Crystal Structure of AlgQ2, a Macromolecule (Alginate)-binding Protein of Sphingomonas sp. A1, Complexed with an Alginate Tetrasaccharide at 1.6-Å Resolution*

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From the Department of Basic and Applied Molecular Biotechnology, Division of Food and Biological Science and the Laboratory of Quality Design of Exploitation, Division of Agronomy and Horticultural Science, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan

Yumiko Mishima†, Keiko Momma‡, Wataru Hashimoto‡, Bunzo Mikami§, and Kousaku Murata¶

Sphingomonas sp. A1 possesses a high molecular weight (HMW) alginate uptake system composed of a novel pit formed on the cell surface and a pit-dependent ATP-binding cassette (ABC) transporter in the inner membrane. The transportation of HMW alginate from the pit to the ABC transporter is mediated by the periplasmic HMW alginate-binding proteins AlgQ1 and AlgQ2. We determined the crystal structure of AlgQ2 complexed with an alginate tetrasaccharide using an alginate-free (apo) form as a search model and refined it at 1.6-Å resolution. One tetrasaccharide was found between the N and C-terminal domains, which are connected by three extended hinge loops. The tetrasaccharide complex took on a closed domain form, in contrast to the open domain form of the apo form. The tetrasaccharide was bound in the cleft between the domains through van der Waals interactions and the formation of hydrogen bonds. Among the four sugar residues, the nonreducing end residue was located at the bottom of the cleft and exhibited the largest number of interactions with the surrounding amino acid residues, suggesting that AlgQ2 mainly recognizes and binds to the nonreducing part of a HMW alginate and delivers the polymer to the ABC transporter through conformational changes (open and closed forms) of the two domains.

ABC transporters are widely found in bacteria to humans. All of them consist of two membrane proteins spanning the cytoplasmic membrane and forming a translation pore with two ATPase molecules providing the energy for accumulation of a substrate inside the cell membrane. Furthermore, ABC importers of Gram-negative bacteria depend on a periplasmic substrate-specific binding protein.

A Gram-negative soil bacterium, Sphingomonas sp. A1, was isolated as a potent producer of an alginate lyase, which catalyzes the depolymerization of a high molecular weight (HMW) alginate (average molecular size, 25,700 Da) (1). Sphingomonas sp. A1 cells are covered with many large plaits (2). When they are assimilating an alginate, pits (0.02–0.1 μm in diameter) are formed on their surface through reconstitution and/or rearrangement of these plaits, and the biopolymer is concentrated in the pits (3). The alginate thus accumulated is then delivered through the action of periplasmic alginate-binding proteins (AlgQ1 and AlgQ2) to a pit-dependent ABC transporter. An alginate-specific ABC transporter consists of AlgM1 and AlgM2 as membrane-spanning permeases and AlgS as an ATP-binding protein (4).

An alginate is a polymer comprising β-D-mannurionate (M) and its C5 epimer, α-L-glucuronate (G) (5). There are block structures that have been shown to cause the gelation and viscosity of the alginate produced by brown seaweed or certain kinds of bacteria (6). The block structures are usually arranged as homopolymeric poly-β-D-mannuronates (MM blocks), homopolymeric poly-α-L-glucuronates (GG blocks), or heteropolymeric saccharides (MG or GM blocks) within the alginate molecule (7).

The HMW alginate-binding proteins of Sphingomonas sp. A1, AlgQ1 and AlgQ2, are members of a large group of periplasmic binding proteins of Gram-negative bacteria (4). AlgQ1 and AlgQ2 exhibit high amino acid sequence similarity to each other (74% homology), although their sequence identity with other binding proteins is less than 30%. Both AlgQ1 and AlgQ2 function in a monomeric form of 57 kDa. Although binding proteins have a diverse set of ligands (e.g. monosaccharides, oligosaccharides, oxanions, amino acids, oligopeptides, and vitamins), an extraordinary feature of AlgQ1 and AlgQ2 is that they bind to a macromolecule with similar high affinities, the $K_d$ values being around $10^{-6}$–$10^{-7}$ M (8).

