OH1 from Orf virus: a new tyrosine phosphatase that displays distinct structural features and triple substrate specificity

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OH1 from Orf virus: a new tyrosine phosphatase

Distinct structural features & triple substrate specificity

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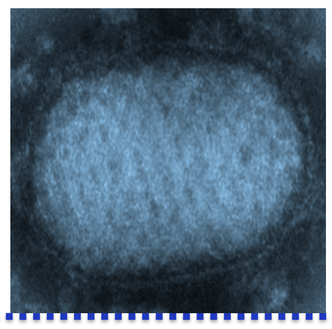
Orf virus -ORFV- is the causative agent of contagious ecthyma. It belongs to the Parapoxvirus genus of the *Poxviridae* family (poxviruses) and infects a wide range of animals. It has been responsible for widespread pandemics, such as Variola virus in humans[1]. The DNA genome of poxviruses contains at least 90 conserved genes essential for viral replication and specific additional genes involved in pathogenesis and interaction with the host[2].

ORFV elicits a short-lived immune response in the host, contributing to multiple reinfections in animals[3]. This feature is further enhanced by the presence of viral genes that modulate the host immune response. Among these regulators, ORFV encodes for a tyrosine phosphatase -named OH1- that is widely conserved in poxviruses. OH1has possibly a role in the inhibition of the host JAK-STAT signaling pathway[4], analogous to the role of the homologous protein VH1 in Vaccinia virus[5-6].

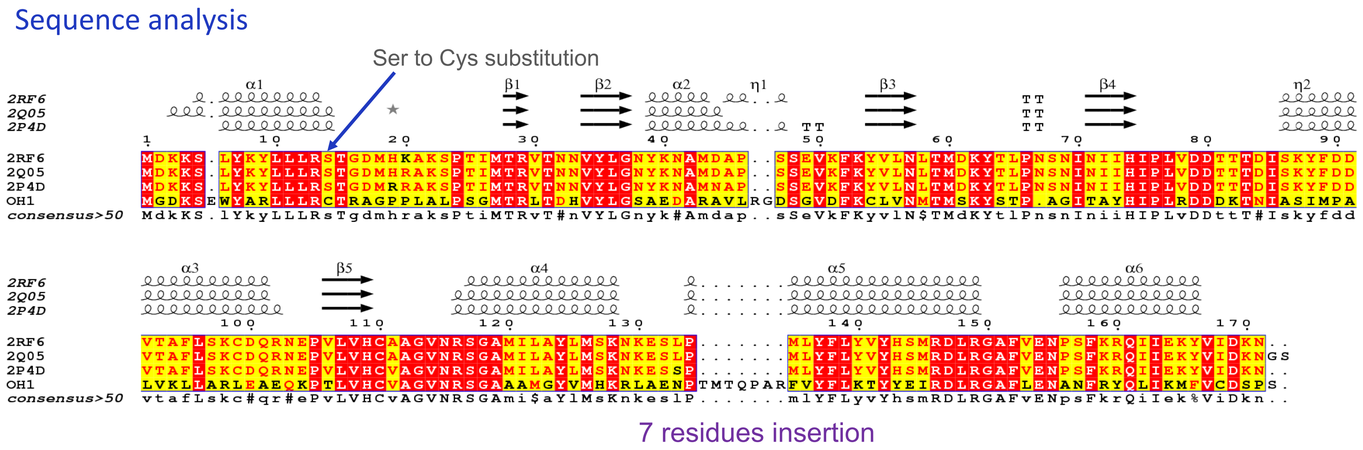
VH1 was structurally characterized in both Vaccinia and Variola virus[7-8] and was shown as the first dual specificity phosphatase DUSP[9]. Vaccinia VH1 structure reveals a typical DUSP fold and a homodimeric quaternary organization with an extensive domain swapping of the N-terminal α-helices stabilized by non-covalent interactions.

This dimerization is proposed to be a structural and mechanistic feature to regulate & recognate its putative substrate STAT1[10].

