Photosensitization of plasmid-DNA loaded with platinum nano-particles and irradiated by low energy X-rays

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Abstract. Damage in DNA plasmids (pBR322) loaded with platinum nanoparticles (NP-Pt) DNA-NP and irradiated with monochromatic X-rays tuned to the resonant photoabsorption energy of the LIII and MIII electronic inner-shell of platinum - respectively 11556 eV and 2649 eV - and off-resonant X-rays - 11536 eV and 2639 eV- is investigated. In all the experiments, an enhancement of the single and double strand break - SSB and DSB - yields is observed when NP-Pt are present. Amplification effects are almost similar for the irradiations performed at on and off the L or M shell resonance suggesting that a non resonant mechanism is responsible for the major part of the DNA breaks enhancement. The amount of DNA breaks measured in the present work is compared to the results in similar experiments made with complexes of plasmid-DNA containing platinum molecule : chloroterpyridine platinum (PtTC). The average number of PtTC molecules in the solution is the same as in the experiments made with NP-Pt in order to study a possible difference in the radiosensitization efficiency when the high-Z atoms are clustered (NP-Pt) or dispersed in the system (PtTC). A mechanism is suggested involving photoelectrons which can efficiently ionize the platinum atoms. These results are consistent with those observed when DNA-NP complexes are irradiated by fast atomic ions. These findings suggest that any nanoparticle made of high-Z atoms might behaves as radiation enhancer whatever the ionizing radiation is electromagnetic or charged particle source.

1. Introduction

The Auger effect is widely used in radiobiology as a tool to generate secondary electrons and charged centers in order to induce molecular damages in the surrounding biomolecules. The underlying idea is that these damages will induce cell death and as a consequence might improves the therapeutic index in radiotherapy. Electron emitting radionucleides such as $^{125}$I, $^{131}$I, $^{32}$P are currently used in the treatment of thyroid tumors or leukemia. Many papers are devoted to the subject and they cannot all be listed here. We refer to a recent review article and references therein [1].

When an inner-shell excitation is induced in an atom, the relaxation of the electronic excited core can occur by photons emission or electron emission - Auger effect -. Both processes exist at the same time, and the branching ratio is in favour of Auger effect for K-shell excitation of relatively low Z atoms ( Z < 30 ) or L, M, N … electronic shell excitation for larger values of the atomic number. For
example the energy relaxation of the L_{III} shell of platinum (Z = 78) is about 80% via the Auger effect, whereas it is only 3% following the relaxation of a K shell excitation in this atom.

Different possibilities exist to induce a hole in an atomic inner shell. The capture of a K shell electron by the nucleus in excess of protons is the mechanism responsible for the Auger effect in the radionucleide ^{125}\text{I}. A more common way to trigger the Auger effect is to induce the excitation of an electron belonging to a K, L, M … shell by photoelectric effect or electronic impact. The absorption of photons by atoms depends of their energies and may exhibit a resonance structure near the ionization threshold. A particular atomic species in a molecule can thus be selected as a target for the primary energy deposition by tuning the photon energy to a specific resonant process.

In the present work we present the results on DNA breaks induction by low energy photons tuned to the L_{III} (11556 eV) and M_{III} (2649 eV) shell of platinum when plasmid DNA are loaded with high-Z atoms included in a molecule (PtTC) or with nanoparticles of platinum (NP-Pt). In the off-resonant X-rays experiments, the energy of the photons was adjusted below the resonant absorption peaks corresponding to the L_{III} and M_{III} shell respectively at 11536 eV and 2639 eV. In addition some experiments were made in presence of a free radicals scavenger, the dimethylsulfoxide (DMSO), in order to disentangle the respective contribution of the indirect effects - mediated by free radicals attacks - from the non mediated by free radicals ‘direct effects’.

2. Materials and methods

2.1. Preparation of the samples

For a simple and rapid quantification of simple and complex damage, plasmid DNA pBR322 provided by TOYBO (Japan) is used in our study. The double-stranded DNA of 4361 base pairs (2.83x10^6 Daltons) is diluted in TE buffer (10 mmol L\text{^{-1}} Tris-HCl (pH = 7.6) and 1 mmol L\text{^{-1}} ethylenediaminetetraacetic acid (EDTA)). Plasmid DNA presents the advantage to have three conformations namely supercoiled, circular, and linear with respectively no break, single strand break, double strand break, which can be separated by migration in agarose gel electrophoresis. Prior to irradiation, DNA samples contain more than 95% supercoiled, 5% circular and no linear forms.

Two types of radiosensitizers are studied in the present work. The first one was the platinum terpyridine chloride (PtTC), used as a platinum containing molecule, whose effect has been studied for long by the group [2]. The molecule PtTC is not an intercalator and does not induce any artefact in the analysis of the plasmids at the concentrations used in this work. It interacts smoothly with DNA through electrostatic interaction and is located close to the DNA. It is thus a good candidate to probe the physical processes, induced at the atomic level in the high-Z atoms bound to the DNA, triggered by X-rays irradiation.

