THERMODYNAMICS AND KINETICS OF ENZYME/INHIBITORS SYSTEMS AND INTERACTION STUDIES OF PEPTIDES AND DMPC VESICLES.

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SPR based biosensor was applied to investigate kinetics and interactions of serine protease inhibitors of two distinct families with trypsin and β-chymotrypsin at low nanomolar concentrations. The technique was also used to evaluate affinities between antimicrobial peptides and DMPC vesicles. In the first case, the immobilized enzymes were exposed to inhibitors concentrations at 5 different temperatures. Data were fitted to a simple bimolecular interaction model. BTCI KD was calculated as 3.42 nM and 2.56 nM for chymotrypsin and trypsin, respectively, while SPCI/chymotrypsin KD was 1.43 nM. The van´t Hoff analysis for BTCI/chymotrypsin interaction gave a ΔG = -11.42 kcal/mol, ΔH = -14.32 kcal.mol^{-1} and ΔS = -9.69 cal.mol^{-1}.K^{-1}, contrasting with the parameters obtained for the SPCI/chymotrypsin interaction: ΔG = -11.97 kcal.mol^{-1}, ΔH = 2.23 kcal.mol^{-1} and ΔS = 47.66 cal.mol^{-1}K^{-1}. As for the peptide/DMPC system, LUVs were immobilized in an L1 sensor chip; the peptides were injected in concentration series ranging from 0.625-10 μM. The resulting sensorgrams for the peptides DS 01 and DShypo 01 show higher signal with increasing peptide concentrations, interactions that tend to a steady-state and very slow dissociation constants. From these data, one can assume that DShypo 01 exhibits a higher affinity for DMPC vesicles when compared to DS 01. In both studies, the data presented here include full comparative analysis with complementary techniques such as ITC and DSC.