Periplasmic binding proteins of ABC transporters are found in various kinds of prokaryotic microbes (8), and the crystal structures of some of these proteins have been determined. Although they exhibit little sequence similarity and are different sizes, ranging from 20 to 60 kDa (8), they have similar overall structures composed of two globular domains with a deep cleft between the domains. The binding of substrates periplasmic proteins favors their closure via large scale hinge bending motions (8). These movements are required for produc-
tive interaction with the membrane permeases. The structures of periplasmic binding proteins of Gram-negative bacteria have many common features as described above, and almost all of them so far analyzed were found to be responsible for the binding of small solutes such as maltose, ribose, amino acids, peptides, and metals (8). However, AlgQ1 and AlgQ2 can bind a macromolecule, and this HMW alginate-specific ABC transporter has two binding proteins (AlgQ1 and AlgQ2), although bacterial ABC transporters generally have one periplasmic binding protein. Because alginate is composed of two sugar monomers (M and G), AlgQ1 and AlgQ2 may have specificity for either sugar or a certain polysaccharide arrangement. Therefore, the structural analysis of a periplasmic binding protein having affinity with macromolecules might provide a new insight into the molecular mechanism underlining macromolecule transport through the action of periplasmic binding protein-dependent ABC transporters.

To elucidate the structural and functional relationship of periplasmic binding proteins in HMW alginate transport, we have determined the x-ray crystal structure of AlgQ2 complexed with an alginate tetrascarhide and revealed the structure of the alginate binding site as well as the mode of alginate binding accompanying a large conformational change.

MATERIALS AND METHODS

Purification, Crystallization, and X-ray Diffraction—The methods used for the purification of AlgQ2 have been described previously (9). Briefly, AlgQ2 was purified from Escherichia coli cells transformed with the algQ2 gene by cation exchange column chromatography, dialyzed against 20 mM sodium HEPES buffer, pH 7.0, and then concentrated to ~15 mg/ml. An alginate tetrascarhide was prepared through depolymerization of HMW alginate with alginate lyase A1-III (10). Cocrystals of AlgQ2 bound with the alginate tetrascarhide were obtained by the hanging drop vapor diffusion method. The solution for a cocrystallization drop was prepared at 20 °C on a siliconized coverslip by mixing 3 μl of the protein solution (15 mg/ml) with an equal volume of mother liquor comprising 30% polyethylene glycol 4000, 0.2 M ammonium acetate, 1 mM alginate tetrascarhide, and 0.1 mM sodium citrate buffer, pH 5.6. Diffraction data for a crystal of AlgQ2 complexed with the tetrascarhide were collected up to 1.6 Å with λ = 0.9 Å, using an Oxford PX210 CCD detector system at beam line BL44XU (Beamline for Macromolecule Assemblies, Institute for Protein Research, Osaka University) at SPring-8 (Hyogo, Japan). The data collection was carried out at the temperature of liquid nitrogen. A complete data set was recorded for a single crystal with an exposure time of 6 s for 1° oscillations. The collected images were processed with program D*Trek (11) and the CCP4 truncate program (12).

Structure Determination and Refinement—The crystal structure of AlgQ2 complexed with the tetrascarhide (holo-AlgQ2) was solved by the molecular replacement method using program CNS (13). The coordinates of the ligand-free form of AlgQ2 (apo-AlgQ2, RCSB Protein Data Bank 14), under accession number 1KWH were used as a search model. Model building was performed with program TURBO-FRODO (AFMB-CNRS, France) on a Silicon Graphics Octane computer. Simulated annealing refinement was carried out with this model using 61.6–2.5 Å resolution data with program CNS (13). Fcalc–Fc and 2Fo–Fc maps were used to locate the correct model. Several rounds of conjugate gradient minimization refinement and B factor refinement, followed by manual model building, were carried out to improve the model by increasing the data to 1.6 Å resolution. Water molecules were incorporated when the difference in density was more than 3.0 e/Å3 above the mean and the 2Fo–Fc map showed a density of more than 1.0 e/Å3. The stereo quality of the model was assessed using program PROCHECK (15).

