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A Super-Channel in Bacteria: Macromolecule Uptake and Depolymerization Systems of *Sphingomonas* sp. A1 with a Special Cell Surface Structure

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Introduction

In a soil isolate of *Sphingomonas* sp. A1, the transport of a macromolecule (an alginate of molecular weight ~ 27 kDa) is mediated by a pit-dependent ATP-binding cassette (ABC) transporter. The transporter is different from other ABC transporters that have been analysed so far in that its function is dependent on the pit, a mouth-like organ formed on the cell surface, but only when the cells are compelled to assimilate macromolecules. This pit facilitates direct import of macromolecules into cells. The ABC transporter coupled with this pit, which functions as a funnel and/or concentrator of macromolecules to be imported, has been designated as the 'super-channel' and, in this review, we discuss the function of the 'super-channel' for the import of macromolecules that have been found for the first time in a bacterium. This review will focus in particular on the three-dimensional structures of the channel, and also on the enzymes involved in the degradation pathway of the imported macromolecule, namely alginate.

Cells of all organisms take in nutrients and physico-chemical/biochemical signals present in the external milieu for harmonious proliferation and differentiation, and

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Abbreviations: ATP, adenosine triphosphate; ABC, ATP-binding cassette; CFTR, cystic fibrosis transmembrane conductance regulator; MDR, multidrug resistance; ORF, open reading frame; K_d, molar dissociation constant; MalE, maltose-binding protein; OAL, oligoalginate lyase.

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excrete biosynthesized, exhausted, and hazardous materials out to maintain cellular conditions suitable for growth and to maintain cellular homeostasis. For both import and export, the transport of biomolecules and signals is usually performed by specific molecules and/or molecular systems, which are localized on cytoplasmic membranes or cell surfaces. The latter molecular systems can be roughly classified into channels for ion-transport and transporters (i.e. carrier proteins, permeases, translocators, and ABC transporters) responsible for the transport of organic compounds (Carafoli *et al.*, 1999).

Among these transporters, ABC transporters are ubiquitous systems found abundantly in all organisms, and they constitute one of the most highly conserved super families (Higgins, 1992). ABC transporters usually consist of a highly conserved ATP-binding protein (ABC-ATPase), which is also termed a traffic ATPase (Ames *et al.*, 1992), and a slightly conserved membrane domain (Higgins, 1992). ABC transporters are indispensable, not only for the supply of nutrients to cells, but also for their protection from hazardous compounds. They have also been implicated in a variety of inherited human diseases such as cystic fibrosis (CFTR, cystic fibrosis transmembrane conductance regulator) (Ames and Lecar, 1992), and in the multidrug resistance (MDR protein) of cancer cells (Hyde *et al.*, 1990) and a wide range of microorganisms.

The remarkable feature of ABC transporters is the extremely wide range of compounds that can be transported outward (export or secretion) or inward (import) using the energy provided by ATP hydrolysis, the latter of which is typical of many prokaryotic systems utilized for the uptake of small solutes (Ehrmann *et al.*, 1998). The ABC transporters in bacteria have been shown to be able to import many compounds, but they are largely restricted to low-molecular weight compounds, for example, histidine, maltose, ribose, phosphate, sulphate, nickel, molybdenum, oligopeptides, fatty acids, and so on and, as far as we are aware, no ABC transporters for the import of high-molecular weight compounds have been reported in the literature.

During a study on the enzymatic depolymerization of biofilm alginate in cystic fibrosis (Ramphal and Pier, 1985), we found that when the cells of a bacterium, *Sphingomonas* sp. A1, were grown on alginate, a mouth-like pit formed on their surface and that a relatively high-molecular-weight substance, an alginate (~ 27 kDa), was directly incorporated into the cells (Hisano *et al.*, 1995). Subsequent analysis of the system involved revealed the association of an ABC transporter that allows the transport of macromolecules into the cytosol (Momma *et al.*, 2000). To the best of our knowledge, this is the first discovery of a bacterium with a pit to be reported in the history of microbiology. Analysis of the pit in connection with macromolecular transport mediated by the ABC transporter may lead to a better understanding of the ABC transporter systems, and the results obtained will give rise to original ideas regarding the transport mechanisms for protein, DNA, and other macromolecules.

In this review, we describe the nature of the transport and degradation pathways for alginate as a representative for such macromolecule transport systems, especially from the standpoint of the three-dimensional structures of the proteins and enzymes that constitute the pathways involved.

Cell surface structure

Alginate is a linear polysaccharide produced by brown seaweed and certain pathogenic bacteria such as *Pseudomonas aeruginosa* (Chakrabarty, 1991). This polymer is composed of β-D-mannuronate and the C5 epimer α-L-guluronate, arranged in three different ways: poly-β-D-mannuronate, poly-α-L-guluronate, and heteropolymeric regions, in which there is random arrangement of the monomers (Harding *et al.*, 1999). Its structure and biotechnological application have recently been reviewed (Tombs and Harding, 1999).

