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Biochemical and Structural Characterization of the Unique Phosphatase of *Orf* Virus

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BACKGROUND

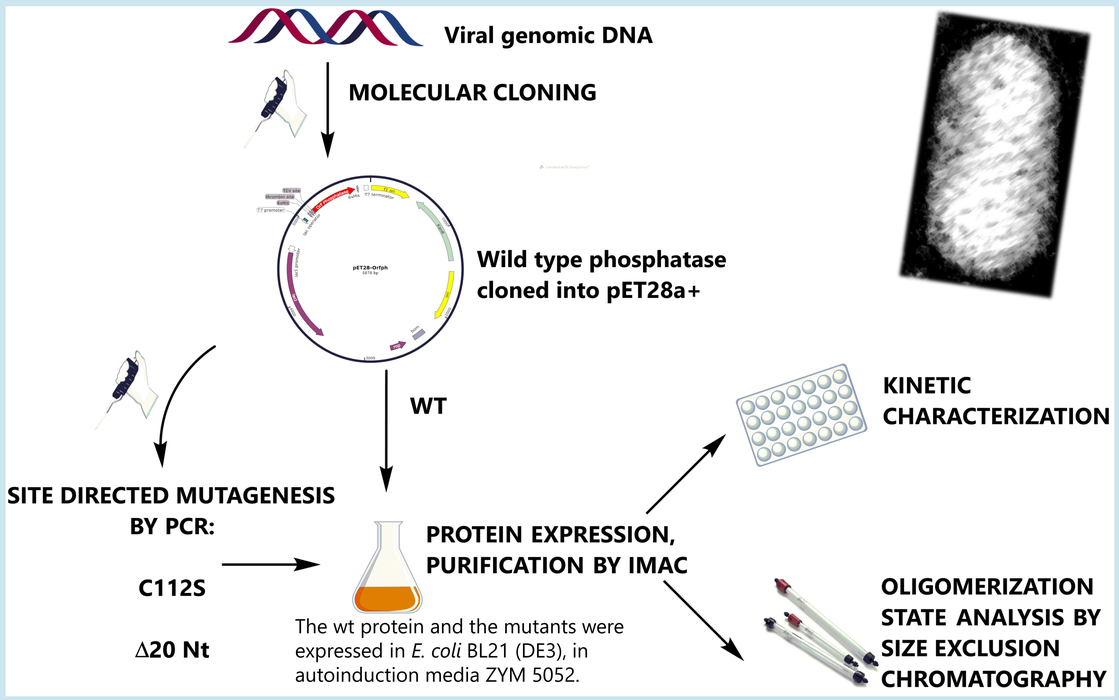
The *Orf* virus is the causal agent of *Contagious Ecthyma*, a pustular dermatitis disease of sheep and goats that occasionally affects humans. *Orf* disease has a relevant significance in the ovine production because of its highly contagious nature and its elevated rate of reinfections. The understanding of viral mechanisms of immune suppression is of great interest for developing new methods to control the disease.

In the genome of *Orf* virus, there is an open reading frame coding for a tyrosine phosphatase, with a 40% sequence homology with the VH1 phosphatase of *Vaccinia virus*. VH1 phosphatase is a virulent factor, crucial for the viability and replication of the viral particle, and the blocking of interferon-ɣ signaling in the host. Recently, it has been shown that the *Orf* virus phosphatase is also capable to modulate the ɣ-interferon pathway.

REASERCH GOALS

- Achieve the recombinant production of the *Orf* virus phosphatase
- Perform an enzymatic characterization
- Modeling of the structure of the *Orf* virus phosphatase

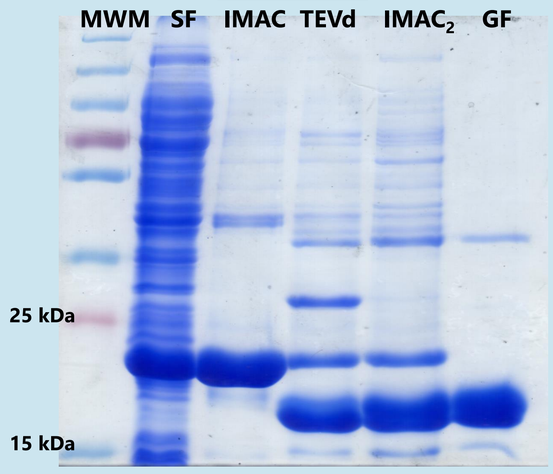
EXPERIMENTAL STRATEGY



RESULTS AND DISCUSSION

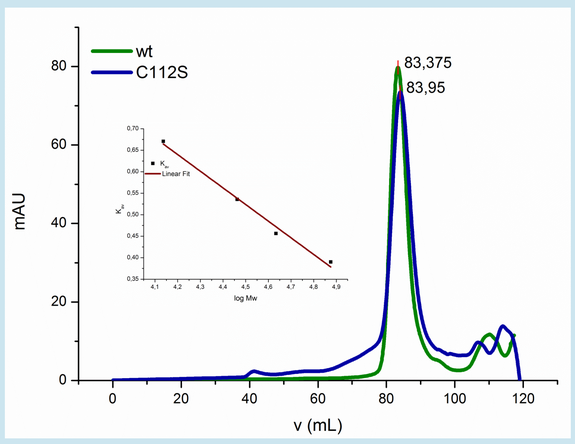
1. Expression and Purification of the *Orf* Virus Phosphatase

SDS - PAGE



- The wt and the activity mutant C112S were purified from the soluble fraction. The Δ20 Nt mutant was insoluble. The His-tag was removed by digestion with TEV protease. After a second IMAC , the proteins were purified by size exclusion chromatography (Superdex 200 16/60).

