

Supplementary Material

The structure of a glycoside hydrolase family 81 endo- β -1,3-glucanase

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Table S1 Primers used in the construction of *RmLam81A* gene

Primers	Primer sequence (5'-3') ^a	Bases (bp)
DP1	TAYAAYGAYCAYCAYTAYCA	20
DP2	TCYTGRTCNCNKCCRTC	17
5'GSP	CCGTCTGGCTTGATGCCTCCTG	22
5'NGSP	TCGCCGTCGTTTGCGTTGTTGA	22
3'GSP	CACTTGGACCCAACATGGAATG	22
3'NGSP	AACAACGCAAACGACGGCGACGAG	24
<i>RmLam81A</i> BamHI ^b	GCCATAGGATCCCAGAGTACAAGTGATGGAGACGAT	36
<i>RmLam81A</i> XhoI ^b	CCGCTCGAGTTAATAAATACGATGCTTGAGATTAAAGGG	39

^a K=G/T, N=A/T/C/G, R=A/G, Y=C/T.

^b Restriction enzyme sites incorporated into primers are underlined.

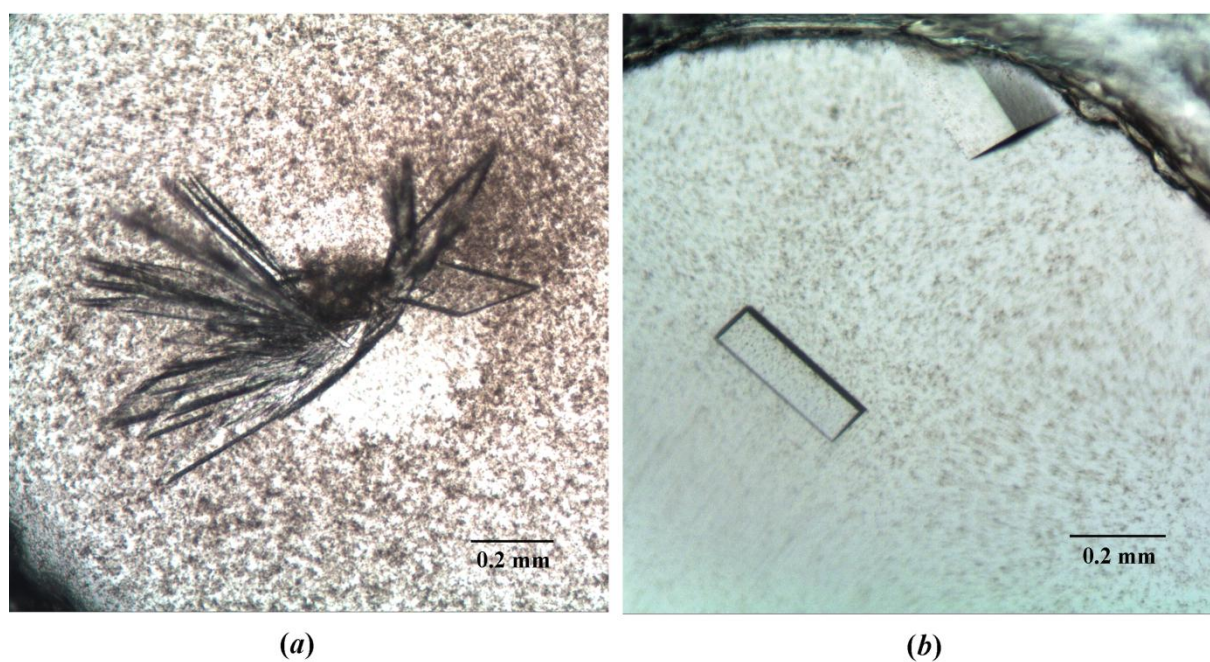


Figure S1

Microphotographs of *RmLam81A* crystals Form I (a) and Form II (b) obtained from conditions: 160 mM Li_2SO_4 , 24% (w/v) PEG4000, 80 mM Tris-HCl pH 8.5 and 6% (v/v) MPD; 24% (w/v) PEG4000, 80 mM Tris-HCl pH 8.5 and 6% (v/v) MPD. The approximate dimensions of Form I and Form II were $0.5 \times 0.2 \times 0.02$ and $0.4 \times 0.2 \times 0.2$ mm, respectively.