In a recent study, a Gram-negative strain, *Sphingomonas* sp. A1, was isolated from a soil sample as a bacterium capable of assimilating alginate as a carbon source. The cells are covered with many large pleats, and a mouth-like pit (0.02–0.1 μm) is formed on the cell surface, possibly through the rearrangement and/or reconstitution of the pleats, when they are grown on alginate as the sole source of carbon (*Figure 5.1*) (Murata *et al.*, 1993; Hisano *et al.*, 1995). Thin sections of alginate-grown cells were seen to have an irregular site where the cell membrane sinks deeply into the cytosol (Hisano *et al.*, 1996). The formation of such pits was found to be reversible and is repressed when cells are grown on glucose, or when alginate-grown cells are transferred to a medium without alginate (Hisano *et al.*, 1995). Cell surface staining for mucopolysaccharides indicated the extensive accumulation of alginate in the pit (Hisano *et al.*, 1996). These results apparently indicated that the pit functions as a funnel and/or concentrator for alginate to be transported into the cells.

Alginate uptake by ABC transporter

A genomic fragment (15 kb) obtained through the complementation of an alginate-uptake deficient mutant of *Sphingomonas* sp. A1 contains 10 possible open reading frames (ORFs) (*Figure 5.2*). As discussed below, five of them (*algS*, *algM1*, *algM2*, *algQ1*, and *algQ2*) constitute an operon, and are considered to encode components of the ABC transporter for alginate import (Momma *et al.*, 2000). About 1 kb upstream of *algS*, the alginate lyase gene, *aly*, is present in the opposite direction, and there are some ORFs encoding amino acid sequences homologous to those of *E. coli*: catalase (Ac. No. p13029; cat), MDR protein (Ac. No. p39843; mdr), and catabolite control protein (Ac. No. p46828; ccp).

PERIPLASMIC BINDING PROTEIN

Analysis of AlgQ1 (526 amino acids: 60 kDa) and AlgQ2 (516 amino acids: 60 kDa) by use of an antibody specific to AlgQ2 revealed that they are exclusively localized in the periplasm. The homology between AlgQ1 and AlgQ2 is appreciably high (74%), although the two proteins show much lower homology (22 and 23%, respectively) with maltose-binding protein (MalE) of *E. coli* (Duplay *et al.*, 1984). Both AlgQ1 and AlgQ2 bind high-molecular-weight alginate (27 kDa) with binding affinity (K_d = 0.1–1 μM), which is comparable with those of other binding proteins (Kellerman and Szmelcman, 1974) such as MalE and HisJ, the latter of which is a histidine-binding protein of *Salmonella typhimurium*.

The crystal structures of AlgQ1 and AlgQ2 have been determined (*Figure 5.2*) (Momma *et al.*, 2002). AlgQ2 has two lobes (N- and C-terminal lobes), each of which

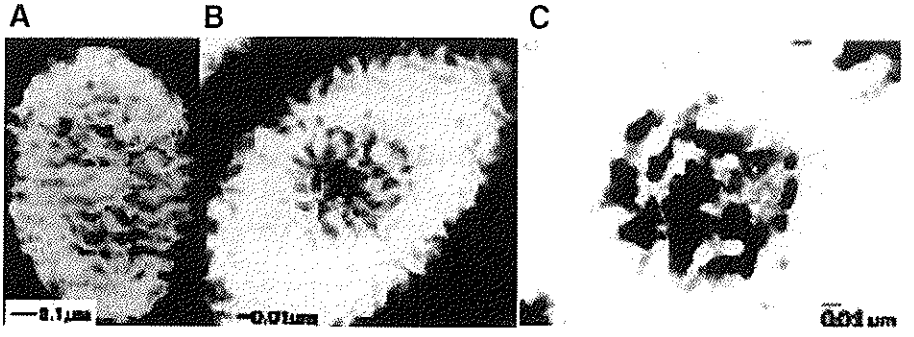

Figure 5.1. Pit formation. Changes in cell surface structure (pit formation) of *Sphingomonas* sp. A1 during growth in the presence of alginate. Culture time (h): A, 0; B, 14; C, 20. The small particles in the pits are alginate gels.

consists of α-helices and β-strands. The overall structure of AlgQ2 resembles those of other substrate-binding proteins, including MalE (Spurlino *et al.*, 1991). As has been observed in the case of the maltose/MalE complex (Sharff *et al.*, 1992), in the absence of alginate, the structures of binding proteins are in the open form so that they can bind a large ligand, namely alginate. Once the ligand is trapped in the cleft formed between the two lobes, the structures of the binding proteins are converted to the closed form, and then they deliver the ligand to the permease of the ABC transporter. Phylogenetic analyses of MalE indicated that the N- and C-terminal lobes each associate with either MalF or MalG, both of which are membrane-bound permease domains of ABC transporters (Hor and Shuman, 1993). Similarly, one lobe of AlgQ1 or AlgQ2 interacts with AlgM1 and the other one with AlgM2. Several kinds of substrate-binding proteins have been characterized, but they are for proteins having affinity toward low-molecular-weight compounds.

