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A Molecular Modeling Study of AAD16034

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Abstract

AAD16034 is an alginate lyase from *Pseudoalteromonas* sp. IAM14594. A very close homologue with known 3D structure exists (marine bacterium *Pseudoalteromonas* sp. strain no. 272). A three-dimensional structure of AAD16034 was generated based on this template (PDB code: 1J1T) by comparative modeling. The modeled enzyme exhibited a jelly-roll like structure very similar to its template structure. Both enzymes possess the characteristic alginate sequence YFKhG+Y-Q. Since AAD16034 displays enzymatic activity for poly-M alginate, docking of a tri-mannuronate into the modeled structure was performed. Two separate and adjacent binding sites were found. The ligand was accommodated inside each binding site. By considering both binding sites, a plausible binding pose for the poly-M alginate polymer could be deduced. From the modeled docking pose (i.e., the most important factor that attracts alginate polymer into this lyase) the most likely interaction was electrostatic. In accordance with a previous report, the hydroxyl group of Y345 was positioned close to the α-hydrogen of β-mannuronate, which was suitable to initiate a β-elimination reaction. K347 was also very near to the carboxylate moiety of the ligand, which might stabilize the dianion intermediate during the β-elimination reaction. This implies that the characteristic alginate sequence is absolutely crucial for the catalysis. These results may be exploited in the design of novel enzymes with desired properties.

Keywords: Homology modeling, Docking, Alginate lyase, AAD16034

Alginate is a viscous gum that is abundant in the cell walls of marine brown algae. It is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (**M**) and its C-5 epimer α-L-guluronate (**G**) (Figure 1). Recently calcium salt alginate fiber has been successfully applied to treat toxic epidermal necrolysis[1]. The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), alternating M and G-residues (MG-blocks) or randomly organized blocks[2].

Polysaccharide lyases (EC 4.2.2.-) are a group of enzymes that catalyze the cleavage of polysaccharide chains via a β-elimination mechanism. Alginate lyases degrade the linear polysaccharide alginate via cleavage of the constituent glycosidic linkages of alginate. This reaction gives rise to unsaturated oligouronic acids having 4-deoxy-L-erythro-hex-4-enopyranosyluronic acid at the nonreducing end[1]. Based on their primary sequences, alginate lyases are grouped into several polysaccharide lyase (PL) families, namely, PL-5, -7, -14, -15 and -18[3]. Most of the PL-5 alginate lyases specifically depolymerize poly-M, while PL-7 alginate lyases depolymerize poly-G. The PL-14 family contains enzymes specific for poly-M or poly-G[4]. Recently, a PL-15 enzyme with an exolytic activity has been isolated and characterized[5,6]. PL-18 lyases are bifunctional, possessing activity for both poly-M and poly-G regions.

Many alginate enzymes from microbes, animals and plants have been studied. Earlier work on alginate lyases focused on using purified enzymes to analyze the fine structure of alginates. More recently,

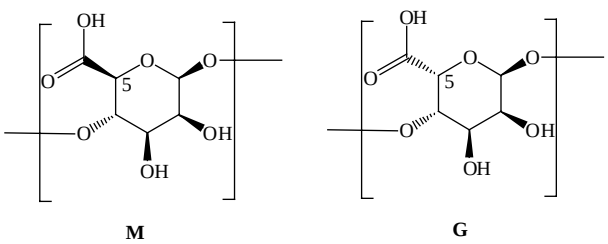
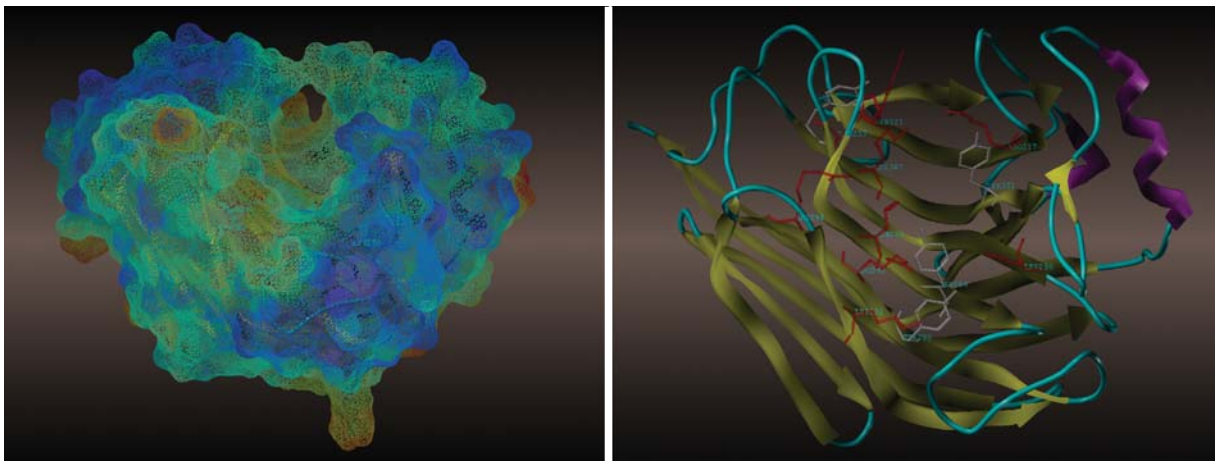


