METHODS FOR RADIATION CONTAMINATION DETERMINATION

Liliana Radu

Department of Molecular Genetics, V. Babes National Institute, Bd. Timisoara 17 A, Bl. 106 A / 56, *Bucharest 061304, Romania, Tel. 00 40 21 4137746, E-Mail : serbanradu@pcnet.ro*

Abstract

The determination of radiation contamination was analyzed in the case of fast neutrons action on chromatin - the complex of deoxyribonuclec acid (DNA) with proteins, that exists in eukaryotic cells nuclei. The effects produced by fast neutrons (0-100 Gy) on chromatin structure were analyzed by (1) ^{1}H-NMR spectroscopy, (2) steady-state fluorescence, (3) time resolved fluorimetry and (4) fluorescence resonance energy transfer (FRET). The fast neutron action on chromatin determines bigger values of the NMR transverse relaxation time, indicating a more injured structure. The chromatin intrinsic steady-state fluorescence decreases on radiation action, proving the destruction of the chromatin protein structure. Time resolved fluorescence measurements shows that the relative contribution of the excited state lifetime of bound ethidium bromide to chromatin DNA diminishes with increasing irradiation dose. This reflects the production of single and double strand breaks of DNA. By FRET method, the distance between dansyl chloride and acridine orange coupled at chromatin was determined. This distance increases upon fast neutron action. As the values of physical parameters analyzed are specific for a determined dose, the establishment of these parameters may constitute a criterion for the radiation contamination at chromatin level.

Introduction

The structure of chromatin, the complex of DNA with proteins, that exists in eukaryotic cells nuclei, is well known (1, 2). DNA is coupled in chromatin with basic proteins (histones) and acidic proteins (nonhistones).

The effects of fast neutrons on DNA have been studied by different methods (3, 4, 5). The damages induced in DNA are single and double strand breaks (SSB and DSB, observed at neutral pH). They are either due to the attack of the sugar moiety (essentially in C4' and C5' positions) and to the modification of the bases by the $OH^{\cdot}$ radical, produced by the radiolysis of water, called indirect effect (the main effect in aerated solutions) or to the direct ionization of DNA constituents, called direct effect. The fast neutrons action on chromatin determines not only damages of DNA, but also of chromatin proteins and of the interaction between DNA and chromatin proteins. A destruction of chromatin proteins takes place and a change in its global structure (6).

In the present paper, we report a study of fast neutrons effects on the chromatin extracted from livers of Wistar rats. Effects on the constitutive DNA and proteins, as well as on the global structure of the chromatin are examined by NMR spectroscopy, steady-state fluorescence, time resolved fluorescence and fluorescence resonance energy transfer (FRET).

As the physical parameters analyzed are specific for a determined dose, the value of these parameters may constitute a criterion for the radiation contamination at chromatin level.

Materials and Methods

The rats were cared for in accordance with legal regulations.

The chromatin was extracted from livers of Wistar rats, according to the standard procedures (7). The chromatin samples with 2.5×10^{-4} M ($\cong$ 100 μg/ml) DNA concentration in 10^{-2} M phosphate buffer, pH=7 were used.

A U-120 classical variable energy Cyclotron was employed as an intense source of fast neutrons produced by 13.5 MeV deuterons on a thick Beryllium target.

The spin-spin relaxation time or the transverse relaxation time (T_2) of chromatin samples was established. The process that determines the arrival of nuclear spins at equilibrium is defined by:

[1] $\Delta \nu_{1/2} = 1/ (\pi.T_2)$

where $\Delta \nu_{1/2}$ is the width at half intensity of the Lorentz line.

The measurements of fluorescence intensity of the chromatin tyrosine (which exists in histones) were performed at 305 nm emission wavelength (λ_{em}) with 280 nm excitation wavelength (λ_{ex}) and for chromatin tryptophan (which exists in nonhistones), at λ_{em} = 345 nm with λ_{ex} = 290 nm.