AlgQ1 and AlgQ2 are therefore the first macromolecule-binding proteins identified for these pit systems. Although the overall structure of AlgQ2 resembles those of other substrate-binding proteins, there are distinct differences between AlgQ2 and MalE of *E. coli* or HisJ of *S. typhimurium*, as follows: i) both AlgQ1 and AlgQ2 are 100 amino acids longer in size than MalE (396 amino acids), although the significance of the difference is obscure; ii) two copies of the alginate-binding protein (AlgQ1 or AlgQ2) are present. Typical bacterial traffic ATPases (transport systems for maltose or histidine) have one copy of the substrate-binding protein, MalE or HisJ, respectively; iii) the two alginate-binding proteins contain calcium ions near their surface (*Figure 5.2*). The function of this bivalent cation is not yet clear.

MEMBRANE PERMEASE

AlgM1 (324 amino acids: 37 kDa) and AlgM2 (293 amino acids: 33 kDa) exhibit a small degree of similarity with UgpA (26%) and UgpE (24%) (Momma *et al.*, 2000), respectively; UgpA and UgpE are both constitutive membrane-bound permease domains of the ABC transporter for the import of glycerol 3-phosphate in *E. coli* (Overduin *et al.*, 1988). The hydropathy profiles indicate that both AlgM1 and AlgM2 contain six putative transmembrane helices.