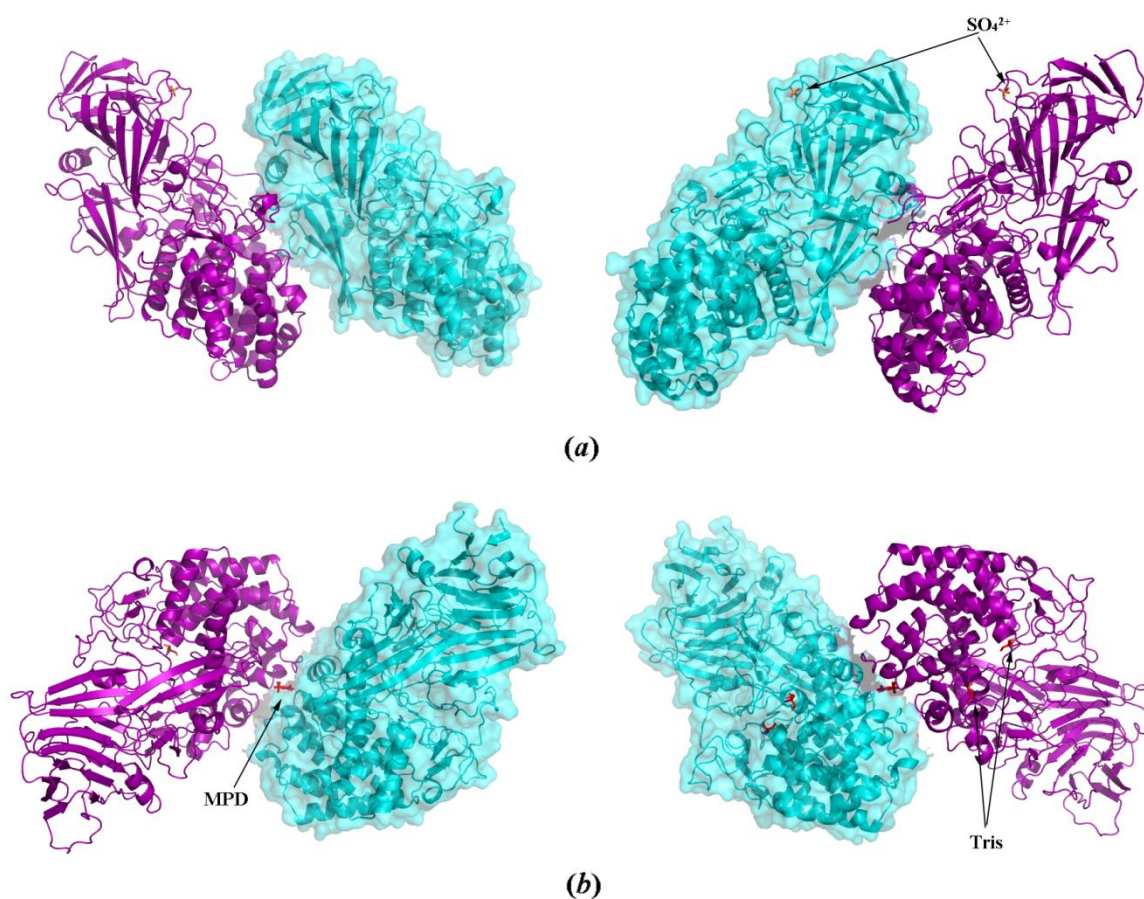


Figure S2

The asymmetric units of *RmLam81A* in Form I (a) and Form II (b). In both structures there are two protein molecules in the asymmetric unit, labeled A and B. Molecule A are shown in purple and Molecule B in cyan. Tris, MPD and sulfate ion are shown as red sticks. All figures were produced using *PyMol* (<http://www.pymol.org/>; V. 1.3; Schrödinger LLC).

