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Modeling Studies of an Exotype Alginate Lyase Atu3025 from Agrobacterium Tumefaciens Strain C58, a Member of Polysaccharide Lyase Family 15

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Abstract

Alginate lyases, also known as alginases or alginate depolymerases, catalyze the degradation of alginate by a β-elimination mechanism that has yet to be fully elucidated. Alginate is a copolymer of α-L-guluronate (G) and its C5 epimer β-D-mannuronate (M), arranged as homopolymeric G blocks, M blocks, alternating GM or random heteropolymeric G/M stretches. Almost all alginate lyases depolymerize alginate in an endolytical fashion via a β-elimination reaction. The alginate lyase Atu3025 from Agrobacterium tumefaciens strain C58, consisting of 776 amino-acid residues, is a novel exotype alginate lyase classified into polysaccharide lyase family 15. Till now there is no crystal structure available for this class of proteins. Since there is no template with high sequence identity, three-dimensional coordinates for exotype alginate lyase (PL 15 family) were determined using modeling methods (Comparitive modeling and Fold recognition). The structures were modeled using the X-ray coordinates from Heparinase protein family (**PDB code: 3E7J**). This enzyme (Atu3025) displays enzymatic activity for both poly-M and poly-G alginate. Since poly-M is widespread; docking of a tri-mannuronate against the modeled structure was performed. We identified some of those residues which are crucial for lyase activity. The results from this study should guide future mutagenesis studies and also provides a starting point for further proceedings.

Key words : Homology modeling, Fold recognition, Docking, Alginate lyase,

1. Introduction

Alginate produced by brown seaweed is commonly used as a thickener in the food industry and as a gelling agent, emulsifier and chelator of metal ions in the pharmaceutical industry.[1] Enzymatically depolymerized alginate oligosaccharides function as a bifidus factor,[2] an elicitor of plant growth,[3] a growth enhancer of human endothelial cells[4] and keratinocytes[5] and an inducer of cytokine production from mouse macrophage cells[6] and human mononuclear cells[7]. The enzymatic production of differently depolymerized alginate oligosaccharides is essential for several medical developments and other industrial uses. 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-D-mannuronate (**M**) and its C-5 epimer α-L-guluronate (**G**). 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.[8] Polysaccharide lyases (EC 4.2.2.3) are a group of enzymes that catalyze the cleavage of polysaccharide chains via a β-elimination mechanism. Based on their primary sequences, alginate lyases are grouped into several polysaccharide lyase (PL) families, namely, PL-5, -7, -14, -15 and -18. Most of the PL-5 alginate lyases specifically depolymerize poly-M, while PL-7 alginate lyases depolymerize poly-G. Alginate lyases A1-IV and A1-IV' from *Sphingomonas* sp. strain A1 and Atu3025 from *Agrobacterium tumefaciens* strain C58 have recently been assigned to a novel family, PL-15.[9] In this current study, exotype alginate lyase from Agrobacterium tumefaciens was selected for insilico studies. Till to date there is no structural report on the PL-15 family of these enzymes or any other exotype alginate lyases. There

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was only one report on the crystallization of this enzyme.[10] The three dimensional structure of exotype alginate lyase was obtained by comparative modeling based on the crystal structure of Heparinase II protein **(PDB code: 3E7J)**. Docking study was performed with alignate (Tri-mannuronate) to identify the residues involves in β-elimination mechanism. Thus we have built a homology model and it helps to predict the residues which may be crucial for lyase activity.

2. Materials and methods

2.1. Construction of the Homology Model

Homology modeling was used to build the model for exotype alginate lyase (Atu3025) protein. The protein sequence (776 amino acids) was retrieved from Uniprot database (http://www.uniprot.org) (UniprotKB accession number: A9CEJ9). The sequence was further used for template identification using FUGUE module in SYBYL 8.1,[11] a molecular modeling package installed in Linux system. PSI- Blast search within FUGUE was utilized to select the templates. The top ranked template (**PDB code: 3E7J)** was aligned with the target sequence. The aligned sequence was taken for the model construction. Homology model was constructed using MODELER,[12] based on the alignment obtained from FUGUE. MODELER is well known comparative modeling software, which generates a refined 3D homology model. The generated model was validated with PROCHECK[13] Ramachandran plot. The root mean square deviation (RMSD) between the main chain atom of the model and template was calculated and the model was used for further proceedings.