RESULTS AND DISCUSSION

Structure Determination—A crystal of holo-AlgQ2 (0.1 mm × 0.1 mm × 0.05 mm) was obtained in 2 weeks by the hanging drop vapor diffusion method. The space group of the crystal was determined to be P21, with unit cell dimensions of a = 95.63 Å, b = 53.88 Å, c = 114.92 Å, and β = 107.53°, and the solvent content was 45.0% assuming two molecules (molecules A and B/asymmetric unit. The results of x-ray data collection and the refinement statistics are summarized in Table I. The structure of holo-AlgQ2 was determined by the molecular replacement method, using apo-AlgQ2 (9) as a search model, and refined at 1.6 Å resolution. All of the polypeptide chain sequence could be well traced, and the electron densities of the main and side chains were generally very well defined on the 2Fo–Fc map. The final R factor was 19.0% for 145,984 data points in the 61.6–1.6 Å resolution range (99.0% completeness). The Rfree value calculated for the randomly separated 10% data was 21.1%. Based on the theoretical curves in the plot calculated according to Luzzati (19), the absolute positional error was estimated to be close to 0.18 Å between 5.0- and 1.6-Å resolution. Judging from the results of Ramachandran plot analysis (20), in which the stereochemical correctness of the backbone structure is indicated by the (φ, ψ) torsion angles, we found that most of the non-glycine residues lie within the most favored regions, the exception being Lys251 (molecule A: φ = 65.0°, ψ = 137.4°; and molecule B: φ = 62.5°, ψ = 139.9°), which is present in a generously allowed region. Lys251 is located next to the terminus of a helix (H12) (9).

Overall Structure of AlgQ2 Complexed with an Alginate Tetrascarhide—Fig. 1a shows a Ca backbone trace of holo-AlgQ2 together with the bound tetrascarhide and a calcium ion. The final model comprises 984 amino acid residues, 2 tetrascarhides, 2 calcium ions, and 821 water molecules/asymmetric unit.

The overall structure of holo-AlgQ2 was similar to that of apo-AlgQ2, except for the disposition of the N and C domains (N domain, residues 1–133 and residues 310–400; C domain, residues 134–309 and residues 401–492). The designation of secondary structure elements of holo-AlgQ2 is the same as that for apo-AlgQ2 (for a description, see Ref. 9). Briefly, holo-AlgQ2 is composed of two globular domains (N and C domains) that form an α/β-structure. The tetrascarhide is bound in the deep cleft between the domains. Both domains exhibit a similar arrangement of the elements of the secondary structure and can be divided further into two parts (N1 and N2 domains, and C1 and C2 domains) (9).

| Crystal Structure | Space group | P21 | a, b, c (Å), β (°) | 95.63, 53.88, 114.92, 107.53 |
|-------------------|-------------|----|-------------------|--------------------------|
| Molecules/asymmetric unit | 2 |
| Data collection | Light source | SPring-8, BL44XU |
| Detector | Oxford CCD |
| Wavelength (Å) | 0.9 |
| Resolution limit (Å) | 61.6–1.6 |
| Measured reflections | 559,708 (47,676) |
| Unique reflections | 175,841 (16,776) |
| Redundancy (%) | 7.4 |
| Completeness (%) | 98.3 (94.6) |
| Rmerge (%) | 7.6 (24.2) |
| Refinement | Resolution (Å) | 61.6–1.6 (1.65–1.60) |
| Used reflections | 145,984 (12,879) |
| Residues/water | 492 × 2821 |
| Calcium ion | 2 |
| Tetrascarhide | 2 |
| Average B factor (Å²) | 12.7 |
| Root mean square deviation |
| Bond (Å) | 0.0053 |
| Angle (°) | 1.27 |
| R factor (%) | 19.0 (22.0) |
| Rfree (%) | 21.1 (24.5) |
Although almost all binding proteins of ABC transporters consist of two domains, and a deep cleft corresponding to a substrate binding site is formed between the two domains, differences in the transitions or crossovers from one domain to the other are observed in the folding topology of the binding protein structures. Based on these differences, the binding proteins are divided into two types (groups I and II) (21). In group I, the transitions from domain to domain are strand to helix for the first two crossovers and strand to strand for the third crossover, e.g., galactose-binding protein (GBP) and L-arabinose-binding protein (ABP), and in group II, the first two are strand to strand and the third is helix to helix, e.g., maltose-binding protein (MBP) and sulfate-binding protein (21). Therefore, AlgQ2, having a sheet topology and crossover connections, is classified into group II.

