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Stratification of surface lysine residues of bovine testicular hyaluronidase on the base of its 3D structure model: Computer aided drug designing of chondroitin sulphate modified enzyme derivative

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Integrity of vascular wall has been underpinned with double protective layer consisting in endothelial cells and their glycocalyx. The injury of vascular wall begins with endothelial glycocalyx degradation. Besides synthesis de novo, the regulators of glycocalyx state are reactive oxygen species, proteolytic and glycosidase enzymes. The latters determine the catabolism of glycosaminoglycan part of glycocalyx. Obstacles of glycosidase study are dealt with niggardly data about these mammalian biocatalysts, their low concentration in organism, small stability, deficiency of structural information. The obtaining of crystal structure of hyaluronidases helps to overcome the mentioned above hindrances.

We used as archetype of the obtained earlier crystal structure of human hyaluronidase-1 in order to construct 3D model of bovine testicular hyaluronidase (BTH). Superposition of 3D BTH model with 3D structure of human hyaluronidase-1 was quite satisfactory, while with 3D structure of bee venom hyaluronidase had some discrepancies (including the presence of epidermal growth factor-like domain in BTH). The active site of 3D BTH model was indicated as well as sorption complex between BTH and minimal substrate (hyaluronan hexamer) and positions of charged amino acid residues (mainly Lys, Asp, Glu). The surface Lys residues were stratificated according to their access for chemical modification (Lys of first, second, third access level and unproductive residues for Lys modification) due to analysis of Lys microenvironment. The destination of BTH modification is the production of stabilized enzyme forms for medical application. According to experimental results, the covalent complex between BTH and chondroitin sulphate (CHS) is more preferable as compared to complexes with other glycosaminoglycans. CHS modified BTH covalent complexes (BTH-CHS) were constructed *in silico* with different degree of Lys modification (on the base of 3D BTH model). Moreover, the 3D model of BTH-CHS was constructed with practically full modified/blocked Lys residues. According to experimental data, such BTH-CHS conjugate had molecular mass 180 kDa and more and it was perspective for medical use. From *in silico* point of view, 140 kDa molecular mass of this conjugate was enough already for fully blockade of Lys residues. The 3D position of CHS chains around BTH globule can be multiform. The fully blockade of surface Lys residues can gain *in silico* with two CHS chains (m.m. 35-50 kDa) or with one CHS chain (m.m. 120-140 kDa). In latter case, BTH globule is located in CHS coat except two sites only without Lys residues. One of these sites is the area around active site of BTH. Such *in silico* results are agreed with appreciable remain endoglycosidase activity of BTH (68-78%) after its deep modification by CHS with different molecular mass. The topography of Lys residues stipulates the preservation of substrate access to active site of modified BTH that determines the Lys residue selection for development of modified enzyme derivatives.

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