

ABSTRACT

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Title of diploma thesis: Oxidative damage to proteins

Hypochlorous acid is a strong oxidant, which is used by phagocytic cells to kill ingested bacteria. Surrounding structures including proteins may be damaged in case the content of a phagocyte is spilled to the vicinity. Catechins belong to a group of flavonoids whose antioxidant properties were demonstrated in many studies. My diploma thesis was focused on a potential effect of eight naturally occurring derivatives of catechin towards oxidative damage of human serum albumin (HSA) by hypochlorous acid. I used two spectrophotometric (determination of carbonyl groups and chloramines) and three electrophoretic methods (native PAGE, SDS-PAGE, immunoblotting). Dependence of an antioxidant effect on its concentration (0-3 mM) was observed during determination of chloramines and obtained data were used for calculation of EC_{50} values of individual catechins using program GraphPad Prism. The most effective compound was catechin whose EC_{50} was 4.8 μ M. Results suggest that antioxidant ability decreases with increasing number of hydroxyl groups in the molecule of flavonoid. Carbonyl groups are a good marker of ongoing protein oxidation. The strongest protective effect possessed catechin, which was able to decrease formation of carbonyl groups by 70.2% at 100 μ M concentration comparing to oxidized control. Some derivatives had rather pro-oxidative effect (epicatechin). Neither the effect of HSA oxidation on its relative mobility (native PAGE) was confirmed nor the aggregation or fragmentation of HSA (SDS-PAGE) was observed. During immunoblotting I found several band containing carbonyl groups (66, 140, 200 and > 250 kDa) in oxidized samples but the same bands were present also in native control.