In contrast to apo-AlgQ2, whose N and C domains are wide open, the complex form with the tetrasaccharide was found to be a closed one as reported for other substrate-binding proteins of ABC transporters. For example, in MBP, hinge bending between the two domains involves rotation of \( \sim 35^\circ \) (22). Between the apo and holo forms, the root mean square deviation of equivalent C atoms that are within a distance of 2.0 Å of one another is 0.67 Å (295 C atoms). These values are 0.42 Å for the N domain (224 C atoms) and 0.47 Å for the C domain (264 C atoms). To characterize the conformational changes that occur upon domain opening and closing, the domain motion was considered separately as rotation and translation (23). The N domains of apo and holo forms were superimposed, and
the rotation and translation required to superimpose the C domains were determined to be 30° and 0.5 Å, respectively (Fig. 1b).

Alginic Binding Site—The binding site for alginate in AlgQ2 is located in the cleft between the two globular domains (N and C domains) (Fig. 1a). AlgQ2 has an especially deep cleft compared with other periplasmic binding proteins. In the open form of AlgQ2, basic residues, mostly Arg, are on the surface around the cleft in each domain, and aromatic residues are located deep in the cleft (Fig. 2). When AlgQ2 is in the closed form, basic residues face the center of the cleft. The positively charged residues in the cleft might facilitate the attachment of the negatively charged alginate molecule in the cleft and hold the substrate in a proper position for binding. These changes show that basic residues, especially Arg, play a role as sensors for alginate and facilitate the efficient binding of alginate.

The bound alginate residues (tetrasaccharide) were identified as ΔM1-M2-G3-M4, from the nonreducing end, where ΔM, M, and G denote unsaturated and saturated β-D-mannuronate and saturated α-L-glucuronate, respectively (Fig. 3), and the sugar binding sites, corresponding to the alginate residues, are designated as S1, S2, S3, and S4, respectively (Fig. 3b). Each alginate residue of the bound tetrasaccharide (ΔM1-M2-G3-M4) was in the 4C1- (M2, M4) or 1C4 -pyranosid form (G3). The sugar difference density of S1 only fits unsaturated mannuronate. The tetrasaccharide used for cocrystallization with AlgQ2 was prepared through depolymerization of HMW alginate with alginate lyase A1-III (10). Depolymerization produces a C4-C5 double bond at the nonreducing terminal mannuronate residues of the products, which are mixtures of MM, MG, and GM blocks (24). However, we found ΔM1-M2-G3-M4 in both molecules A and B, which may indicate that ΔM1-M2-G3-M4 was the predominant species of tetrasaccharide obtained in the present experiment. The bound tetrasaccharide adopts a linear shape (Fig. 3a). The torsion angles of the glycosidic bonds, defined as O5'-C1'-O4'-C4 and C1'-O4-C4-C5 (φ), between ΔM1 and M2, M2 and G3, and G3 and M4 of each molecule, are shown in Table II. The torsion angles between ΔM1 and M2 in both molecules are in the lowest energy region of the isoenergy map. According to the report by Braccini et al. (25), the adiabatic maps of glucuronic and mannuronic acid dimers are almost identical, and the energy minima are similar, with identical (φ, ψ) values and comparable relative energies. The torsion angles between M2 and G3, and G3 and M4 are also in the lowest energy region of the isoenergy maps of both mannuronic and glucuronic acid dimers. These results suggest that bound tetrasaccharides are in a stabilized conformation.

