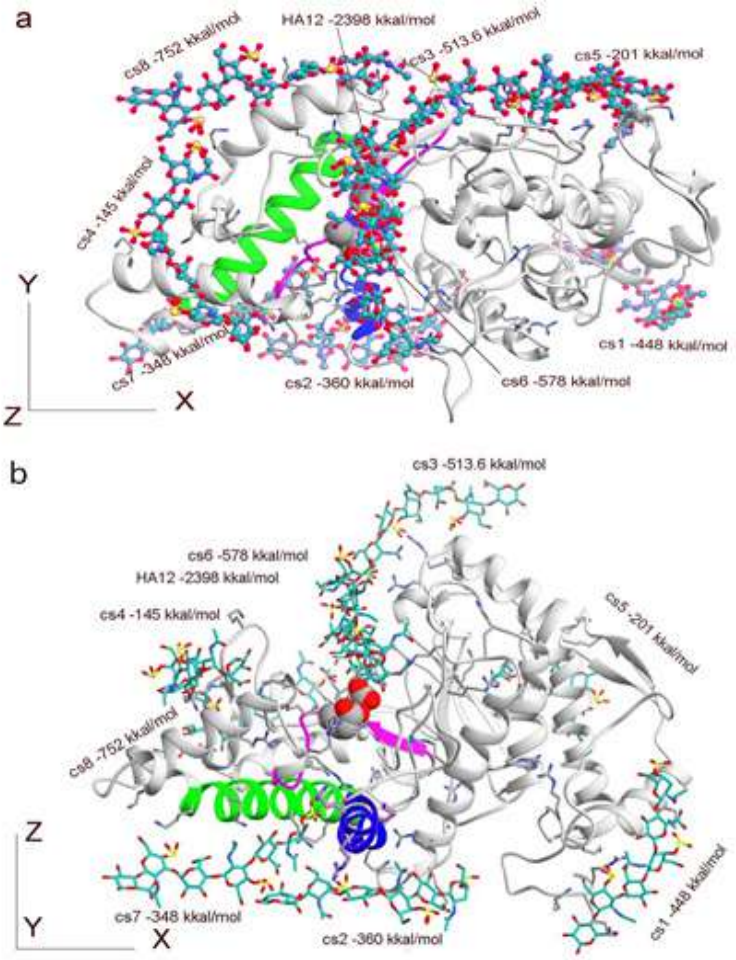

Figure 3: Location of eight CHS binding sites (cs1, cs2, cs3, etc.) in 3D model of BTH with free binding energy values at 0 K. The image is given in projection formed by principle axes of inertia for hyaluronan, a dodecasaccharide fragment of the substrate (HA12, center of the image), and for BTH (horizontal axis X). **a** Principle axis of inertia for hyaluronan passes vertically, Y. **b** Principle axis of inertia for hyaluronan Y is perpendicular to

the plain of the figure. The active site loop between Asp 139 and Ala 156, including Asp 147 and Glu 149 is highlighted in light violet, alpha-helix from Ala 185 to Gly 197 is highlighted in green, and helix from Glu243 to Glu256 in highlighted in blue.

Protective effects of CHS ligand and inhibiting/inactivating effects of heparin with/without electrolyte environment were computed with inclusion of varied number of CHS ligands and heparin positioning into active center of the enzyme. Ligand binding energy which depends primarily on electrostatic interactions between GAG and arginine and lysine amino acid residues of BTH was determined as the difference between energy of the total complex before and after removal of the ligand followed by filling the released space with solvent. It should be noted that the enzyme-substrate complex is stable in any position with any number of associated CHS or heparin ligands. We further analyzed the stability of substrate-free enzyme, assuming that BTH is stable in the presence of eight CHS ligands in their binding sites.

Electrostatic Forces for Docking

Electrostatic interactions are a necessary condition for docking and recognition of GAG ligands [27]**.** Electrostatic gradient of protein is a driving force aimed at ligand relocation along the gradient vector applied to the mass center of the ligand. Our computational data clearly demonstrate changes in surface electrostatic potential of free BTH (Figure 2), during interaction with eight CHS trimers (Figure 4).

