Ovalbumin related protein X is a heparin-binding glycoprotein exhibiting antimicrobial activities

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OVALBUMIN-RELATED PROTEIN X IS A HEPARIN-BINDING GLYCOPROTEIN EXHIBITING ANTIMICROBIAL ACTIVITIES

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INTRODUCTION

► The egg white is a nutritious reserve and a source of bioactive molecules for embryo

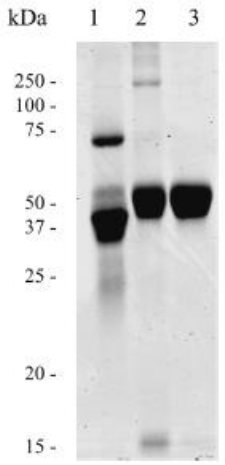
► Characterize the biological activities of egg white proteins

► Ovalbumin-related protein X (OVAX) belongs to the ovalbumin family which consists of 3 related genes : Ovalbumin, OVAX and OVAY which have evolved from a common ancestor gene

► Ovalbumin is the major egg white protein (about 50 mg/mL of egg white) of still unknown function

► OVAX and OVAY not yet characterized

► egg white OVAX purification method developed (Estimated concentration : 0,3 mg/mL)



INTRODUCTION

Author's Choice

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 288, NO. 24, pp. 17285–17295, June 14, 2013

Ovalbumin-related Protein X Is a Heparin-binding Ov-Serpin Exhibiting Antimicrobial Activities*[S]

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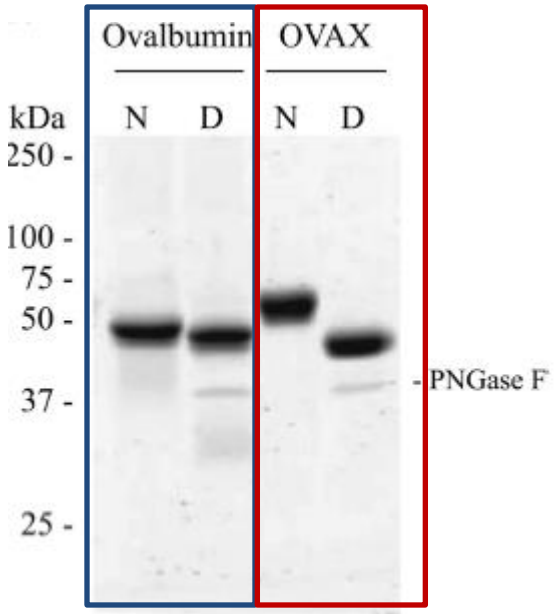
Biochemical characterization

Ovalbumin-related protein X glycosylation



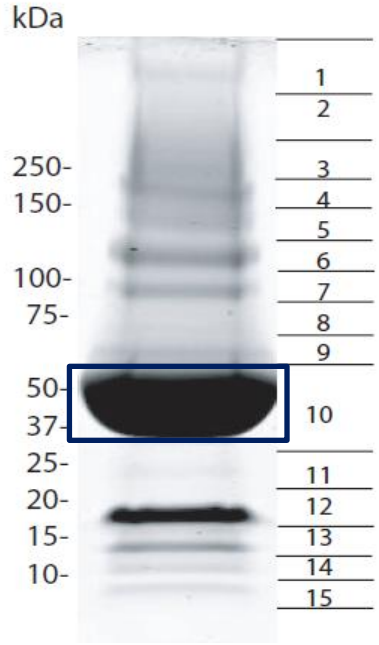
Ovalbumin
1 glycosylation site

OVAX is highly glycosylated

5 predicted sites
3 confirmed by mass spectrometry

Biochemical characterization

Ovalbumin-related protein X has a high affinity for heparin (a negatively charged glycosaminoglycan)

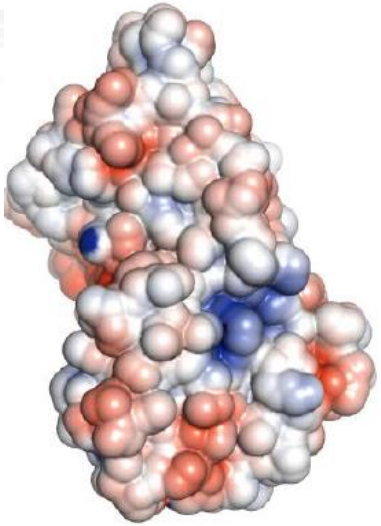


OVAX is the main heparin-binding protein of egg white

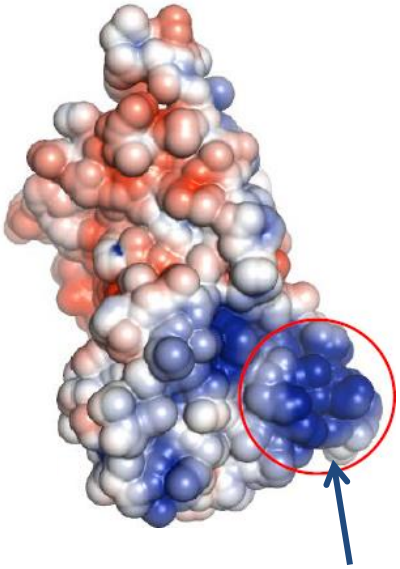
In constrast, ovalbumin has no affinity for heparin although both proteins share 60 % protein sequence identity

3D structure modeling of OVAX

Ovalbumin



OVAX



Presence of a cluster of exposed positive charges= heparin-binding domain

Identification of the sequence of the potential heparin-binding site

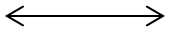
STQTKVQKPKCGKSVNIHLLFKELLSDITASKANYSLRIANRLYAEKSRPILPIYLKCVKK

→ Antimicrobial activity ?

