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INVITED REVIEW

Alginate Lyase: Structure, Property, and Application

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Abstract Alginate is a linear polysaccharide in which β-D-mannuronate (M) and its epimer, α-L-guluronate (G), are covalently (1-4)-linked in different sequences. Alginate is mainly used as a food additive to modify food texture due to its high viscosity and gelling property. Alginate lyase can degrade alginate by cleaving the glycosidic bond through a β-elimination reaction, generating oligomer with 4-deoxy-L-erythro-hex-4-enepyranosyluronate at the non-reducing end. Alginate oligosaccharides have been shown to stimulate the growth of human endothelial cells and the secretion of cytotoxic cytokines from human macrophage. Alginate can be converted into unsaturated monosaccharide by saccharification process using endolytic and exolytic alginate lyases, thus alginate lyases have potential as key biocatalyst for application of alginate as a renewable source for biochemicals and biofuels in near future. In this paper, structures and functions of various alginate lyases are reviewed. Prospects on future applications of alginate lyases are also discussed.

Keywords: alginate, alginate lyase, alginate oligosaccharides, unsaturated monosaccharide

1. Introduction

Alginate is a linear polysaccharide in which β-D-mannuronate (M) and α-L-guluronate (G) are covalently (1-4)-linked in different sequences [1]. α-L-Guluronate is C5 epimer of β-D-mannuronate [2]. The uronic acid monomers are linked to form polymannuronate block (polyM-block), polyguluronate block (polyG-block) and random copolymer (polyMG-block) (Fig. 1). Alginates are a quite abundant in nature. In brown algae, they are produced as a structural component, comprising up to 40% of dry weight [3]. Some bacteria can also synthesize alginates [4,5]. Currently, alginates are mainly used as food additives to modify food texture [6]. They are also used as the media for cell and tissue immobilizations [7,8]. Commercial alginates are produced by extraction from biomass of marine macroalgae such as *Laminaria hyperborea*, *Macrocystis pyrifera*, *Laminaria digitata*, *Ascophyllum nodosum*, *Laminaria japonica*, *Eclonia maxima*, *Lessonia nigrescens*, *Durvillea antarctica*, *Sargassum* sp. and *etc*.

Alginate lyases can degrade alginate through β-elimination of the glycosidic bond [9-11]. They yield various oligosaccharides with unsaturated uronic acid at the non-reducing terminus and unsaturated uronic acid monomers. Various alginate lyases have been found and isolated from algae, marine invertebrates, marine and some soil microorganisms. Alginate lyases can be characterized as polyM-, polyG-, and polyMG-specific lyases based on the substrate specificity. Alginate lyases have either endo- or exo-degradation activity with the corresponding substrate specificity.

Alginate oligosaccharides have been shown to have some interesting biological activities [12]. They can stimulate the growth of human endothelial cells and the secretion of cytotoxic cytokines from human macrophage [13,14]. Thus, alginate lyases have attracted much attention as biocatalysts for preparation of functional oligosaccharides. Alginate lyases in themselves can be used as pharmaceuticals for enhancing antibiotic killing of mucoid *Pseudomonas aeruginosa* in cystic fibrosis [15]. Unsaturated alginate

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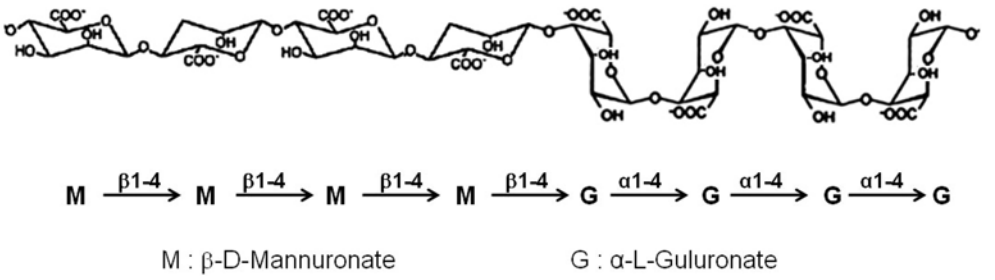

Fig. 1. Chemical structure of alginates. Alginate is a linear polysaccharide in which β-D-mannuronate (M) and its C5 epimer, α-L-guluronate (G), are covalently (1-4)-linked in different sequences.

oligosaccharides and unsaturated uronic acid monomers can be obtained from alginate by alginate lyase-catalyzed saccharification. In this paper, alginates, alginate lyases and their applications are reviewed. Especially, structure and functions of various alginate lyases are analyzed and compared. Prospects on future applications of alginate and alginate lyases are also discussed.