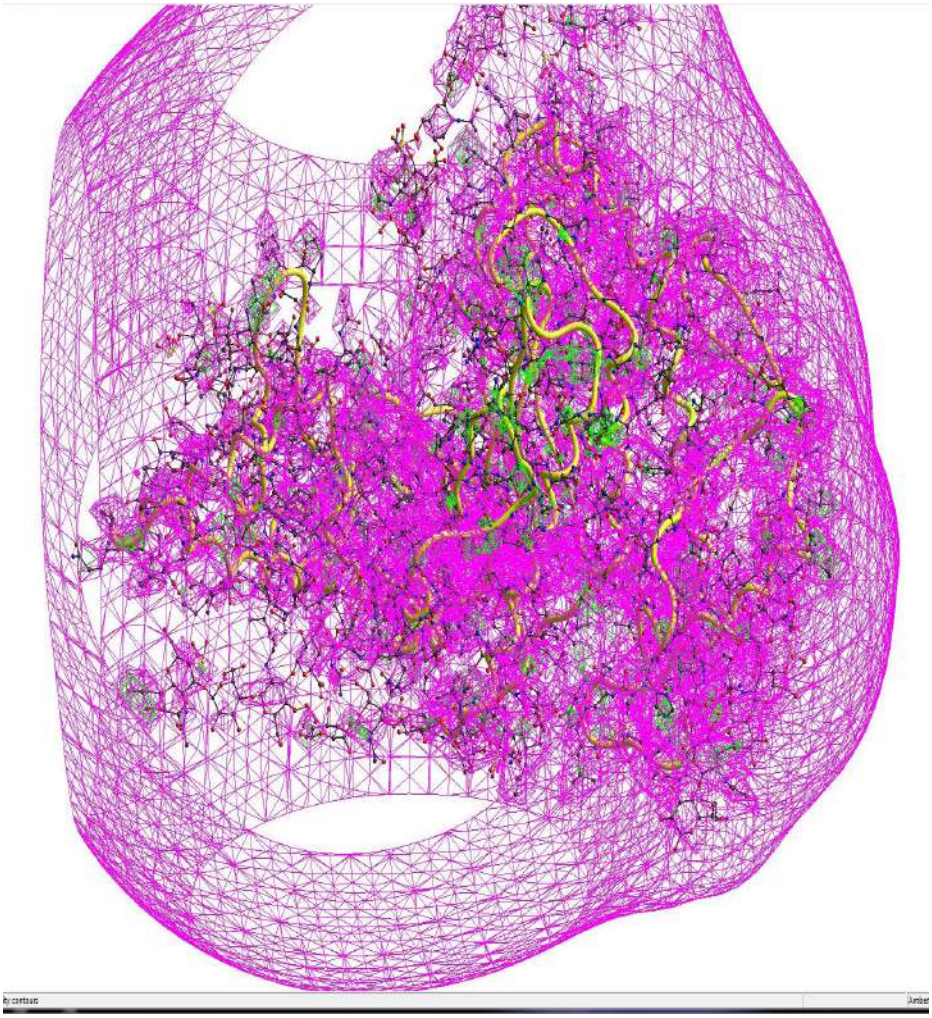

Figure 4: Surface electrostatic potential of bovine testicular hyaluronidase during interaction with eight CHS trimeric ligands stabilizing enzyme structure.

Electric field generated by the enzyme with 8 ligands envelopes the entire protein complex and nearly coincides with molecular relief of the enzyme. Free BTH is positively charged (Figure 2), while in CHS shield its charge is negative (Figure 4). In both cases the system remains stable, and heparin inclusion in the active center produces no significant effect on it. The situation changes after fixation of CHS ligands at cs1, cs2, cs3 and cs4 positions and heparin tetramer at cs6 position (Figure 5).