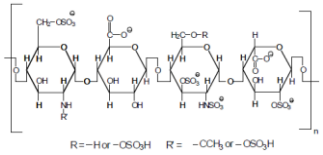
Biological activities

Biological activity of Ovalbumin-related protein X : antibacterial ?

Heparin-binding proteins
Cluster (s) of exposed positives charges



Heparin
Negatively charged glycosaminoglycan

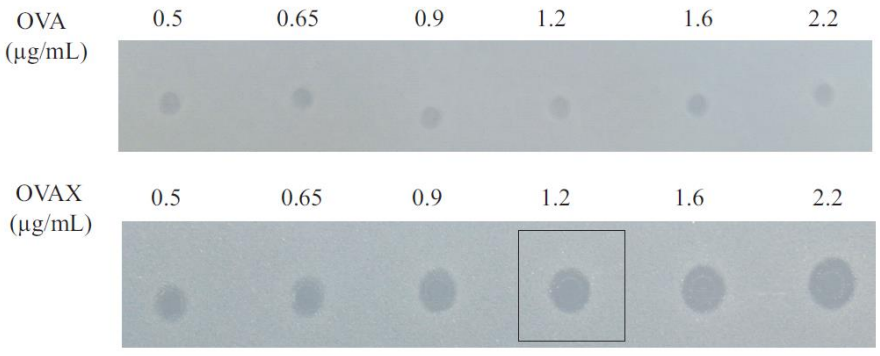


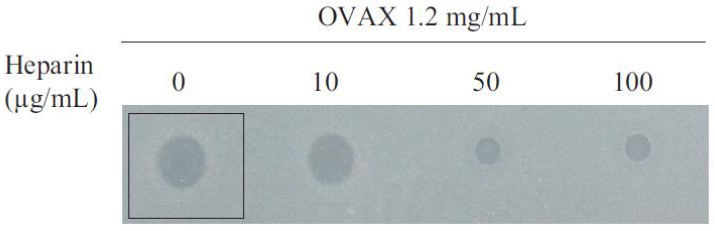
Other negatively charged surfaces
Bacterial lipopolysaccharide
Peptidoglycan

Biological activities

Antibacterial activity

Radial diffusion Lehrer assay on 2 Gram positives and 2 Gram negative bacteria





► Anti-*Salmonella* activity depends on heparin-binding site(s)

Minimal active concentration of purified OVAX, ovalbumin, and av-BD11 (positive control)

MAC indicates minimum active concentration (corresponding to a 0.5-mm clear zone). Gram+, Gram positive; Gram−, Gram negative.

Bacterial group/strains	MAC (µM) ± S.D.		
	AvBD11	Ovalbumin	OVAX
L. monocytogenes (Gram+)	0.90 ± 0.83	>28	1.90 ± 0.49
S. aureus ATTCC 29740 (Gram+)	7.73	>37	>37
S. enterica sv. Enteritidis ATCC 13076 (Gram−)	0.79 ± 0.49	>51	10.02 ± 1.36
E. coli ATCC 25922 (Gram−)	1.6	>37	>37

OVAX

- OVAX is a heparin –binding glycoprotein with antibacterial activities
- The antibacterial activity depends on heparin-binding site(s)

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PCT

(10) Numéro de publication internationale
WO 2011/151407 A1

Peptides derived from heparin-binding domain of OVAX might have antimicrobial activity of increased value as compared to OVAX ?

Eur. J. Biochem. **271**, 1219–1226 (2004) © FEBS 2004 doi:10.1111/j.1432-1033.2004.04035.x

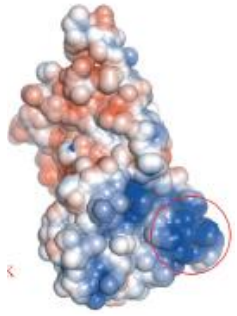
Antimicrobial activities of heparin-binding peptides

Emma Andersson[1], Victoria Rydengård[1], Andreas Sonesson[1], Matthias Mörgelin[2], Lars Björck[2] and Artur Schmidtchen[1]

OVAX derived peptides

Predicted amino acid sequence of the heparin-binding site of OVAX

STQTKVQKPKCGKSVNIHLLFKELLSDITASKANYSLRIANRLYAEKSRPILPIYLKCVKK



Conception and Characterization of OVAX-derived peptides *(Beauclercq et al. 2013)*