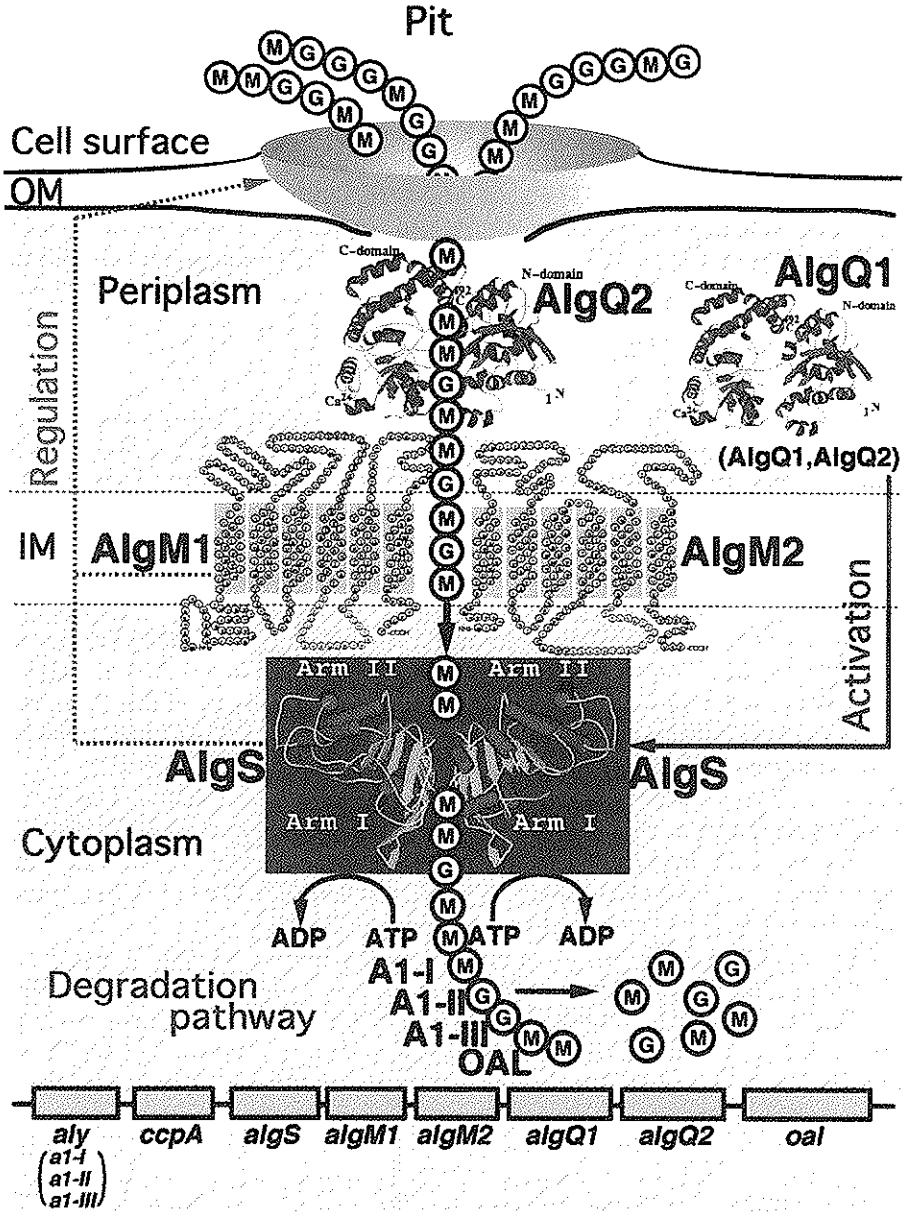

Figure 5.2. Pit-dependent macromolecule import system 'super-channel' in *Sphingomonas* sp. A1. The mechanism for the transport of alginate, which is given as a representative among macromolecules to be imported, is described in the text. Solid line, activation of AlgS by binding proteins AlgQ1 and AlgQ2 (ribbon model showing the position of Ca^{++}); dotted line, regulation of pit formation by the ABC transporter (AlgM1/AlgM2:AlgS-AlgS); OM, outer membrane; IM, inner membrane; G, guluronate; M, mannuronate; *aly*, gene for alginate lyases (A1-I, -II, and -III); *ccpA*, catabolite-control protein gene; *algS*, *algM1*, and *algM2*, ABC transporter genes for alginate import; *algQ1* and *algQ2*, genes for alginate-binding proteins; *oal*, oligoalginate lyase gene.

Most membrane-bound permease domains of bacterial ABC transporters have an EAA motif of approximately 20 amino acids (EAA...G.....I.LP) at a distance of about 100 amino acid residues from the C-terminus, which leads to the formation of a loop in the direction of the cytoplasmic fraction and to contact with the ATP-binding protein of the ABC transporter (Saurin and Dassa, 1994). A sequence matching the consensus permease EAA motif (EAA...G.....I.LP) is found in both AlgM1 (**ESAQVDGATRWQMITRITLP**) and AlgM2 (**EAA**RMDG**A**NDLQILWKVY**IP**) (Momma *et al.*, 2000). Therefore, AlgM1 and AlgM2 are thought to interact with ATP-binding proteins (AlgS), and to constitute the ABC transporter for alginate import as membrane-bound permeases.

ATP-BINDING PROTEIN

AlgS (363 amino acids: 40 kDa) contains two short, highly conserved consensus sequences, i.e. the Walker A (GXXGXGKST) and B (hhhhDEPT; h, hydrophobic amino acid) motifs that constitute a nucleotide-binding pocket. AlgS shows significant homology with ATP-binding domains (ATPase) of bacterial ABC transporters, such as UgpC (Overduin *et al.*, 1988) (52%) and MalK (Gilson *et al.*, 1982) (48%) of *E. coli*, which are components of the ABC transporters for the import of glycerol 3-phosphate and maltose, respectively. Furthermore, a C motif of the SGG region (LSGG), which is thought to interact with bound Mg^{2+}-ATP (Jones and George, 1999), is between 'Walker' motifs A and B of AlgS (Walker *et al.*, 1982; Momma *et al.*, 2000). AlgS expressed in *Sphingomonas* sp. A1 is localized in the membrane fraction and exhibits apparent constitutive ATPase activity in the form of a homodimer in the presence of divalent cations such as Mg^{2+}, Mn^{2+}, and Co^{2+} (Momma *et al.*, 2000). These results indicate that AlgS is the ATP-binding domain of an ABC transporter. In general, ABC transporters in prokaryotic cells are composed of two ATP-binding domain molecules interacting with two membrane-bound permeases. Therefore, the ABC transporter for alginate import in *Sphingomonas* sp. A1 probably consists of two molecules of AlgS and two permeases (AlgM1 and AlgM2).

The crystal constants have been established (Mishima *et al.*, 2001), and X-ray crystallographic analysis is now well under way. A three-dimensional model of AlgS has been constructed by means of a run of the SWISS-Model program using HisP (Hung *et al.*, 1998) (ATP-binding protein of the histidine transporter of *S. typhimurium*) as a template (*Figure 5.2*) (Peitsch, 1996). AlgS appears 'L' shaped, consisting of two arms, I and II. Arms I and II are composed of β-strands and α-helices, respectively. Comparison of the structure of the AlgS model with that of the HisP/ATP complex (Hung *et al.*, 1998) has indicated that 'Walker' motifs A and B are localized in Arm I. The mechanism of binding of AlgS to ATP is assumed to be as follows. The sequence (GCGK) in 'Walker' motif A directly interacts with the β-phosphate of ATP and two amino acids (DE) in 'Walker' motif B associate with the γ-phosphate *via* a water molecule. Recently, several amino acid residues in the α-helices of Arm II in MalK (ATP-binding protein of the maltose transporter of *E. coli*) have been reported to interact with the EAA loops of MalF and MalG, which are membrane-bound permeases of the transporter (Hunke *et al.*, 2000). Therefore, Arm II of AlgS is also thought to be responsible for the association with the EAA loops of AlgM1 and AlgM2.

