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Alpha Hemolysin Induces an Increase of Erythrocytes Calcium: a Fluorescence Lifetime Imaging Microscope Study

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α -hemolysin (HlyA) from *Escherichia coli* is considered as the prototype of a family of toxins called RTX (repeat in toxin), a series of proteins that share genetic and structural features. HlyA is an important virulence factor in *E. coli* extraintestinal infections, such as meningitis, septicemia and urinary infections. High concentrations of the toxin causes the lysis of several cells as erythrocytes, granulocytes, monocytes, endothelial cells and renal epithelial of different species and low concentrations induces the production of cytokines and apoptosis. Eriptosis, the apoptosis process in erythrocytes, can be induced by several toxins and the increase in calcium concentration inside the cell is being postulated as the trigger of this process. In this context, we followed the calcium concentration inside the erythrocytes while incubating with sublytic concentrations of HlyA; calcium concentration was monitored following the changes in lifetime of the calcium indicator Green 1 using fluorescence lifetime imaging microscopy (FLIM). Data were analyzed using the phasor representation.

In this report we present evidences that, at sublytic concentrations, HlyA induces an increase in Calcium concentration in rabbit erythrocytes in the first 5 minutes. Results are discussed in relation to the difficulties of measuring Calcium concentrations in erythrocytes where hemoglobin is present, the contribution of the background, the heterogeneity of the response observed in different cells and how the phasor approach for lifetime measurements analysis can solve these challenges successfully.

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