ABSTRACT

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Title of Doctoral Thesis **Interaction of proteins with low-molecular substances in vitro. Effect of glycation, substances of plant origin, and combination of these factors on function and spectral properties of selected proteins.**

Plant polyphenolic compounds naturally occurring in human diet possess a wide range of biological and pharmacological properties. They can interact with various proteins including enzymes. Their interaction with serum albumin has a great significance because of its ability to bind, transport and store many endogenous and exogenous low-molecular compounds present in blood circulation. One of the objectives of this thesis was to study chemical structure-binding affinity relationships of two important groups of polyphenolic substances, namely hydroxycinnamic acids and catechins (flavanols), with the molecule of serum albumin in the in vitro models using spectroscopic (UV-Vis absorption spectroscopy, fluorescence quenching method) and electrophoretic (native and SDS PAGE) methods. Some polyphenols caused the changes in protein conformation and the relationships between their structures and obtained binding affinities to serum albumin were noticed.

Green tea catechins (flavanols) represent one of the most important plant polyphenolic substances. Interaction between these compounds and ERp57 enzyme in the in vitro model has been studied by fluorescence quenching method. Effects of green tea catechins on disulfide reductase activity of ERp57 enzyme in the in vitro model was monitored by sensitive fluorescent assay using dieosin glutathione disulfide as a fluorescent probe. This probe displays low fluorescence at the excitation/emission wavelength of 520 nm/545 nm, respectively, which significantly increases on reduction of its disulfide bonds (i.e. the presence of ERp57). Relationships between structures of the studied catechins and their binding affinities or their inhibitory effects on reductase activity of ERp57 were discussed.

Alpha-dicarbonyl compounds (e.g. methylglyoxal) are formed during various metabolic processes and also by non-enzymatic protein glycation by glucose. They are thought to be principal precursors in generation of AGEs in vivo. Methylglyoxal can readily react with amino groups of proteins to form covalent cross-links and reactive oxygen species (ROS). It contributes to the onset and progression of many human diseases including diabetes mellitus and its related complications. Plant polyphenolic compounds can reduce formation of AGEs and thus slow down the process of non-enzymatic glycation by different mechanisms such as trapping of reactive α-dicarbonyl compounds or ROS. Another aim of this thesis was to study the effects of green tea catechins on methylglyoxal-mediated non-enzymatic glycation of serum albumin in the in vitro models using fluorescence methods for determination of total (“non-specific”) AGEs (λex/λem = 330/410 nm) and argpyrimidine as specific AGE (λex/λem = 320/380 nm). Antiglycating activity of the studied catechins was dependent on their structure and concentration.

Glutathione S-transferases (GSTs) represent a group of enzymes which catalyze conjugation of reduced glutathione to various hydrophobic compounds. They are important enzymes in detoxification of various xenobiotics. Effect of methylglyoxal on the structural and catalytic properties of GST from Schistosoma japonicum was investigated in the in vitro model using spectroscopic (UV-Vis absorption and fluorescence spectroscopy) and electrophoretic (native PAGE, SDS PAGE/western blotting) methods. It was found out that methylglyoxal reduced catalytic activity of GST and caused changes in the molecular charge of the enzyme as well as changes in its structure (i.e. conformational changes, formation of AGEs and cross-links) in dependence on the concentration.