Abstracts

– Imaging and Biospectroscopy –

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Antifungal defensin Psd1 increases membrane roughness and promotes apoptosis in Candida albicans

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*Psd*1 is a defensin, isolated from *Pisum sativum* seeds, previously shown to have a strong interaction with fungal-specific membrane components [1]. *Candida albicans* is an important human pathogen, causing oral, genital and systemic opportunistic infections, which are especially relevant clinically in immunocompromised patients, such as HIV-infected individuals. We tested the effects of this antimicrobial peptide, comparing it with the antifungal drugs amphotericine B and fluconazole, at the minimal inhibitory concentration (MIC) and at a 10-fold higher concentration. By atomic force microscopy (AFM) imaging we assessed morphological changes on *C. albicans* cells. SYTO-9 and propidium iodide allowed us to image live and dead cells by confocal microscopy and to quantify their ratio. Our results show that, with increasing incubation times and *Psd*1 concentrations, there is an increased cell death and surface roughness, with the appearance of apoptotic features, such as membrane blebs, cell size alterations, membrane disruption and leakage of cellular contents. Thus, we were able to visualize the action of *Psd*1 against a relevant fungal human pathogen, aiming at its possible use as a natural antimycotic agent.

[1] Gonçalves *et al.* (2012) *Biochim Biophys Acta* 1818:1420

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Cytoprotective bile acids are high affinity ligands for the apoptotic protein BAX

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Hydrophilic bile acids (such as ursodeoxycholic acid, UDCA) can inhibit apoptosis in both hepatic and non-hepatic cells. The mechanism associated with this effect seems to be related with the blockage of a series of processes that converge on mitochondrial damage. Bax is a pro-apoptotic member of the Bcl-2 family that is involved in pore formation on mitochondrial membranes. Submicellar concentrations of cytoprotective bile acids have been shown to modulate Bax translocation to mitochondria, suggesting that these molecules could interact directly with the protein. In this study, our objective was to evaluate the affinity of bile acids to recombinant Bax protein, making use of fluorescence methodologies. Here, we show that cytoprotective bile acids bind to recombinant Bax protein with significantly higher affinity than apoptotic bile acids. Notably, the binding site for UDCA seems to be located in a hydrophobic pocket of the protein. This interaction could be responsible for the disruption of Bax translocation to the mitochondrial outer membrane in the presence of UDCA.

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Interaction of cytotoxic and cytoprotective bile acids with membranes of living cells

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Deoxycholic acid (DCA) is an apoptotic bile acid at submillimolar concentrations, while ursodeoxyhcolic acid (UDCA) prevents apoptosis in the same concentration range. The mechanisms that trigger these opposite signaling effects are still unclear. We have recently shown that these bile acids exhibit low partition to cholesterol-rich (liquid ordered,l_o) membranes, and that DCA and other apoptotic bile acids partially disrupt the ordering of lipid model membranes by cholesterol in liquid disordered membranes (l_d).

Using fluorescence microscopy methodologies, we show that fluorescent derivatives of DCA and UDCA are present at very low concentrations in the plasma membrane of both HEK293 and hepatocyte living cells, possibly as a consequence of low partition of bile acids to cholesterol-rich membranes. Additionally, both cytotoxic and cytoprotective unlabeled bile acids have no effect on the fluidity of the plasma membrane at apoptotic concentrations. However, fluorescent derivatives of bile acids are found significantly enriched in the mitochondrial membrane of hepatocytes. These results suggest that the modulation of apoptosis by bile acids is not the result of modulation of plasma membrane structure and are likely associated with mitochondria damage/protection.

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Origin of A0, A1 and A3 conformational substates of carbonmonoxy myoglobin

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Temperature dependence of infrared absorption spectra of complex of myoglobin with carbon monoxide (MbCO) showed that MbCO has at least three conformational substates, characterized by different spectra of infrared absorption in the region of C-O absorption. However, structures of these substates were unknown.

At the same time recent results of the high-resolution X-ray study of MbCO showed presence of three different substructures of MbCO.

To check if these sub-structures correspond to the substates observed in the infrared spectra we performed DFT quantum chemical calculations of the MbCO active center with its closest distal environment, which correspond to each of the refined sub-structures. These calculations revealed the dependence of vibrational frequency of the coordinated C-O ligand on the changes in the structure of the heme environment. The calculations showed, that the observed different X-ray structures correspond to the A_0, A_1, and A_3 substates. It was also shown that electronic structure of different parts of the heme environment notably depends on the electrostatic interactions between them. This conclusion questions reliability of results of the standard molecular dynamics approach to determination of the structure and dynamics of the heme environment.