2. Alginate

Alginic acid is an anionic polysaccharide consisting of polyM-block, polyG-block or polyMG-block with pK_a value of 1.5 ~ 3.5 [11,16,17]. The biological function of alginate is structure supporting materials in brown algae. The mechanical rigidity and flexibility of alginate is controlled by the content of guluronic acid monomer. As the content of guluronic acid of alginate increases, gel strength increases. A low content of guluronic acid gives a more flexible texture of alginate.

Commercially available alginates are extracted from seaweeds and are widely used in food industry and for biotechnological applications. Alginate is used as a gelling agent in the production of gel-like foods because it can absorb water and form gel quickly in the presence of calcium ion. It is used to increase viscosity of ice cream and cosmetics as a thickener. It can be used as a weight-loss functional food ingredient because alginate is not digested in human gastric-intestinal tract and cannot be used as an energy source.

Alginate can bind divalent cations such as Ca^{2+} and Mg^{2+}, resulting in formation of gel. Due to the temperature-independent gel-forming ability and biocompatibility of alginate, it is used for the immobilization of cells for many biotechnological applications. Generally, a high content of guluronic acid gives strong gel strength. The pH of the solvent significantly affects the gel formation because variation in pH changes the ionic form of the uronic acid residues.

Alginate has some medical applications. Alginate is used in pharmaceutical formulations such as indigestion tablets. It is used in wound and burn dressings because it can be removed with less pain. It is also used as an impression-making material in dentistry [18]. Alginate has some other applications. It can be used for removing heavy metals and radioactive toxins in human body as a good chelator. Alginate can be used in reactive dye printing as thickening agent because it does not react with dyes.

3. Alginate Lyase

3.1. Biological function of alginate lyase in biosynthesis and biodegradation of alginate

Various alginate lyases have been discovered, cloned and characterized from marine gastropods, brown algae, Chlorella virus, marine and soil microorganisms [19-25]. Alginate lyases have found both in non-alginate-synthesizing and alginate-synthesizing organisms. In the non-alginate-synthesizing organisms, alginate lyases play important roles in assimilation of alginate as a carbon source. Marine and soil microorganisms possess more than one alginate lyase for degradation of complex structured alginate. Some marine animals have alginate lyase in their digestive tracts to degrade and use alginate as a carbon source [26]. Alginate lyases play certain roles in both biosynthesis and biodegradation of alginate in alginate-synthesizing microorganisms [27]. Genetic studies on alginate lyase-producing microorganisms have revealed that the alginate lyase genes are clustered with other alginate biosynthetic gene locus [28]. In the biosynthesis of alginate, alginate lyase appears to play a certain role in control of alginate polymer length and optimization of polymerization reaction [29]. In the mucosal alginate-synthesizing *Pseudomonas aeruginosa*, alginate lyase facilitates dissemination of the bacteria by degrading the alginate in biofilm, thus allowing the bacteria to spread out [30]. The alginate lyase of *Azotobacter vinelandii* is involved in cyst germination [31]. Gel-forming alginate is a part of the protective cyst coat in a resting cell stage, and destabilization of the cyst coat by alginate

