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Determination of thiol peroxidase activity in the rat blood serum using *tert*-butyl hydroperoxide and homocysteine

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Abstract

Thiol peroxidases include glutathione peroxidases (Gpx) and peroxiredoxins and catalyze the reduction of inorganic (H_2O_2) and organic hydroperoxides. Thiol peroxidases of Gpx family utilize reduced glutathione (GSH) as a reductant. Early studies recognized that the extracellular selenium-dependent glutathione peroxidase (Gpx3) from human plasma has a wide thiol specificity and may utilize low-molecular-weight thiols, such as cysteine, mercaptoethanol, and dithiothreitol, instead of GSH (Takebe et al., *J. Biol. Chem*., 2002, 277: 41254-41258). In our previous studies we obtained results confirming the hypothesis that Gpx3 in rat plasma may utilize the reduced homocysteine (Hcy-SH) instead of GSH in a thiol peroxidase reaction with H_2O_2 (Razygraev et al., *Biomed. Khim*., 2016, 62: 431-438). In the present study performed using Hcy-SH, we replaced H_2O_2 with *tert*-butyl hydroperoxide (TBHP) and developed a new variant of the Ellman's-reagent-based method for determining the thiol peroxidase activity in rat serum. Using reaction medium consisted of 0.073 M Tris/HCl buffer (pH 8.5), 0.25 mM EDTA, 17.1 mM NaN_3, 0.45 mM DL-Hcy-SH, 1.6 mM TBHP, and diluted rat serum (incubation temperature was 37 °C), we found, that the rate of serum-catalyzed oxidation of Hcy-SH in the presence of TBHP is proportional to amount of a biomaterial in a reaction mixture; the rate of the enzymatic reaction is constant during more than 40 s of incubation. Hcy-SH:TBHP oxidoreductase reaction stoichiometry (2:1 mole ratio) was confirmed for the catalyzed interaction between Hcy-SH and TBHP. With 0.45 mM DL-Hcy-SH, the TBHP concentration of 1.6 mM is the saturating concentration for enzyme, that was revealed in the kinetic study. In our opinion, Hcy-SH and TBHP is the optimal combination of substrates for determination of thiol peroxidase (probably Gpx3) activity due to a relatively slow rate of spontaneous (nonenzymatic) reaction of these compounds with each other in the reaction conditions described above.