Title: Histatin 1 Interaction with Salivary Proteins using Surface Plasmon Resonance

Trainee Name: Karla Crosara

Supervisor(s): Dr Walter Siqueira

Structured Abstract:

Introduction: Protein-protein interaction (PPI) is a common cellular event where two or more proteins have close, specific physical contact. Because of such PIPs, the participating proteins may be activated, repressed, degraded or protected. For this reason, huge interest has been drawn to identifying and understanding PIPs, specially by the pharmaceutical industry. Surface plasmon resonance (SPR) is a very promising technique to study PIPs. In SPR the real-time binding between the two molecules can be observed, and binding affinities and association/ dissociation kinetics can be determined, with low sample consumption. Histatin 1 is a protein found uniquely in human salivary that has been extensively studied due to its many functions including antimicrobial activities. The objective of this study is to investigate the binding affinities and association/dissociation kinetics of histatin 1 interaction with human serum albumin and lysozyme.

Methods: Surface plasmon resonance (SPR) was used in all experiments. A total of 30 ug of histatin 1 was re-suspended in 200 ul of 0.1 M citrate buffer at pH 6.0, and immobilized on the COOH SPR sensor chip. Aliquots of 400 nM of albumin or lysozyme were used as analytes. Flow rate was set to 20 ul/min, allowing 4 minutes of interaction. PBS and HEPES buffers were used at pH 7.4. Regeneration, when needed, was achieved with NaOH up to 10 mM. All data were acquired in duplicate, and double referencing was used to compensate for any nonspecific binding. TraceDrawer software was used for data analysis.

Results: After subtraction of the curve relative to the nonspecific binding, interaction between histatin 1 and lysozyme was observed for all tested buffers. Notable, the interaction between histatin 1 and lysozyme was stronger in PBS milieu than when in HEPES buffer. In addition, it was observed an affinity reduction between histatin 1 and lysozyme when different calcium concentrations were added to the HEPES. Surprisingly, albumin did not demonstrate any direct binding behavior with histatin 1 despite of the tested buffer or ionic concentration.

Discussion: Our results demonstrated that lysozyme directly interacts with histatin 1. The same direct interaction between histatin 1 and albumin was not observed. This data suggest that lysozyme could participates in the first shell of the histatin 1 protein complex by binding directly to it. Moreover, this type of study assists in the elucidation how salivary proteins bind and interact with each other to form the acquired enamel pellicle on the tooth surface.