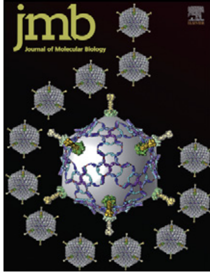

OH1 from Orf Virus: A New Tyrosine Phosphatase that Displays Distinct Structural Features and Triple Substrate Specificity

Danilo Segovia[1], Ahmed Haouz[2], Darío Porley[1,3], Natalia Olivero[3], Mariano Martínez[4], Mahendra Mariadassou[5], Mabel Berois[3], Gwenaëlle André-Leroux[5] and Andrea Villarino[1]

*1 - **Sección Bioquímica y Biología Molecular,** Facultad de Ciencias, UdelaR, 11400 Montevideo, Uruguay*
*2 - **Institut Pasteur,** Plate-forme de Cristallographie, CNRS-UMR 3528, 75724 Paris, France*
*3 - **Sección Virología,** Facultad de Ciencias, UdelaR, 11400 Montevideo, Uruguay*
*4 - **Institut Pasteur,** UMS, CNRS-UMR 3528 and Université Paris Diderot, 75724 Paris, France*
*5 - **MaIAGE,** INRA, Université Paris-Saclay, 78350 Jouy-en-Josas, France*

***Correspondence to Mabel Berois, Gwenaëlle André-Leroux and Andrea Villarino:** mabber@fcien.edu.uy; gwenaelle.andre-leroux@inra.fr; avillarino@fcien.edu.uy*
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Abstract

Viral tyrosine phosphatases such as VH1 from Vaccinia and Variola virus are recognized as important effectors of host–pathogen interactions. While proteins sharing sequence to VH1 have been identified in other viruses, their structural and functional characterization is not known. In this work, we determined the crystal structure of the VH1 homolog in the Orf virus, herein named OH1. Similarly to Variola and Vaccinia VH1, the structure of OH1 shows a dimer with the typical dual-specificity phosphatase fold. In contrast to VH1, the OH1 dimer is covalently stabilized by a disulfide bond involving residue Cys15 in the N-terminal helix alpha-1 of both monomers, and Cys15 is a conserved residue within the Parapoxvirus genus. The *in vitro* functional characterization confirms that OH1 is a dual-specificity phosphatase and reveals its ability to dephosphorylate phosphatidylinositol 3,5-bisphosphate, a new activity potentially relevant in phosphoinositide recycling during virion maturation.

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Introduction

Orf virus (ORFV), the causative agent of contagious ecthyma, belongs to the Parapoxvirus genus of the *Poxviridae* family (poxviruses). This family infects a wide range of animals and has been responsible of widespread pandemics, such as the case of Variola virus in humans [1]. The DNA genome of poxviruses contains at least 90 conserved genes essential for viral replication and a variable set of additional genes that are specific and involved in pathogenesis [2]. The replication cycle takes place entirely in the cell cytoplasm in a way that is independent from the cell nucleus. The virions are enveloped particles whose lipid components directly derive from the host cell endoplasmic reticulum and the Golgi apparatus [3].

ORFV elicits a short-lived immune response in the host, contributing to multiple reinfections in animals. This feature is further enhanced by the presence of viral genes that modulate the host's immune response [4]. Among these regulators, ORFV encodes a tyrosine phosphatase (defined in this work as OH1) that is widely conserved in poxviruses. A recent study suggested that this phosphatase has a role in the inhibition of the host's JAK–STAT signaling pathway [5], analogous to Vaccinia virus VH1 [6,7]. VH1 has been structurally characterized [6,8] and was the first dual-specificity phosphatase (DUSP) described in the literature [9]. The structure of Vaccinia VH1 revealed a homodimeric quaternary structure involving an extensive domain swapping of the N-terminal α-helices stabilized by non-covalent interactions. Dimerization of VH1 is a structural and mechanistic feature proposed to regulate the activity and recognition of its putative physiological substrate STAT1 [10].

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Table 1. Data collection and refinement statistics.