Ethidium bromide - EtBr -(Sigma) was used as a DNA intercalating agent, at a concentration of 6.25×10^{-5} M and its binding was monitored using time–resolved fluorescence measurements (measuring of the lifetimes of fluorescence). The fluorescence response function (8) is:

[2] $I(t) = A + B_1 \exp. (-t/\tau_1) + B_2 \exp. (-t/\tau_2)$

where I(t) is the time dependent fluorescence intensity, A, B_1, B_2 are constants, and τ_1, τ_2 are the lifetimes for bound and free states of the ligand.

In FRET method, a donor fluorophore is excited by incident light and if an acceptor is in close proximity, the excited state energy from the donor can be transferred to it (9). Double fluorescent labeling of chromatin was performed with dansyl chloride (Sigma) (λ_{ex} = 323 nm, λ_{em} = 505 nm) and acridine orange (Gurr) (λ_{ex} = 505 nm, λ_{em} = 530 nm). The dansyl chloride reacts, under mild alkaline conditions, with α - and ε -amino groups of the proteins, the cysteine sulphydryl group, the histidine imidazole group and tyrosine phenolic group, while the acridine orange is intercalated between DNA base pairs.

The Förster energy transfer efficiency is defined as:

[3] $E_f = (I_A^D / I_A - 1) \cdot \varepsilon_A / \varepsilon_D$

where I_A and I_A^D are the fluorescence intensities of the acceptor, in the absence and in the presence of the donor, respectively; ε_A and ε_D are the molar extinction coefficients of the ligands at excitation wavelength.

The energy transfer efficiency of this process depends on the inverse sixth distance, *r*, between donor and acceptor, by the expression:

[4] $E_f = r^{-6} / (r^{-6} + R_0^{-6})$

where R_0 represents the Förster critical distance at which 50% of the excitation energy is transferred to the acceptor. If the molar absorption coefficient is in the usual chemical units, $M^{-1}cm^{-1}$, wavelength in cm, and the units of J are $M^{-1}cm^3$, the expression of R_0^6 is:

[5] $R_0^6 = 8.79 \times 10^{-25} (n^{-4} Q_d k^2 J)\ cm^6$

where n is the refractive index of the medium, Q_d is the fluorescence quantum yield of the donor in absence of the acceptor, k^2 is the orientation factor for dipole – dipole interaction and J is the normalized spectral overlap integral. For this pair of fluorochromes R_0 = 29.72 Å.

The RMN determinations were performed with a NKr Aroma 78 spectrometer. A time-resolved fluorimeter FL 900 CD, Edinburgh Analytical Instruments was used for time resolved fluorimetry measurements and an Aminco Bowman SPE 500 fluorimeter was used for steady-state fluorescence and FRET determinations.

Results and Discussions

(1) ^{1}H-NMR spectroscopy

The dependence of the transverse relaxation time (T_2), obtained by NMR spectroscopy, on fast neutrons dose is represented in Fig. 1.

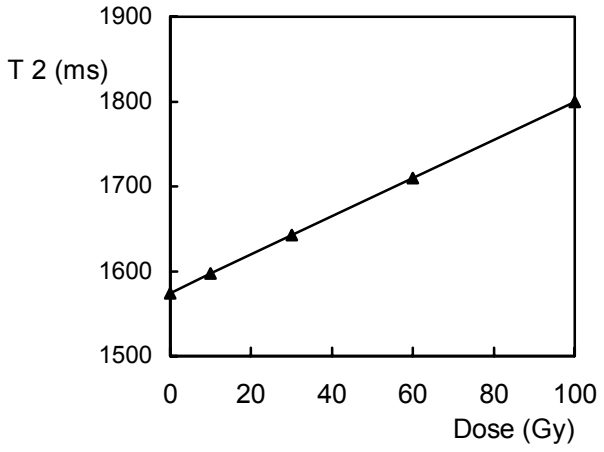

Fig. 1. The chromatin transverse relaxation time versus radiation dose

The dependence of chromatin transverse relaxation time (T_2) on fast neutron dose, D, is linear:

[6] $T_2(ms) = 2.25\ D + 1{,}575$

The increase of T_2 values on fast neutrons action indicates less bound water in chromatin, therefore a more injured chromatin structure.

