CORRELATION OF CONFORMATIONAL CHANGE WITH NOVEL REDOX REGULATION OF A CYSTEINE PROTEINASE FROM *PORPHYROMONAS GINGIVALIS*

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Arg-specific (RgpA, RgpB) and Lys-specific (Kgp) cysteine proteinases generated by the black-pigmented, anaerobic *Porphyromonas gingivalis* have been implicated in the onset and progression of adult periodontitis. Investigation of the catalytic mechanisms of these enzymes would benefit the development of enzyme-specific inhibitors for clinical use.

In this study, RgpB purified from P. *gingivalis* HG66 culture fluid was pretreated with 1 mM cysteine for different periods of time (10, 20 and 60 min) before being added to a peptide substrate histatin 5. After incubation for various time intervals, an aliquot of the digest was analysed by MALDI MS. Histatin 5 is a salivary peptide of 24 amino acids (DSHAKRHHGYKRKFHEKHHSHRGY) containing multiple arginine and lysine residues. The MS data indicated that cysteine was required for full RgpB proteinase activity but the activation of the enzyme decreased significantly (80 %) within 1-h pretreatment with cysteine under the experimental conditions. Although only arginine specificity was initially observed after 10-min cysteine pretreatment as documented in the literature, a novel finding was that RgpB also showed a preference for lysine cleavage, which was evident after 20 min and became dominant after 60 min cysteine pretreatment. The RgpB enzyme when pretreated with Cys for 10 min did not show any significant Lys activity even after prolonged digestion of the substrate histatin 5, indicating that the Lys activity observed was not derived from a minor contaminant proteinase. Furthermore, the Lys activity could not be attributed to the presence of the Kgp proteinase as the activity was abolished by the addition of the Rgp-specific proteinase inhibitor, leupeptin. Also, the cleavage of a Kgp-resistant Lys-13 residue in histatin 5 by the Lys activity derived from RgpB was not consistent with the presence of Kgp.

Native PAGE analyses of RgpB in the absence and presence of cysteine revealed a correlation of conformational change with the change of the enzyme specificity, presumably due to disulfide formation. A model of possible disulfide formation in oxidised RgpB based on the released X-ray crystal structure is presented and its correlation with the specificity change is currently under investigation.