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 (1.92–1.88)
R_{merge}	0.141 (2.117)
R_{pim}	0.094 (1.538)
Unique reflections	26,622
Completeness (%)	95.0 (50.8)
Multiplicity	3.2 (2.4)
Mn(*I*) half-set correlation CC(1/2)	0.989 (0.174)
$I/\sigma(I)$	4.4 (0.3)
Refinement	
Total no. of observations	84,484
No. of reflections	26,404 (2617)
Resolution (Å)	17.73–1.89 (1.97–1.89)
R-value, working set	0.1872 (0.2241)
R-free	0.2169 (0.2262)
Protein atoms	2730
Solvent atoms	157
RMSD	
Bond (Å)	0.01
Angles (°)	1.05
Mean *B*-factors ($Å^2$)	36.71
Ramachandran plot (%)	
Preferred regions	97.1
Allowed regions	2.9
Outliers	0
Clash score	3

Numbers in parentheses refer to the highest-resolution data shell.

The DUSP family shares with classical protein tyrosine phosphatases (PTPs) a similar three-dimensional fold and a two-step catalytic mechanism, which involves residues from two spatially distinct loops. The first loop is the phosphate-binding loop (P-loop), which contains the PTP signature motif HCXXGXXRS(T) with the catalytic cysteine, and the second loop is the D-loop, which contains a conserved aspartate critical for catalysis [11]. In contrast to classical PTPs, which possess a deep and P-Tyr-specific active site pocket, the active site of DUSPs consists of a 6-Å deep flat pocket, shallow enough to accommodate both P-Tyr and P-Ser/P-Thr residues. In addition, some DUSPs present a wider active site, which can accommodate the sugar head group of phosphoinositides (PtdIns) [12].

In this work, we report the structural and functional characterization of ORFV OH1. Remarkably, and in contrast to VH1 of Variola and Vaccinia, the homodimer of OH1 is covalently stabilized by a disulfide bond involving the N-terminal Cys15 from both monomers. This quaternary structure is probably a prototypical structure of Parapoxvirus genus phosphatases. In addition, the substrate specificity analysis confirms that OH1 is a DUSP and reveals its ability to dephosphorylate phosphatidylinositol bisphosphate [PtdIns-(3,5)P_2], a new activity potentially relevant in PtdIn recycling during virion maturation.

Results and Discussion

OH1 forms a covalent dimer with distinct structural features compared to VH1

OH1-wt and the catalytically inactive mutant OH1-C112S were produced in *Escherichia coli* and purified to homogeneity. While no crystals were obtained for OH1-wt, OH1-C112S crystallized within 3 days in space group $P2_1$ and diffracted to 1.89 Å (Table 1). The three-dimensional structure of OH1-C112S was solved by molecular replacement using the homologous structure of Vaccinia VH1 (3CM3) as a search model [6]. OH1-C112S adopts the regular elongated shape of a PTP [12], further enhanced by the dimer formation that positions the active sites 44 Å from each other, which is in the range measured in the *Vaccinia* VH1 dimer [10] (Fig. 1a). Each active site of OH1 accommodates a phosphate ion that mimics the position of a phosphosubstrate. The residues of the P- and D-loops of the OH1 active site are strictly superimposed to the P-and D-loops of the active Variola/Vaccinia VH1, respectively (RMSD 0.1 Å). Most of the P-loop contains small residues that wrap around every oxygen of the phosphate group through nitrogen atoms of the backbone (Fig. 1a, inset). Thus, the structure of OH1 phosphatase from ORFV shows a preserved overall monomer topology associated with a conserved active site that defines the classical fold of an active PTP. Nevertheless, the structure of OH1 reveals critical changes in the dimer organization, with respect to previously characterized viral VH1

Fig. 1. Overall structure of ORFV OH1. (a) Cartoon representation of ORFV OH1 dimer with chains A and B and its surface shown partially transparent. Helices α1 from chain A and chain B are colored orange. For each monomer, the phosphatase active site is shown with the P- and the D-loops in blue and magenta, respectively. The phosphate ion is shown as orange sticks and residues closely interacting with the phosphate are shown in stick and labeled (details are inset). (b) Cartoon representation of the ORFV OH1 dimer stabilized by a disulfide bond involving the two C15 of helices α1A and α1B and shown as orange sticks, and a six-residue insertion shown in blue. Inset: details of the electron density of the disulfide bond mapped from a F_o–F_c mesh contoured at 2σ zoomed and centered on the disulfide bridge C15–C15. The labeled residues stabilizing the OH1 dimer are shown as sticks. (c) Cartoon representation of the Vaccinia VH1 dimer with helices α1 involved in domain swapping colored gold. Inset: the position of helix α1A in OH1 (orange) is incompatible with the α1B helix from VH1 (gold) as shown by the steric clash represented by the red arrow.

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