2.2. Fold Recognition

Fold recognition was done using different fold recognition servers such as Bioinfo modeler (http://meta.bioinfo.pl), FFAS (http://ffas.ljcrf.edu/) and PSI-PRED (http://bioinf4.cs.ucl.ac.uk:3000/psipred). The structural predictions were used to identify the spatial locations of functionally important residues, such as active sites and the sites of disease-associated mutations.

2.3. Molecular Docking

Docking study was performed by using SYBYL 8.1[11] molecular modeling package installed in Linux system. Protein structure was prepared by using biopolymer module of SYBYL 8.1. Hydrogen atoms were added to structure, atom types and charges were assigned using AMBER7 FF99 force field and side chain amides were modified. Alginate (Tri-mannuronate) was sketched by using sketch module in SYBYL 8.1 and the molecule was fully minimized by using Tripos force field and Powell method with termination gradient set at 0.05 kcal/mol. The molecule was fully minimized with Gasteiger-Hückel charges. Docking study was performed by using Surflex-Dock module of SYBYL 8.1, which uses empirical scoring function to score ligand and protomol[14] guided docking.

3. Results

3.1. Sequence Analysis of Exotype Alginate Lyase

Sequence alignment is the prerequisite for homology modeling. The sequence conservation of the query (exotype alginate lyase) with the template structure (**PDB code: 3E7J**) was examined with the alignment obtained from FUGUE. The results obtained from FUGUE module is shown in Table 1. The alignment of query sequence with the template is shown in Figure 1. The sequence identity between the query and the template was 15%. Though the sequence identity is low, we followed the concept proposed by Tramontano in which sequences with low sequence identity can still lead to a useful model. Sequence analysis of template and query sequences indicated that both of them having Heparinase II - III like domain, whereas the domain regions in the template and query sequences overlaps each other. The domain region in the query (exotype

Table 1. Scores obtained from FUGUE

Profile Hit	PLEN	RAWS	RVN	ZSCORE	ZORI	AL
3E7J A	743	-428	768	38.86	41.97	0
2FUQ A	746	-583	614	28.40	31.52	0
1QAZ A	351	-284	138	10.57	13.41	2
1JRH I	95	-14	35	9.54	12.37	2
1HN0 A	971	-1276	172	8.16	11.10	0
3K7X A	339	-331	78	7.86	10.57	2