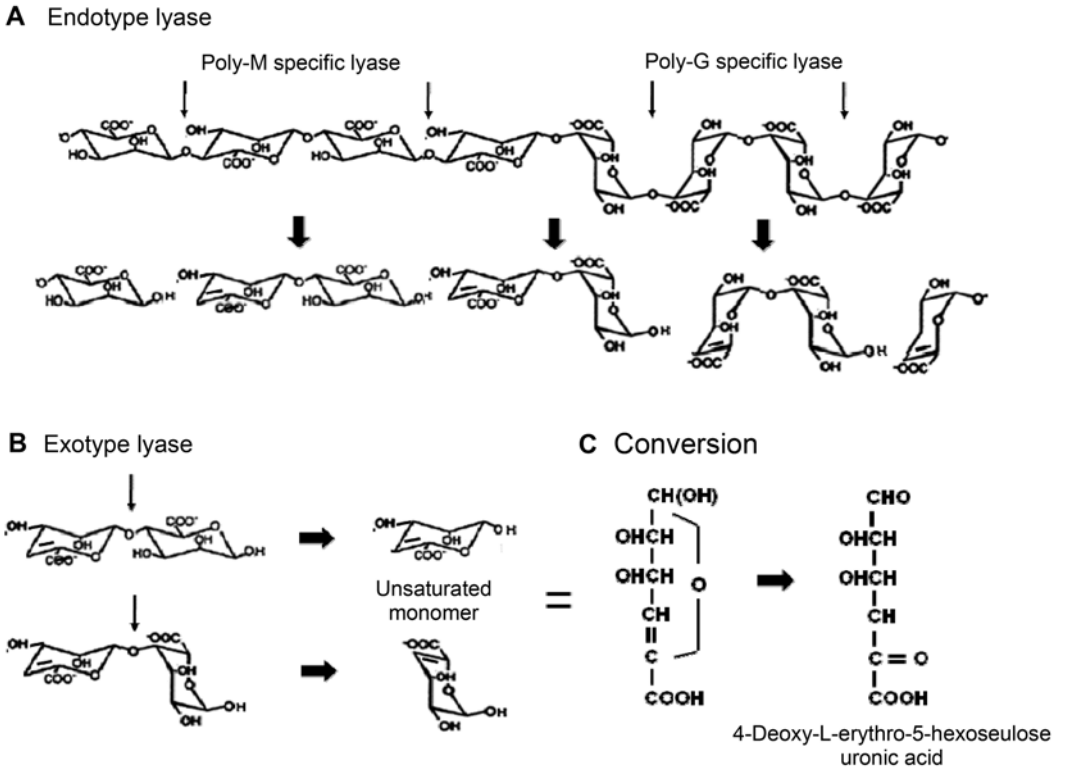

Fig. 2. Alginate degradation by endolytic (A) and exolytic (B) alginate lyases-catalyzed β-elimination reactions. Unsaturated monosaccharides are released from alginate or oligosaccharides by exolytic alginate lyase, and the monosaccharide is nonenzymatically converted into 4-deoxy-L-erythro-5-hexoseulose uronic acid (C). Thin arrows indicate the cleavage sites for the corresponding alginate lyases.

lyase is required at the germination stage.

Some microorganisms can use alginate as a carbon source. In this case, microorganisms are dependent on the depolymerization activity of alginate lyase that degrades 1-4 glycosidic linkage using a β-elimination reaction, leaving oligomer with 4-deoxy-L-erythro-hex-4-enepyranosyluronate (denoted as Δ) at the nonreducing end [32]. Alginate polymer is degraded into oligomer with an unsaturated uronic acid on the non-reducing end by the action of endo-type alginate lyase (Fig. 2). Endo-type alginate lyases can exhibit polyM-, polyG-, or polyMG-specific activity. The alginate oligomers are further depolymerized into unsaturated monosaccharide by exo-type alginate lyase. The constituent monosaccharide is nonenzymatically converted to 4-deoxy-L-erythro-hexoseulose uronic acid (DEH), and then the α-keto acid is converted to 2-keto-3-deoxy-D-gluconic acid (KDG) by NADPH-dependent DEH reductase [33]. The KDG is proposed to be converted to 2-keto-3-deoxy-6-phosphogluconic acid (KDPG). Then, the KDPG is split into pyruvate and glyceraldehydes-3-phosphate, the key intermediates of glycolysis pathway, by KDPG aldolase.

In general, microorganisms that grow on alginate use extracellular alginate lyases to degrade alginate and then transport the degraded product into cytoplasm to assimilate them *via* cellular metabolism. Some bacteria such as *Sphingomonas* sp. strain A1 can uptake alginate polymer directly using a periplasmic alginate binding protein-dependent ATP binding cassette (ABC) transporter. The outer-membrane proteins responsible for import of alginate were identified to have a pit on the cell surface [34,35]. The transport channel consists of three components, a pit with a diameter of 0.02 ~ 0.1 μm on the cell surface, alginate binding proteins in periplasmic space and ABC transporter on the cytoplasmic membrane. After the alginate is transported into cytoplasm, it is degraded by endo- and exo-type alginate lyases in cytoplasm. In *Sphingomonas* sp. A1, various alginate lyases are located in the cytoplasm, while other alginate lyases from *A. vinelandii* are located in the periplasmic space.