1 GGTTAGGCCATTATTGTAAGCAATTATCAGATTTCAAGTATAGTTGCCGCCGCCACGATTACTATGATTACCGATTATTCAGGAGTGGCGTCGACAGATACAAGTGAAGGAG 120
-25 M R F Q V I V A A A T I T M I T S Y I E G V A S Q S T S D G 5
121 ACGATCTGTTGTCCTGTATCGAATTTTGTATCCCAAGAGTATTTCCCGGAGATCAAGCATCCTTTGAACCCATGTATGCGAATACAGAAATGGCAAAATTTGTACCAACCAATTCGT 240
6 D D L F V F V S N F D P K S I F F E I K H P F E F M Y A N T E N G K I V P T N S 45
241 GGATCAGCAATCTGTTTACCCGAGCGCGGATAACTTGGCACCTACGACTCCAGATCCTTACACTTTACGTTTACGATGGCTACGAGGTACCCCTGGAGTACGATTAGACAACCTT 360
46 W I S N L F Y P S A D N L A P T T F D P Y T L R L L D G Y G G N F G L T I R Q P 85
361 CAGCCAAGgtatgtatgaaggtttggagcgcataataatactaaaggtatgaattatgtttgaagcaaaaaatgaactttataggtGCTTGTGTTCTCTCTCTACGAAACGAGTGCCTAC 480
86 S A K Intron 1 V L G S Y F P T N D V F Y 101
481 ACAGATGCAGGCTACATGATTAAACAGTGTAGTCTGTGACCTTCGCTTACGAGCAGCAATGGAGCGATGTGGTGCCAGATCGCCAAAGTTACCGACTGGGATCATTTAAGCGCAAAATTTA 600
102 T D A G Y M I N S V V V D L R L T S S E W S D V V P D R Q V T D W D H L S A N L 141
601 CGCTTATCAACGCTCAGGACAGCAATAGTACATTTGCAATTTGCCGCGGCATATATACTGCCAATTATAACAACCTTACGCCCGAGTTCTTACGCCAATACGCGATA 720
142 R L S T P Q D S N S Y I D F P I V R G M A Y I T A N Y N N L T P Q F L S Q H A I 181
721 ATATCTGTCCAGGCTGATGAAGAATCAGACGATAATACATCAACGTTCTCCGCTCGTAAATCACCATGAACGACGATCCAACTTCTACATTTATTATCTCCCTTGGT 840
182 I S V E A D E K K S D D N T S T F S G R K F K I T M N D D F T S T F I I Y S L G 221
841 GACAAGCCCCCTTGAGCTTCGCAAGCAAGCAATTCCAACTTGTGAGCTCCAGCCGTACACGGGTGTTATCCGTGTAGTAACTTCCCGCGCGAGTTCGAGACATTTGCTTGACGA 960
222 D K F L E L R K Q D N S N L V A S K P Y T G V I R V A K L P A P E F E F E T L D A 261
961 AGTCAGCAGTGTGGCTCAGGAGCGATATTTACGCTGTTGCGACGATAACAACGGGCGATCTACACGATAAAATGGAGACAAACAGCAACGAGGCTCTCTCTCACATACGCA 1080
262 S R A V W P T G G D I S A R S D D N N G A S Y T I K W K T N S N E A P L L T Y A 301
1081 TAAGCACATTTGATAGTACGATGACAGCAATGTAAACGACCGACATGACTCTCCAGTCTGCGACTAAAGGGCCCATGACAGCGTGTGGTGGCAACGAAATGGACATTTGCGTGA 1200
302 Y A H H L T S I D D S N V K R T D M T L Q S A T K G P M T A L V G N E W T L R E 341
1201 ACGAATGAGCCCTGTAGAGTGTGTACCACTACAGCAGCTCCAAACCTTACGACTATCAACGAGATTAACAGGATATCGCTAGTAACTATACAGGAAACAGCC 1320
342 T E L P V E W L P Q A A P N P T T I N E I M T E I N K D I A S N Y T Q E T A 381
1321 AAAGAGGACAATTACTTTTCGCGCAAGGATTCGCAAAATTCGCCATGCTTGCTGATGCTTCAACAAGTCGGATCAGACACAGCTTCGCAACCCAGAGTTGGCGCAAAATCGCGTACG 1440
382 K E D N Y F S G K G L Q K F A M L A L I L N K S D Q T Q L R N P E L A Q I A L D 421
