

GLUTATHIONE BINDS TO ALBUMIN WITH A SMALL CONFORMATIONAL CHANGE IN PHYSIOLOGICAL MEDIUM

Alves, M.A., Brigagão, M.R.P.L., Schneedorf, J.M.

Departamento de Ciências Exatas – Universidade Federal de Alfenas, Minas Gerais, Brazil.

Reduced glutathione (GSH) and serum albumin are known to play an important role in oxidative stress in animals, being considered the major intra and extra cellular antioxidant among them, respectively. The present study is concerned with the mechanism involved in the uptake of GSH by albumin (BSA). The interaction was studied by second-derivative UV-spectrophotometry and first-derivative linear sweep voltammetry (LSV). The spectroscopy data were obtained in PBS solution at pH 7.4 varying both GSH and protein, also blocking Cys-34 residue with 3:1 N-ethylmaleimide per protein molecule. LSV was carried out with DHME, Ag/AgCl and Pt electrodes in 5ml of PBS buffer at 200 mV/s ranging from 0 to -800mV. The results from UV-derivative spectra showed a slightly conformational change for BSA with 1:1 stoichiometry prior a non-specific linkage. Data from LSV presented two classes of binding sites for the interaction with $n_1 = 1.1 \pm 0.0$, $K_{ass} = 6.3 \times 10^5 \pm 20.9$ and $n_2 = 3$, $K_{ass} = 1,9 \times 10^{18}$. The binding increases with pH values from 6.5 up to 9.0 with an optimum value at 8.0, and with no of NaCl concentration influence up to 1.0 M. The overall results point to the binding of GSH with BSA as a Langmuir model with two non-interacting sites, without electrostatic effects, and presenting a minor transconformation on the protein surface.

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