ABSTRACT

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Title of diploma thesis: Carbonylation of human serum albumin by glycation agents: effect of carbonyl reductase 1

This diploma thesis is concerned with a formation of carbonyl groups in the molecules of human serum albumin (HSA) as a result of selected glycation agents activity and the ability of enzyme carbonyl reductase 1 (CBR1) to influence this process. Initial experiments were devoted to find optimal conditions for quantification of carbonyl groups in the model of HSA glycated *in vitro* by fructose and methylglyoxal using spectrophotometric assays and the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with subsequent immunoblotting. The glycation of HSA was monitored also by the measurement of fluorescent AGE products. Then the effect of CBR1 on the formation of carbonyl groups in the samples of HSA glycated with reactive carbonyl compounds fructose, methylglyoxal, glyoxal, and glyceraldehyde was studied. Spectrophotometric assays, SDS-PAGE with immunoblotting and enzyme immunoassay ELISA were employed. CBR1 was not able to remove already formed carbonyl groups under given conditions. Also the ability of CBR1 to decrease the amount of carbonyl groups during the incubation of HSA with the carbonyl compounds was not confirmed. Glycation proceeded at the highest rate at pH 7,4 compared to the glycation at pH 6 or pH 8. The variable amount of CBR1 which was added at the beginning of incubations of HSA with carbonyl compounds did not considerably affect amount of carbonyls at these pH values. Nevertheless, mild increase in carbonyl content was noticable with increasing amount of CBR1 at pH 8. That could be caused by the altered CBR1 activity to its dehydrogenase activity as a result of alkaline pH. Assumption, that CBR1 is involved in the process of removing reactive carbonyl groups was not proven.