PLEN - Profile length
RAWS - Raw alignment score
RVN - Raw score-Raw score for NULL model
ZSCORE - Z-score normalized by sequence divergence
ZORI - Original Z-score
AL - Alignment algorithm used for ZORI/Alignment calculation
0-GLOBAL, 2-GloLocSeq (No sequence termini gap penalty)

```
           1                                                  50
3E7JA      .......... .......... .......... .......... ..........
A9CEJ9     MRPSAPAISR QTLLDEPRPG SLTIGYEPSE EAQPTENPPR FSWLPDIDDG

          51                                                 100
3E7JA      .......... .......DVV WKDV...... ......DG.. ..........
A9CEJ9     ARYVLRISTD PGFTDKKTLV FEDLAWNFFT PDEALPDGHY HWCYALWDQK

         101                                                 150
3E7JA      .......... .......... VSMPIPPKT. ........HP RLYLREQQVP
A9CEJ9     SATAHSNWST VRSFEISEAL PKTPLPGRSA RHAAAQTSHP RLWLNSEQLS

         151                                                 200
3E7JA      DLKNRMNDPK LKKVWADMIK MQ.EDW.... ...KPADIPE VKDFRFYFNQ
A9CEJ9     AFADAVAKDP NHCGWAEFYE KSVEPWLERP VMPEPQPYPN NTRVATLWRQ

         201                                                 250
3E7JA      KGLTVRVELM ALNYLMTKDP KVGREAITSI IDT..LETAT FKPAGDISRG
A9CEJ9     MYIDCQEVIY AIRHLAIAGR VLGRDDLLDA SRKWLLAVAA WDTKGATSRA

         251                                                 300
3E7JA      I...GLFMVT GAIV..YDWC YDQLKPEEKT RFVKAFVRLA KML.......
A9CEJ9     YNDEAGFRVV VALAWGYDWL YDHLSEDERR TVRSVLLERT REVADHVIAH

         301                                                 350
3E7JA      .ECGYPPVKD KSIVGAASEW MIMRDLLSVG IAIYDEFPEM YNLAAGRFFK
A9CEJ9     ARIHVFPYDS HAVRSLSA.. .VLTPACIAL QGESDEAGEW LDYTVEFLAT

         351                                                 400
3E7JA      ....EHLVAR NWFYPSHNYH QGMSALNVRF TNDLFALWIL DRMGAGNVFN
A9CEJ9     LYSPWAGTDG GWAEGPHYWM TGMAYL.IEA ANLIRSYIGY DLYQRPFFQN

         401                                                 450
3E7JA      PGQQFILYDA IYKRRPD.GQ ILAGGDVDYS RKKPKYYTMP ALLAGSYYKD
A9CEJ9     TGRFPLYTKA PGTRRANFGD DSTLGDLPGL KLGYNVRQFA GVTGNGHYQ.

         451                                                 500
3E7JA      EYLNYEFLKD PNVEPHCKLF EFLWRD.... ........TQ LGSRKPDDLP
A9CEJ9     ..WYFDHIKA DATGTEMAFY NYGWWDLNFD DLVYRHDYPQ VEAVSPADLP

         501                                                 550
3E7JA      LSRYSGSPFG WMIARTGWGP ES..VIAEMK VNEYSFLNHQ HQDAGAFQIY
A9CEJ9     ALAVFDD.IG WATIQKDMED PDRHLQFVFK SSPYGSLSHS HGDQNAFVLY

         551                                                 600
3E7JA      YKG.PLAIDA GSYTGSSGGY NSPHNKNFFK RTIAHNSLLI YDPKETFSSS
A9CEJ9     AHGEDLAIQS GYYV....AF NSQMHLNWRR QTRSKNAVLI G.........

         601                                                 650
3E7JA      GYGGSDHTDF AANDGGQRLP GKGWIAPRDL KEMLAGDFRT GKILAQGFGP
A9CEJ9     .......... .......... GKGQYAEKDK ALARRA...A GRIVSV....

         651                                                 700
3E7JA      DNQTPDYTYL KGDITAAY.. .SAKVKEVKR SFLFLNLKDA KVPAAMIVFD
A9CEJ9     .EEQPGHVRI VGDATAAYQV ANPLVQKVLR ETHFVN.... ..DSYFVIVD

         701                                                 750
3E7JA      KVVASNPDFK KFWLLHSIEQ PEIKGNQITI KRTKNGDSGM LVNTALLPDA
A9CEJ9     EVECSEPQ.E LQWLCHTLGA PQTGRSSFRY NGRKAGFYGQ FVYSSG....

         751                                                 800
3E7JA      ANSNITSIGG KGKDFWVFGT NYTNDPKPGT DEALERGEWR VEITPKKAAA
A9CEJ9     GTPQISAVEG FP........ .......... .......... .DIDPKEFEG

         801                                                 850
3E7JA      EDYYLNVIQI ADNTQQKLHE VKRIDGDKVV GVQLAD..RI VTFSKTSETV
A9CEJ9     LDIHHHVCAT V..PAATRHR LVTL....LV PYSLKEPKRI FSF.IDDQGF

         851                                                 900
3E7JA      DRPFGFSVVG KGTFKFVMTD LLPGTWQVLK DGKILYPALS AKGDDGALYF
A9CEJ9     STDIYFSDVD DERFKLSL.. ..PKQF.... .......... ..........

         901                                                 950
3E7JA      EGTEGTYRFL R
A9CEJ9     .......... .
```

Fig. 1. Sequence alignment between A9CEJ9 (exotype alginate lyase) and 3E7J generated by FUGUE (PSI-BLAST)

alginate lyase) was identified (500-647 residues) (http://www.ebi.ac.uk/interpro/ISpy?mode=sigle&ac=A9CEJ9). The domain region in the template was identified and it spans from 379-549 (http://www.ebi.ac.uk/interpro/IEntry?ac). It was found that the domain region of both query and template overlaps in the alignment.

3.2. Fold Recognition

Fold recognition study was performed to identify the sequences of functionally important residues. Fold recognition was performed using online servers with default parameters. The template **[PDB code: 3E7J]** was reported as the top template in each server. The results are shown in Table 2. Since 3E7J was reported as the top template by all the servers, this template was used confidently for modeling purposes.