In both molecules A and B, M4 is an α-anomeric, and M4-O1 is wholly axial (Fig. 3a). No other electron density is present in the binding site. Hydrogen bonds are formed between M4-O1 and a tetrasaccharide.
and two water molecules (molecule A, WAT36 and WAT325; molecule B, WAT559 and WAT617). WAT36 is hydrogendoined to Asp^{77}OD1, -OD2, and Gln^{55}NE2. This preferential binding of the \( \alpha \)-anomer to AlgQ2 is because of the water-bridged hydrogen bonds between axial M4-O1 and the amino acid residues.

The bound tetrasaccharide molecule undergoes interactions with the surrounding amino acid residues (Fig. 4). The hydrogen bond interactions between the bound tetrasaccharide and AlgQ2 are listed in Table III. Consistent with the involvement of many charged residues in sugar binding sites, basic and acidic side chains provide many of the nitrogen and oxygen atoms capable of hydrogen bond formation, respectively. Side chains with polar planar groups (Asn, Asp, Glu, Arg, and His) are the ones most often used for hydrogen bonding to carbohydrates because of the great abundance of aromatic residues. Furthermore, a typical characteristic of uronic acids is that C6 is oxidized to carboxyl acid. Thus, these residues (O61 and O62) may confer the specificity to AlgQ2.

All residues binding with \( \Delta M1 \) (Ser^{77}, Asp^{77}, Tyr^{77}, and Glu^{396}) are located in helices, all residues binding with M2 (Tyr^{129}, Arg^{186}, and Trp^{399}) in \( \beta \)-sheets, and all residues binding with G3 and M4 (His^{187}, Arg^{313}, and Asp^{21}) in loops. In MBP, most residues binding with maltose are located in loops of the N domain, and its localization may participate in the hinge bending motion (27). But in AlgQ2, most of these residues are in the helices of both domains and \( \beta \)-sheets, with a few in loops.

The C-C contacts between AlgQ2 and the tetrasaccharide are listed in Table IV. The number of C-C contacts is 39 (\( \Delta M1, 23; M2, 7; G3, 6; \) and M4, 3). In holo-AlgQ2, almost all the nonpolar-nonpolar contacts between AlgQ2 and the tetrasaccharide are mainly through aromatic side chains (Trp^{77}, Trp^{99}, and Tyr^{129}). Indeed, the close proximity of aromatic side chains to bind a tetrasaccharide is a recurring feature of proteins/enzymes that bind tetrasaccharides. The structure of AlgQ2 is notable among those of proteins/enzymes in that AlgQ2 binds saccharides because of the great abundance of aromatic residues, 16 of which are located in or near the binding site groove.

Comparison of the structures showed that two water molecules (molecule A, WAT36 and WAT325; molecule B, WAT559 and WAT617). WAT36 is hydrogendoined to Asp^{77}OD1, -OD2, and Gln^{55}NE2. This preferential binding of the \( \alpha \)-anomer to AlgQ2 is because of the water-bridged hydrogen bonds between axial M4-O1 and the amino acid residues.

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domain. In particular, the aromatic Trp\textsuperscript{270} residue in the C domain exhibits the most favorable stacking interactions with ∆M1. Such stacking interactions between a sugar and aromatic side chains are quite common in protein-carbohydrate complexes (28). Of special significance regarding AlgQ2 is the finding that some of the van der Waals contacts are confined to a nonpolar cluster of nonpolar atoms within the sugar. In the alginate molecule, the disposition of both the axial OH2 and equatorial OH3 of ∆M1 on the hydrophilic side of the ring creates a cluster of nonpolar atoms (C3, C4, and C5) on the opposite side. This nonpolar cluster is stacked with Trp270 (Fig. 4). Because MM and MM blocks seem to be bound at the S1 position by computational simulation. If glucuronate is present instead of the S1 subsite may bind saturated and unsaturated mannurionate acids selectively.

Hinge Bending Motion of AlgQ2—The rotations around main chain dihedrals in the three interdomain connections are related to the opening and closing of AlgQ2. Movements in the three hinge segments are greatest at residues 133–136 (L-SA1:SC3), 294–314 (L-SC4:SA3), and 399–401 (L-H19:H20) (L, loop; SA, β-sheet in N1 domain; SC, β-sheet in C1 domain; H, α-helix; these notations have been defined previously (9)).

