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Supporting information for article:

Elucidation of the mechanism of interaction between *Klebsiella pneumoniae* pullulanase and cyclodextrin

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Table S1 Interaction between residues of CBM41 and symmetric molecules.

Hydrogen bond (*a*) and C-C contact (*b*) distances were in the ranges of 2.45 Å to 3.24 Å and 3.95 Å to 4.44 Å, respectively. Distances were calculated using Contact from the CCP4 program suite (Collaborative Computational Project, Number 4, 1994). The apostrophe indicates a residue in the symmetric molecule.

(*a*)

		Hydrogen bond (Å)				
CBM41	Symmetry	1 mM α-CD	1 mM β-CD	1 mM γ-CD	10 mM α-CD	10 mM γ-CD
Glu45 N	Ser1082’ O	2.86	2.87	2.84	2.82	2.65
Ala46 O	Ser1081’ OG	2.89	3.07	3.00		2.95
Ser50 OG	Gly1077’ O	2.77	3.12		2.73	2.61
Gln53 NE2	Asp1024’ O	3.02	3.11		3.06	3.18
Ser105 OG	Asp982’ OD2	3.02	3.20			
Lys107 N	Gly802’ O	3.18	3.12	3.15	3.16	3.20
Glu45 O	Ser1081’ OG		2.81	2.94		
Gln53 NE2	Ala1026’ O		3.02	2.80		2.92
Thr68 OG1	Gln1037’ OE1					3.19

(*b*)

	C-C contacts				
	Symmetry molecules				
CBM41	1 mM α-CD	1 mM β-CD	1 mM γ-CD	10 mM α-CD	10 mM γ-CD
Gly44	Lys1083’		Lys1083’	Lys1083’	Ser1083’
Gly44	Ser1082’	Ser1082’		Ser1082’	Ser1082’
Glu45	Ser1081’	Ser1081’	Ser1081’	Ser1081’	Ser1081’
Ala46		Ser1081’	Ser1081’	Ser1081’	
Val47	Ser1081’	Ser1081’		Ser1081’	Ser1081’
Val47	Arg977’	Arg977’	Arg977’	Arg977’	Arg977’
Gln48	Pro1079’	Pro1079’	Pro1079’	Pro1079’	Pro1079’
Gln48	Ser1081’	Ser1081’		Ser1081’	Ser1081’
Ser50			Pro1079’		Thr1028’
Arg52		Gly1027’	Gly1027’	Gly1027’	Gly1027’
Gln53		Thr1028’			Thr1028’
Thr68	Gln1037’	Gln1037’	Gln1037’		Gln1037’
Thr103	Gln1023’	Gln1023’	Gln1023’	Gln1023’	
Thr103	Asp1024’	Asp1024’	Asp1024’	Asp1024’	Asp1024’
Asp106	Gly802’	Gly802’	Gly802’	Gly802’	Gly802’
Asp106	Ile798’	Ile798’	Ile798’	Ile798’	Ile798’
Lys107	Gly802’	Gly802’	Gly802’	Gly802’	Gly802’
Lys107	Ala803’				Ala803’
Val113	Asp1024’	Asp1024’	Asp1024’	Asp1024’	Ala1024’
Pro115		Gly1027’	Gly1027’		
Pro115	Ala1026’	Ala1026’	Ala1026’	Ala1026’	Ala1026’
Asn132		Ala468’	Ala468’	Ala468’	Ala468’

Table S2 Sugar puckering parameters and torsion angles.

The tables of Cremer-Pople parameters and torsion angles for CDs bound to CBM41 (*a*) and the active site (*b*). Cremer-Pople parameters were calculated using a Web-site calculator (http://enzyme13.bt.a.u-tokyo.ac.jp/CP/). The torsion angles were calculated using the COOT program (Emsley and Cowtan, 2004). Torsion angles were defined as O5 – C1 – O4' – C4' (Φ) and C1 – O4' – C4' – C5' (ψ) between Glc 1 (include C1 atom) and Glc 2 (include O4 atom; dash number).