Table 2. Template selected by different fold recognition servers with their scores based on each server

Server	Template	Score (Obtained from each server)
FFAS server	3E7J A	4e-57
Bioinfo server	3E7J A	-121
PSI-PRED	3E7J A	202.549

Table 3. Models generated using MODELER, with various scores (Dope, Molpdf, and GA341)

Model No	Dope Score	Molpdf	GA341
Model 1	-69632.52344	5683.30273	0.78858
Model 2	-70510.36719	6706.35958	0.88226
Model 3	-70925.23438	6945.76123	0.97765
Model 4	-70069.07031	7061.98779	0.95791
Model 5	-71185.05469	5802.12256	0.96029

3.3. Homology Modeling and Model Validation

Homology modeling was performed using MODELER. Five models were generated with default parameters. The results are shown in Table 3. All the models were more or less similar. The generated models were quite similar without much variance in their DOPE score, Molpdf and GA341values. Model 1 was selected for further studies. The selected model is shown in Figure 2. The selected model was validated by using PROCHECK. The Ramachandran plot ϕ/ψ distribution of backbone conformational angles for each residue of the refined structure revealed that 80.1%, 16.0%, 2.8%, and 1% of the residues are located in the most favorable, additionally allowed, generously allowed, and disallowed regions, respectively. Moreover, overall the PROCHECK G-factor value -0.26 also indicates the higher quality of the constructed model. The PROCHECK results reveal that the generated model is satisfactory, and is thus considered as a reliable source for the rest of the study. The distribution of residues in the Ramachandran plot is shown in Figure 3. The RMSD

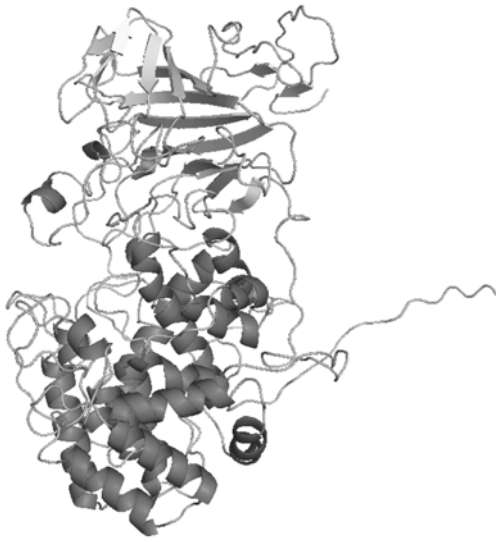

Fig. 2. Model generated by homology modeling (MODELER), Helices in red, Sheets in green, and Loops in yellow.

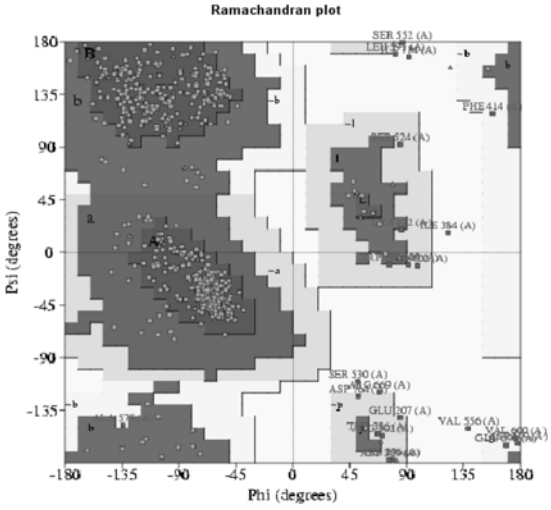

Fig. 3. Ramachandran plot of exotype alginate lyase. The plot calculation on the three-dimensional (3D) model of exotype alginate lyase protein was calculated with the PROCHECK program.

between the main chain atom of model and template was found to be 1.510Å. The superposition of the template and model structure is shown in Figure 4.

3.4. Docking of Alginate Trimer into the Docking Site

Alginate (Tri–mannuronate) was docked in to the active site of the generated model, to identify the residues which are crucial for lyase activity. The results are shown in Figure 5. Hydrogen bonding interactions are formed between alginate trimer and Arg249, Ala585. Moreover, hydrophobic interactions are also seen between alginates and Ser 530, His531, Tyr555, Phe558, and Asp295.

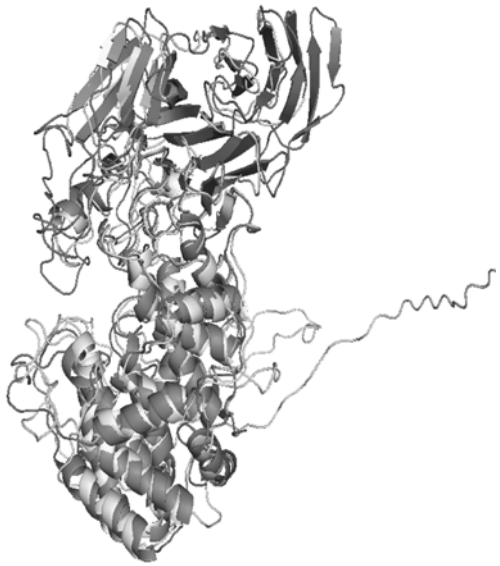

Fig. 4. Representation of superimposed structure of model (oligo alginate lyase) on the template (3E7J). Model in yellow color and the template is in Magenta.

