S4-2

Glycosaminoglycan Lyases of *Bacteroidal* Microbes in Human Intestine

Yeong Shik Kim[1*], Wan-Seok Kim[1], and Dong-Hyun Kim[2]
[1]Natural Products Research Institute, College of Pharmacy, Seoul National University
[2]College of Pharmacy, Kyunghee University

Heparin-like glycosaminoglycans (HLGAGs) play an important role in the extracellular matrix, regulating a wide variety of biological functions. HLGAGs are highly sulfated and acidic polysaccharides having disaccharide units of uronic acid (L-iduronic or D-glucuronic acid) and D-glucosamine residues connected through 1→4 linkages. HLGAGs are heterogeneous due to the varying degree of modification of the functional groups in the disaccharide unit. Heparin, heparan sulfate, chondroitin sulfate, hyaluronic acid, and keratan sulfate are typical examples and they are obtained from animal tissues. Recently, a new glycosaminoglycan named acharan sulfate, was isolated and characterized from the African giant snail *Achatina fulica*. This GAG is neither heparin nor heparan sulfate, but instead represents an unusual repeating sequence, → 4)-2-acetamido-2-deoxy-α-D-glucopyranose(1→4)-2-sulfo-α-L-idopyranosyluronic acid (1→ [→GlcNAc→IdoA2S→] (1).

Three types of heparin lyase that can degrade HLGAGs (heparin and heparan sulfate) have been studied from *Flavobacterium heparinum*; 1) heparin lyase I (heparinase I, EC 4.2.2.7), acting primarily at the → 4)-α-D-GlcNS(6S or OH)(1→4)-α-L-IdoA2S (1→ linkages present in heparin; 2) heparin lyase II (heparinase II or heparitinase II), acting at the →4)-α-D-GlcNS(6S or OH)(1→ 4)-α-L-IdoA(2S or OH) or -β-D-GlcA(1→ linkages present in both heparin and heparan sulfate; and 3) heparin lyase III (heparinase III or heparitinase I, EC 4.2.2.8), acting on the →4)-α -D-GlcNS(or Ac) (1→4)-β-D-GlcA (or IdoA) (1→ linkages found exclusively in heparan sulfate. Heparin lyases (heparinases) have aided not only in understanding the heterogeneous structure related to the important physiological roles of heparin and heparan sulfate, including anticoagulation, potentiation of angiogenesis and the modulation of cellular proliferation but also in clinical applications such as in the monitoring of heparin levels and the neutralization of heparin in blood. They are also very useful for the preparation of low molecular weight heparins. Heparin lyase I and -II were applied as potent inhibitors of neovascularization.

While we are studying the metabolisam of acharan sulfate, polysaccharide lyases that can degrade glycosaminoglycans (GAGs) were identified in an anaerobic strain living in the human intestine. The strain was isolated from the stool of a healthy male and identified as *Bacteroides sp.* strain HJ-15. A detailed taxonomical study indicated the species is a strain of *Bacteroides stercoris* (2). The isolate

was cultured and the polysaccharide lyases were purified. These enzymes could act on GAGs containing either glucosamine or galactosamine suggesting the presence of both heparinases and chondroitinases. Various GAGs were incubated with the partially purified enzyme and the products formed were analyzed by strong anion-exchange high performance liquid chromatography and proton nuclear magnetic resonance spectroscopy. These studies demonstrated the presence of at least two types of polysaccharide lyases: heparin lyase and chondroitin sulfate lyase. The eliminative mechanism of these lyase enzymes was confirmed through the isolation of unsaturated disaccharide products. The heparin lyase acted on both heparin and acharan sulfate. The *Bacteroides* chondroitin lyase, acted on chondroitin sulfates A, B (dermatan sulfate), and C, resembling chondroitin lyase ABC. Each enzyme has been purified to homogeneity by a combination of ion-exchange, hydroxyapatite, and gel-filtration chromatographic steps. The molecular weights of heparinase I, heparinase II (acharan sulfate lyase), heparinase III, chondrotitinase ABC, and chondrotinase AC were determined as 48, 83, 70, 116, and 84 kDa by SDS-PAGE, respectively. Their physicochemical properties were characterized and compared from *Flavobacterial* enzymes (3-6).