(2) Intrinsic steady-state fluorescence

The variation of the chromatin intrinsic (tyrosine and tryptophan) fluorescence intensities on radiation dose are indicated in Fig. 2 and 3.

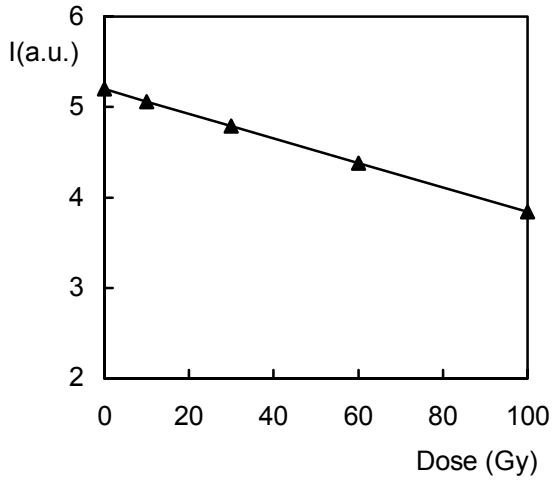

Fig. 2. The chromatin intrinsic fluorescence, corresponding to tyrosine, versus radiation dose

The dependence of the chromatin intrinsic (tyrosine) fluorescence relative intensity (I) on fast neutrons dose (D) is linear :

[7] $I = -\ 0.0136\ D + 5.2$

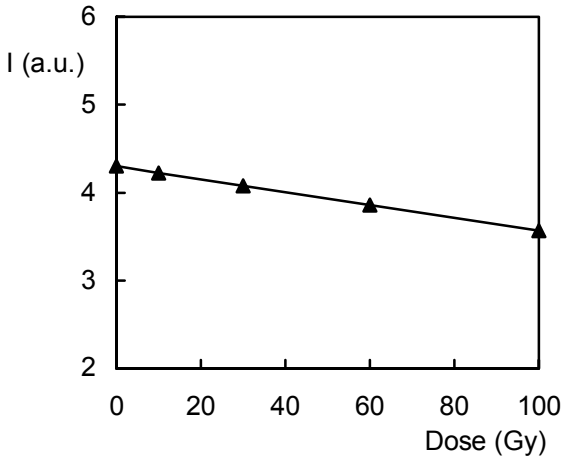

Fig. 3. The chromatin intrinsic fluorescence, corresponding to tryptophan, versus radiation dose

The dependence on dose, in the case of chromatin fluorescence intensity, corresponding to tryptophan is:

[8] $I = -\ 0.0073\ D + 4.3$

The modification of the intrinsic chromatin fluorescence indicates a destruction of chromatin protein structure, due to radiation action. As the protein structure is damaged, the fluorescence intensity of its fluorescent aminoacids is reduced.

(3) Time resolved fluorescence

The time resolved fluorescence measurements supplied the lifetimes of the excited states of free (2 ns) and bound (24 ns) EtBr to chromatin. The dependence of the relative contribution, f (%), of excited state lifetime of bound EtBr to chromatin on fast neutrons dose is presented in Fig. 4.

