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Grant Title: Studies on novel enzymes for degradation of fragmented glycosaminoglycans

Abstract: Glycosaminoglycans (GAGs: i.e. hyaluronan, chondroitin sulfate, heparin, heparan sulfate) in mammalian extracellular matrix molecules become typical targets for some pathogenic bacteria to attach and/or invade to host cells. Polysaccharide lyases produced by bacteria such as streptococci and clostridia depolymerize GAGs through β-elimination reactions, and the resultant unsaturated disaccharides are subsequently degraded to constituent monosaccharides (i.e. unsaturated uronic acid and amino sugar) by unsaturated glucuronyl hydrolases (UGLs). Streptococcal UGL has been reported to be involved in bacterial attachment to host cells. Based on the glycoside bonds, unsaturated chondroitin and heparin disaccharides are classified as 1,3- and 1,4-type substrates, respectively, for UGL. In this study, we aimed to clarify structure and function of bacterial UGLs, and the data obtained here will contribute to molecular design of inhibitors for bacterial UGLs.

The GAG-degrading *Pedobacter heparinus* encodes 13 UGLs (Phep_0790, 0960, 1296, 2238, 2649, 2694, 2830, 2839, 2845, 3864, 3866, 3963, and 4097) in the bacterial genome. Recombinant Phep_2238, Phep_2649, and Phep_2830 were enzymatically characterized. Phep_2238 preferred unsaturated heparin disaccharides and also degraded unsaturated chondroitin disaccharides. Phep_2649 showed a low activity toward every substrate. Phep_2830 was specific for unsaturated heparin disaccharides. Tertiary structure of Phep_2830 was determined at 1.35 Å resolution by X-ray crystallography. A pocket-like cleft and lid loop at subsite +1 were specifically observed in Phep_2830 in comparison with streptococcal and bacillus UGLs with a substrate specificity to unsaturated chondroitin disaccharides. Unsaturated heparin disaccharides were subjected to docking simulation into Phep_2830, and the complex structure revealed that the direction of substrate pyranose rings was distinct from that in unsaturated chondroitin disaccharides (Fig. 1). Phep_2830 mutants with a mutation at the pocket and lid loop exhibited significantly reduced enzyme activity. These data suggest that the pocket-like cleft and lid loop are structural determinants for the recognition of 1,4-type substrates by UGLs.

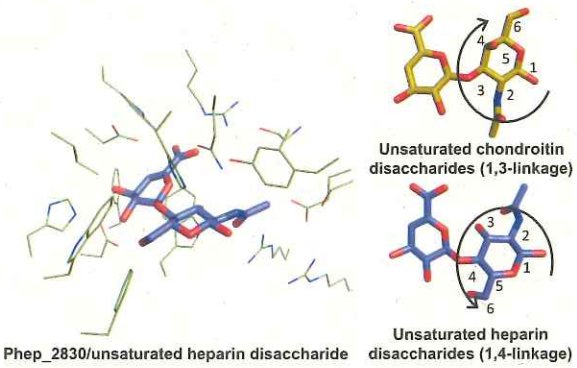

Fig. 1. Structure of Phep_2830

The enzyme activity of streptococcal UGL was inhibited by glycine in a dose-dependent manner. The resultant unsaturated uronic acids derived from GAG were metabolized to 2-keto-3-deoxy-D-gluconic acid by isomerase and reductase in streptococcal cells.

1. Y. Maruyama, S. Oiki, R. Takase, B. Mikami, K. Murata, and W. Hashimoto. Metabolic fate of unsaturated glucuronic/iduronic acids from glycosaminoglycans: Molecular identification and structure determination of streptococcal isomerase and dehydrogenase. ***J. Biol. Chem.*, 290(10),** 6281-6292 (2015).

2. Y. Nakamichi, B. Mikami, K. Murata, and W. Hashimoto. Crystal structure of a bacterial unsaturated glucuronyl hydrolase with specificity for heparin. ***J. Biol. Chem.*, 289(8),** 4787-4797 (2014).