1441 AAACCAAGGCTGCGTCTTACCATATCTCCAGATGAGCAAGCTGATCCATTCAGATATGATACCTATACAAAGGCAATTTGTCGCAAGGCTGAGTTGCCGACTTCAATGGAGGTACA 1560
422 K L K A A F L P Y L Q N E Q A D P F R Y D T L Y K G I V A K A G L P T S M G G T 461
1561 GATGATCTGTCTGCGAGTGTGTGCTATCTTACTACAGTATCATCACTACCAAGTATTTGTTGTTACAGgttaaatctgaattttctctcatctaaatatggaattctctttttgt 1680
462 D D L S A E E F G H S Y Y S D H H Y H Q G Y F V V T 486
1681 tagcggtatgaagagattttcatgtcttttactgaacattgaaactaatatatagtGCTGCCATTATCCATCACTTGGACCCCAACATGGAATGCTGATCGACTCAAGGCTTGGACGGAAGGCG 1800
487 Intron 2 A A I I H H L D F T W N A D R L K A W T E A 508
1801 TTATTCGAGACGTCAACAACGCAACGCGACGAGTACTTTGCGAGCATTCAGAACTGGGATTGGTTTGTGCTCAGAGCTGGGCGAGGACATCAAGCCAGACGGTGACCTGGACG 1920
509 L I R D V N N A N D G D E Y F A A F R N W D W F A G H S W A G G I K P D G A L D 548
1921 GTCGTGACCAAGAGTCTGCTCCCGAGgtttgtcatgtgaacacagtttcaaatctgaagaattttccatcttttccactgagagaagtaaaatttttaattgttgcagTGGTCAACTT 2040
549 G R D Q E S V P E Intron 3 S V N F 561
2041 TTATTTGGGCGCAAAATTTTGGGCTTAGCTACGCGCAACAGCCTCTCACCAAGCTTGTCTGCTGCACTGGCAGTTACCAACGAAACAGTACGAAATTTCTGATGCTTGTATGG 2160
562 Y W G A K L W G L A T G N T F L T K L A S L Q L A V T K R T T Y E Y F W M L D G 601
2161 TAACAATAATCGACCAAGAAATATTTGTGAGAACAAGGTTATCGGTATCTACTTTGAGCAAAAGACGGATTATgtaaaggtattcgaattgggaacgsggtcatgttgaatatcttttggct 2280
602 N K N R P E N I V R N K V I G I Y F E Q K T D Y Intron 4 625
2281 aacattatttcaacattcttttgaacttagACAACCTACTTTGAGCAGTACCTTGTAGTATATTCATGGCATTCAACAGTTACCAATGACTCCAGAGCTAATGGAATATATCGTACCOCT 2400
626 T T Y F G R F L E Y I H G I Q L P M T F E L M E Y I R T P 655
2401 GAGTTTgcagataataactgttttactctgcataatgtaaaagtctataaagaataatttcgacttagGTATCTCAAGAAATGGGACGAAAGCTAGGTGCTATTGACCGACCGCTCCAGT 2520
656 E F Intron 5 V S Q E W D E K L G A I A P T V Q 674
2521 CACCGTGGGCAAGTGTCTTACCTCAATTATGCAATTATCACTGACAGGCTTATCCAGCCCTCAGAAAGGTGCAAAATGGACGATGGCCAGACTCGCTCCTCTCTCTTGTACCTTA 2640
675 S P W A G V L Y L N Y A I I N P A E A Y P A L R K V Q M D D G Q T R S Y S L Y L 714
2641 CAGCAACTCGTCTCTTCTTCGCTGCGAgtatgtatcctcgaagttctttggcattttattacagtccttttaacgttttaagcgcccttttgcgttagGCTTTTGGCAGCTTTGGCTC 2760
715 T A T R P H F F R R Intron 6 S L L A A L A 731
2761 GACATGGGAGTACCGAAGACCTCTCTTCTCTTGTGACGACGACAGCAGAGATGTTTCTCTCTGCGATTCGCTCGACTCAATCCCTTAACTCTCAAGCATCGTATTATAT 2880
732 R H G S T R R P S L P S S G D D D K H E D G F L L R F R R L N F F N L K H R I Y 771
2881 AA TCTCTATAAAAAAGTGAGAGAAAGATATAAAGTCTATATGTACATAAAAAAAAAAAAAAAAAAAAA 2951
771 * 771

