PROTEIN-THIOL SUBSTITUTION OR PROTEIN DETHIOLATION BY SH/SS ECHANGE REACTIONS: THE ALBUMIN MODEL

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Dethiolation experiments of thiolated albumin with thionitrobenzoic acid and non-protein SH (glutathione, cysteine, homocysteine) were carried out to understand the albumin role regulating plasma thiol and disulfide species concentration by thiol/disulfide (SH/SS) exchange reactions. During these experiments we observed that thiolated albumin underwent thiol substitution (XSSP+RSH←→RSSP+XSH) or dethiolation (XSSP+XSH←→PSH+XSSX), depending on the different pKa values of thiols involved in protein-thiol mixed disulfides (XSSP). It appeared in these reactions that the compound with lower pKa in mixed disulfide was a good leaving group and that the pKa differences dictated the kind of reaction (substitution or dethiolation). Thionitrobenzoic acid, bound to albumin by mixed disulfide (TNB-ALB) underwent rapid substitution after thiol addition, forming the corresponding XSSP (peaks at 0.25-1 minutes). In turn, XSSP were dethiolated by the excess of remaining non-protein SH groups, due to the lower pKa value in mixed disulfide with respect to that of other thiols. Dethiolation of XSSP was accompanied by formation of XSSX and PSH up to equilibrium levels at 35 minutes, which were different for each thiol. Structures by molecular simulation of four thiolated albumin by the various thiols, carried out for understanding the role of sulfur exposure in mixed disulfides in dethiolation process, evidenced that the sulfur exposure is important for the rate but not for determining the kind of reaction (substitution or dethiolation). Our data underline the contribution of SH/SS exchanges towards the distribution of thiol redox species in human plasma. These reactions may be important to use thiols as therapeutical agents to decrease plasma levels of totally Hcy that is considered an important risk factor for outbreak of cardiovascular and other diseases.