(*a*)

		GLC 1	GLC 2	GLC 3	GLC 4	GLC 5	GLC 6	GLC 7	GLC 8
	Φ, θ (°)	111, 6	58, 17	357, 28	75, 16	118, 33	72, 23		
1 mM	Q	0.56	0.56	0.57	0.48	0.55	0.53		
α-CD	conformation	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$		
	Φ, Ψ (°)		111, -130	105, -108	117, -123	108, -98	109, -108	109, -86	
	Φ, θ (°)	50, 13	358, 9	103, 17	344, 18	337, 5	26, 10	39, 14	
1 mM	Q	0.56	0.55	0.60	0.55	0.57	0.53	0.53	
β-CD	conformation	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	
	Φ, Ψ (°)		114, -130	129, -102	104, -117	109, -120	120, -112	118, -127	104, -116
	Φ, θ (°)	21, 7	258, 10					28, 21	119, 16
1 mM	Q	0.57	0.58					0.63	0.52
γ-CD	conformation	$^4C_1$	$^4C_1$					$^4C_1$	$^4C_1$
	Φ, Ψ (°)		101, -117					107, -91	129, -116
	Φ, θ (°)	109, 7	66, 15	19, 17	77, 14	119, 38	67, 26		
10 mM	Q	0.54	0.55	0.53	0.54	0.51	0.50		
α-CD	conformation	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$		
	Φ, Ψ (°)		110, -130	106, -98	110, -122	106, -101	114, -115	112, -86	
	Φ, θ (°)	21, 8	61, 7	92, 15	336, 21	116, 13	103, 6	338, 10	126, 33
10 mM	Q	0.55	0.53	0.54	0.52	0.56	0.54	0.56	0.51
γ-CD	conformation	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$
	Φ, Ψ (°)		101, -128	135, -101	110, -133	129, -128	122, -119	126, -101	113, -123	120, -108

(*b*)

		GLC 1	GLC 2	GLC 3	GLC 4	GLC 5	GLC 6	GLC 7	GLC 8
	Φ, θ (°)	30, 10	34, 11	97, 17	17, 10	33, 10	72, 8	342, 13	
1 mM	Q	0.57	0.55	0.56	0.58	0.54	0.47	0.57	
β-CD	conformation	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	
	Φ, Ψ (°)		106, -129	118, -103	121, -105	114, -121	108, -118	118, -109	124, -119
	Φ, θ (°)	28, 15	14, 17	29, 19	101, 11	47, 17	44, 10		
10 mM	Q	0.55	0.53	0.60	0.51	0.57	0.53		
α-CD	conformation	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$		
	Φ, Ψ (°)		140, -131	107, -104	117, -120	110, -110	89, -99	117, -106	
	Φ, θ (°)	56, 12	82, 16	160, 7	77, 15	47, 10	58, 9	111, 27	305, 10
10 mM	Q	0.58	0.52	0.59	0.58	0.54	0.53	0.50	0.57
γ-CD	conformation	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$	$^4C_1$
	Φ, Ψ (°)		104, -137	128, -102	123, -112	130, -112	93, -124	124, -105	123, -108	117, -121

Table S3 Interaction between residues of CBM41 and CDs.

Hydrogen bond, C-C clash contacts and C-C contacts distances were in the ranges of 2.45 Å to 3.24 Å, 3.25 Å to 3.94 Å and 3.95 Å to 4.44 Å, respectively. Distances were calculated using Contact from the CCP4 program suite (Collaborative Computational Project, Number 4, 1994). The complex structures observed the C-C contacts and C-C clash contacts were written in parentheses. The apostrophe indicates a residue in the symmetric molecule.