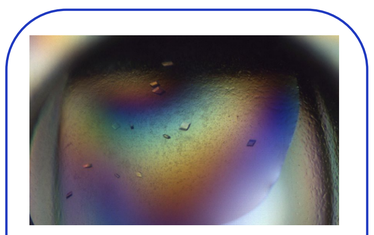
Sequence analysis and homology modeling of OH1, using VH1 as 3D template, revealed both a 7 residues gap insertion and a critical Ser to Cys substitution that could impede the quaternary dimeric organization. We investigate *in vitro* and *in silico* OH1 as a virulence factor phosphatase.



Sequence analysis



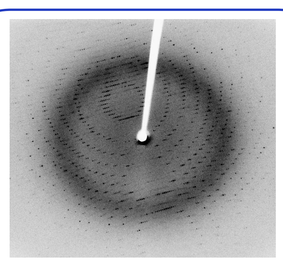
7 residues insertion



Cristal conditions:
200mM $NH_4 2SO_4$
100 mM NaAc, pH 4.6
PEG MME 2K 30%
Cryo-protected with oil

Data collected at PX1

SOLEIL SYNCHROTRON



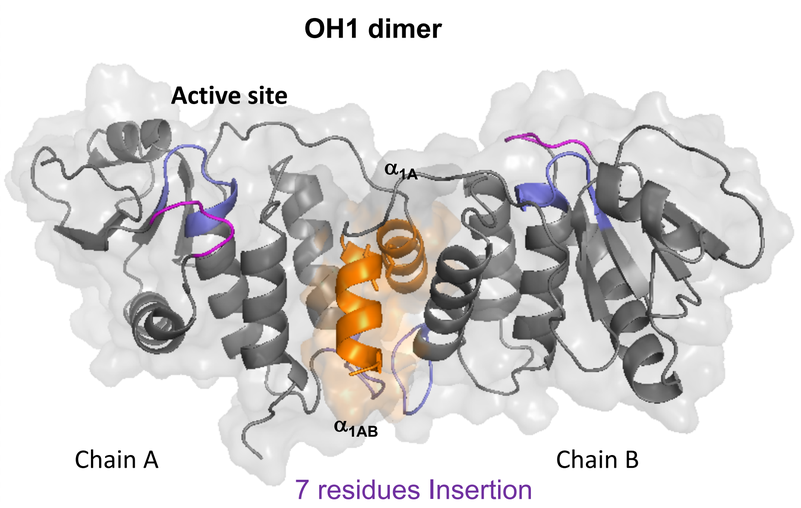
Data statistics	
Space group	$P2_1$
Cell dimensions	
a, b, c (Å)	49.56, 63.55, 55.39
α, β, γ (°)	90.00, 97.07, 90.00
Resolution (Å)	41.58-1.88 (1.97-1.89)

Homology modeling

Crystal structure resolution at 1.89 Å

Homodimer : remarkably stabilized by a covalent disulfide bond

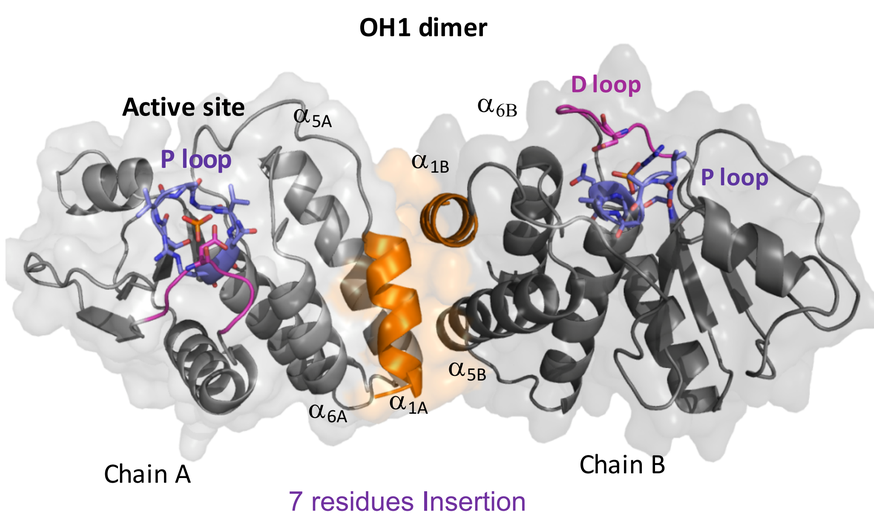
OH1 dimer



7 residues Insertion

Chains A and B modeled with modeler mod9.13 with Vaccinia VH1 as template.
Reconstruction of the homodimer using PyMOL –align. Check with Coot.

