

POSTER PRESENTATION

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The effector protein ExoY secreted by *Pseudomonas aeruginosa* is a nucleotidyl cyclase with preference for GTP

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Background

Pseudomonas aeruginosa is an important opportunistic pathogen causing serious pulmonary, urogenital and systemic infections. *P. aeruginosa* injects four effector proteins into host cells via the type III secretion system. ExoY is one of these proteins.

ExoY was originally classified as adenylyl cyclase with homology to the typical calmodulin-stimulated adenylyl cyclase exotoxins CyaA from *Bordetella pertussis* and edema factor from *Bacillus anthracis*, but the pathophysiological function of ExoY has remained elusive [1,2]. Recently, our group showed that CyaA and edema factor possess a rather broad base specificity (ATP >> CTP > UTP) [3,4], raising the question of whether ExoY may also bind and metabolize nucleoside 5'-triphosphates other than ATP.

Methods

We determined cyclic nucleotide concentrations in cells transfected with ExoY plasmid or infected with *P. aeruginosa* with a highly sensitive HPLC-MS/MS method. Moreover, we determined the catalytic activity of purified ExoY.

Results

In mammalian cells transfected with ExoY plasmid and infected with ExoY-encoding *P. aeruginosa*, massive production of cGMP and cUMP was observed, with little production of cAMP. Purified ExoY was a highly effective nucleotidyl cyclase with the substrate preference GTP >> UTP ~ ATP > CTP. Fluorescence resonance energy transfer studies with methylanthraniloyl-substituted nucleotides corroborated the preference of ExoY for GTP. In contrast to ExoY, CyaA induced accumulation

Table 1 Comparison of ExoY with CyaA and EF

Parameter	ExoY	CyaA	EF
Bacterial source	<i>Pseudomonas aeruginosa</i>	<i>Bordetella pertussis</i>	<i>Bacillus anthracis</i>
Secretion type	III	I	II
Function as toxin	Unknown, "antitoxin?"	yes	yes
Activation mechanism	Cytosolic cofactor (mammalian cells and <i>D. discoideum</i>)	calmodulin	calmodulin
Substrate-specificity purified enzyme	GTP >> UTP ≥ ATP > CTP	ATP >> CTP > UTP > GTP	ATP >> CTP > UTP > GTP
Substrate-specificity intact cells	UTP ~ GTP > ATP > CTP (except for B103 cells)	ATP >> CTP > UTP >> GTP (ineffective)	ATP >> CTP >> UTP and GTP(ineffective)

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of cyclic nucleotides in the order cAMP > cCMP > cUMP in mammalian cells, and edema factor induced only cAMP- and cCMP accumulation.

Conclusion

ExoY is a nucleotidyl cyclase with preference for GTP, and the substrate-specificity of ExoY is clearly different from that of CyaA and edema factor. Our data open the door for future studies aiming at the elucidation of the as yet unknown pathophysiological function of ExoY and which role cCMP, cGMP and cUMP play in this process.

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