3.2. Structure of alginate lyase

Alginate lyase is a member of the polysaccharide lyases (PLs, EC 4.2.2.-). PLs can be classified into 22 families based on the analysis of hydrophobic cluster of primary structures. Alginate lyases belong to seven families, PL-5, -6, -7, -14, -15, -17, and -18 in the Carbohydrate Active enZYme database (http://www.cazy.org/) [36-39]. Most of endolytic bacterial alginate lyases are assigned to PL-5 and

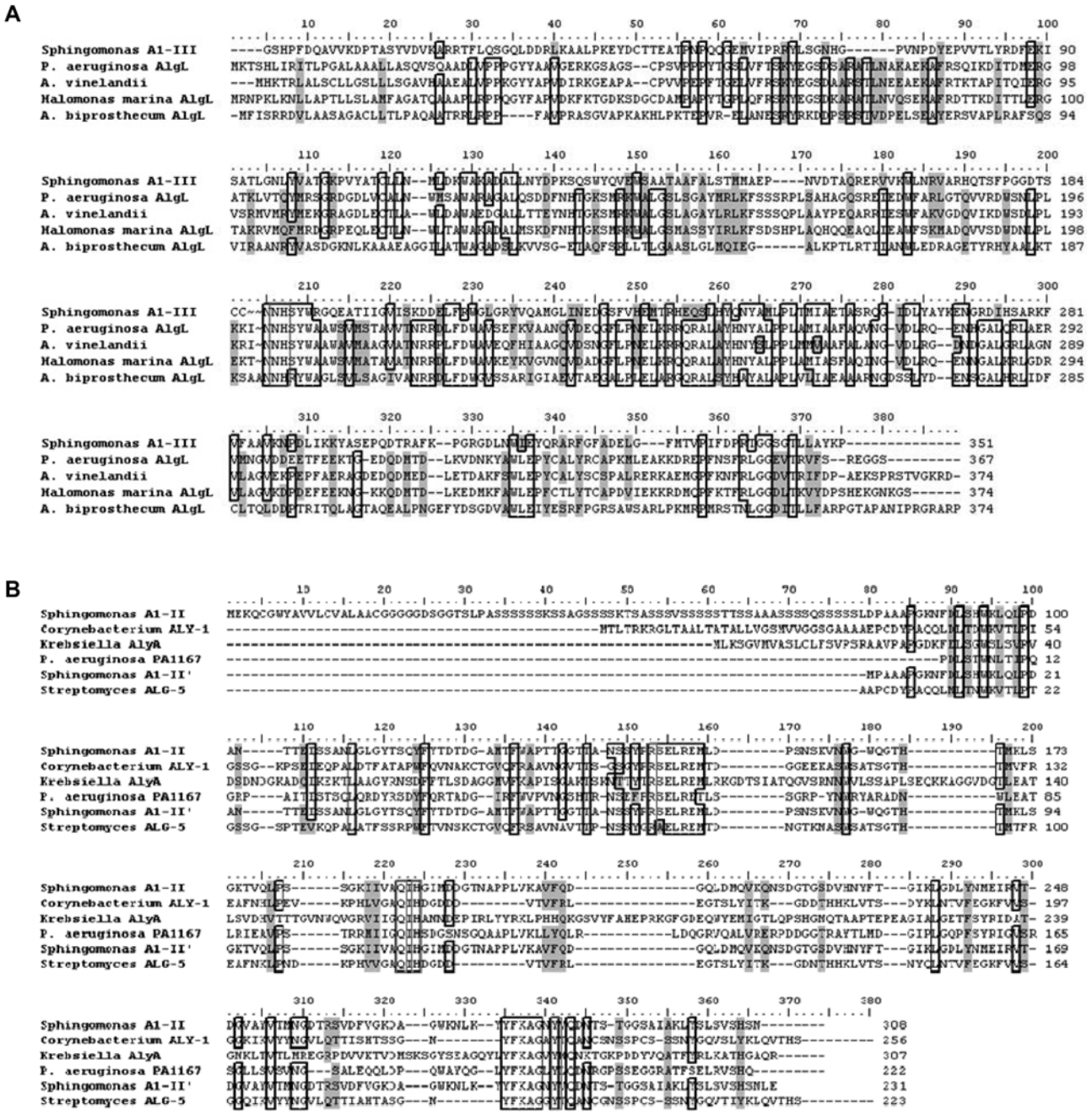

Fig. 3. Multiple sequence alignments of PL-5 alginate lyases (A) and PL-7 alginate lyases (B). The red box indicates key amino acids of Arg, Asn/Gln, His and Tyr in the active sites.

PL-7 [40-42]. Two exolytic alginate lyases, A1-IV from *Sphingomonas* sp. strain A1 and Atu3025 from *Agrobacterium tumefaciens* strain C58, are grouped to PL-15 [43-45]. Based on the cloning and sequencing of various alginate lyases, multiple sequence alignments of PL-5 alginate lyases and PL-7 alginate lyases are done and their sequence similarities are analyzed (Figs. 3A and 3B). Multiple sequence alignments reveal that there is relatively low similarity between PL-5 and PL-7 families. However, the catalytically important amino acids of Arg, Asn (for PL-5, Gln for PL-7), His and Tyr are significantly conserved in the active sites.

Three dimensional structures of A1-III (PL-5) and A1-II' (PL-7) from *Sphingomonas* sp. A1, ALY-1 (PL-7) from *Corynebacterium* sp. and Atu3025 (PL-15) alginate lyase from *A. tumefaciens* strain C58 have been solved by X-ray crystallographic analysis. The alginate lyase A1-III from *Sphingomonas* sp. A1 consisting of 351 amino acid residues

