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Radiation Effects On Mammalian DNA - PAC Method And Theory

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Abstract

The flexibility of DNA and its molecular dynamics have been the subject of various studies. One of the techniques that have been employed is the PAC method, which is considered as a valuable tool for such studies. In PAC experiments we can detect the angular correlation of two γ rays emitted in succession by a radioactive probe. Using the hyperfine interaction of the probe with its surrounding electric field gradient we can obtain information about the dynamics of the molecules to which the probe is attached. In this work, for the PAC measurements, we use ^{111}In which is added in a buffered solution of calf thymus DNA, exposed to various doses (0 - 80 Gy) of γ -rays.

1 Introduction

The probability of emission of a radiation by an excited nucleus depends on the orientation of nuclear spin axes relative to the direction of emission. In an ordinary radioactive source, the nuclei have their spins distributed randomly in space and therefore the radiation emitted is isotropic.

In angular correlation work, the nuclear orientation in a system composed of many radioactive nuclei, which decay to the ground state with the emission of a number of γ -rays in cascade, is obtained simply by selecting only those nuclei which have emitted the first γ -ray, γ_1 in a fixed direction \mathbf{k}_1 . The intermediate nuclear states will have their spins aligned relative to \mathbf{k}_1 , and therefore, the second γ -ray, γ_2 , detected in coincidence with the first one, is not isotropic. This explains why the emission probability of γ_2 is a function of the angle theta between γ_1 and γ_2 , that is to say, why the γ_1 and γ_2 are correlated in space [1,2].

From the experimental point of view, the angular correlation is observed by measuring γ_1 and γ_2 in coincidence, as a function of angle between the two counters: a fixed one which detects γ_1 and a mobile one which measures γ_2 in different angles relative to the direction of γ_1 . For this reason fast - slow coincidence circuits are used. Two types of measurements are performed, depending on the mean life, τ , of the intermediate level and on the resolution time t_c of the coincidence circuit. The most valuable information is obtained from the time differential angular correlation (TDAC) measurements [3]. For such measurements to be possible, the resolution time must be much smaller than the mean life of the intermediate level.

The ^{111}In isotope has a half life of the intermediate level ≈ 84 nsec while the resolution time of the system (two NaI detectors) with which we have obtained the results shown below was 5 nsec. However, our system has been recently improved (two BaF_2 detectors) and the resolution time is now ≈ 0.5 nsec.

2 The PAC Method

In a time differential angular correlation experiment, a suitable radioactive probe - usually ^{111}In - as in the present case - is attached to the molecule of interest and the probability $W(\theta, t)$ of emission of two successive γ -rays is measured, as a function of the relative angle and the time interval between the two emissions. The correlation function has the form:

$$W(\theta, t) = \text{const} \cdot \exp(-t/\tau) \cdot \sum_{\nu} A_{\nu\nu} G_{\nu}(t) P_{\nu}(\cos\theta) \quad (1)$$

$$\nu = 0, 2, 4, \dots$$

where τ is the mean lifetime of the intermediate state, $A_{\nu\nu}$ depend on the spins and multiplicities of the transitions, $P_{\nu}(\cos\theta)$ are the Legendre polynomials and $G_{\nu}(t)$ are the perturbation factors which contain the influence of extranuclear fields [1,4,5]. Thus, in a PAC measurement, all the information of physical interest is contained in these perturbation factors.

If the intermediate nuclear state between the two successive emissions has a spin $I = 5/2$, as is the case of ^{111}In , terms above $\nu = 2$ in equation (1) can be neglected. A measurement of the angular correlation $W(\theta, t)$ at 180° and 90° can yield the perturbation factor $G_2(t)$ from

$$G_2(t) = \frac{2}{A_2} \left[\frac{W(180^\circ, t) - W(90^\circ, t)}{W(180^\circ, t) + 2W(90^\circ, t)} \right] \quad (2)$$

Theoretically, if the perturbation is caused by a static electric quadrupole interaction, the perturbation factor can be written to second order as

$$G_2^*(t) = S_{20} + \sum_{n=1}^3 S_{2n} \exp(-\frac{1}{2}\omega_n^2 \delta^2 t^2) \cdot \exp(-\frac{1}{2}\omega_n^2 \tau^2) \cdot \cos(\omega_n t)$$

where ω_n are the quadrupole interaction frequencies which are harmonics of a minimum frequency ω_0 and the exponential damping terms account for the distribution of ω_n and the finite experimental resolving time. The coefficients S_{2n} depend on the geometry of the radioactive sample and have been tabulated for several special cases [6].