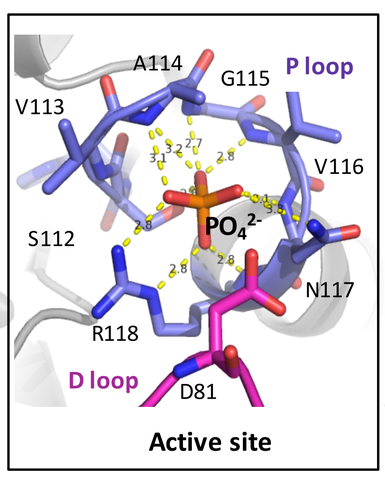
OH1 dimer



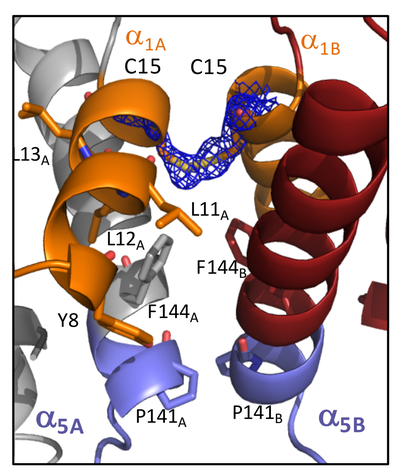
7 residues Insertion

Refinement using CCP4-Phaser, buster and coot, Rwork/Rfree 18.72/21.69

5ncr pdb deposited



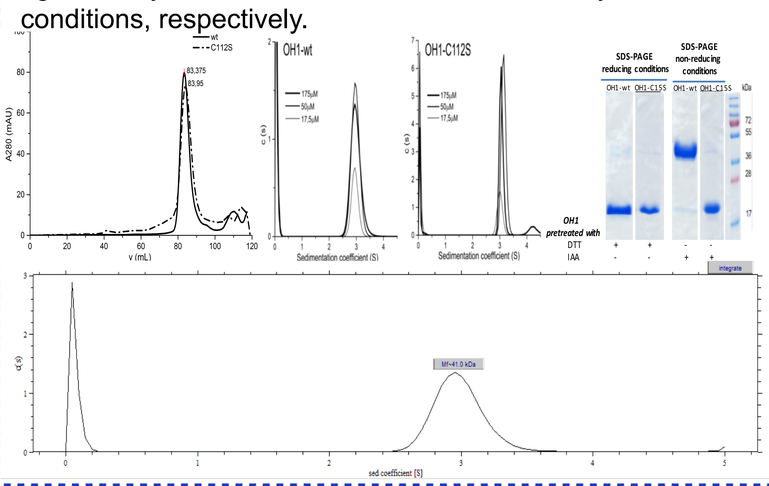
A conserved active site

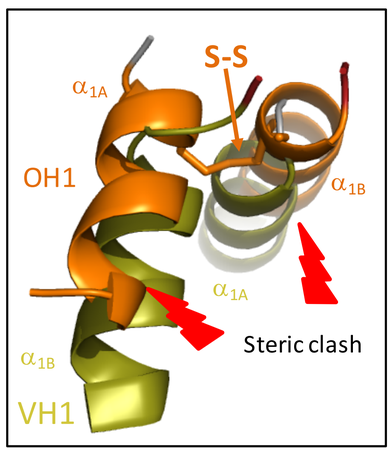


7 residues Insertion
Extra helical turn
Ser to Cys: disulfide bridge

Covalent dimerization: disulfide bridge incompatible with domain swapping of α1

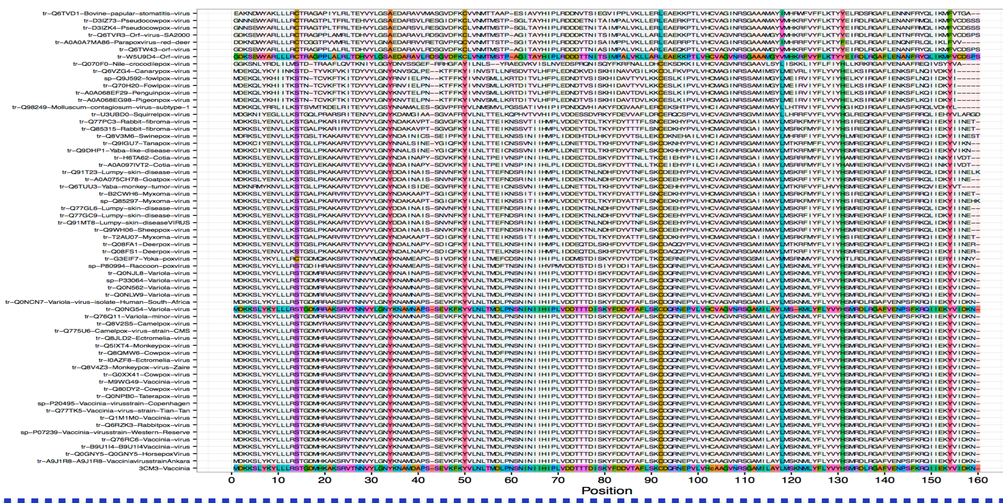
To confirm that Cys 15 is involved in the covalent dimerization of OH1 in solution, an OH1-C15S mutant was produced and purified, and its covalent dimer formation capacity was evaluated. OH1-wt and OH1-C15S proteins were pretreated with a reducing (DTT) or an alkylating (IAA) agent of Cys, and the results were evaluated by SDS-PAGE under reducing and non-reducing conditions, respectively.





Phylogenetic studies: how parapoxvirus associates Ser 15 to Cys substitution with other aa changes

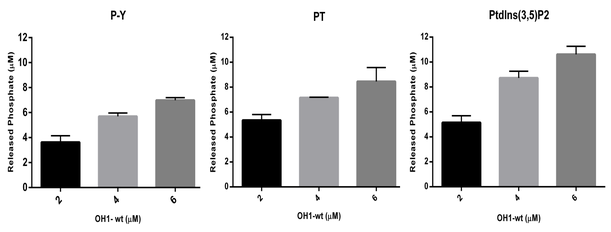
OH1 could represent Parapoxvirus genus phosphatases, since Cys15 is only conserved within this group, and is absent in all members of the Orthopoxvirus genus, such as Vaccinia/Variola VH1, and several other members of the Poxviridae family.