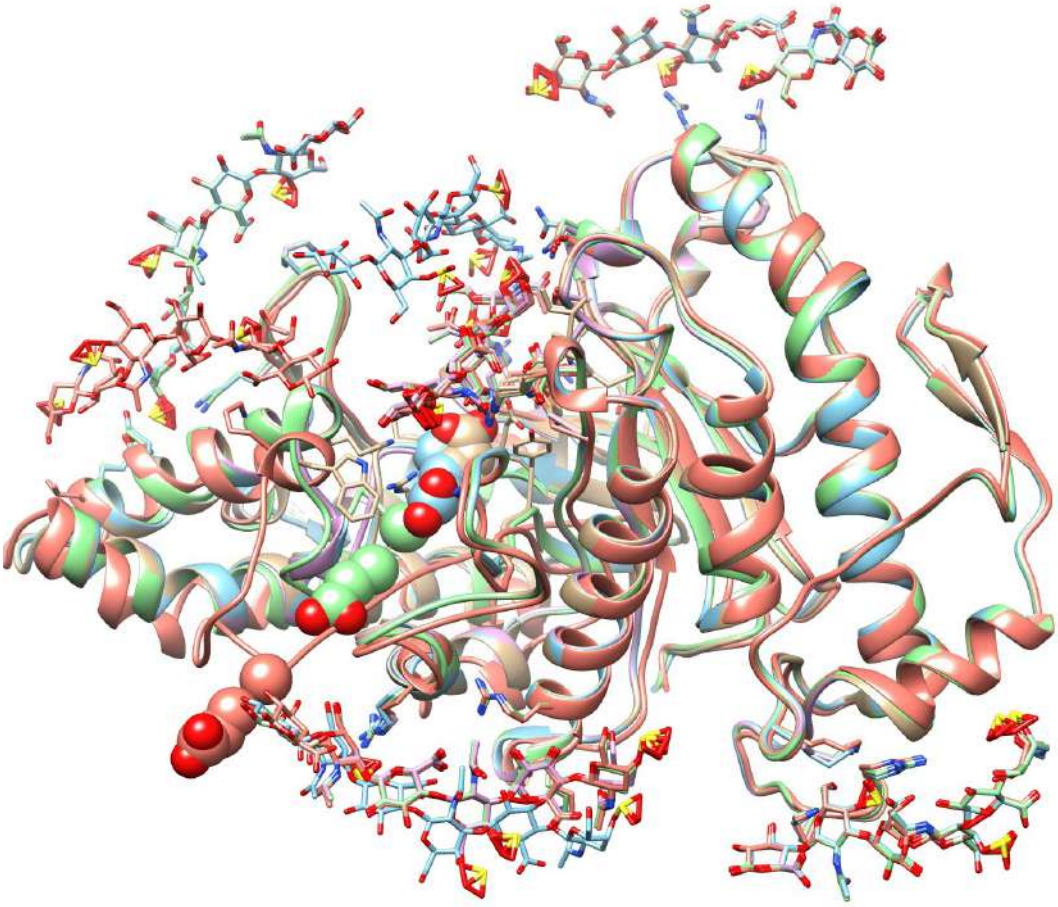

Figure 5: Irreversible conformational changes in bovine testicular hyaluronidase after coupling with four CHS ligands at cs1, cs2, cs3 and cs4 positions and heparin tetramer at cs6 position. Superposition of protein structures surrounded by CHS ligands corresponds to the shape of the complex at 20th, 60th, 100th, 140th and 180th ps of the dynamics and the enzyme structure highlighted in beige, blue, green and light brown, respectively.

The electric field intensity created along its potential pushes heparin ligand into BTH active site. Being not stabilized by disulfide bonds between Asp 139 and Ala 156, the active site loop bearing Asp 147 and Glu 149 which are responsible for protein activity (highlighted in violet) is pushed out into the gap between alpha coil from Ala 185 to Gly 197 (green) and the coil from Glu 243 to Glu 256 (blue, Figure 3). This leads to irreversible molecular deformation and inactivation of BTH (Figure 6).

