ABSTRACT

The gastrointestinal tract represents the interface between body and external environment. It is equipped with sophisticated mechanisms that regulate the function and implement effective strategies for its protection relying on consecutive defense lines characterized by specific factors involved in different roles. Among these elements, during the last 30 years, three small secreted proteins, TFF1, TFF2 and TFF3 that constitute the so-called family of " Trefoil factors" revealed their prominent role. These proteins share a compact and protease-resistant structure, characterized by the presence of a trefoil domain stabilized by three disulfide bridges. Each protein is able to form dimers and heterodimers through a fourth intermolecular disulfide bond involving a conserved cysteine residue present in the C-terminal tail. They are promptly induced in response to insults involving gastric epithelia, and show their activity in the epithelial "restitution", the important process of repair of the damaged epithelium. Along with their physiological expression and function, the aberrant presence of these peptides characterize pathological contexts such as inflammatory and neoplastic processes.

Our research group showed for the first time the ability of TFF1 monomers to bind copper through its C-terminal tail, and the ability of the metal to induce a conformational change in the protein structure that favors the formation of dimers, thus affecting its biological activity .

Following these findings, first aim of the investigation was the structural and functional study of the Cu- TFF1 complex. The characterization of the interaction of copper and the recombinant proteins hrTFF1 hrTFF3 in monomeric and dimeric forms was carried out, and both forms showed a selective binding with the metal cation. Characterization was extended to the evaluation of the thermodynamic parameters of the Cu-TFF1complex by isothermal titration calorimetry.

Experiments were carried out in order to evaluate the influence of copper on secretion pathway and cellular localization of the protein. It was shown that copper overload results in a reduced secretion and increased intracellular localization, mainly into the trans-Golgi compartment. Furthermore, copper load increases the protein up-take of gastric cells. This evidence let us hypothesize the involvement of the protein in the mechanisms of metal homeostasis, although further investigations are needed to shed light on this uncharacterized role of the protein.

Interestingly, recent studies showed that TFF1 dimers are able to bind *H. pylori* lipopolysaccharides, thus mediating their binding to the gastric mucosa. Our more recent data indicate that copper causes an improved colonization of gastric cells hyper-expressing TFF1. In fact, we show that the TFF1 C-terminus is involved in the interaction with the pathogenic bacterium, as confirmed by SPR analysis and colonization assays of gastric cells.

TFF1 and mucins, both main components of the protective mucous layer, modulate its rheological properties, thus exerting an additional protective role. The pathogenic bacterium mainly localizes into the adherent mucus layer of the

gastrointestinal epithelia, and interacts with TFF1 and mucin MUC5AC. The results of our rheological analyses show that copper is able to harden the mucus produced and layered onto the surface of HT29-E12 intestinal goblet cell culture. Viscoelastic properties of the mucus could be affected by the balance of dimer production influenced by copper, thus favoring the interaction with mucin MUC5AC and increasing the number of docking sites for bacterial adhesion.

The results here presented provide further information for a better understanding of the role played by the TFF1 trefoil peptide in the physio-pathological processes of the gastrointestinal tract. In addition, the clear involvement of the copper-TFF1 complex in the processes of adhesion and colonization of the pathogenic bacterium *Helicobacter pylori* provides new useful molecular details of the host-pathogen interactions characterizing the colonization and survival of bacteria in the gastric tissue.