When a probe, such as ^{111}In , is attached to a slowly rotating molecule with rotational correlation time τ_c , the static perturbation factor is modified as follow

$$G_2(t) = G_2^* \cdot \exp(-t/\tau_c) \quad (3)$$

However, for the case of a rapid molecular motion where τ_c is short compared to the mean lifetime, this modification takes the simpler form

$$G_2(t) = \exp(-\lambda_2 t) \quad (4)$$

where the inverse time (relaxation) constant $\lambda_2 = c\omega^2\tau_c$ with $c = 3.6 \cdot [4I(I+1) - 7]$.

If the radioactive probe can be attached to more than one system of hosts with different perturbing characteristics, as in an actual experiment, the experimentally determined $G_2(t)$ (Eq. 2) is in fact the weighted sum of the individual perturbation factors which can be of any type described by equations (3) and (4). In the present case, the general form assumed was

$$G_2(t) = f_0 + f_1 G_2(t)(Eq.3) + f_2 G_2(t)(Eq.4) + f_3 G_2(t)(Eq.3) \quad (5)$$

where $f_0 + f_1 + f_2 + f_3 = 1$ and the fractions correspond to the unbound ^{111}In ions, the bound ones to DNA, the present ions in the form of aggregates with polar water molecules and the bound ions to the glass container respectively [7,8].

Experimentally, as is now clear, we get two coincidence spectra at 180° and 90° and, since $N(\theta, t) = \bar{N} \cdot W(\theta, t)$, we obtain $G_2(t)$ (Eq.2). The next step is to use a minimizing procedure and try to fit the experimental $G_2(t)$ with

the theoretical one varying $f_i (i = 0, 1, 2, 3), \omega_0, \tau_c, \eta, \delta$. In most experiments $f_3 = 0$. The computer program used is the "MINUIT" and can be obtained from CERN.

These parameters are interpreted as follow:

f_i : Gives the information about the fraction of radioactive nuclei being bound to the macromolecules of interest. In a comparative set of measurements one might obtain useful information as for the condition of binding.

ω_0 : Gives the information about the strength of interaction. Again only a comparative set of measurements can lead to useful information (change of the environment of the macromolecule etc.).

τ_c : Includes the information about the flexibility of the macromolecule. If one assumes Debye's formula $\tau_c = \frac{4\pi r^3}{3kT} \xi$ (where r is the effective molecule radius and ξ is the viscosity), the "mean radius" of the molecules can be obtained. Otherwise, a comparative analysis can yield information about the changes in the flexibility of the macromolecules.

η : Is the anisotropy of the field gradient.

δ : Is the statistical distribution of ω .

Both η and δ give information about the binding sites.

3 Experiment

The radioactive label ^{111}In , which decays to ^{111}Cd , was obtained as carrier free $^{111}\text{InCl}_3$ in HCl. The angular correlation measurements were performed on the 173 - 247 keV transitions of ^{111}Cd , using the computer controlled correlation apparatus at Demokritos with two $3\text{in} \times 3\text{in}$ NaI (Tl) detectors.

For the irradiation of the calf thymus DNA samples, a ^{60}Co source of 15 Ci was employed. The ^{111}In was added to each individual sample in a final concentration of $5 \times 10^{-1}\text{M}$. A total of 5 mCi were used over a period of 4 weeks, with a fresh sample placed in the apparatus every 24 h [7].

4 Results

As it is seen from the Fig. 1 [7], the PAC method can differentiate the five groups of samples, i.e. the buffer, the non-radiated DNA (control) and the DNA exposed to 20, 40 and 80 Gy of γ radiation. After fitting these perturbation factors to the theoretical function of Eq.(5) using the program MINUIT, the corresponding rotational correlation times and quadrupole frequencies were obtained.

The rotational time, τ_c , as defined in Eq.(3), is a direct measure of the rate

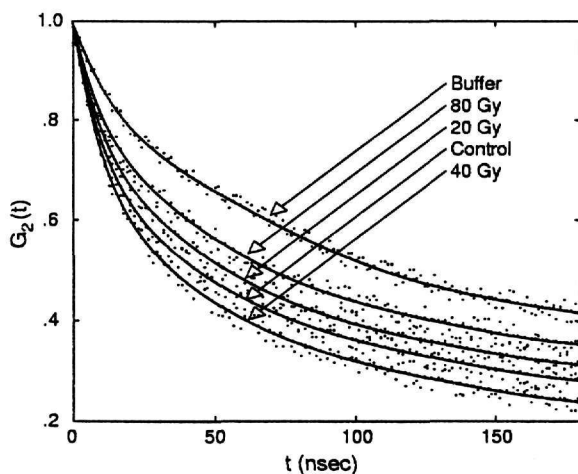


Fig. 1. Plots of the perturbation factor, $G_2(t)$, for the five different groups of samples (i.e. buffer, control, and DNA exposed to 20, 40 and 80 Gy of γ -radiation) as a function of the time lapse between the successive emission of the 173 and 247 keV γ -rays of ^{111}Cd .

