

STRUCTURAL AND BIOLOGICAL ALTERATIONS OF CROTOXIN FOLLOWING IRRADIATION IN THE PRESENCE OF THIOL GROUPS

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Aqueous solutions irradiated with gamma rays leads to free radicals formation, mainly OH* and hydrated electron. When irradiating proteins in solution, those reactive species react with the proteic molecule, resulting in structural alterations leading to partial or total inactivation or even loss of function. These properties of ionizing radiation have been employed by us to attenuate toxins and produce immunogenic toxoids. In the present work, we investigated the influence of thiol compounds, well known OH* scavengers, in structural, toxic and enzymatic alterations resulting from irradiation of crotoxin, the main toxic component of *Crotalus durissus terrificus* venom. During the experiments, 2 mg/ml crotoxin solutions in 150 mM NaCl, 50 mM DTT or 100 mM cysteine were irradiated with ⁶⁰Co gamma rays with a 2000 Gy dose. Controls were native and irradiated without scavengers crotoxin as well as crotoxin incubated with the scavengers. All samples were dialysed against water and structural alterations were investigated by HPLC size exclusion chromatography. Toxic and phospholipase A₂ activities were determined by LD₅₀ and indirect hemolysis respectively. The results showed that incubation of the samples with the scavengers led to dissociation of the polypeptidic chains as a result of S-S bonds reduction. However, when irradiating these samples and removing the scavengers, a large amount of aggregates could be observed, suggesting reassociation via RS* radicals. The aggregates amount was higher in those samples than in irradiated control. Toxicity was not reached although we assayed it till 27,8 LD₅₀ of native crotoxin. Enzymatic activity decreased as a consequence of incubation with the scavenging substances and no further inactivation could be observed following irradiation.