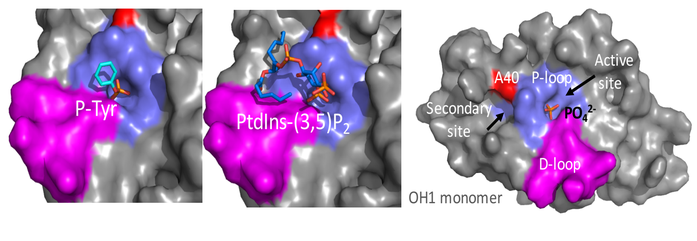


Multiple sequence alignment of 64 phosphatases with 6 sequences from the Parapoxvirus genus (top 6 sequences). The position of conserved residues specific to parapoxvirus that strongly correlated to the N-terminal Ser 15 to Cys substitution is highlighted.

Functional characterization and docking of substrates

OH1 is a dual specificity phosphatase.
OH1 unexpectedly reveals its ability to dephosphorylate phosphatidylinositol 3,5 biphosphate.





Docking analysis, using ADT, of several phosphatidylinositol phosphates confirm that they can be accommodated in the active site of OH1. This new activity could be relevant in phosphoinositide recycling during virion maturation.

Conclusions and perspectives

- OH1 displays structural features compared to viral VH1 phosphatases.
- Orf virus OH1 phosphatase is a covalent dimer involving the N-terminal Cys15.
- OH1 possibly depicts the structure of Parapoxvirus genus phosphatases.
- OH1 is a dual specificity phosphatase that presents activity towards PInsP *in vitro.*
- By analogy with VH1, the homodimer could recognate & regulate its host STAT1.
- Pull-down experiments of OH1 with STAT1, and protein-protein docking of OH1 complexed to STAT1 are in progress.

Bibliography

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