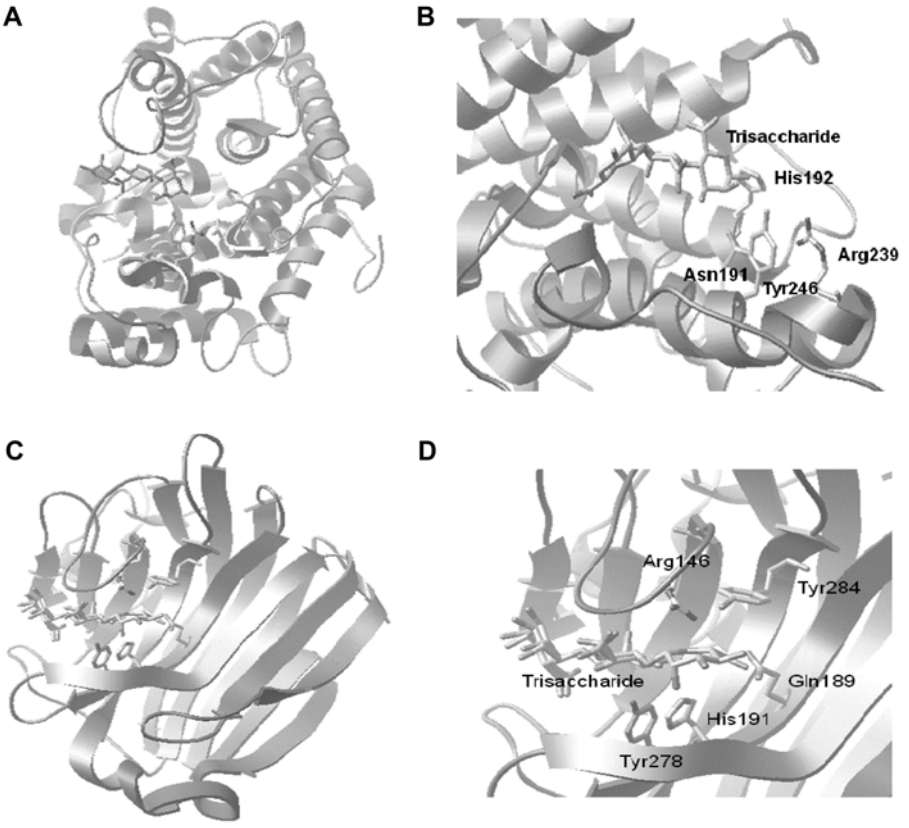

Fig. 4. Overall 3-D structures (A, C) and active site with trisaccharide (DMG or MMG) substrate (B, D) of A1-III (1HV6.pdb; a member of PL-5 class) and A1-II' (2Z42.pdb; a member of PL-7 class) alginate lyases from *Sphingomonas* sp. A1. A1-III has almost entirely of α-helical structure and a deep tunnel-like cleft in a $(\alpha/\alpha)_6$-barrel structure, whereas A1-II' is abundant in β-strands and a deep cleft in a β-sandwich fold. Both of them have Tyr, His, Asn (Asn for PL-5 or Gln for PL-7) and Arg in the active centers. Protein structures were visualized with Phyton Molecular Viewer (PMV-1.5.4).

is known to possess polyM-specific activity. When the three-dimensional structure of A1-III alginate lyase was revealed, it consists almost entirely of α-helical structure and a deep tunnel-like cleft in a $(\alpha/\alpha)_6$-barrel structure (Figs. 4A and 4B) [46]. The characteristic $(\alpha/\alpha)_6$-barrel was similar to those found in glucoamylase and cellulase. Compared to A1-III alginate lyase, the structure of PL-7 alginate lyases (ALY-1 from *Corynebacterium* sp. and A1-II' from *Sphingomonas* sp. A1) is abundant in β-strands and has a deep cleft in a β-sandwich fold (Figs. 4C and 4D) [47]. The fold is similar to the β-jellyroll fold in β-glucanase. The alginate lyase of A1-II' from *Sphingomonas* sp. A1 was further characterized by mutational analysis [42]. The structure of A1-II' was abundant in β-sandwich fold like ALY-1. The hydrogen bond networks and stacking-like associations of three adjacent β-strands at the center of the active cleft were proved to be important to maintain its activity by site-directed mutagenesis. In the catalytic active sites of the alginate lyases in ALYIII, ALY-1 and A1-II', Tyr, His, Asn (or Gln) and Arg were highly conserved and expected to form the catalytic active center.