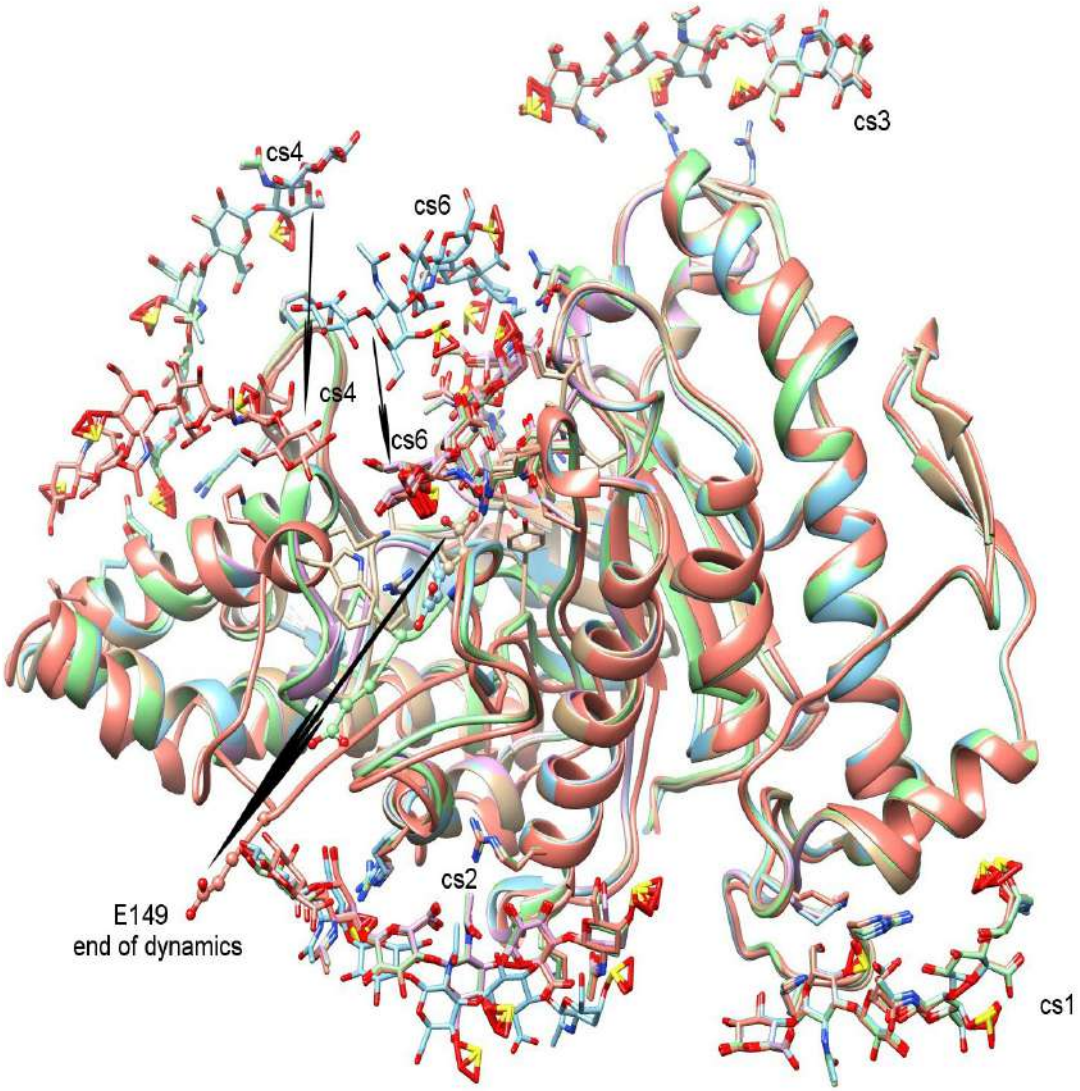

Figure 6: Irreversible conformational changes in bovine testicular hyaluronidase after peripheral dislocation of Glu149 and Asp147 which are responsible for enzyme activity. Arrows indicate initial and end positions of Glu149 during transformational transition. Significant changes in active site leading to the enzyme inactivation. Location of the ligands at cs4 and cs6 positions after inclusion in active site. During transition the ligands at cs1 and cs3 are virtually immovable relative to the protein and move at cs2, cs4 and cs6 positions. Highlighting is the same as in Figure 5.