Figure S3

Nucleotide and deduced amino acid sequences of the full-length cDNA and flanking regions of *RmLam81A* from *Rhizomucor miehei*. The translational initiation codon, ATG and termination codon, TAA are boxed. Six intron sequences are shown in lower case letters with dotted underline. A poly (A+) is double lined. Conceptual translation of the ORF to amino acids is shown in a one-letter code below the respective codon. A putative signal peptide is underlined. The asterisk indicates the stop codon. Three N-glycosylation sites are indicated by underline and the Asp residues are highlighted in grey.

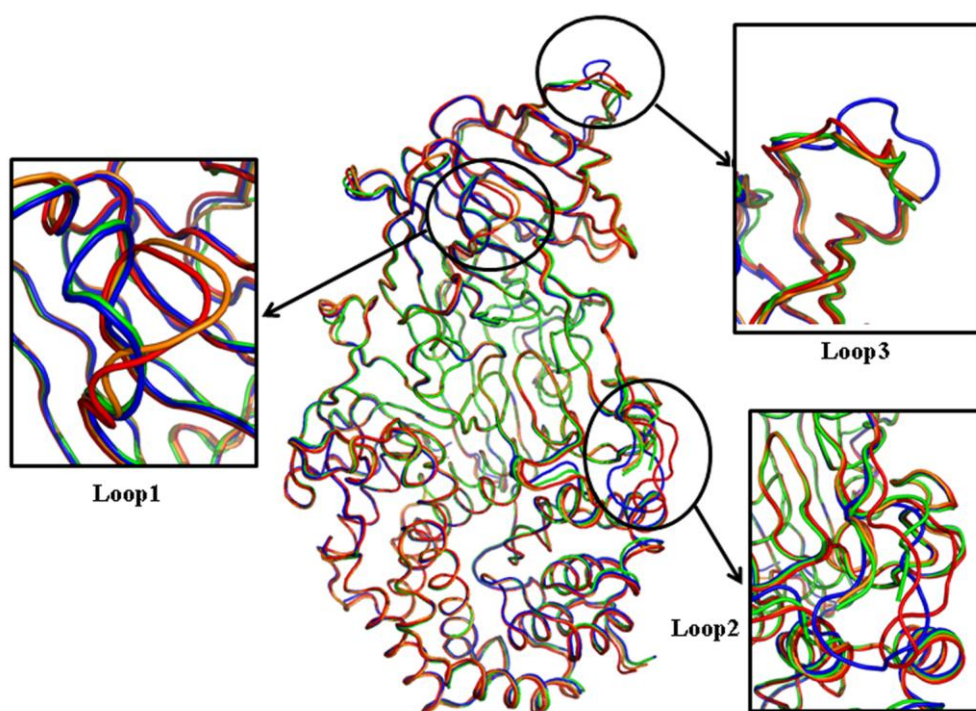


Figure S4

Superposition of the present four structures of *RmLam81A*. Colour code: Form I molecule A, blue; molecule B, green; Form II crystal structure molecule A, red; molecule B, orange. Loop1: $\beta 3$ - $\beta 4$ (Leu71-Pro78), Loop2: $\beta 5$ - $\beta 6$ (Ser92-Gly105) and Loop3: $\beta 13$ - $\beta 14$ (Glu187-Thr197).

All Figures were prepared with *PyMOL* (v.1.3; Schrödinger LLC).