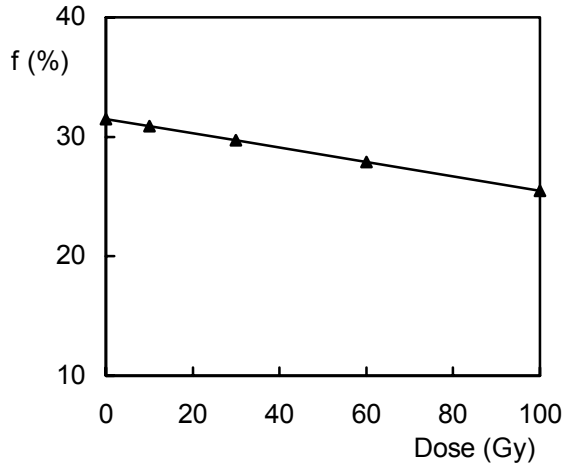

Fig. 4. The relative contribution of the excited-state lifetime of bound ethidium bromide in chromatin versus radiation dose

The relative contribution, f(%), of excited state lifetime of bound EtBr to chromatin on fast neutron dose, D, varies linearly:

[8] $f(\%) = - 0.06\ D + 31.5$

Since in a free DNA, one EtBr molecule gets intercalated between 2 base-pairs (4 nucleotides), in the experimental conditions used, all EtBr molecules are bound to a free DNA. In the case of EtBr binding to chromatin, in the same conditions of concentrations, only a part of the ligand is bound to DNA, due to the masking of DNA binding sites by chromatin proteins (10).

The diminution with fast neutrons dose of the relative contribution of the excited state lifetimes of bound ligand constitutes an evidence of the reduction of chromatin DNA double strand structure, due to the single and double strand breaks produced in DNA structure.

3) Fluorescence resonance energy transfer (FRET)

In Fig. 5 is represented the average distance between dansyl chloride and acridine orange coupled at chromatin versus fast neutrons dose.

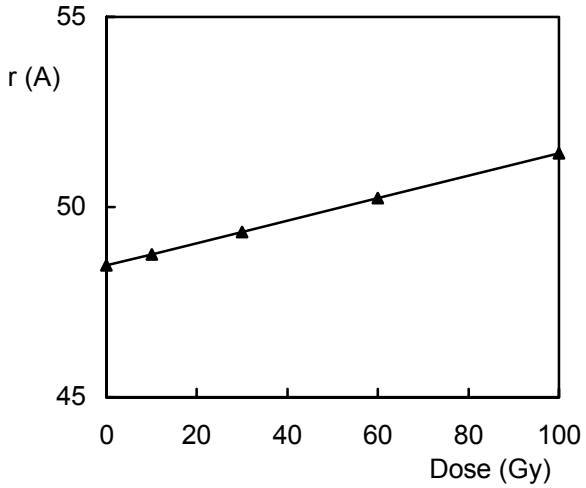

Fig. 5. The average distance between dansyl chloride and acridine orange coupled at chromatin versus radiation dose

The distance r (Å), between donor chromophore and acceptor chromophore, varies linearly with the fast neutrons dose (D) :

[10] $r(Å) = 0.0296\ D + 48.46$

The increase of the average distance between the two ligands, under the action of fast neutrons, reflects the growth of the distance between chromatin proteins and DNA, which suggests a loosening of the chromatin structure.

Conclusions

The greater values of the RMN transverse relaxation time (T_2), on fast neutrons action, indicate less bound water in chromatin, therefore a more injured structure.

The chromatin intrinsic fluorescence decreases on radiation action, proving the destruction of chromatin protein structure.

By the time resolved fluorescence was established that the relative contribution of the excited state lifetime of bound ethidium bromide to chromatin DNA diminishes with the fast neutrons dose. This denotes single and double strand breaks produced in chromatin DNA structure.

By FRET method it was observed that the mean distance between chromatin proteins and DNA increases upon fast neutron irradiation.

The parameters analyzed: the RMN transverse relaxation time of chromatin (T_2), the intrinsic chromatin fluorescence (I), the relative contribution of the excited state lifetime of bound ligand for chromatin-ethidium bromide complexes (f), established by time-resolved fluorescence and the average distance between dansyl chloride and acridine orange, coupled at chromatin (r), determined by fluorescence resonance energy transfer method (FRET) vary linearly with the fast neutrons dose.

The values of physical parameters determined are specific for a determined dose. The analysis of these parameters may constitute a criterion for the radiation contamination determinations.

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