ABSTRACT

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Title of diploma thesis: Modification of Glutathione S-transferase by selected glycating agents

Glycation is a non-enzymatic reaction, which leads to the binding of carbonyl compounds to free amino groups of amino acids, peptides, proteins or other biomolecules. This process is closely related to impaired protein function in diabetes mellitus, during aging, and in neurodegenerative diseases. The main purpose of this thesis was to monitor the impact of several glycating agents on the properties of the enzyme glutathione S-transeferase (GST, EC 2.5.1.18) which plays a key role in the detoxification of many exogenous as well as endogenous compounds. Methylglyoxal (0.5–2 mM), glyceraldehyde (0.5–10 mM), glucose (50 mM), and fructose (50 mM) were selected as glycating agents. The course of protein glycation was evaluated by following methods: GST catalytic activity assessment, determination of primary amino groups, assessment of AGEs formation using fluorescence and Western blotting, generation of high molecular cross-links and aggregates by denaturationg polyacrylamide gel electrophoresis (SDS-PAGE), changes in GST molecular charge by native PAGE. Methylglyoxal and glyceraldehyde exerted the strongest glycation potential, whereas glucose and fructose modified GST only minimally. Glycation in the presence of methylglyoxal 2 mM led to a noticeable decline in GST activity (by 27.6% after 180-min incubation at 37ºC), a loss of 14 primary amino groups, which led to a change in mobility of GST molecule during native PAGE. Furthermore, formation of high molecular cross-links with molecular weight ranging from 50 to 200 kDa occured. In contrast, glycation in the presence of glyceraldehyde 10 mM resulted in minor decrease in enzyme activity (by 10.9% after 180-min incubation at 37ºC), but to a loss of 26 primary amino groups. Cross-links with molecular weight ranging from 65 to 100 kDa were formed during glycation by glyceraldehyde.