Characterization of Quercetin Binding to Bovine Beta-Trypsin

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Quercetin is one of the most common flavonoids present in foods, but little information is available about its interaction with food constituents (proteins) or with digestive enzymes. Trypsin is a well-studied serine protease that digests proteins in duodenum. Quercetin was shown to inhibit trypsin activity *in vitro*, albeit neither binding mode nor inhibition type was defined yet. Quercetin also was shown to undergo oxidation in alkaline pH forming electrophilic compounds that can damage proteins and DNA. In this context, the objectives of the present work are to evaluate quercetin stability in solution and to characterize the interaction between quercetin and bovine beta-trypsin by CD, UV-visible spectrum, x-ray crystallography and enzymatic kinetics. Quercetin was shown to exhibit wide and fast oxidation under pH 8 and pH 9 buffer solutions and precipitation under pH 7 and pH 6. Preliminary kinetic results performed under pH 8, indicate a competitive character of inhibition. Crystals of beta-trypsin grown in media containing quercetin (cocristallization) or soaked in quercetin rich solution were collected at the Brazilian Synchrotron Light Laboratory (LNLS). After structure refinement, the ligand was not found in the active site of the enzyme, although in the cocristallized crystal a covalent modification was found at the reactive serine residue and was assigned as a possible sulphonyl group. CD spectrum in far-UV region shows changes in secondary structure of the enzyme in the presence of quercetin and UV-visible spectrum is being conducted to study possible shifts in specific enzyme absorption peaks due to quercetin binding.

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