The PtTC molecules were obtained from FLUKA - Sigma Aldrich Chemie GmbH Schnelldorf Germany - and used without any further purification. As shown previously by the group, a ratio of 1 platinum atom per 15 phosphate atoms (7 or 8 base pairs) corresponds to the optimized quantity of platinum to get efficient sensitization [2]. The concentrations of PtTC solutions were determined by spectrophotometry, at \(\lambda = 278\) nm. The absorption coefficient \(\epsilon\) is equal to 25100 L.mol\text{^{-1}}.cm\text{^{-1}} [3] at this wavelength.

Platinum nanoparticles (NP-Pt) coated with polyacrylic acid (PAA Sigma-Aldrich) were synthesized by radiolytic reduction of platinum complexes Pt(NH\text{3})\text{4Cl\text{2}H\text{2}O (Sigma Aldrich10^{-4} mol L\text{^{-1}) in aqueous solution containing PAA 0.5 mol L\text{^{-1}}. For more details in the protocol see reference [4]. The solutions were deaerated prior to the irradiation by bubbling with nitrogen. The irradiation was carried out in a panoramic source ^{60}\text{Co source, at a dose rate of 2.2 kGy h\text{^{-1}} (1 Gray = 1 J kg\text{^{-1}}. The irradiated solutions were protected from light and stored at 4°C. Transmission electron microscopy (TEM) observations were performed on a JEOL JEM 100 CXII transmission electron microscope at accelerating voltage of 100 kV. The nanoparticles have almost the same size, about 3 nm, and each contains around 1000 atoms. The solutions were finally diluted down to a concentration in metallic atoms similar to the concentration reached with PtTC.
A solution of DNA and PtTC or DNA and nanoparticles was prepared with a defined ratio \( r \) (ratio \( r = \frac{\text{number of Pt atoms}}{\text{number of P atoms in DNA}} \)). In the present work we have taken \( r = 1/15 \). No study with different ‘\( r \)’ values have been made in the present work. Such study can be found in a previous work [5] and shows that the number of DSB increases along the values of \( r \). The DNA was incubated with radiosensitizer for one hour prior to irradiation. When PtTC molecules or nanoparticles NP-Pt were added to the samples the concentrations of platinum atoms remain constant. The concentration of DNA and salty species remains also constant for every samples. The addition of any of the radiosensitizers had no deleterious effects on DNA, as shown systematically with controls. In order to investigate the role of the water radicals, the free radical scavenger DMSO was added in some samples at a concentration equal to 1.0 mol.L\(^{-1}\).

2.2. Irradiations

Irradiations by X-Rays were performed at the Photon Factory on beamlines 27 A and B, Tsukuba, Japan. A sample chamber for irradiation was made of acrylic resin with a Kapton film (7.9 \( \mu \text{m} \)) window. The window area was \( 8 \text{ mm} \) (in height) \( \times \) \( 10 \text{ mm} \) (in width) and the depth was \( 2 \text{ mm} \). A sample solution of 100 micro liter was put into the vessel. After appropriate doses of irradiation under an aerobic condition, small aliquots were taken out of the sample, and analyzed with gel electrophoresis. More details about the experimental setup can be found in the references [2, 6]. The doses ranged from 0 to 250 Grays when DMSO is not present and up to 800 Gy in presence of DMSO. Dose rates are a few Gy/min at BL-27 A and about 10 Gy/min at BL-27 B.

2.3. Plasmids analysis

The samples were loaded with 1 \( \mu \text{L} \) loading dye solution. The electrophoresis was performed in a 1.7 % agarose gel, in an electric field of 10 \( \text{V cm}^{-1} \), at 4\(^\circ\)C during 3h30. After migration, the gel was stained with ethidium bromide (1\( \mu \text{g mL}^{-1} \)), and the DNA lines were revealed under UV light (302 nm) and recorded by a CCD camera. The free of access image analysis software (National Institute of Health Image) was used to quantify the intensity of DNA lines of the different DNA conformations. Considering that the supercoiled plasmid (S) is binding the ethidium bromide 1.42 times less than the relaxed (R) and the linear (L) forms, the percentage of the three different conformations of DNA is determined. The average fraction of single and double strand breaks per plasmid (SSB, DSB) is calculated according to an earlier report [7]. In the equations below L and S represent respectively the normalized amount of linear and supercoiled forms of plasmids.

\[
\text{SSB} = \ln[(1 - L)/S] \quad \text{and} \quad \text{DSB} = L/(1 - L) \quad (1)
\]

3. Results and discussion

It is important to remember that the average number of platinum atoms is the same in all experiments presented here. The numbers of SSB and DSB per plasmid as a function of the dose (figures 1–4) increases in a linear way. The numerical values of the slope of the lines resulting from the fit are reported in the table 1. In all experiments an enhancement of the number of SSB and DSB is observed when the plasmids are in presence of NP-Pt or PtTC.