The hinge region of AlgQ2 is shown in Fig. 5. The hydrogen bond between Gly\textsuperscript{396} (which is an element of H19 in the N domain) and Arg\textsuperscript{309} (which is an element of L-SC4:SA3) is maintained in both the opened and closed forms and thus stabilizes the interaction between H19 and L-SC4:SA3 (Fig. 5 and Table V). In the closed form, H19 and L-SC4:SA3 are close to the tetrasaccharide in the cleft, and the hydrogen bond mediated hydrogen bond between Gly\textsuperscript{396} and Arg\textsuperscript{309} greatly increased (Δψ = 45°, Δψ = 33°), and hinge bending motion occurred in L-SC4:SA3. As a result of this movement, the indirect water-mediated hydrogen bond between Gly\textsuperscript{133} (L-SA1:SC3) and Gln\textsuperscript{310} (L-SC4:SA3) via a water molecule (WAT715) is lost, and both the ϕ and ψ angles of Gly\textsuperscript{133} and Gln\textsuperscript{310} change greatly.

### Table IV

| Sugar atoms | Protein atoms | Distance\textsuperscript{a} (Å) | Element/domain |
|-------------|---------------|-------------------------------|----------------|
| M1 C1       | Trp\textsuperscript{270} CD2 | 4.0              | SC2/C          |
| M1 C1       | Trp\textsuperscript{270} CE2 | 4.1              | SC2/C          |
| M1 C1       | Trp\textsuperscript{270} CE3 | 4.0              | SC2/C          |
| M1 C1       | Trp\textsuperscript{270} CH2 | 4.0              | SC2/C          |
| M1 C1       | Trp\textsuperscript{270} C2  | 4.2              | SC2/C          |
| M1 C1       | Trp\textsuperscript{270} C3  | 4.3              | SC2/C          |
| M1 C3       | Glu\textsuperscript{396} CD  | 4.0              | H19/N          |
| M1 C4       | Trp\textsuperscript{270} CE3 | 4.0              | SC2/C          |
| M1 C4       | Glu\textsuperscript{396} CD  | 3.8              | H19/N          |
| M1 C4       | Trp\textsuperscript{270} CH2 | 4.4              | L-H19:H20/N—C |
| M1 C5       | Trp\textsuperscript{270} CB   | 3.8              | SC2/C          |
| M1 M5       | Trp\textsuperscript{270} CD2  | 3.6              | SC2/C          |
| M1 C5       | Trp\textsuperscript{270} CE3  | 3.5              | SC2/C          |
| M1 C5       | Trp\textsuperscript{270} CG   | 3.8              | SC2/C          |
| M1 C5       | Trp\textsuperscript{270} C2   | 4.2              | SC2/C          |
| M1 C6       | Trp\textsuperscript{270} CB   | 3.3              | SC2/C          |
| M1 M6       | Trp\textsuperscript{270} CD2  | 4.0              | SC2/C          |
| M1 C6       | Trp\textsuperscript{270} CE3  | 4.2              | SC2/C          |
| M1 C6       | Trp\textsuperscript{270} CG   | 3.7              | SC2/C          |
| M1 C6       | Ser\textsuperscript{373} CB   | 3.7              | H14/C          |
| M1 C6       | Trp\textsuperscript{299} CH2  | 4.2              | L-H19:H20/N—C |
| M2 C2       | His\textsuperscript{187} CE1  | 3.8              | L-SC1:H9/C     |
| M2 C3       | Trp\textsuperscript{270} CE2  | 4.3              | SC2/C          |
| M2 C4       | Trp\textsuperscript{270} CE2  | 4.0              | SC2/C          |
| M2 C5       | Trp\textsuperscript{270} C2   | 3.9              | SC2/C          |
| M2 C6       | Tyr\textsuperscript{129} CE2  | 4.0              | SA1/C          |
| M2 C6       | Tyr\textsuperscript{129} CZ   | 4.2              | SA1/C          |
| M2 C6       | Arg\textsuperscript{313} CD   | 4.2              | L-SC4:SA3/C—N |
| G3 C2       | Arg\textsuperscript{313} CZ   | 4.3              | L-SC4:SA3/C—N |
| G3 C3       | Arg\textsuperscript{313} CZ   | 3.9              | L-SC4:SA3/C—N |
| G3 C4       | Arg\textsuperscript{313} CG   | 4.3              | L-SC4:SA3/N    |
| G3 C5       | Arg\textsuperscript{290} CG   | 4.1              | L-SC4:SA3/N    |
| G3 C6       | Arg\textsuperscript{290} CD   | 4.4              | L-SC4:SA3/N    |
| G3 C6       | Arg\textsuperscript{290} CG   | 4.2              | L-SC4:SA3/N    |
| M4 C2       | Asp\textsuperscript{313} GE   | 3.7              | L-SC4:SA3/N    |
| M4 C3       | Arg\textsuperscript{290} CA   | 4.2              | L-SC4:SA3/N    |
| M4 C5       | Asn\textsuperscript{375} CG   | 4.4              | L-SC4:SA3/N    |