Very recently, the complete 6.26-Mb genome sequence of the Gram-negative anaerobe, *Bacteroides thetaiotaomicron,* which is a predominant member of the normal human distal small intestinal and colonic microbiota, has been reported (7). The predicted sequence represents a diverse array of glycosylhydrolases (α-galactosidases, β-galactosidases, α-glucosidases, β-glucosidases, β-glucuronidases, β-fructofuranosidases, α-mannosidases, amylases, and endo-1,2-β-xylanases, plus 14 other activities) and GAG degrading enzymes including heparin, heparan sulfate and chondroitin sulfate. Based on the genome sequence, the cloning of the glycosaminoglycan degrading enzyme genes was performed using the polymerase chain reaction (PCR). The PCR amplification of the glycosaminoglycan degrading enzymes from the original encoded genome sequence used synthetic 5' and 3' primers that contained mismatches to introduce *EcoR* I and *Xho* I restriction sites. The genes are located immediately 3' to a His_6-tag to facilitate purification by Ni^{2+}-affinity chromatography. The glycosaminoglycan degrading enzymes were cloned into the *EcoR* I/*Xho* I sites of plasmid pET. The recombinant heparin lyase I, heparin lyase III, chondroitin sulfate lyase ABC and chondroitin sulfate lyase AC were expressed in *Escherichia coli* using the T7 polymerase pET expression system. The recombinant glycosaminoglycan degrading enzymes were purified to homogeneity by combination of DEAE-Sepharose, CM-Sephadex C-50 and Ni-affinity column chromatography. A milligram quantity of each enzyme could be obtained in a 1 L culture. These enzymes will be very useful for the medical and industrial applications in the future.

References

1. Kim, Y.S. Jo, Y.Y., Toida, T., Park, Y.M. Linhardt, R.J. (1996) A New Glycosaminoglycan from the Giant African Snail from *Achatina fulica. J. Biol. Chem.* 271,11750-11755
2. Ahn, M.Y., Shin, K.H., Kim D.H, Jung, E.A, Toshihiko, T., Linhardt, R. J., Kim, Y. S. (1998) Characterization of *Bacteroides* species from human intestine that degrades glycosaminoglycans.

Can. J. Microbiol. 44, 423-429

3. Kim, B.T. Kim, W.S. Kim, Y.S. Kim, D.H. (2000). Purification and characterization of a nobel heparinase from *Bacteroides stercoris* HJ 15. *J. Biochem* (Tokyo) 128:323-328.
4. Kim B.T., Hong S.W., Kim W.S., Kim Y.S., Kim D.H. (2001) Purification and characterization of acharan sulfate lyase, two novel heparinases, from *Bacteroides stercoris* HJ-15., *Eur. J. of Biochem.* 268: 2635-2641
5. Hong S.W., Kim B.T., Shin H.Y., Kim W.S., Lee K.S., Kim Y.S., Kim D.H.(2002) Purification and characterization of novel chondroitin ABC and AC lyases from Bacteroides stercoris HJ-15, a human intestinal anaerobic bacterium. *Eur. J. Biochem.* 269: 2934-2940.
6. Hong, S.W., Shin, H.Y., Kim, Y.S. Kim, D.H. (2003) Purification and characterization of novel salt-active acharan sulfate lyase from *Bacteroides stercoris. Eur. J. Biochem.* **270**, 3168-3173
7. Xu, J., Bjursell, M.K., Hooper, L.V., Gordon, J.I. (2003) A genomic view of the human-*Bacteroides thetaiotaomicron* Symbiosis. Science, 299, 2074-2076