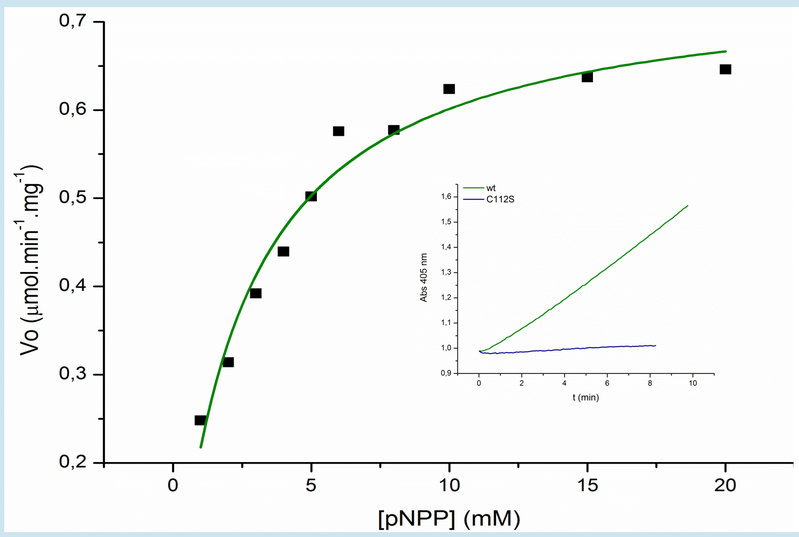
SIZE EXCLUSION CHROMATOGRAPHY



- The wt and C112S eluted with an apparent molecular weight of 40 kDa, consistent with a dimeric protein.

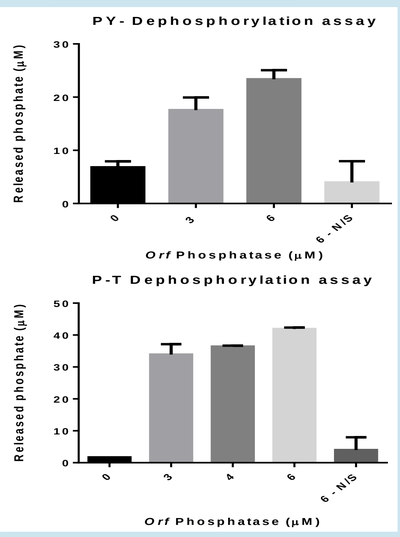
2. Enzymatic characterization

KINETIC PARAMETERS



- The kinetic parameters were determined with the pNPP substrate.
- For the wt protein V_{max}: 0,74 µmol of pNP.min^{-1}mg^{-1}, K_m: 2,43 mM
- The mutant C112S didn't shown any phosphatase activity.

DUAL SPECIFICITY



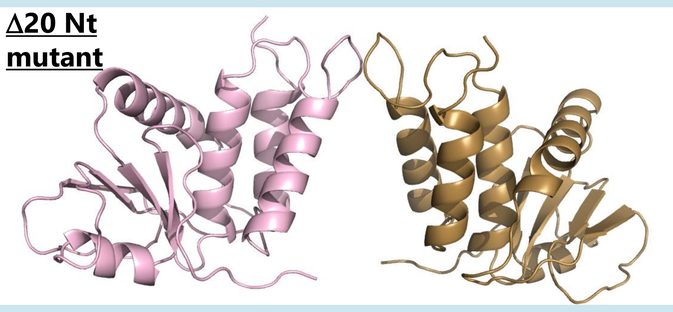
- PY/PThr peptides were used as substrates and the release of Pi was detected with Malachite Green.
- The wt phosphatase has a dual specificity

3. Molecular modeling

Wt Phosphatase



Δ20 Nt mutant



The *Orf* virus wt phosphatase and the Δ20 Nt mutant were homology-modeled, using the crystal structures of VH1 phosphatase from *Vaccinia virus* as a 3D template. The wt and the mutant C112S show a stable dimer *in silico*, with the N-terminal helix α1 of chain A that swapsover C-terminal helices α 5 and α 6 of chain B and vice-versa.

The Δ20 Nt mutant encompasses a complete deletion of helix α1. We hypothesize that such deletion should preclude the dimer formation, because it does not display enough interface with helices α 5 and α 6 of the second protomer to stabilize the dimer.

CONCLUSIONS AND OUTLOOK

- The wild type *Orf* virus phosphatase and the mutant C112S are dimers in solution, as suggested by molecular modeling
- The Cys 112 is essential for the activity, a signature of PTPs
- The wild type protein is a dual specificity phosphatase, as VH1
- Even though the Δ20Nt mutant was insoluble, molecular modeling strongly suggests it would be a monomer

- Currently, our team is working in obtaining the soluble form of the Δ20Nt mutant, and in developing a cell cultured model to study the *Orf* virus phosphatase in a cellular context
- Crystallization assays were initiated with the wild type protein and the mutant C112S



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