The figure 1 displays the induction of SSB in free DNA and complexes of DNA loaded with PtTC molecules or NP-Pt particles following irradiation by photons tuned to the M\( _{\text{III}} \) energy shell of platinum. The law of DNA breaks induction can be considered to be linear. The number of breaks is almost the same whether the photon energy is tuned to the M\( _{\text{III}} \) shell threshold (2649 eV) or just below (2639 eV). The number of SSB induced in similar conditions in DNA complexes with PtTC molecules is slightly larger than the latter one. The results obtained in experiments with PtTC irradiated with photons at 2639 eV (off resonance) are not shown for sake of clarity. They have also almost the same values than those measured at 2649 eV.
Figure 1. Number of SSB per plasmid versus dose induced by photons tuned on and off the M III inner-shell energy. Full triangles stand for irradiation of plasmid DNA added with PtTC. Open squares and diamonds stand for irradiation of plasmids added with nano-particles. Crosses represent the number of single breaks in free DNA.

Figure 2. Number of SSB versus dose induced in plasmid by photons tuned on the L III inner-shell energy. Full triangles stand for irradiation of plasmid DNA added with PtTC. Diamonds stand for irradiation of plasmids added with nano-particles. Crosses represent the number of single breaks in free DNA.

The figure 2 above displays the results on the SSB induction by photons at an energy corresponding to the L III energy shell of platinum. We can check in the table 1 that in all experiments the number of SSB per plasmid at the energy corresponding to the L III shell is larger than the number of SSB induced by photons tuned to the M III shell energy. However, when the nanoparticles are present, the specific enhancement $\Delta m$ (equation 2) is smaller at this energy than to the value for the M III shell energy. We propose below an explanation of this finding.

$$\Delta m = m_{SSB}(DNA + nanos) - m_{SSB} (free DNA)$$ (2)
Figures 3 and 4 show the number of DSB induced by photons tuned respectively to the M\textsubscript{III} and L\textsubscript{III} shell energy. The law for the DSB induction is seen to be linear and the numerical values of the slopes are reported in the table 1. It is important to notice that for all experiments made in presence of platinum atoms contained in PtTC molecules or nanoparticles the number of DSB is increased significantly.

The values reported in table 1 show that the number of DSB induced at L\textsubscript{III} or M\textsubscript{III} shell energy are close to each other and have almost the same values when the DNA is added with nanoparticles (9x10\textsuperscript{-5} breaks per plasmid per Gray). The values of the DSB-L shell obtained in previous experiments [2, 8] have been also reported in table 1. The values of reference 2 are relative to previous experiments with photons at L\textsubscript{III} energy and similar to the present one and show a good agreement with the present measures. The induction of DSB with C\textsuperscript{6+} carbon ions is studied in [8]. In the latter experiments with C\textsuperscript{6+} ions the values of the slope in presence of PtTC (19x10\textsuperscript{-5}) and without (9x10\textsuperscript{-5}) are larger than the slope measured when photon are used (13x10\textsuperscript{-5}, 6.7x10\textsuperscript{-5}). However one can remark that in all cases - DSB-M shell, DSB-L shell and DSB-C\textsuperscript{6+} - the ratio m(DSB, PtTC)/m(free DNA) is always near 2, independent of the photon or particle energy.
In the table 1 we report also the slopes of the SSB and DSB induction in presence of DMSO at the concentration of 1 M. The large decrease of the values of the slopes indicates that the SSB and DSB are mainly due to the attack of free radicals hydroxyl radicals HO• following radiolysis of water induced by secondary electrons. The Auger effects in platinum atoms induced by secondary electrons, like photoelectrons, results mainly in HO• free radicals production [2].

It is generally admitted that the SSB are induced by free radicals HO• that have migrated through the medium, whereas DSB are the consequence of production of dense free radicals in the proximity of the DNA [9]. The stronger Auger effect - more energy relaxed in LIII shell than in the MIII shell - induces more free radicals HO•. The local recombination of the HO• radicals in a dense cluster tends to decrease the number of migrating HO•, with the consequence that less SSB breaks are induced. Such discussion has been already presented in reference [10].

