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High-resolution Crystal Structure of a Truncated ColE7 Translocation Domain: Implications for Colicin Transport Across Membranes

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ColE7 is a nuclease-type colicin released from Escherichia coli to kill sensitive bacterial cells by degrading the nucleic acid molecules in their cytoplasm. ColE7 is classified as one of the group A colicins, since the N-terminal translocation domain (T-domain) of the nuclease-type colicins interact with specific membrane-bound or periplasmic Tol proteins during protein import. Here, we show that if the N-terminal tail of ColE7 is deleted, ColE7 (residues 63–576) loses its bactericidal activity against E. coli. Moreover, TolB protein interacts directly with the T-domain of ColE7 (residues 1–316), but not with the N-terminal deleted T-domain (residues 60–316), as detected by co-immunoprecipitation experiments, confirming that the N-terminal tail is required for ColE7 interactions with TolB. The crystal structure of the N-terminal tail deleted ColE7 T-domain was determined by the multi-wavelength anomalous dispersion method at a resolution of 1.7 A °. The structure of the ColE7 T-domain superimposes well with the T-domain of ColE3 and TR-domain of ColB, a group A Tol-dependent colicin and a group B TonB-dependent colicin, respectively. The structural resemblance of group A and B colicins implies that the two groups of colicins may share a mechanistic connection during cellular import.

Crystal Structure of ColE7 Translocation Domain

Table 1. Data collection, phasing and refinement statistics of ColE7 T-domain

A. *Diffraction data statistics*			
Space group		$P3_121$	
Cell constants (Å and deg.)		$a=59.04, b=59.04, c=132.18\ \alpha=90, \beta=90, \gamma=120$	
Wavelength (Å)	λ_1, 0.9795	λ_2, 0.9797	λ_3, 0.9805
Resolution range (Å)	40–1.7	40–1.7	40–1.7
Observed reflections	315,209	304,670	288,166
Unique reflections	29,971	29,940	29,879
Completeness (%)[a]	99.3 (100.0)	99.1 (100.0)	98.8 (100.0)
$\langle I \rangle / \langle \sigma I \rangle$[a]	68.8 (11.8)	67.2 (12.3)	64.9 (13.1)
R_{merge} (%)[a,b]	8.5 (27.2)	6.3 (24.7)	6.2 (22.9)
B. *Phasing statistics*			
Number of Se sites	4	4	4
Phasing power (centric/acentric)	3.54/3.47	3.61/3.52	2.18/2.15
Figure of merit (centric/acentric)		0.75/0.70	
C. *Refinement statistics*			
Resolution range (Å)			40–1.7
Reflections (working/test)			26,591/2959
R_{work}/R_{free} (%)			18.1/20.9
Number of atoms (protein/water)			1704/303
Average *B*-factor (Å^2) (protein/water)			21.4/33.8
RMS deviations (bond length (Å)/bond angle (degree))			0.015/1.759

[a] The numbers in parentheses are for the last shell in the resolution range of 1.76–1.70 Å.
[b] $R_{merge} = \sum_h \sum_i |I_{hi} - \langle I_h \rangle| / \sum_h \sum_i I_h$, where $\langle I_h \rangle$ is the mean intensity of i observations for a given reflection h.

TAABLE 1

T-domain was then produced and crystallized. Three sets of X-ray diffraction data were collected using synchrotron radiation at different wavelengths at beamline NW12 in the KEK Photon Factory, Tsukuba, Japan. The structure was solved by the MAD method using anomalous signals from four Se-atoms.

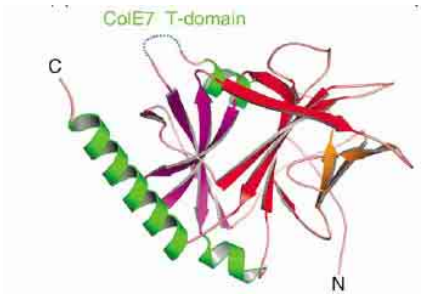

FIGURE 1

Structures of the ColE7 T-domain and its superposition onto ColE3 and ColB. (a) The crystal structure of the ColE7 T-domain contains three central layers of b-sheets (in magenta, red and gold) flanked by three a-helices. The N-terminal end (residues 60–75) and a loop region from residues 120–123 (displayed in a broken line) are disordered and not seen in the structure.

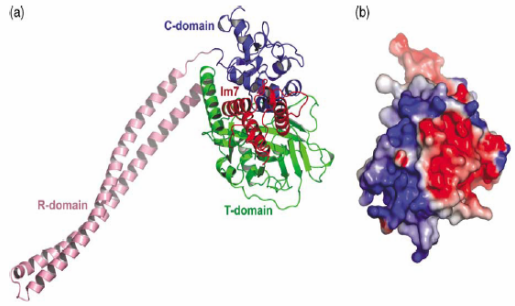

FIGURE 2

Structural model of ColE7/Im7 complex. (a) The crystal structure of the ColE7 T-domain was docked with the crystal structure of the C-domain/Im7 complex (PDB entry, 1MZ8) by ZDOCK.28 The full-length ColE7/Im7 model was constructed using the crystal structure of ColE3 (PDB entry, 1JCH) as a template to assemble the R-domain with T/C domains by Modeller 8.1.41 (b) The molecular surface of the ColE7 T-domain is mapped with electrostatic potential (electropositive charges in blue and negative in red). This view is rotatedw1808 vertically from the one in (a) to show the convex surface, which is facing outward, not blocked by the C-domain and Im7. The left region of the T-domain in this view is positively charged and this surface is highly conserved among ColE2, E3, E6, E7 and E9. This conserved basic region is suggested to be tested for a role in protein or membrane interactions during colicin import.