The effect is enhanced by inclusion of cs8 into the system Electric field created by ligands at cs1, cs2, cs3, cs4 and cs6 (Figure 7) does not resemble that of BTH stabilized by eight CHS ligands (Figure 4). Our data indicate that there is no specific coupling of heparin tetramer and CHS trimer to BTH active site. Formation of major contacts results from electrostatic interactions realized via arginine residues on BTH surface, while lysine residues are important for covalent modification of the enzyme [8].

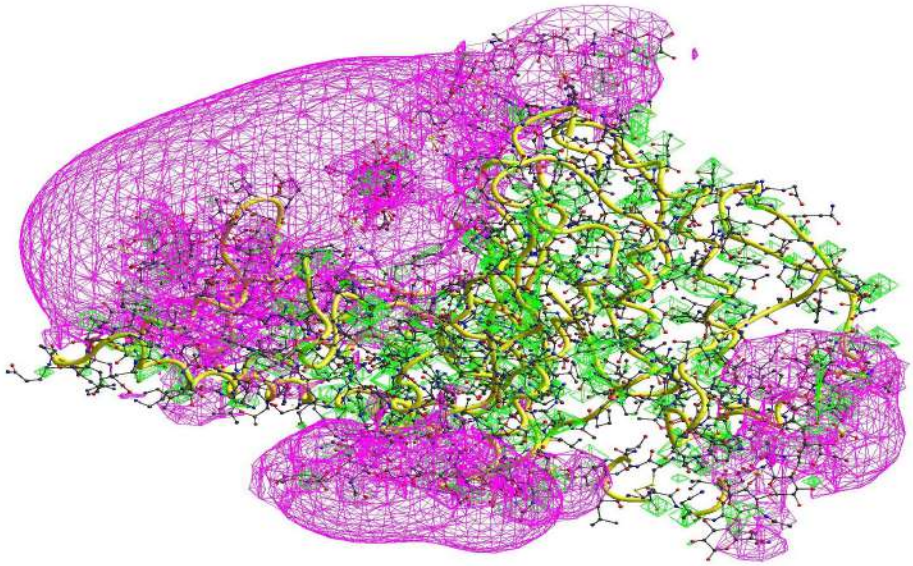

Figure 7: Surface electrostatic potential of bovine testicular hyaluronidase surrounded by five glycosaminoglycan ligands: CHS trimers at cs1, cs2, cs3 and cs4 positions and heparin tetramer at cs6 position, which results in enzyme inactivation.

Covalent Modification for Hyaluronidase Stabilization

It should be noted that cs8, cs3, cs1 and cs2 positions are most preferred for electrostatic coupling of CHS ligands. By contrast, CHS inclusion at cs7 positions, which has low affinity but is the best for covalent modification due to direct contact with four lysine residues, prevents BTH deformation by leveling the field (Figure 8) and sterically closing the gasp mentioned above (Figure 3). Thus, covalent binding of CHS trimer to cs7 can be sufficient for prevention of heparin-induced BTH inactivation.