\textsuperscript{a} Distance ≤ 4.4 Å.
(Gly^{133}, \Delta \phi = -49^\circ \text{ and } \Delta \psi = -27^\circ; \text{ Gln}^{310}, \Delta \phi = 37^\circ \text{ and } \Delta \psi = 41^\circ). \text{ In the closed form, Gln}^{310} \text{ forms hydrogen bonds with residues of the C domain (Fig. 5 and Table V). In particular, Asp}^{78} \text{ and Asn}^{207} (L-H9:SD1) \text{ are in the loop connecting the C1 and C2 domains. These interactions may stabilize AlgQ2 in the closed form.}

Therefore, the hinge motion of AlgQ2 can be predicted to be as follows. When Glu^{396} forms a hydrogen bond with M1, a conformational change occurs in L-SC4:SA3, which interacts with Glu^{396} through hydrogen bonds. As a result of this conformational change, the association via a water molecule (WAT715) between L-SA1:SC3 and L-SC4:SA3 is lost, and both loops undergo a dynamic hinge bending motion. A water molecule (WAT715) and the interaction between Glu^{396} and L-H9:SD1 via L-SC4:SA3 are important for the hinge motion of AlgQ2.

CONCLUSIONS

In periplasmic binding protein-dependent transport systems, a soluble binding protein is the first component to interact with the substrate to be transported, acting as a high affinity receptor for the substrate in the periplasm. A little understanding of how binding proteins function in transport has been obtained through studies on the MBP system (22, 26–28). In this system, MBP undergoes a ligand-induced conformational change that has been observed in both the presence and absence of a ligand, and MBP becomes tightly bound to the membrane transporter.

The way in which a tetrasaccharide and HMW alginate bind to AlgQ2 is of interest. Because an alginate is a highly polar molecule, hydrogen bonding is the dominant interaction in protein-carbohydrate complex formation. Thus, on complex formation, the solvation shell of water is exchanged for the polar groups responsible for hydrogen bond interactions in the binding site of the protein. As in the cases of MBP and other periplasmic binding proteins, AlgQ2 has two globular domains connected by a flexible hinge, and in the ligand-bound structure, the ligand is buried deep within the cleft between the two domains. In the absence of a ligand, the cleft between the domains is more open and exposed to the solvent.

However, there are significant differences in protein-sugar interactions between AlgQ2 and MBP. The S1 subsite seems to be the most important one for the binding of alginate in AlgQ2, and it may be specific for mannuronate in this structure. The conformations of MM and GG blocks are known to be linear and helical, respectively (24). Based on the conformation of alginate, mannuronate is favorable at the S1 subsite. Although in

