ABSTRACT

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Title of thesis: Optimization of electrophoretic methods for study of glycation of aspartate aminotransferase by methylglyoxal

Main scope of this thesis was the optimization of several electrophoretic methods for monitoring of the course of glycation of aspartate aminotransferase (AST) by methylglyoxal as well as of the effect of selected antioxidants at this process. I observed formation of high-molecular protein aggregates using denaturing electrophoresis (SDS-PAGE) and Western blotting and changes in the molecular charge of AST during glycation using native PAGE. In both types of electrophoresis, I searched for suitable concentration of the separation gel and the amount of loaded protein. Further, I compared three routinely used staining methods (Silver Staining, Coomassie Blue R-250 and G-250). I found out that the high-molecular aggregates with molecular weight about 130 and 160 kDa, which corresponds to tri- and tetramers of AST, are formed with increasing concentration of methylglyoxal. Using immunochemical detection, I detected glycation products with molecular weight mainly about 70 and 130 kDa and with molecular weight about 160 kDa at higher concentrations of methylglyoxal. Glycation by methylglyoxal caused progressive loss of positive charges in the molecule of AST, which was manifested by increased migration of these samples during native PAGE. Silver Staining turned out to be the most sensitive staining method. Staining using colloidal Coomassie Blue G-250 was more sensitive and faster than by R-250. The greatest protective effect among studied antioxidants possessed aminoguanidine. Hydroxycitric acid only partially defended against glycation, while uric acid exerted no positive effect. The electrophoretic methods, which will be further used for study of compounds with potential antiglycation properties in the *in vitro* model of protein glycation, were successfully optimized.