Figure 1. C-5 epimeric configuration of M and G monomers.



(A) Electrostatic Surface Model (B) Ribbon Model

Figure 2. AAD16034 homology model.

the emphasis has shifted towards elucidating the structure-function relationship of alginate lyases and the bacterial biochemical pathways for alginate degradation and utilization. Alginate appears to play a key role in the stabilization of biofilms formed by *Pseudomonas aeruginosa* and some other pseudomonas, with most Pseudomonas strains producing large amounts of extracellular alginates[7]. Because of the high molecular mass and negative charge of bacterial alginate, the polysaccharide is highly hydrated and viscous. Alginates occupy the majority of the extracellular polymeric substance (exopolysaccharide; EPS) of mucoid *P. aeruginosa* and have been implicated in the development and maintenance of the mechanical stability of biofilms[8]. Alginate lyase removes the EPS from the surface of mucoid *Pseudomonas*[9] and reduces attachment of mucoid strains of *P. aeruginosa*[10].

In contrast to algal alginate, these bacteria produce polysaccharides that are often substituted with O-acetyl groups on the 2 and/or 3 positions of D-mannuronate. However, most alginate lyases from brown algae cannot degrade the O-acetylated bacterial alginate. To date, only a few lyases that are effective on acetylated alginate have been identified; all are capable of degrading M-blocks, but not all of these M-specific lyases exhibit activity against acetylated bacterial alginat[2].

Results

We used the SYBYL ver. 7.0 composer module using the alignment shown in Figure 3. Because there

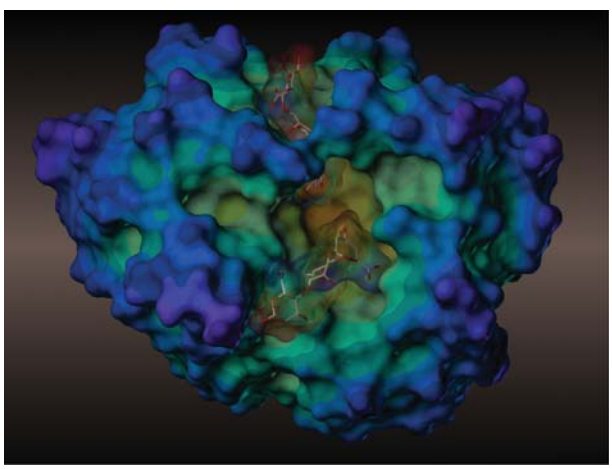


Figure 3. Docking of a tri-mannuronate (Two binding sites are shown together).

was no gap in the aligned sequences, the structure of the partial sequence (226-398) could be built with confidence. After constructing the model backbone, side-chain orientation was determined and then adjusted to avoid steric clash. The amino acid charges were determined followed by the initial setup of the modeled structure.

The side chain structures were optimized with a distance dependent dielectric constant with the backbone structure intact[11]. Figure 2A shows an electrostatic surface map of the model indicating that the inside of the channel is positively charged (red color). Figure 2B shows the residues responsible for this positive charge in red; the involved residues are mostly **R** and **K**. In the middle, three **Y**s are present (de-

noted in white) along the cleft, which is expected to be important in the catalytic mechanism. In a previous report, the importance of tyrosine was suggested by sulfate ion binding, and the proximity to the tyrosine hydroxyl group and α-hydrogen of tri-mannuronate[12]. Therefore, the resultant model clearly implies a positively charged binding cleft for the negatively charged alginate polymer.

