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Effects of ^{60}Co radiation on the molecular strucure of crotamine ☆

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Abstract

Crotamine is a 4882 daltons basic polypeptide with myotoxic activity. This toxin induces skeletal muscle spasms leading to spastic paralysis of the hind limbs in mice. Ionizing radiation has been successfully employed to attenuate toxins, preserving their immunogenic properties. The molecular alterations suffered by irradiated biomolecules are not yet fully characterized and much work remains to be done within this field. In the present work, we used crotamine as a model to investigate the effects of gamma radiation on the structure of polypeptides. Toxin samples were irradiated with 400, 2000 or 10,000 Gy, doses at a 5.17 kGy/h dose rate in a gammacell ^{60}Co source. After irradiation, the samples were analyzed by mass spectrometry in positive mode. Also, structural changes were investigated by solvent-mediated quenching and UV spectroscopy.

Keywords: Crotamine; Gamma radiation; Spectroscopy

1. Introduction

Crotamine is a strongly basic polypeptide ($pI = 10,3$), with myotoxic activity and a molecular weight of 4882 Da from the South American rattlesnake *Crotalus durissus terrificus* (Laure, 1975). It is composed of 42 amino acid residues, without free sulfhydryl groups and reticulated by three disulfide bonds (Boni-Mitake et al., 2001).

This toxin affects the functioning of voltage-sensitive sodium channels of skeletal muscle sarcolemma, inducing a sodium influx, resulting in depolarization and contraction of the skeletal muscle. These effects induce necrosis of the muscle fibers characterized by extensive vacuolization of the sarcoplasmatic reticulum and disruption of actin and myosin filaments (Smith and Schmidt, 1990).

In the present work, we used crotamine as a model to investigate the effects of gamma radiation on the structure of polypeptides.

2. Materials and methods

2.1. Crotamine irradiation

Crotamine was dissolved in 0.15 M NaCl solution to a final concentration of 2 mg/ml and irradiated with 400, 2000 or 10000 Gy doses with a 5.17 kGy/h dose rate using gamma rays emitted by a ^{60}Co source (Gammacell, 220–Canada).

2.2. Ultraviolet spectra

The native and irradiated samples were submitted to UV scanning from 200 to 360 nm. The blank employed for baseline subtraction consisted of 0.15 M NaCl.

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2.3. Mass spectrometry

Aliquots of native and irradiated crotamine were injected into an electrospray ion source fitted to a Micromass Q-TOF mass spectrometer in positive mode. The mass was obtained by scanning the instrument from 800 to 2000 m/z at a rate of 2 s per scan.

2.4. Solvent-mediated fluorescence quenching

Aliquots of 5 μg/ml in 50 mM ammonium phosphate pH 7,8 of the native and irradiated samples were analyzed on a Hitachi F-2000 fluorescence spectrophotometer at 25°C. The excitation wavelength was fixed at 275 nm and the emission was scanned from 300 to 500 nm.

3. Results and discussion

3.1. Ultraviolet spectra

The UV spectra (Fig. 1) indicate changes in the chromophores exposure. The toxin irradiated with 2000 Gy presented a higher absorbance spectrum than the other samples. However, all the samples presented similar absorbance at 280 nm, indicating that the chromophores were not destroyed during the irradiation process. Thus, the spectral differences may be ascribed to the unfolding of the polypeptide chain. These data suggest that irradiation leads to changes in the primary and tertiary structure of the toxin. These results are in agreement with the data reported about crotalic venom and crotamine irradiated with 2000 Gy (Costa and Rogero, 1988; Boni-Mitake et al., 2001).

3.2. Mass spectrometry

The native and 400 Gy irradiated toxins showed a mass of 4882,1 Da matching the molecular weight described in the literature (Laure, 1975).

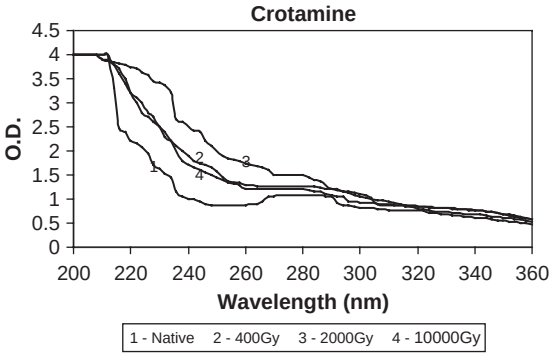

Fig. 1. Analysis of UV spectra of native and irradiated toxins.

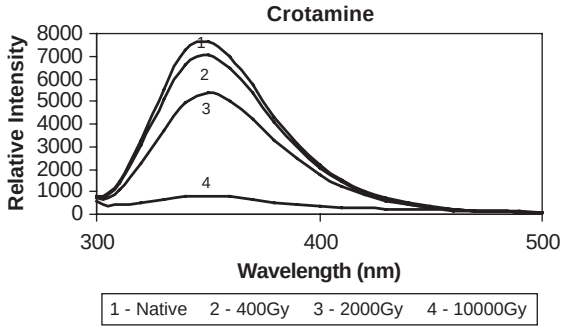

Fig. 2. Analysis of fluorescence quenching of native and irradiated toxins.

When the toxin irradiated with 2000 and 10,000 Gy were analyzed, the mass envelopes became more complex, suggesting oxygen adducts, formation. With 2000 Gy dose, an envelope corresponding to a crotamine dimer was detected ($mass = 9765,8$), while with the 10,000 Gy sample, a trimeric molecule ($mass = 14632,5$) was observed when the spectrum was scanned between 700 and 1500 m/z.

3.3. Solvent-mediated fluorescence quenching

Since the UV data indicate that irradiation did not affect the tryptophan residues, the dose-dependent decrease in emission at 350 nm may be due to the exposure of the fluorophores to the solvent. This alteration can indicate the unfolding of the molecule (Fig. 2).

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