Spectroscopic characterization of the effects of gamma radiation on crotoxin

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Ionizing radiation has been successfully used to attenuate the biological activity of proteic toxins. This approach has been explored by our group to investigate the potential of irradiated venoms as an alternative for the production of anti-ophidic sera, using an antigen with a much lower toxicity but, with preserved immunogenicity, resulting in high levels of neutralizing antibodies. All our results indicate that irradiated venoms and toxins undergo structural modifications, but further characterization of the irradiated product are still required in order to explain the detoxification process. Previous data, using crotoxin as a model, indicate that this protein, following irradiation, undergoes unfolding and/or aggregation resulting in a much lower toxic antigen, but the exact mechanisms and structural modifications leading to aggregation are not clear yet. In the present work, we compared native, purified crotoxin, with its 2 kGy irradiated counterpart. Analytical procedures included fluorescence spectroscopy, circular dichroism and dynamic light scattering. Our results indicate that after irradiation, the soluble protein presented higher fluorescence, associated with losses of secondary structure and formation of soluble aggregates. The analyses by light scattering indicate that the irradiated crotoxin formed multimers with an average molecular radius 100 folds higher than the native toxin. Taken together, our data indicate that changes in secondary (and possibly tertiary) structure, as well as oligomerization, led to shielding of previously exposed tryptophans, resulting in increased fluorescence. Concluding, irradiation leads to progressive changes in the structure of the toxin, which could explain the decrease in toxic activity.

Word Keys: gamma radiation, crotoxin, spectroscopy

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