The mechanism of action of alginate lyase has been proposed previously[13]. To complete the elimination of the 4-O-glycosidic bond of alginate, a positively charged group of amino acid residues in the active site of the enzyme is required to interact with the carboxylate of alginate. The role of Y in the elimination reaction by *Sphingomonas* sp. alginate lyase (A1-III) involving the proton at C5 has been demonstrated using X-ray crystallographic analysis[14].

Discussion

A 3D structure of an alginate lyase (AAD16034) was generated based on the template structure (1J1T). The modeled enzyme has a jelly-roll like structure, which is very similar to its template structure. Since AAD16034 degrades poly-M alginate, docking a trimannuronate to the modeled structure was performed. By considering docking poses, a plausible binding pose for poly-M alginate polymer could be deduced. The most important factor responsible for the attraction of the alginate polymer into this lyase might be an electrostatic interaction. As in the case of other alginate lyases, the hydroxyl group of tyrosine (Y345) may be responsible for initiation of the β-elimination reaction. A lysine was also very near to the carboxylate of the ligand (K347). This lysine may be responsible for the stabilization of the dianion intermediate. The characteristic alginate sequence is also present in the modeled structure. These results may be valuable in the further design of novel enzymes with more desirable properties.

Figure 4. Two domains in AAD16034.

Materials & Methods

AAD16034 (EC 4.2.2.3) is an extracellular poly-**M** lyase obtained from *Pseudoalteromonas* sp. IAM-14594[15]. It possesses 398 amino acid residues and belongs to the PL-18 family (another 1J1T) that is characterized by a β-jelly roll fold[16]. A search of the Pfam web site (http://pfam.sanger. ac.uk/search?tab= searchSequence Block) reveals two domains for this sequence. One domain is a carbohydrate binding motif (CBM), and the other is alginate lyase 2 domain (Figure 4). This family forms an all-β fold and is different from an all-α fold. Because we are mainly interested in the catalytic effect of this enzyme rather than binding interaction, we further aligned the sequence with the sequence from *Pseudoalteromonas* sp. Strain No. 272, which also belongs to the PL-18 family (Figure 5). The alignment score was very high (expectation value, 6e-111). Furthermore, the sequence identities were high (82 %), with 192 of 233 residues being identical. Even more encouragingly, three-dimensional (3-D) constructs were achieved, since the sequence alignment did not possess any gaps. A consensus sequence YFKhG1Y-Q, where h represents a hydrophobic residue and 1 neutral residue, is present in the C-terminal region of several alginate lyases[1], and was presently also detected in the C-terminus of AAD16034.

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AAD16034  166  DTGSGSGIASNITNGSIFDLEGNNPHPLVNSNTLEFVPLEARHITPNGNGWRHEYKVKES  225
               D  +GS I S+IT+GSIFDLEG+NP+PLV+  +TL FVPLEA+HITPNGNGWRHEYKVKES
1J1T        1  DNSNGSTIPSSITSGSIFDLEGDNPNPLVDDSTLVFVPLEAQHITPNGNGWRHEYKVKES  60

AAD16034  226  ARAAMTETYEVFEATVKVEMSDGGKTIISQHHASDTGTISKVYVSDTDESGFDDSVAGNG  285
               R AMT+TYEVFEATVKVEMSDGGKTIISQHHASDTGTISKVYVSDTDESGF+DSVA NG
1J1T       61  LRVAMTQTYEVFEATVKVEMSDGGKTIISQHHASDTGTISKVYVSDTDESGFNDSVANNG  120

AAD16034  286  IFDVYVRLRNTSGKEEKHALGTIRSGGSFNLKVVNNYGDVDVTALGTTFGIPVEDDSESY  345
               IFDVYVRLRNTSG EEK ALGT+ SG +FNL+VVNNYGDV+ VTA G + FGIPVEDDS+SY
1J1T      121  IFDVYVRLRNTSGNEEKFALGTMTSGETFNLRVVNNYGDVEVTAFGNSFGIPVEDDSQSY  180

AAD16034  346  FKFGNYLQSQDPYTLDECGESGNSDSFKECFKDLGITKAKVTMTDVSYTRRTN  398
               FKFGNYLQSQDPYTLD+CGE+GNS+SFK CF+DLGIT++KVTMT+V+YTR TN
1J1T      181  FKFGNYLQSQDPYTLDKCGEAGNSNSFKNCFEDLGITESKVTMTNVTYTRETN  233
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Figure 5. Sequence alignment between AAD16034 and 1J1T (blastp).

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