4. Discussions

The main aim of this study is to identify the residues which would be crucial for lyase activity. Domains that are conserved in the proteins of the same family will have more similar functions. Thus, the model was built with more confidence by selecting the template on the basis of domain concept which will be more reliable, not only structurally but also functionally. Since there is no template with high sequence identity, we did modeling by comparative as well as threading concepts. Modeling with FUGUE and fold recognition servers indicates that 3E7J (Sugar binding protein) is the recognized template with low sequence identity. Sequence analysis of the query and template sequence identifies that both of them having Heparinase II-III like domain in common. The alignment obtained from FUGUE reveals that both the domains match accordingly. Domain analysis indicates that the function

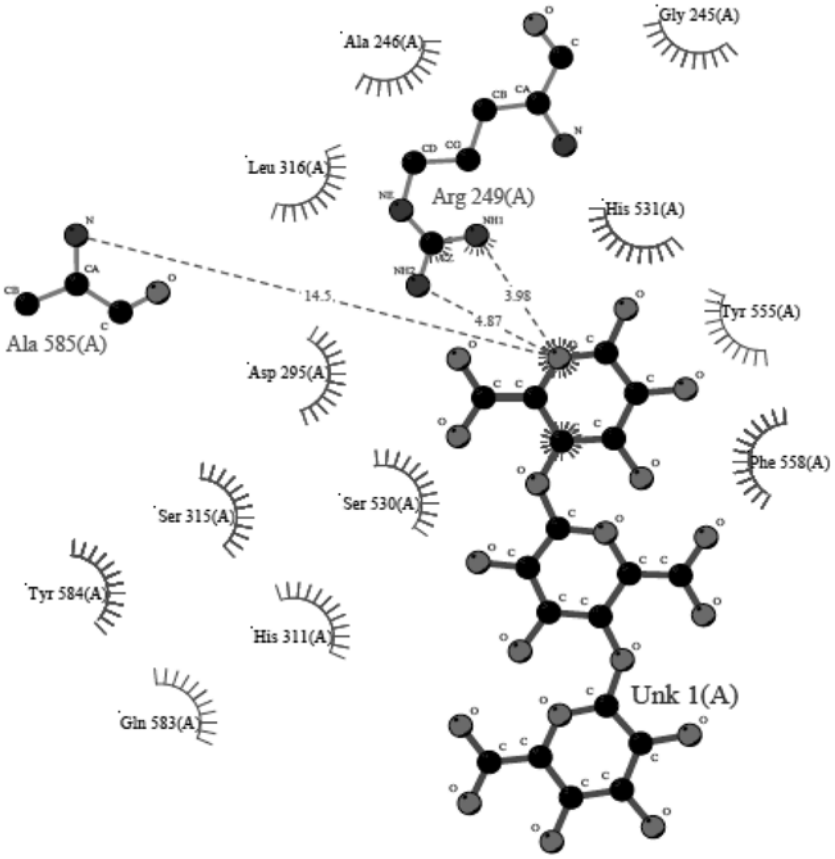

Fig. 5. Hydrogen and Hydrophobic interactions between model and alginate trimer generated by Ligplot.

of Heparinase II-III like protein is similar to that of alginate lyases. Since both query and template sequences share the same domain we thought that this domain would be the functional part. In addition, the structural information obtained from PDB for the template (3E7J) reveals that the residues in which the sugar products binds are from this domain (Heparinse II-III). Therefore we modeled the protein based on the alignment obtained from modeling concepts. Docking studies revealed that the residues Arg249, Ser530, His531, Tyr555, Phe558, may be crucial since it form hydrophobic and hydrogen bonding interactions with alignate trimer. These results would be useful for further studies and we suggest that mutagenesis studies on these residues might be effective.

5. Conclusion

To the best of our knowledge, the first molecular modeling report of the exotype alginate lyase from Agrobacterium tumefaciens has been presented here based on modeling techniques such as fold recognition and comparative modeling. Modeling was done on the basis of common domain (Heparinse II-III) though it has low sequence identity. Docking was done to identify the functional residues. The residues which are crucial for lyase activity are predicted. The results obtained from this study would be useful for mutagenesis experiments.

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