Regulation of the import system

The alginate import system or 'super-channel' consists of a pit, binding proteins (AlgQ1 and AlgQ2), and an ABC transporter (permeases, AlgM1 and AlgM2; and ATP-binding protein, AlgS), which might be linked to degradative enzyme processes (alginate lyases, A1-I, -II, and -III; and oligoalginate lyase, OAL) (Yonemoto *et al.*, 1991; Hashimoto *et al.*, 2000). The import system and depolymerization process may be closely and simultaneously regulated in a unified manner for efficient incorporation of macromolecules, as easily seen from the clustering of the relevant genes in an operon (*Figure 5.2*).

It appears that some regulation mechanisms are operating in the ABC transporter for alginate import (*Figure 5.2*). First, the disruption of *algM1* and/or *algS* results in the failure of cells to form a pit in the presence of alginate (Momma *et al.*, 2000). This indicates that the ABC transporter controls the formation of a pit in the presence of alginate (*Figure 5.1*). Secondly, although AlgS is active *in vitro*, even after separation from the transport complex, the ATPase activity of AlgS is substantially enhanced in the presence of both permeases (AlgM1 and AlgM2) and periplasmic binding proteins (AlgQ1 and AlgQ2) complexed with alginate (*Figure 5.2*) or other macromolecules, such as polygalacturonate, gellan, and xanthan (unpublished results). This observation is supported by the results of Nikaido *et al.* (1997) and Morbach *et al.* (1993). They have demonstrated that purified HisP and MalK show constitutive ATPase activity in the absence of transport substrates, histidine and maltose, respectively, but their ATPase activities are enhanced in the presence of binding proteins and their substrates.

However, the mechanism of the activation of AlgS (ATPase) in the presence of periplasmic binding proteins (AlgQ1 and AlgQ2) and permeases (AlgM1 and AlgM2) has not yet been elucidated. A fascinating finding has, however, been made by Ehrmann *et al.* (1998) through analysis of a structural model of maltose-binding protein MalE of *E. coli*. They have indicated that periplasmic binding proteins come into direct contact, across the cytoplasmic membrane, with ABC-ATPase in order to activate or trigger ATP hydrolysis. However, in the alginate transport system, activation of the ABC-ATPase by periplasmic binding proteins requires the participation of permeases, which thus indicates that the information regarding the structural change induced in the binding protein through the binding of alginate is transferred to the ABC-ATPase by way of mediation by permeases.

Notwithstanding, this is of great significance for characterization of the prokaryotic import system since, in the export system, no substrate-binding proteins are utilized, and substrates are thought to come into direct contact with the membrane domains. Determination of the three-dimensional structure of permeases is essential for elucidating the intrinsic mechanism underlying the activation of ABC-ATPases.

Depolymerizing enzymes

Alginate incorporated into cells through the 'super-channel' is depolymerized through consecutive reactions catalyzed by endo- and exo-type alginate lyases (A1-I, -II, and -III, and OAL) present in the cytosol (Yonemoto *et al.*, 1991, 1993; Hisano *et al.*, 1993a,b, 1994; Hashimoto *et al.*, 1998a, 2000). The genes for all the proteins required

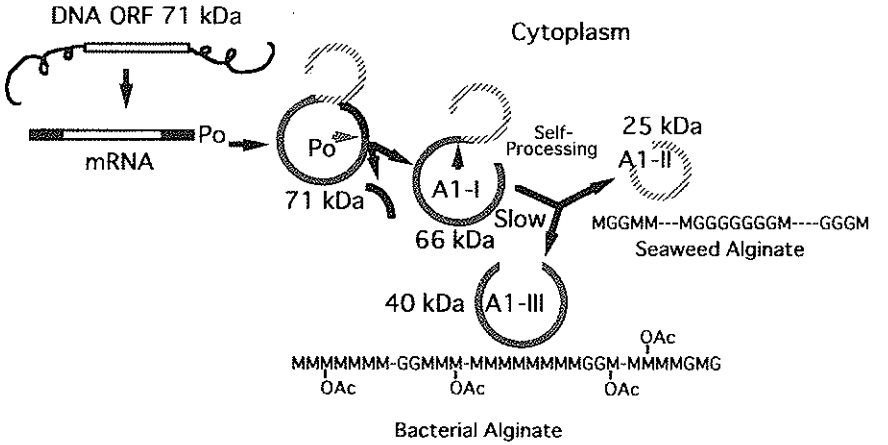

Figure 5.3. Formation of alginate lyase species through protein structure modifications. The N-terminal peptide (5 kDa) of precursor protein (Po) is excised to generate protease activity in the product A1-I lyase, which is subsequently split into two lyases, A1-II and A1-III, with different substrate specificities and increased specific activities.

for the import and depolymerization of alginate, as well as the regulator gene, are coded in a cluster (*Figure 5.2*), and they are considered to be transcribed simultaneously. Detailed description of the properties of relevant proteins (enzymes) is, however, not the purpose of this review. We report only the properties of alginate lyases (Yoon *et al.*, 2000a), and the three-dimensional structure of alginate lyase A1-III (*Figure 5.4a*) (Mikami *et al.*, 1994; Yoon *et al.*, 1999, 2000b; Yoon, 2001), since the enzyme is useful for liquefying the alginate biofilm produced by *P. aeruginosa* infecting the lungs of cystic fibrosis cases (Murata, 1994).

