

## **A disulphide tethered occluding loop confers elastase-like activity on the subtilisin-like protease AprV2**

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Ovine Footrot is primarily caused by the Gram-negative, anaerobic bacterium, *Dichelobacter nodosus*. Footrot is a debilitating and contagious disease that causes severe economic loss to the sheep industry and is characterised by the separation of the hoof from the living epidermal tissue. The severity of ovine footrot can range from virulent to benign and is dependent upon the causative *D. nodosus* isolate.

Both the benign and virulent isolates of *D. nodosus* excrete several acidic and basic proteases. In particular, the acidic subtilisin-like protease AprV2 from virulent strains of *D. nodosus* is a potent elastase that is postulated to participate in connective tissue destruction. Interestingly, the equivalent protease isolated from benign strains (AprB2) differs by only a single amino acid (Tyr92==>Arg) yet displays markedly reduced activity against elastin. We have used structural studies to understand the molecular basis for this difference.

Both AprV2 and AprB2 crystallised in space group P1 ( $a = 43.1 \text{ \AA}$ ,  $b = 46.0 \text{ \AA}$ ,  $c = 47.2 \text{ \AA}$ ,  $\alpha = 97.8^\circ$ ,  $\beta = 115.2^\circ$ ,  $\gamma = 113.9^\circ$  and  $a = 42.7 \text{ \AA}$ ,  $b = 45.8 \text{ \AA}$ ,  $c = 45.7 \text{ \AA}$ ,  $\alpha = 98.4^\circ$ ,  $\beta = 114.0^\circ$ ,  $\gamma = 114.6^\circ$  respectively). The crystals of AprV2 diffracted to  $2 \text{ \AA}$  and those of AprB2 to  $1.8 \text{ \AA}$ . Both structures were determined by Molecular Replacement using the PHASER algorithm. The structures reveal a conserved subtilisin catalytic core domain with a unique disulphide tethered solvent exposed loop that overhangs and partially occludes the active site. The single Tyr92==>Arg substitution is located at the tip of this loop, and does not contribute to the primary specificity determining subsites. Accordingly, activity assays using chromogenic peptides revealed no significant difference in enzyme activity between the two proteases. However, using an elastin competition assay we also show that AprV2 binds more efficiently to elastin than AprB2. Together, these data suggest that Tyr 92 in AprV2 forms part of a substrate exosite required for efficient binding to elastin.