Recently, the structure of the exolytic alginate lyase Atu3025 in PL-15 family from *Agrobacterium tumefaciens* was determined (Fig. 5) [48,49]. Atu3025 exolytically degrades alginate and oligosaccharides and releases unsaturated monosaccharides. Atu3025 consisted of a central $(\alpha/\alpha)_6$-barrel and anti-parallel β-sheet as a basic scaffold. A short α-helix in the α/α-barrel and a conformational change at the interface between the central domain and C-terminal domain were proved to be essential for the exolytic degradation.

3.3. Property of alginate lyase

Alginate lyases possess different substrate specificities. The substrate specificities of alginate lyases are dependent on the differences in their amino acid sequences and distributing of monosaccharide residues in substrate. Alginates are composed of four different types of linkage such as M-M, M-G, G-M, and G-G with various extent of each linkage. Alginate lyases can be classified according to their preferred substrate specificities. PolyG-specific lyases preferentially degrade polyG-block [50,51], while polyM-specific lyases have a preference for polyM-block [52]. Some alginate lyases have been identified as polyMG-

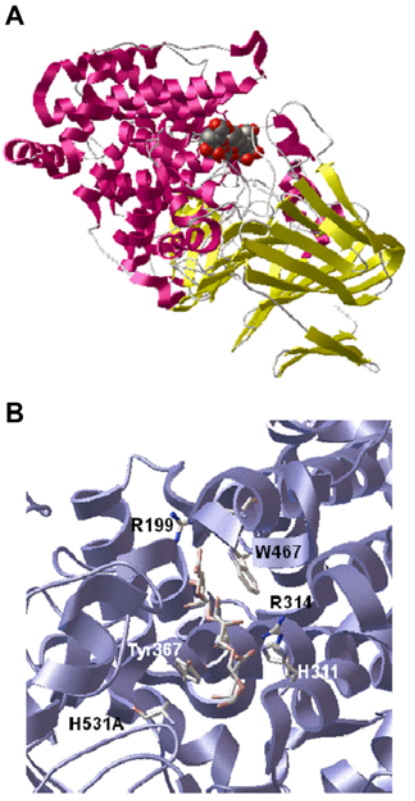

Fig. 5. Overall 3-D structures (A) and active site with trisaccharide (ΔGG) substrate (B) of alginate lyase H531A mutant (3AFL.pdb, a mutant of Atu3025) in PL-15 family from *Agrobacterium tumefaciens*. Atu3025 has a central α/α-barrel and anti-parallel β-sheet as a basic scaffold.