In *Sphingomonas* sp. A1, alginate lyases (A1-I, -II, and -III) are produced through successive post-translational processing of the primary gene product (*Figure 5.3*) (Murata *et al.*, 1993; Yonemoto *et al.*, 1993). Namely, the enzyme is first produced as a preproform Po (641 amino acids: 71 kDa), the N-terminal peptide (53 amino acids: 5 kDa) of which is then removed to give rise to a precursor A1-I (588 amino acids: 66 kDa). A1-I is split into two mature alginate lyases, N-terminal domain A1-III, (359 amino acids: 40 kDa) and C-terminal domain A1-II (229 amino acids: 25 kDa), each of which carries different substrate specificity (*Figure 5.3*). A1-III is specific to actylated alginate and A1-II to non-acetylated one. Similar post-translational processing mentioned above is also observed for polysaccharide lyases: xanthan (Hashimoto *et al.*, 2001) and gellan (Hashimoto *et al.*, 1998b) lyases in *Bacillus* sp. GL1, and hyaluronate lyase in *Streptococcus agalactiae* (Gase *et al.*, 1998).

Alginate lyases (A1-I, -II, and -III) release oligoalginates with degrees of polymerization between 2 and 4 as final reaction products (Hashimoto *et al.*, 1998a; Yoon *et al.*, 2000a), which are then further degraded to monosaccharides by oligoalginate lyase (OAL) (761 amino acids: 86 kDa) (Hashimoto *et al.*, 2000). Among these alginate lyases, A1-III depolymerizes alginate endolytically into di- and trisaccharides as the final products, and recognizes the tetrasaccharide as the minimum substrate