at which the particular site - to which the radioactive probe is attached - changes its direction and, therefore, can be associated with the flexibility of the molecule. As shown in Fig. 2, the variation of the rotational correlation time as a function of the irradiation dose clearly indicates that there is a change in the flexibility of the irradiated DNA. However, the relatively large experimental errors in the τ_c values do not allow for a clear comparison between the three different radiation doses.

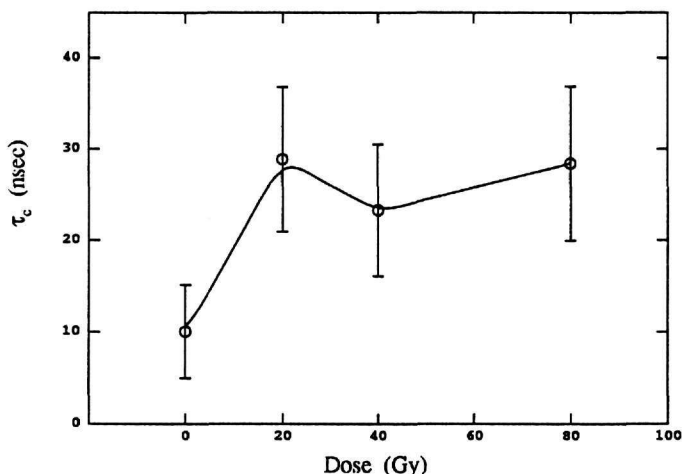


Fig. 2. The variation of the correlation time τ_c as a function of the dose.

The quadrupole frequency is a measure of the strength of the perturbing interaction and is related to the topology of the electric field gradient at the

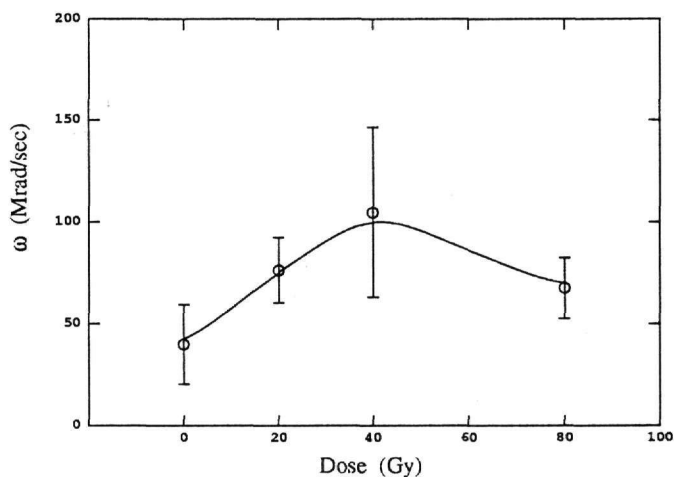


Fig. 3. The variation of the quadrupole frequency ω_n as a function of the dose.

binding site of the ^{111}In probe. It is expected that the breaking of any bond at the binding site will result in steeper field gradients, thus increasing the quadrupole frequency. In Fig. 2 we can see that our experimental results follow this expectation. In addition, the values of ω_n , although they also suffer from relatively large experimental errors, indicate that the breakage of the bonds indeed result in steeper field gradients [7].

As for these large errors, we hope that our new device with the two BaF_2 detectors will improve the results. More measurements will also be obtained in different doses.

Most of the damage induced on the DNA molecules exposed to ionizing radiation in aqueous solutions evolves from the interaction with water free radicals. The result is the partial or total destruction mainly of the nitrogenous bases of the DNA molecule. These alterations lead to the appearance of single strand breaks (SSB). Accumulation of SSBs results in the breakage of the hydrogen bonds among the opposite strands and the formation of double strand breaks (DSBs).