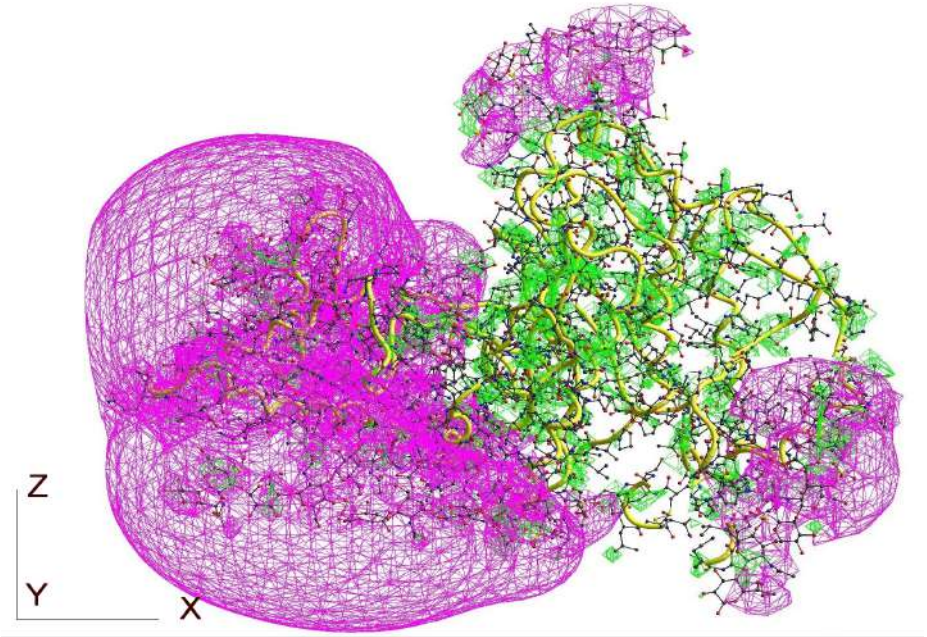

Figure 8: Surface electrostatic potential of bovine testicular hyaluronidase with six CHS trimers coupled at positions designated as cs7, cs1, cs2, cs3, cs4, cs8 and heparin tetramer located at cs6 position. Structural stabilization is achieved by CHS coupling at cs7 position.

Electrostatic interactions which regulate dislocation of heparin or CHS ligands included in the active site (cs6) determine location of sites for their attachment. Binding energy for a ligand at cs6 position in the absence of substrate is almost the same at that in a solvent. According to the docking data, the ligand is rapidly coupled at cs3 and cs8 positions. The third ligand molecule consecutively added to cs6 migrates to cs4 position without any irreversible conformational deformation of BTH. The enzyme stability is specified by electrostatic field created by GAG ligands, when their location on molecular surface dominates over their number. Inclusion of a fourth molecule into cs6 position (when previous ligands are distributed among cs3, cs8, cs4 and cs5 positions) induces dislocation of the ligand to the active site. This results in irreversible BTH inactivation due to deformation of the active site loop. Modeled immobilization of CHS ligands at cs1 and cs2 positions did not prevent BTH inactivation. Only covalent immobilization of the ligands at cs7 or cs7, cs1, cs5 (docking data) completely protected BTH against heparin-induced inactivation. Thus, our findings demonstrate regulatory role of electrostatic interactions (enzyme inactivation or stabilization under varied conditions) upon molecular docking of BTH with GAG ligands. It is noteworthy that degradation of non-sulfated chondroitin by testicular hyaluronidase in comparison with hyaluronan is more efficient at higher pH [28]. 3D structures of hyaluronan and chondroitin differ only by C-4 position in hexosamine residue. These delicate structural differences can be important in the structure-function regulation of hyaluronidases.

Conclusion

Computational analysis demonstrates structural stabilization of BTH upon interaction with six CHS ligands at cs7, cs1, cs2, cs3, cs4 and cs8 positions and heparin ligand at cs6 position. The difference of surface electrostatic potential of the protein (Figure 8) from that of stable BTH with eight CHS ligands (Figure 5) emphasizes delicate/fine formation of electric field of the enzyme complex which is associated with the significance of slight structural differences in ligands regulating the enzyme function. Computational docking reveals reversible and irreversible conformational changes in a 3D model of BTH after electrostatic coupling of GAG ligands, which leads to inactivation (Figure 7) and stabilization of BTH (Figures 5 and 8) under these conditions.

The affinity of GAG binding to enzyme is determined by electrostatic and hydrophobic interactions [29] which are important for enzyme modifications [30]. Analysis of electrostatic field dynamics in relation to the type of ligand bound to BTH and evaluation of the contribution of hydrophobic interactions is reasonable and prospective for the development of experimental strategy for the research into the enzyme structure-function relationships.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

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