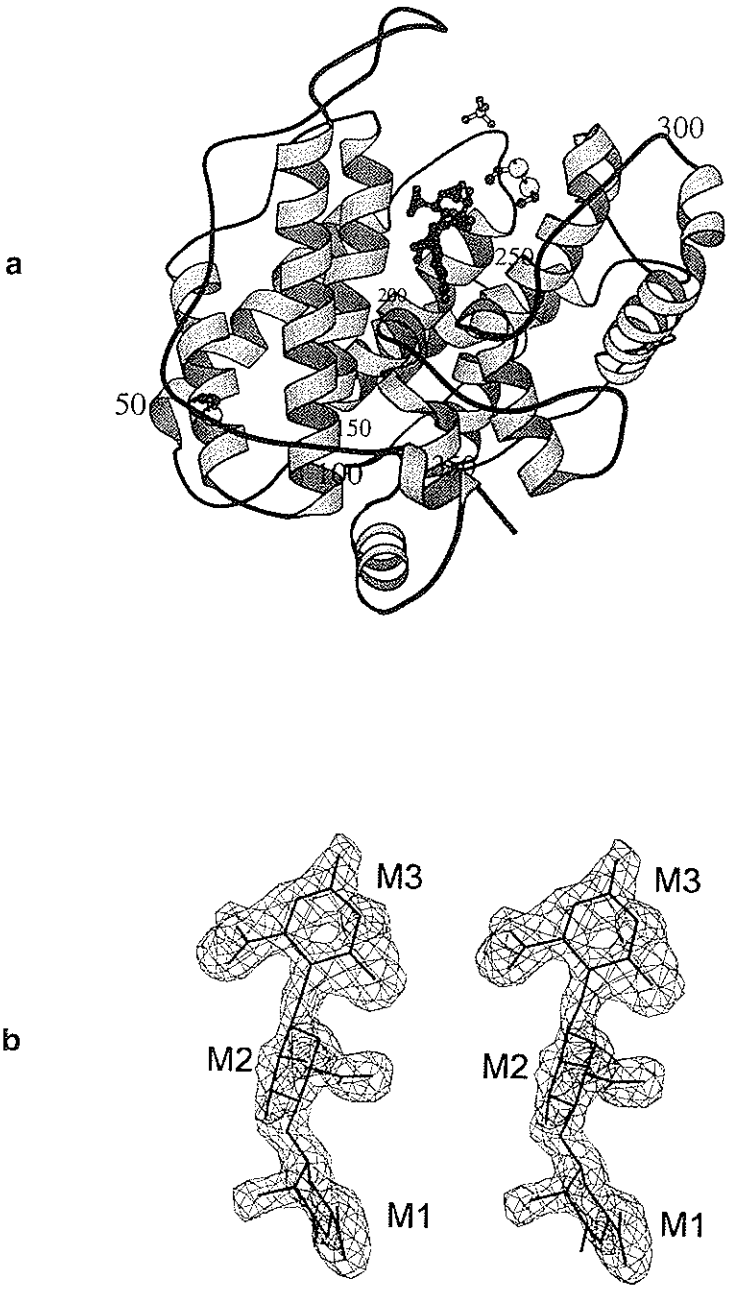

Figure 5.4. a) Overall structure of A1-III with trisaccharide product (ribbon diagram). The figure shows loops, 12 α-helices, one sulphate ion, and two S–S bridges, Cys49–Cys112, Cys188–Cys189 and a trisaccharide product. The bound trisaccharide product, sulphate ions and the cystine residues are represented as ball-and-sticks. This figure was prepared using the programs MOLSCRIPT and Raster3d. b) Omit map of A1-III without trisaccharide product. The omit map and trisaccharide molecule are shown as thin and thick lines, respectively. This figure was prepared using the programs TURBO-FRODO (Bio-Graphics) on a Silicon Graphics INDY computer. The details are written by Yoon *et al.* (2001).

(Hashimoto *et al.*, 1998a). This indicates that A1-III has subsites occupied by the tetrasaccharide and cleaves the middle of the glycosidic bond of the compound.

The crystal structure of alginate lyase A1-III has been determined (*Figure 5.4*). The enzyme A1-III is only composed of 12 α-helices, i.e. there are no β-sheets, and has an α6/α5-barrel supersecondary structure. Since a 'tunnel-like' cleft exists in the barrel consisting of the helices, alginate possibly enters the cleft and then interacts with the catalytic centre of A1-III, of which the structure has been solved by means of an X-ray crystallographic study of the A1-III product complex (Yoon *et al.*, 2001). As anticipated, the trisaccharide product was positioned at the centre of the deep cleft (*Figure 5.4a*). The configuration of the trisaccharide was such that the non-reducing end (ΔM – 3, residue 401) was outside and the reducing end (M – 1, residue 403) was inside of the cleft. The bound trisaccharide product in the active site was in the $^4C_1$ – pyranosid forms, except for the sugar with a double bond at the reducing end (*Figure 5.4b*).

Figure 5.5 shows the predicted molecular mechanism of A1-III action. The reaction follows the mechanism of lyase specific for the substrate with an axial-equatorial relationship as proposed by Linhardt *et al.* (1986), except that the base residue and the proton donor is the same Tyr residue. In this process, the negative charge on the C–5 carboxylate group is first neutralized by Arg239 and Asn191. Subsequently, the C–5 proton can more easily be removed and, in the next step, Tyr246 extracts a proton from C–5 of the mannuronic acid, resulting in the formation of a carboxylate dianion intermediate. It seems inevitable that His192 stabilizes the intermediate. Then, Tyr246 donates a proton to the oxygen of the glycosidic bond, resulting in the formation of a double bond between C–4 and C–5 and the cleavage of the glycosidic bond.

Our hypothesis that the side chain of Tyr acts as both a proton acceptor and donor is quite different from previously reported ideas concerning the mechanism of polysaccharide lyases. Greiling *et al.* (1975) had proposed that the mechanism of hyaluronate lyase action was based on a single histidine residue, which could act successively as a proton acceptor and proton donor. Gacesa (1987) had also proposed a three-step mechanism of alginate lyase. Based on a manually reoriented substrate model of hyaluronate lyase determined by X-ray crystallographic analysis, Ponnuraj and Jedrzejas (2000) proposed a mechanism involving three residues, His399, Tyr408 and Asn349, in which His399 and Tyr408 act as a proton acceptor and a proton donor, respectively. These authors assume that His residue acts as a proton acceptor. Our hypothesis that Tyr246 acts as a proton acceptor and a proton donor is based on the structure of an extended mannuronic acid residue from a bound trisaccharide. In order to prove the present hypothesis, we are now trying to determine X-ray crystal structures of the mutants of A1-III, in which Tyr246 and His192 are converted to Phe246 and Ala192, respectively. The structural analysis of the mutant enzymes/substrate complex will clarify the details of the mechanism of the enzyme.

The X-ray crystallographic study of alginate lyase A1-III can contribute towards our understanding of not only the nature of β-elimination reactions, but also the common structural rules that all the polysaccharide lyases so far analysed recognize uronic acid residues and split the polymers at the sites. The α/α-barrel supersecondary structure has also been observed in the cases of cellulase (Juy *et al.*, 1992) and glucoamylase (Aleshin *et al.*, 1992). Recently, *N*-acyl-D-glucosamine 2-epimerase, a

