

Le donne, l cavalieri, le armi e gli amori or: how bacteria defend themselves

Annalisa Pastore

Matisse's women...



Musco et al. (1997) Nat Struct Mol Biol Musco et al., (2000) Structure

I share my time between



The Wohl Institute, King's College London



Scuola Normale Superiore, Pisa

An institute for neuroscience





A different encounter...



...outside neurodegeneration

Bacteria: a world by their own...



Bacterial capside



The importance of sporulation



Capsules



A shield against ennemies



In most cases the capsule is made of saccharides



In other cases... Poly gamma glutamate (PGA)







Glutamate = umami





Poly gamma glutamate = Natto



Fermented soy beans

The properties of PGA

Viscous

Able to englobe water and metals

Robust

Difficult to hydrolase

PGA nanoparticles

γ-PGA



Chitosan

These properties make PGA important for:

- Fight against antibiotic resistance

- Drug delivery

- Food industry

- Soil protection

The pathway of PGA



Schematic representation of the regulatory system of g-DL-PGA synth

subtili



A new family of PGA hydrolases



RESEARCHARTICLE

γ-PGA Hydrolases of Phage Origin in *Bacillus* subtilis and Other Microbial Genomes

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Pgh proteins from B. anthracis and B. subtilis





Stereoselectivity

Cleavage of D-PGA, L-PGA or DL-PGA



Removal of capsule from encapsulated *B. anthracis* by CapD and PghP



PghZ is unresponsive



We selected PghL of B. subtilis



A non toxic bacillus A good model for new studies A well behaved protein

PghL secondary structure and stability



A monodispersed protein No difference between Zn bound and Zn free Zinc does not contribute to the protein stability

Analysis of the pattern of degradation



Mechanism of cleavage

Endopeptidase

The smallest fragments are penta and hexa-peptides

23 Chromatogram А 40000 30000 M 20000 10000 20.0 40.0 60.0 Chromatogram 50000 В 40000 30000 [h] ity 1 20000 10000 20.0 40.0 60.0 0.0 Chromatogram 2 70000 С 60000 50000 40000 30000 20000 3 10000

20.0

0.0

40.0

60.0

The presence of EDTA does not affect cleavage



0 10 50 100 200 10 50 100 200 EDTA(mM)

Getting crystals of PghL



Very minor difference between PghL and PghP



We were lucky...



Serendipity: Molecular dimension idea

Features of The PGA Screen:

- A stand-alone new protein precipitant.
- Easy mixing properties with other PEGS.
- Suitable for soluble and membrane protein crystallization.
- Non-toxic and non-denaturing.
- Compatible with liquid—handling robots.



Examples of lysozyme crystals grown from a variety of PGA-LM conditions.

PghL structures



Zinc free

Zinc bound

Zinc and PGA bound

The answer to several important questions

What is the role of Zinc?

How do Pgh recognize PGA?

What determines the minimal cleavage peptide?

What determines specificity of PGA hydrolases

What determines stereoselectivity?

PghL is a Zinc dependent hydrolase



Zinc coordination



Zinc is not structurally important

Superposition of the three structures



Zinc has no structural role No activation is needed

Superposition of PghL and PghP



Superposition with CPA



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The structural determinant of the interactions



Interactions between PGA and PghL



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PghP	DPDN	VNR	CA <mark>SG</mark> ł	< <mark>GVC</mark>	LEI	SSA	GRRAF	FCN	NDVS	KAN	- <mark>G</mark> N	/T <mark>CEF</mark>	LD-	YAEA	KEAE	AEY	YG	208
YmaC	NAEN	NNG	ETG	_ <mark>SI</mark> C	LEI	SRE	GREAF	FD-	- DF \	YK NF	KYTH	(N<mark>S</mark>EF	Y <mark>A</mark> -	YVS A	i <mark>k</mark> gvl	EKE	Y S	235
YoqZ	NEGN		IS<mark>TG</mark>I	V <mark>SI (</mark>	LEI	STE		/F	NT _{F1}				YD ÿ	YI AV	LTKFI	NEN	VY <mark>CNAGVAP</mark>	27
YjqB	HPNN	VNR 1	'K <mark>TE</mark>	VGLC	LE\	/STA		_FR-	- NFC		(S <mark>YI (</mark>		DR-	YVEA'	VKRGF	YK-		200
YndL	S <mark>P</mark> NN	ANKS	6K <mark>TG</mark> I	_ <mark>SI</mark> C	I EN	<mark>/Stg</mark> f	RKSL	_F <mark>C</mark> -	- FS	SLKSF	AVTO	INERF	YE-	FTEV	NFR- F	FL <mark>KN</mark>	SY	252
	. 220.		23	30		24	10 .		. 250)	2	260		27	0		280	

A single mutation is sufficient to abrogate activity



The answer to several important questions

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What determines stereoselectivity?

Conclusions

We have solved the structures of a new PGA hydrolase in three different states

Zinc has no structural role

The PGA bound structure explains specific features

Structure can inform mutations to test activity

Acknowledgments

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Thank you for your attention!



Lele Luzzati, Zauberflute