The Auger effect in LIII or MIII shell occurring in proximity of the DNA plasmid is always sufficient to induce a DSB. It can be said that there is some kind of saturation of DSB induction in that case. The number of DSB induction due to nanoparticles is less than the induction due to PtTC molecules. It should not be concluded that PtTC molecules are more efficient than nanoparticles to generate double DNA breaks. In the present experiments the nanoparticles have not the same binding capacity to DNA in comparison to the PtTC molecules as mentionned above. They are statistically located at a larger distance than PtTC from DNA. This different location in the medium is less favorable to induce the DSB as already mentioned [9].This simple model explains well why the specific enhancement of SSB due to nanoparticles decreases when the energy of the photons is larger, whereas the number of DSB is practically independent of the photon energy, at least in the present range of energies. The proposed model interprets also the fact that the major part of the SSB is scavengable by DMSO due to the migration of HO• in the buffer, whereas the DSB are less scavengable because the HO• produced close to DNA are less easily scavenged by DMSO.

The nanoparticles are widely considered nowadays to be a possible approach to improve the therapeutic index of radiotherapy. The present experiments suggest that no large difference in DNA damages induction is observed when nanoparticles (NP-Pt) or PtTC molecules are used. The soft X-rays used here will hardly present any interest for medical protocols in radiotherapy because they are strongly absorbed by the matter and cannot penetrate into the tissues. Radiation using X-rays in the 100-200 keV have been proposed by different groups see for example [11] and references therein. These photons can penetrate in a deeper location in tissues. However they are strongly absorbed in the entrance channel and can induce severe damages like radiodermitis in the healthy tissues.

In our group we have shown that the combination of radiation with ions (He2+, C6+) and PtTC or NP-Pt can enhance the cell death when they are loaded with PtTC [8] or DSB when plasmids [12] are added with NP-Pt. The atomic ions have an interesting dose deposition profile, preserving the entrance channel and they are able to deliver a large dose at a deep seated location (Bragg peak). For this reason they should be particularly efficient in the combination with radiosensitizers such as nanoparticles.

|          | PtTC on | Nanos on | Nanos off | Free DNA |
|----------|---------|----------|-----------|-----------|
| SSB-L shell x10³ | 13.6 ± 3.4x10⁻¹ | 8.6 ± 1.9x10⁻¹ | 8.4 ± 1.9x10⁻¹ | 7.7 ± 3.7x10⁻¹ |
| SSB-M shell x10³ | 6.5 ± 7x10⁻² | 5.5 ± 10⁻¹ | 5.4 ± 10⁻¹ | 2.9 ± 10⁻¹ |
| DSB-L shell x10⁻⁵ | 13 ± 5x10⁻¹ | 9 ± 4x10⁻¹ | 8.4 ± 2x10⁻¹ | 6.7 ± 4x10⁻¹ |
| DSB-L shell [2] | 12.7x10⁻⁵ | 9 ± 6x10⁻¹ | 8.6 ± 6x10⁻¹ | 5.5 ± 5x10⁻¹ |
| DSB-M shell x10⁻⁵ | 10.6 ± 4x10⁻¹ | 9 ± 6x10⁻¹ | 8.6 ± 6x10⁻¹ | 9x10⁻⁵ |
| DSB C⁺⁺ [8] | 19x10⁻⁵ | 9 ± 6x10⁻¹ | 8.6 ± 6x10⁻¹ | 5.5 ± 4x10⁻¹ |
| SSB-M shell x10⁻⁴ | 6.8 ± 5x10⁻¹ | 6.4 ± 4x10⁻¹ | 7.4 ± 5x10⁻¹ | 5.7 ± 4x10⁻¹ |
| DMSO | 1.4 ± 10⁻¹ | 1.5 ± 1.2x10⁻¹ | 1.5 ± 1.2x10⁻¹ | 1.6 ± 1.2x10⁻¹ |

Table 1. Numerical values of the slopes m of the lines in the figures 1 – 4.
made with high-Z atoms. The key point is to design nanoparticles of high-Z atoms (platinum, gold …) able to target with a good selectivity the tumor. A great deal of works toward this goal is made in different groups. For example in the reference [13] one can find different considerations about this challenge.

4. Conclusion
Most of the experiments dealing with the study of radisensitization by nanoparticles made of heavy atoms (platinum, gold) make use of photons X-rays in the 100 keV energy range. The present experiments demonstrate that low energy electron can also trigger the enhancement of DNA breaks by a non resonant process mediated by secondary electrons like photoelectrons. The mechanisms involving secondary electrons presented here suggests that any ionising radiation can induce the activation of heavy atoms contained in nanoparticles or molecules via the secondary electrons emitted along the track of the ionising particles. This hypothesis is confirmed by experiments made in similar conditions with fast carbon ions C⁶⁺ particles (290MeV/amu).

Considering the property of dose deposition profile in matter by the atomic ions, the analysis presented in this work suggests that the combination of irradiation by ions and suitable nanoparticles presenting a significant selective uptake in tumor might be an efficient tool to improve the therapeutic index of radiotherapy.

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