CAMP (http://www.bicnirrh.res.in/antimicrobial/) *(Thomas et al., 2010)*
Predict antimicrobial activity of peptides based on a collection of known antimicrobial peptides

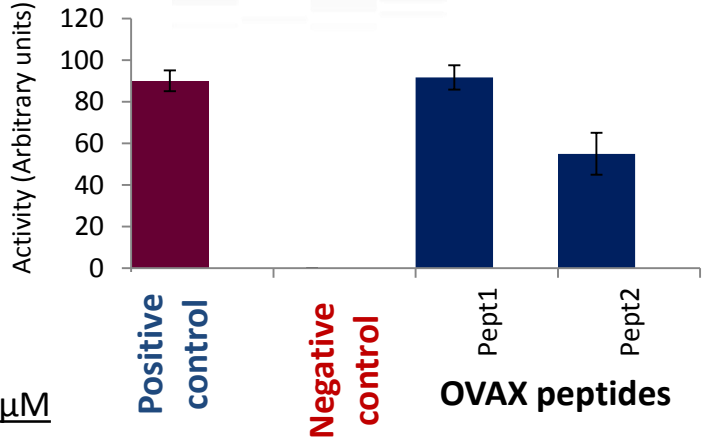
	Name	Sequence	CAMP (SVM)
Positive control	AKK15	AKKQRFRHRNRKGYR	0.953
Negative control	KNN15	KNNQKSEPLIGRKKT	0
OVAX	Peptide 1	17 aa (heparin-binding site)	0.929
	Peptide 2	13 aa (heparin-binding site)	0.778

Andersson *et al. 2004*

OVAX derived peptides

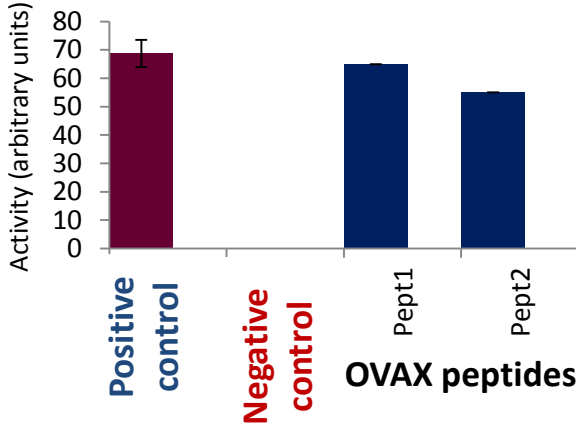
Antibacterial activity

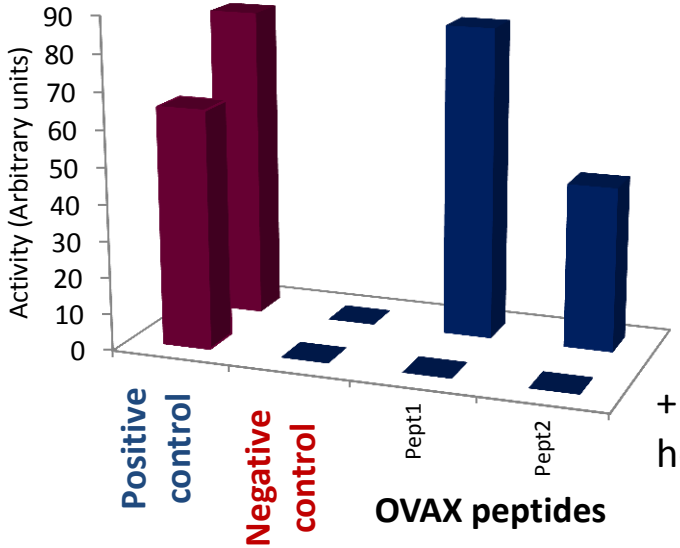
Activity against *Listeria monocytogenes*



100 µM

Activity against *Salmonella* Enteritidis





+ 100 µg/mL heparin

- Activity depends on the heparin-binding site

Conclusions

- OVAX is a heparin –binding glycoprotein with antibacterial affinity
- The antibacterial activity depends on heparin-binding site(s)
- OVAX-derived peptides containing heparin-binding sites are antibacterial

FUTURE STUDIES : the MUSE Project

Région Centre

- to confirm experimentally the heparin-binding site of OVAX
- to determine affinity constants of peptides to heparin (Surface Plasmon Resonance BIAcore)
- to screen antimicrobial activities of OVAX and derived peptides (strains with resistance to antibiotics, parasites, viruses) and assess other biological activities (immunomodulatory, anticancerous…)

Potential use and added values of identified molecules with biological activities → food and non food use of egg

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IMPACT (2013-2017)
(Identification of Matrix Proteins Affecting Calcite Texture in chicken and guinea fowls eggshells)

Scientists


Joël GAUTRON (DR2)


Yves NYS (DR1)


Sophie REHAULT -GODBERT (CR1)


Nicolas GUYOT (CR2)

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Maryse MILLS


Jean-Claude POIRIER


Magali BERGES

ITAVI


Angélique TRAVEL (Engineer)

SABRE
CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

SABRE
(2006-2010)