The observed increase in the rotational correlation time, even at small doses where SSBs are predominant, can be understood in the light of the "segmental flexibility" proposed by Alisson and Schurr [9]. In their model, the DNA can be viewed as consisting of a series of identical rigid rods connected at their ends by torsion springs. It has been suggested that the torsion springs could be attributed to the action of absorbed polycations. However, this does not seem likely since the segmental flexibility described by this model has also been observed in experiments where aqueous solutions of DNA were used without any proteins being present. The measurements already shown with the method of PAC resulted in relatively short correlation times (< 20 nsec) in agreement

with the rapid decay of the fluorescence polarization anisotropy of ethidium bromide bound to DNA. It is therefore essential to search for alternative ways to explain this segmental flexibility other than the one offered by Alisson and Schurr since the latter could be justified only under certain conditions not existing in all observed cases. Furthermore, if such an alternative way could be envisioned, it should form the basis describing, in quantitative terms, other phenomena related to structural changes of DNA, such as thermal denaturing, as the existing models for describing the thermally induced helix to coil transitions in DNA have certain limitations.

5 Discussion

As in all theoretical approaches DNA is considered as a quasi-one-dimensional lattice composed of N base pair units. Under ordinary conditions, it forms a double helix (B form). This double-stranded structure is held together by hydrogen bonds between complementary base pairs (A-T or G-C) and stacking (hydrophobic) interactions between the nearest neighbor bases on same strands. When the excitation modes of an individual nucleotide are considered, it is seen that the energy required for a transition between the two lowest states is of the order of 80 kcal/mole, while transitions between vibration levels require energies of the order of 10 kcal/mole. Therefore, thermal energies at room temperature could only induce transitions between rotational states of the individual nucleotides since the spacing of their energy levels is of the order of 1 kcal/mole. In addition, the "stacking potential" will allow energy to be stored in the form of vibrations between nearest neighbor bases at same strands. However, it is difficult to visualize that this part of the internal (thermal) energy of such structure could be stored in the form of coherent vibrations covering the whole length of the formation constituting a DNA molecule. As more likely, local coherent vibrations covering a few tens or hundreds of base pairs would prevail. Each group of coherently vibrating stacked base pairs along the DNA axis can constitute a "rod". An increase in temperature and, therefore in internal energy, will result in an increase of the vibrating amplitudes, eventually leading to unstacking and a subsequent weakening of the hydrogen bonding between the complementary base pairs. This will in turn facilitate the disruption of the hydrogen bonds leading to the denaturation or melting of DNA, forming single stranded chains. In the framework of this overall picture, one can attempt to develop a quantitative description of the behavior of DNA under varying external conditions, such as temperature, or when subjecting DNA to any form of ionizing radiation, such as γ rays.

5.1 Model Description

One of the most frequently used methods to investigate the melting transition in DNA is the measurement of the variation in the UV absorbance of a dilute DNA solution as a function of increasing temperature. The basic assumption of the proposed theoretical description is that the observed hyperchromicity (i.e. increased UV absorbance) in the melting transition of DNA is directly proportional to the number of disrupted base pairs [10].

Assuming that the UV absorbance has a linear dependence on the number of disrupted base pairs, the above expression can be transformed into

$$A(\theta) = A_{st} + A_u \cdot (1 - e^{-\frac{c(\theta-\theta_o)^{N+1}}{N+1}}) \quad \text{for } \theta > \theta_o \quad (6)$$

$$A(\theta) = A_{st} \quad \text{for } \theta < \theta_o \quad (6a)$$

where A_{st} is the UV absorbance of the intact DNA helix and A_u is the hyperchromicity (i.e. the increase in the absorbance corresponding to a fully denatured DNA molecule). The subscripts “st” and “u” stand for “stacked” and “unstacked” respectively. The parameter N is related to the possible paths along which energy is conducted. The power form is readily understood if each such path is considered as having an independent contribution while the overall effect is the composite result. Bearing in mind that it is generally assumed that DNA can be viewed as a quasi-one-dimensional lattice, N must be relatively small, which is in contrast to the case of a crystalline structure of any solid where N must tend to infinity. However, as it is shown in Fig. 4 [10], in this limit Eq. (6) does become a step function, describing correctly the behaviour of an ordinary crystal with definite melting point. Thus, in this proposed model a special importance is assigned to the variation in the value of N when associated structural variations are studied.

When DNA is exposed to any ionizing radiation, for example γ -rays, SSBs (Single Strand Breaks) and DBSs (Double Strand Breaks) are formed. At small doses, where SSBs are dominant, the formation of SSBs disrupts the thermal energy flow along the long axis of DNA. This can be best understood if we consider that it is through a coupling of the stacked base pairs that coherent energy flow through this mode of transfer. The remaining thermally accessible mode of energy transfer is the transition of the G-C and A-T pairs to higher rotational levels, as stated previously. Additional modes of energy transfer, for example the coupling of vibrational levels or transitions among electronic states, require higher energies than are available and, as such, will not be observed.