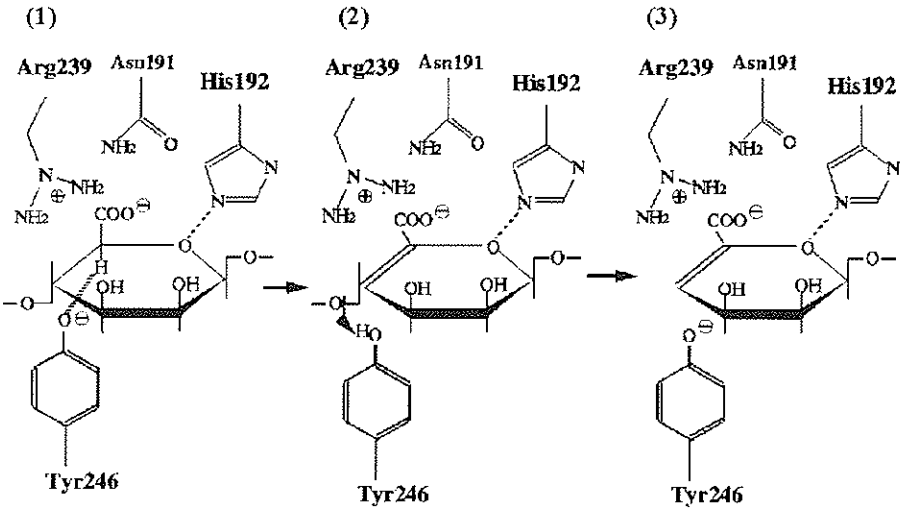

Figure 5.5. Schematic presentation of polymannuronic acid degradation mechanism. 1) Arg239 interacts with the carboxyl group of M + 1, which was designated according to nomenclature for sugar-binding subunits (Davies *et al.*, 1997), and with Tyr246 to stabilize the negative charge of the ionized side chain. His192 is hydrogen-bonded to O–5 of the sugar. Tyr246 is positioned close to O–4 and C–5. 2) Tyr246 extracts the proton of C–5, resulting in the formation of a carboxylate dianion intermediate. 3) Tyr246 donates a proton to the glycoside oxygen, resulting in the cleavage of the glycosidic bond and the formation of a double bond between the C–4 and C–5 atoms.

rennin-binding protein, was also clarified to have the α6/α6-barrel structure (Itoh *et al.*, 2000). Therefore, it appears that sugar-metabolizing enzymes have developed from a common ancestor protein (Itoh *et al.*, 2000), although they catalyze different reactions, i.e. lyase, hydrolase, or epimerase, and no similarities have been found among their primary structures.

Features of macromolecule import

The alginate import pathway 'super-channel' in *Sphingomonas* sp. A1 is illustrated in *Figure 5.2*, together with the depolymerization processes for the polymer imported. The bacterium forms a pit through rearrangement and/or reconstitution of pleat molecules on the cell surface and concentrates the polymer in the pit, although the mechanism underlying targetting of alginate to the pit is not yet clear. The periplasmic binding proteins may be responsible for this process, and are expected to be localized just under the pit. The concentrated alginate is trapped by AlgQ1 and AlgQ2 present in the periplasm and then delivered to the ABC transporter. The ABC transporter directly incorporates alginate into cells through the action of permeases (AlgM1 and AlgM2) by use of energy generated through the hydrolysis of ATP by AlgS. As described above, the ABC transporter regulates the formation of the pit, and AlgS is activated through contact with AlgQ1 and/or AlgQ2 through an unknown mechanism. The pit-dependent macromolecule import system is universal, at least among the sphingomonad bacteria, and we have confirmed that cells of *Sphingomonas mali*, when grown on alginate, form a pit on their cell surface and incorporate alginate through the pit (unpublished results).

Two kinds of molecular systems for the transport of huge molecules across the inner and outer membranes have been elucidated in bacterial systems. One is the TolC-dependent haemolysine export system in *Escherichia coli* (Andersen *et al.*, 2000). The other one is the alginate import system presented in this review. However, the alginate system is apparently different from the haemolysin system in both transport orientations and molecular machineries involved in the system.

The pit-dependent macromolecule import system found in *Sphingomonas* sp. A1 is unique compared with the import systems so far found in other bacteria. First, this system requires no complicated apparatus for the secretion of extracellular depolymerization enzymes or for the import of depolymerization products. Secondly, this system promises highly efficient utilization of biopolymers. This is often impossible, especially when a biopolymer is depolymerized outside of cells by extracellular enzymes, since some of the depolymerized products are dispersed through diffusion.

Furthermore, the pit-dependent macromolecule import system bears a striking resemblance to endo- and phagocytoses, in that these systems accompany drastic changes in the structure of the cell surface and/or cell membrane. The bacterium *Sphingomonas* sp. A1 contains glycosphingolipids in place of lipopolysaccharides, a major component of the membranes of Gram-negative bacteria. Glycosphingolipids are a ubiquitous component of cell membranes in eukaryotes, and, in that respect, the membrane structure of *Sphingomonas* sp. A1 is essentially similar to that of eukaryotes. Therefore, phylogenetic analysis of the pit-dependent system would be useful for elucidating the common features, as well as the evolutional origins, of endo- and phagocytoses.

The crystal structures of bacterial ATP-binding proteins HisP (Hung *et al.*, 1998) and MalK (Diederichs *et al.*, 2000) and many substrate-binding proteins have now been clarified. With determination of the three-dimensional structures of membrane domain units (permeases), the entire structures and overall functions of ABC transporters will be solved by one effort.

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