Sugar atom	Protein atom	1 mM α-CD	1 mM β-CD	1 mM γ-CD	10 mM α-CD	10 mM γ-CD	C-C contacts	C-C Clash contacts
GLC 1								
O3	Lys133 NH	2.80	3.04	2.64	2.91	2.68	Trp95	Trp95
O2	Lys133 NH		2.91	3.23	3.18	3.14	Lys133 (1α,1β,1γ,10γ)	Lys133
GLC 2								
O6	Tyr78 OH	2.67	2.59	3.07	2.94		Tyr78 (1β,1γ,10α,10γ)	Tyr78 (1α,1β,10γ)
O2	Asp138 OD1	2.95	3.19	3.21	2.88	2.93	Trp80	Trp80 (1α,1γ,10α,10γ)
O2	Asp138 OD2	3.07		2.80	3.08	2.77	Trp95 (1β, 10γ)	Ile126 (1α,1β,10α,10γ)
O3	Asp138 OD2		2.74	3.10	2.64	2.67	Ile126 (1β,1γ) Asp138 (1α,1γ,10α)	Asp138 (1α,10α,10γ)
GLC 3								
O2	Trp80 NE2		2.94				Trp80 (1β,10γ)	
O2	Trp80 NE1					3.03	Asn82 (1β,10γ)	Trp80 (10γ)
O2	Asn82 ND2		3.00			3.22	Asp138 (1β)	
GLC 4								
GLC 5								
O3	Asp594’ OD1	3.19					Gln593’ (1α)	Gln593’ (1α,10α)
GLC 6								
O2	Asp594’ OD1	2.66 2.89			2.78		Gln593’ (1α,1β,10α)	
O3	Asp594’ OD1		3.02				Asp594’	Gln593’
O3	Gln455’ NE2		2.71				(1α,10α)	(1β,10γ)
O6	Gln593’ NE2		2.97				Glu404’ (10γ)	
GLC 7								
O2	Asp594’ OD1		2.47				Gln593’	
O3	Gln455’ NE2			2.74		2.64	(1β,1γ,10γ)	Asp594’
O3	Asp594’ OD1					2.78	Asp594’	(1β)
O6	Wat			3.06			(1β,1γ,10γ)	
GLC 8								

O2	Asp594' OD1	2.75	2.57	Gln593' (10γ) Asp594' (10γ)

Table S4 Interaction between residues of the active site and CDs.

Hydrogen bond, C-C clash contacts and C-C contacts distances were in the ranges of 2.45 Å to 3.24 Å, 3.25 Å to 3.94 Å and 3.95 Å to 4.44 Å, respectively. Distances were calculated using Contact from the CCP4 program suite (Collaborative Computational Project, Number 4, 1994). Arrow shows the water-mediated hydrogen bond. The complex structures observed the C-C contacts and C-C clash contacts were written in parentheses.

Sugar atom	Protein atom	1 mM β-CD	10 mM α-CD	10 mM γ-CD	C-C contacts	C-C Clash contacts
GLC 1						
O2	Asp734 OD2	2.77	2.84	2.67	Trp708	
O2	Arg737 NH2	2.99	2.96	3.05	Asp734	Trp708
O3	Arg737 NH1	2.97	2.74	2.85	Arg737 (10α)	Pro745 (1β)
O6	Wat	2.50	2.56		Pro745 (10α,10γ)	
O6	Wat←Asp709		2.79		Phe746 (10α)	
GLC 2						
O2	Asn835 ND2	3.03				
O2	Asn835 OD1		2.92	3.02		
O3	Asn835 OD1	3.24				
O3	Asn835 ND2		3.19	3.17	Trp708	Trp708 (10α)
O3	Wat→Asp834		3.21		Phe746 (10α,10γ)	Leu678 (10γ)
O6	Wat←Glu706	2.59	2.92			
O6	Wat←Thr642	2.74				
O6	Wat←Ala641		3.05			
GLC 3						
O2	Wat←Asn835	3.12			Phe746 (10α)	Phe746 (10α)
O3	Arg889 NH2			2.67		
GLC 4						
O6	Wat		3.10		Phe746 (10α)	Phe746 (10α)
GLC 5						
					Phe746 (1β, 10α)	Phe746 (10α)
GLC 6						
O3	Wat←Phe746	2.98			Phe746	Phe746 (1β, 10α)
GLC 7						
O2	Wat←Asp738	2.94			Phe746 (1β)	
O3	Wat←Asp738	2.53			Asn813 (1β)	
GLC 8						
					Asn813 (10γ)	