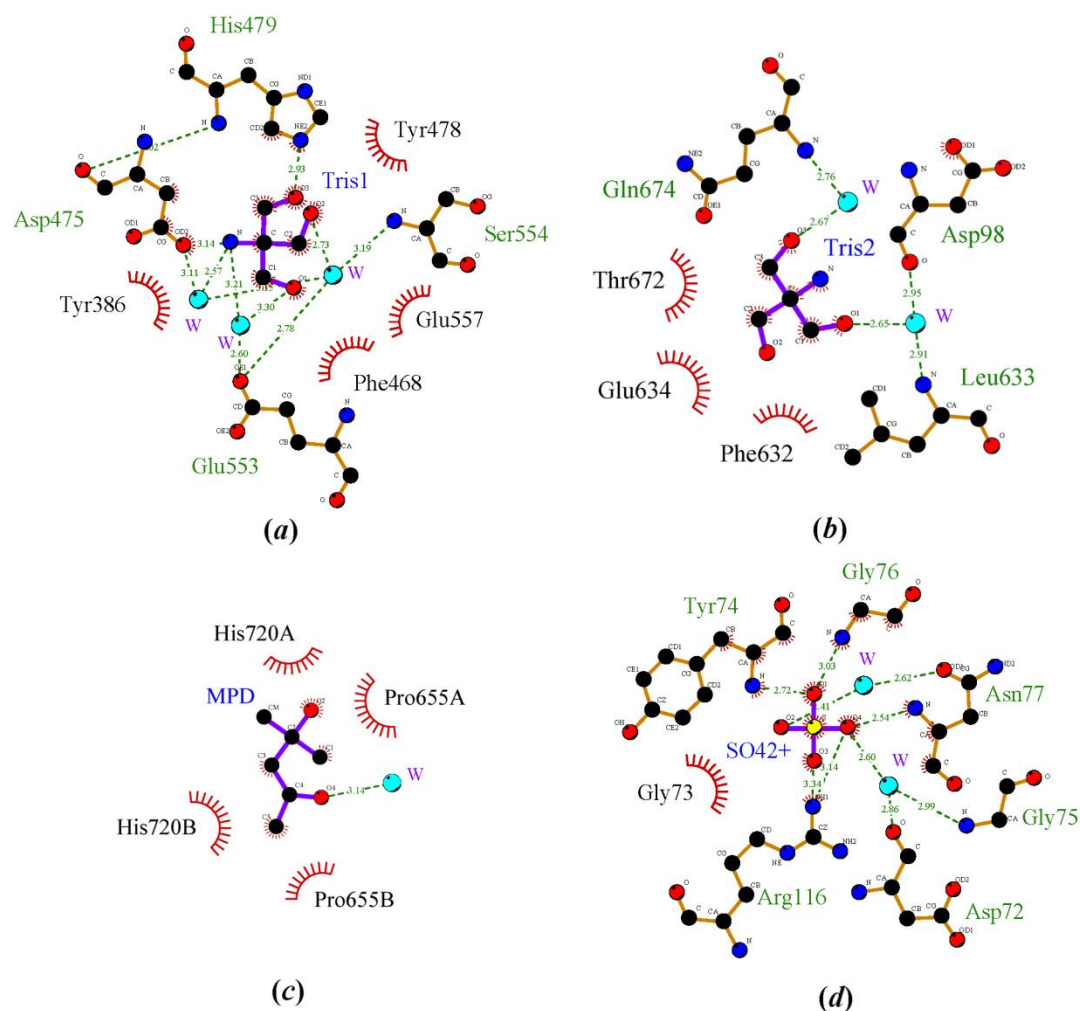


Figure S5

Schematic representation of the interactions between the enzyme and (a) the primary Tris molecule (Tris1), (b) the second Tris molecule (Tris2), (c) MPD molecule and (d) sulfate ion.

This picture was obtained using *LigPlot* (Wallace *et al.*, 1995). The atoms involved in hydrogen bonds (with distances) or hydrophobic contacts are depicted.