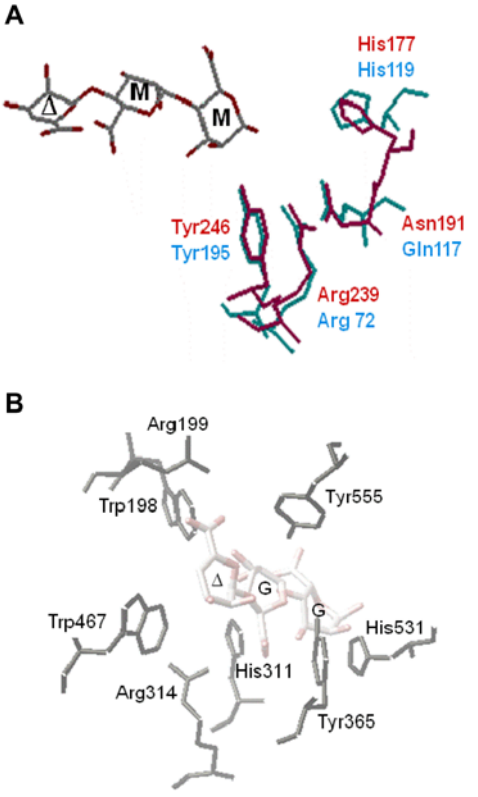

Fig. 6. Proposed active sites of endolytic and exolytic alginate lyases. (A) The key amino acids on the catalytic active sites of A1-III (1HV6.pdb, PL-5 family) and ALY-1 (1UAI.pdb, PL-7 family). The Arg239, Asn191, His192, and Tyr246 of A1-III (shown in red) are superimposed well on the corresponding Arg72, Gln117, His119, and Tyr195 of ALY-I (shown in cyan) with trisaccharide ΔMM (Δ and M represent unsaturated mannuronate and saturated mannuronate, respectively) by using RasMol software. (B) The catalytic site of Atu3025. The catalytically important amino acids surrounding trisaccharide (ΔGG) with unsaturated nonreducing end are shown. Substrate molecules were expressed by CPK coloring (carbon and oxygen are shown as gray and red, respectively).

specific lyases [53]. To date, majority of alginate lyases are characterized to have polyM-specific lyase activity. Although alginate lyases can be classified as polyM-, polyG- and polyMG-specific lyases, they usually exhibit low activity for another block. Some alginate lyases show a bifunctional activity for both of polyM-block and polyG-block [54]. Alginate lyases can also be classified as endo- and exo-type lyases on the basis of mode of cleaving site. Many of alginate lyases possess endo-type lyase activity. Recently, a few examples of exo-type lyases have been reported and characterized [55,56].

The catalytic active sites of various alginate lyases have been analyzed (Fig. 6). A possible catalytic mechanism of alginate lyase has also been proposed [9,57,58]. In the first step, the carboxyl negative charge is neutralized by Arg and Asn. The following step, the proton on C5 is abstracted by a catalytic residue such as Tyr *via* general base catalysis. The resulting carboxylate dianion is stabilized by His. In the final step, a donation of a proton from Tyr to form a double bond between C4 and C5 and cleavage of the glycosidic bond result in the formation of unsaturated monomer in the non-reducing end of oligomer. A detailed mechanism of action needs to be elucidated to understand and manipulate the catalytic characteristics of alginate lyase for preparation of tailored alginate oligosaccharides.

