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<https://doi.org/10.1021/bi800353g>

Biochemistry, 47, 25, pp. 6650-6661, 2008-05-31

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Characterization of Chondroitin Sulfate Lyase ABC from *Bacteroides thetaiotaomicron* WAL2926[†]

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Received February 29, 2008; Revised Manuscript Received May 14, 2008

ABSTRACT: Chondroitin sulfate ABC lyase (ChonABC) is an enzyme with broad specificity that depolymerizes via β -elimination chondroitin sulfate (CS) and dermatan sulfate (DS) glycosaminoglycans (GAGs). ChonABC eliminates the glycosidic bond of its GAG substrates on the nonreducing end of their uronic acid component. This lyase possesses the unusual ability to act on both epimers of uronic acid, either glucuronic acid present in CS or iduronic acid in DS. Recently, we cloned, purified, and determined the three-dimensional structure of a broad specificity chondroitin sulfate ABC lyase from *Bacteroides thetaiotaomicron* (BactnABC) and identified two sets of catalytic residues. Here, we report the detailed biochemical characterization of BactnABC together with extensive site-directed mutagenesis resulting in characterization of the previously identified active site residues. BactnABC's catalysis is stimulated by Ca^{2+} and Mg^{2+} cations, particularly against DS. It displays extremely low activity toward hyaluronic acid and no activity toward heparin/heparan sulfate. Degradation of CS and DS by BactnABC yields only disaccharide products, pointing to an exolytic mode of action. The kinetic evaluations of the active-site mutants indicate that CS and DS substrates bind in the same active site, which is accompanied by a conformational change bringing the two sets of active site residues together. Conservative replacements of key residues suggest that His345 plays the role of a general base, initiating the degradation by abstracting the C5 bound proton from DS substrates, whereas either Tyr461 or His454 perform the equivalent role for CS substrates. Tyr461 is proposed, as well, to serve as general acid, completing the degradation of both CS and DS by protonating the leaving group.

Glycosaminoglycans are highly sulfated, linear polysaccharides composed of alternating hexosamine (glucosamine or galactosamine) and uronic acid (D-glucuronic or L-iduronic) moieties. Most GAGs¹ are linked through a serine residue to a core protein forming a proteoglycan (PG). PGs are ubiquitous within the extracellular matrix (ECM) and are present on the cell surfaces of vertebrates. Through their interaction with a wide variety of proteins, PGs modulate fundamental biological processes including cell adhesion,

proliferation, differentiation, signaling, inflammation, and infection (1–8).

Dermatan sulfate (DS) and chondroitin sulfate (CS) are structurally related GAGs, composed of alternating 1,4- β -D-N-acetylgalactosamine (GalNAc) and 1,3- α -L-iduronic acid (DS) or 1,3- β -D-glucuronic acid (CS) (5, 9). The uronic acid moiety in CS is exclusively β -D-glucuronic acid (GlcA), whereas DS contains a mixture of α -L-iduronic acid (IdoA) and GlcA epimers (Figure 1) (10). During their biosynthesis, CS and DS polysaccharides undergo sulfation at various positions, generating their chemical diversity. Chondroitin sulfate is primarily characterized by O-sulfation at either the C4 position (chondroitin 4-sulfate, CS-A) or the C6 position (chondroitin 6-sulfate, CS-C) of GalNAc, whereas DS is primarily sulfated at the C4 position of the galactosamine. Additional, less frequent O-sulfation is found at the C2 position of IdoA in DS, creating disulfated disaccharide units that have been implicated in regulating the biological functions of this polymer (3).

Specialized microorganisms express GAG-degrading lyases using their terminal products as a source of carbon. Unlike the eukaryotic GAG hydrolases, these prokaryotic enzymes depolymerize GAGs through a β -elimination mechanism characterized by the removal of a relatively acidic proton from the C5 carbon (the chiral center) of the uronic

[†] This research was supported by the Canadian Institutes of Health Research (CIHR) grant MOP-74725 to M.C., by the KOSEF grant RO1-1999-2-209-010-5 to Y.S.K., and by the grant from National Institute of Agricultural Biotechnology to B.-S.H.

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¹ Abbreviations: GAG, glycosaminoglycan; CS, chondroitin sulfate; DS, dermatan sulfate; CS-A, chondroitin 4-sulfate; CS-C, chondroitin 6-sulfate; GalNAc, N-acetyl-D-galactosamine; GlcA, β -D-glucuronic acid; IdoA, α -L-iduronic acid; ChonABC, chondroitin sulfate ABC lyase; BactnABC, chondroitin sulfate ABC lyase from *Bacteroides thetaiotaomicron*; PvulABC1, chondroitin sulfate ABC I lyase from *Proteus vulgaris*; PvulABCII, chondroitin sulfate ABC II lyase from *Proteus vulgaris*.