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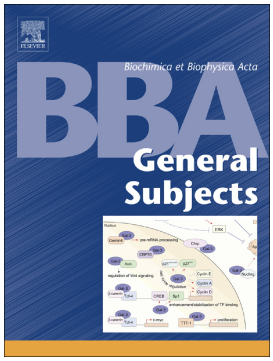

S-glutathionylation of glyceraldehyde-3-phosphate dehydrogenase induces formation of C150-C154 intrasubunit disulfide bond in the active site of the enzyme

K.V. Barinova, M.V. Serebryakova, V.I. Muronetz, E.V. Schmalhausen

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S-glutathionylation of glyceraldehyde-3-phosphate dehydrogenase induces formation of C150-C154 intrasubunit disulfide bond in the active site of the enzyme

Barinova K.V.[1,2], Serebryakova M.V.[1], Muronetz V.I.[1,2], Schmalhausen E.V.[1]*

[1]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, 119234, Russia;
[2]Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, 119234, Russia.

*Corresponding author: Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, 119234, Russia; E.mail: shmal@belozersky.msu.ru

Abstract

Background

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic protein involved in numerous non-glycolytic functions. S-glutathionylated GAPDH was revealed in plant and animal tissues. The role of GAPDH S-glutathionylation is not fully understood.

Methods

Rabbit muscle GAPDH was S-glutathionylated in the presence of H_2O_2 and reduced glutathione (GSH). The modified protein was assayed by MALDI-MS analysis, differential scanning calorimetry, dynamic light scattering, and ultracentrifugation.

Results

Incubation of GAPDH in the presence of H_2O_2 together with GSH resulted in the complete inactivation of the enzyme. In contrast to irreversible oxidation of GAPDH by H_2O_2, this modification could be reversed in the excess of GSH or dithiothreitol. By data of MALDI-MS analysis, the modified protein contained both mixed disulfide between Cys150 and GSH and the