4. Ongoing and Promising Application of Alginate Lyase

4.1. Preparation of bio-functional alginate oligosaccharides

Alginate-degraded products containing 3 ~ 7 monomers and up to 20 ~ 25 monomers are generally considered as alginate oligosaccharides. Alginate oligosaccharides prepared from alginates by alginate lyases have been reported to possess various biological activities. Alginate oligosaccharides have been shown to exhibit probiotic properties *in vivo*. The proportions of bifidobacteria such as *Bifodobacterium* and *Lactobacillus* in rats were increased from 2.5 to 5-fold when the rat was fed with unsaturated alginate

oligosaccharides [59]. Alginate oligosaccharides produced by polyG-preferred lyase of *Streptomyces* sp. strain A5 promoted the growth of banana plantlets, red amaranth and other plants [60-64].

Oligosaccharides have many biological activities that can be applied in therapeutics and biotechnology [65]. Alginate oligosaccharides have been shown to stimulate the secretion of cytotoxic cytokines from human macrophage [13]. Alginate oligosaccharides stimulate VEGF-mediated growth and migration of human endothelial cells [14]. When oligosaccharides were injected in mouse, they induced an increase of the granulocyte colony-stimulating factor [66]. Oligosaccharides with 3 ~ 9 DP (degree of polymerization) increased tumor necrosis factor-a (TNF-a) secretion from the macrophage cell line RAW264.7 by 10-fold than the alginate polymer [67]. The mixture of oligomannuronates with DP 7 and DP 8 inhibited the ROS production from immune cells [12]. In the study on the biological activity of alginate oligosaccharides, it has been suggested that the activities are strongly dependent on the composition, size and conformation of alginate oligosaccharides. The mechanisms of action of alginate oligosaccharides need to be elucidated in more mechanistic basis.

4.2. Medical applications of alginate lyase enzyme

Exopolysaccharide biofilm is one of the important virulence factors for lung infections by pathogenic bacteria. In the bacterial alginate synthesized by *P. aeruginosa*, the C2 and/or C3 positions on the some of the M residues are *O*-acetylated. This acetylation makes the alginate more resistant to degradation, causing *P. aeruginosa* cells to be more resistant to phagocytic cells and/or antibiotics [68].

Co-administration of alginate lyase can increase the efficacy of antibiotic in the respiratory tract because alginate lyase eliminates the extracellular alginate produced by *P. aeruginosa*. Recently, the putative effects of co-administration of alginate lyase with antibiotics on cystic fibrosis patients have been investigated [15,69]. When alginate lyase was co-administered with antibiotics such as gentamicin, the killing efficiency of mucoid *P. aeruginosa* in the respiratory tract increased. Therefore, alginate lyases are expected to become useful protein biopharmaceuticals for the treatment of bacterial mucoid biofilm-dependent diseases, providing that antigenicity of the enzymes should be lowered.

4.3. Potential of alginate lyase-derived monosaccharides for the production of biofuels and biochemicals

Recently, algal biomass has attracted much attention as the raw materials for biohydrogen, bioethanol and biodiesel [70-72]. The lipids and storage carbohydrate of algae can be used for the production of biodiesel and bioethanol, respectively. Alginate has a potential as renewable materials for the production of biofuels and biochemicals. Alginate makes up to 40% of dry weight in brown algae, indicating that the method to use alginate for production of biofuels and biochemicals needs to be developed. In order to use alginate as a renewable source, development of efficient alginate saccharification technologies is prerequisite [73]. As described in this article, alginate can be converted into unsaturated monosaccharides by using endo- and exo-type alginate lyases. The unsaturated monosaccharides can be splitted to pyruvate and glyceraldehydes-3-phosphate, and then the pyruvate can be converted into various biofuels and biochemicals by introduction of relevant genes and metabolic engineering. To evaluate the feasibility, the metabolic pathways and relevant enzymes of alginate catabolism need to be elucidated.

5. Conclusion

Food industry demand on alginate is considered saturated. To expand alginate's applications to specialty chemicals market, tailored alginates and alginate oligosaccharides with different degrees of polymerization and monomer composition need to be produced in large quantity. For the application of alginates as renewable sugar for the production of biofuels and biochemicals, the developments of efficient saccharification technology and metabolic-engineered cell factory that can use non-fermentable alginate are required. The key step to meet these ends is to understand the relationship between structure and function of alginate lyases including exact molecular catalytic mechanism and structural determinants for recognizing substrate specificity. Continuing developments and characterizations of alginate lyases will drive alginate to enter into the specialty chemicals market in the near future.

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