TABLE V  Hydrogen bonds between the N and C domains of apo- and holo-AlgQ2

| Protein atoms/N Elements | Protein atoms/C Elements | Distance Å |
|-------------------------|-------------------------|------------|
| Lys^{77} NZ | H3 | Asp^{425} OD1 | L-H20:H21 | 2.9 |
| Arg^{155} O | H6 | Glu^{490} NE2 | H23 | 3.0 |
| Asp^{78} OD2 | H3 | Arg^{431} NH2 | H21 | 2.8 |
| Glu^{396} OE1 | H19 | Arg^{309} NH1 | L-SC4:SA3 | 3.0 |
| Glu^{396} OE2 | H19 | Arg^{309} NH2 | L-SC4:SA3 | 2.6 |

| Protein atoms/N Elements | Protein atoms/C Elements | Distance Å |
|-------------------------|-------------------------|------------|
| Asp^{78} OD1 | H3 | Tyr^{434} OH | H21 | 2.7 |
| Glu^{310} OE1 | L-SC4:SA3 | Asp^{306} OD1 | L-H9:SD1 | 3.0 |
| Gln^{310} NE2 | L-SC4:SA3 | Asp^{307} OD1 | L-H9:SD1 | 3.0 |
| Lys^{311} NZ | L-SC4:SA3 | Glu^{422} OE1 | L-H20:H21 | 2.7 |
| Glu^{396} OE1 | H19 | Arg^{309} NH1 | L-SC4:SA3 | 2.9 |
| Glu^{396} OE2 | H19 | Arg^{309} NH2 | L-SC4:SA3 | 3.0 |

* N, N domain; C, C domain.

* Distance ≤ 3.2 Å.
other periplasmic binding proteins, a reducing terminal sugar binds at the S1 subsite, AlgQ2 binds a nonreducing terminal sugar at the S1 subsite (22, 26). Furthermore, AlgQ2 interacts strongly with a nonreducing terminal sugar, although a glucose positioned at the S2 subsite participates in the greatest number of hydrogen bonds and van der Waals contacts in MBP complexed with an oligosaccharide (28). Compared with other periplasmic binding proteins, the sugar binding cleft of AlgQ2 extends farther toward the core of the protein. As judged on computational simulation, at least hexasaccharide can be bound without exposure to the solvent.

A ligand-induced conformational change is believed to be crucial for the function of a periplasmic binding protein in both transport and chemotactic processes. Such a conformational change generates the appropriate stereochemistry for specific interaction of the liganded protein with membrane-bound protein components, in preference to the unliganded form, thus initiating the translocation process.

On going from the open cleft, unliganded structure to the closed structure, there is a concerted shift of the Glu396 side chain, which moves up into the cleft as a result of sugar binding (Fig. 5). This ligand-induced movement of Glu396 may be the triggering mechanism for the motion that enables the other domain to participate in ligand binding and ultimately to engulf the bound tetrasaccharide. The major driving force for the hinge closing in AlgQ2 is probably the result of exclusion of a water molecule from the binding site. The shift in the equilibrium on sugar binding may also be enhanced by the interaction of the sugar with Glu396, which results in the perturbation of the hinge favoring the closed form. Because Glu396 is hydrogen bonded to M1-O3 in the S1 subsite in the closed form, it also may reflect the importance of S1 subsite for binding alginates.

Two or three water molecules function as hinge water in the ABP and RBP (29, 30) belonging to group I judging from transitions from domain to domain. In ABP and RBP, hinge water molecules play an important role in hinge motion and are also involved in the stabilization of each form. However, in AlgQ2, only one water molecule (WAT715) is responsible for hinge motion, and the loss of WAT715 is likely to cause dynamic hinge movements. Because this is the first example of a hinge water molecule of group II binding proteins, the water-mediated hinge motion observed in AlgQ2 should facilitate the clarification of the conformational changes in group II binding proteins.

This is the first reported description of the structural and functional relationship of a periplasmic binding protein of a bacterial ABC transporter which binds macromolecules. Further analyses of the structural features of AlgQ2 complexed with oligo- and polysaccharides and comparison with AlgQ1, which resembles AlgQ2 on alignment, should provide new insights into the mode of binding an acidic polysaccharide.

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Crystal Structure of AlgQ2, a Macromolecule (Alginate)-binding Protein of Sphingomonas sp. A1, Complexed with an Alginate Tetrasaccharide at 1.6-Å Resolution

Yumiko Mishima, Keiko Momma, Wataru Hashimoto, Bunzo Mikami and Kousaku Murata

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