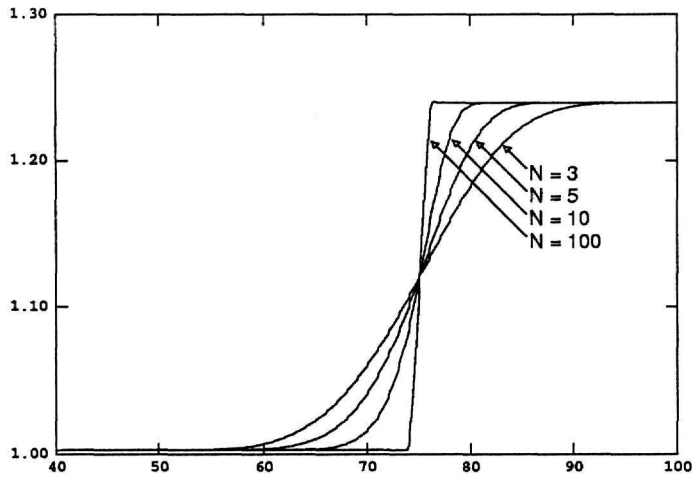


Fig. 4. The variation of $A(\theta)$ for different values of N . It is apparent that when N tends to infinity $A(\theta)$ becomes a step function.

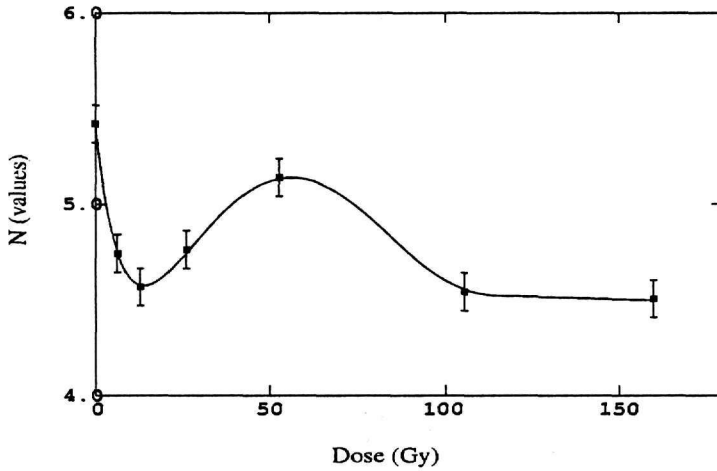


Fig. 5. The variation of the N parameter as a function of the dose.

The disruption of the thermal energy flow would result in decreasing values of N . However, when the radiation dose is increased, the contribution of DSBs also increases. The production of DSBs tends to form fragments as compared to the intact DNA. As a result, N is expected to show a tendency to increase again due to an increased conduction of thermal energy from the solution to the DNA and, as it is shown in Fig. 5 this notion is supported. Consequently, the variation in the power parameter N appears to be very sensitive to variations in the formation of SSBs and DSBs.

In addition, within the region around 50 Gy, the values of N maximize the irradiated samples. A similar trend within this dose region has been found when

thermodynamic parameters were estimated through work with inverse gas chromatography, indicating a high efficiency in the induction, at this particular region of exposure, of extensive structural alterations of the DNA molecule in aqueous solutions. This is also supported by the perturbed angular correlation measurements which were obtained from non irradiated DNA and DNA exposed to radiation under the same experimental conditions. Thus, the variation of the N parametrize the variation of $A(\theta)$ values observed in our experimental results. An explanation of this behaviour might be that, under our experimental conditions, the concentration of short double stranded DNA segments, originating from effective DSBs (i.e. DSBs that lead to fragmentation), maximizes within the region of 50 Gy resulting in an increase of the value of exponent N . The exponent N , according to the model, is associated not only with the possible paths of energy propagation along the DNA molecule but also with the surface to volume ratio of a molecule because this affects the rate of energy transfer from the solution to that molecule. This ratio is higher in short double stranded DNA segments than in the long ones of the non-irradiated material and will tend to drive N to higher values. However, at higher doses (above the region of 50 Gy), extensive damage and disruption of the DNA moieties restricts the possible paths of energy propagation but simultaneously lessens the effectiveness of additional induction of DSBs on the DNA molecules. As a result, the value of N will again tend to decrease and reach asymptotically its lowest value.

6 Conclusions

According to these first results of the TPAC measurements we believe that we can get valuable information about the structural changes of macromolecules such as DNA. In addition, with our improved detecting system, we hope to obtain more accurate measurements and be able to correlate the structural changes with radiation dose, particularly in the low radiation region.

Conclusions concerning the flexibility of DNA can also be obtained by the thermal transition results offering an additional tool so that to explain qualitatively the structural changes of irradiated DNA.

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