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Induction of strand breaks in DNA films by low energy electrons and soft X-ray under nitrous oxide atmosphere

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ABSTRACT

Five-monolayer (5 ML) plasmid DNA films deposited on glass and tantalum substrates were exposed to Al K_α X-rays of 1.5 keV under gaseous nitrous oxide (N_2O) at atmospheric pressure and temperature. Whereas the damage yields for DNA deposited on glass are due to soft X-rays, those arising from DNA on tantalum are due to both the interaction of low energy photoelectrons from the metal and X-rays. Then, the differences in the yields of damage on glass and tantalum substrates, essentially arises from interaction of essentially low-energy electrons (LEEs) with DNA molecules and the surrounding atmosphere. The *G*-values (i.e., the number of moles of product per Joule of energy absorbed) for DNA strand breaks induced by LEEs (G_{LEE}) and the lower limit of *G*-values for soft X-ray photons (G_{XL}) were calculated and the results compared to those from previous studies under atmospheric conditions and other ambient gases, such as N_2 and O_2. Under N_2O, the *G*-values for loss of supercoiled DNA are 103 ± 15 nmol/J for X-rays, and 737 ± 110 nmol/J for LEEs. Compared to corresponding values in an O_2 atmosphere, the effectiveness of X-rays to damage DNA in N_2O is less, but the *G* value for LEEs in N_2O is more than twice the corresponding value for an oxygenated environment. This result indicates a higher effectiveness for LEEs relative to N_2 and O_2 environments in causing SSB and DSB in an N_2O environment. Thus, the previously observed radiosensitization of cells by N_2O may not be only due to $OH^\bullet$ radicals but also to the reaction of LEE with N_2O molecules near DNA. The previous experiments with N_2 and O_2 and the present one demonstrate the possibility to investigate damage induced by LEEs to biomolecules under various types of surrounding atmospheres.

1. Introduction

Nitrous oxide (N_2O) is a molecule, which has been widely studied for many reasons. It is a minor component of the atmosphere, known as the 'laughing gas', having many beneficial actions, but it is also a strong 'greenhouse gas' and its emission into the atmosphere should be strictly controlled (Leont'ev et al., 2001). It is about 200–300 times more powerful in global warming potential than of carbon dioxide (CO_2) and contributes to ozone loss (Bange, 2000). Nitrous oxide has been used in many radiation chemistry studies to scavenge solvated electrons formed by the radiolysis of water. This property allows increasing the production of $OH^\bullet$ to show its importance in the lethality of the cells. When irradiated, dissolved N_2O reacts rapidly to eliminate e^-_{aq} and increases the yield of $OH^\bullet$, via $e^-_{aq} + N_2O \xrightarrow{H_2O} N_2 + OH^\bullet + OH^-$ (Watanabe et al., 1981; Ewing et al., 1991). Since $OH^\bullet$ radicals are major contributors to lethality, it is therefore logical to expect N_2O to be a radiosensitizer. Powers et al. (1972) first observed the sensitizing action of N_2O on spores of *Bacillus megaterium* to X-rays. Samuni and Czapski (1978) then irradiated *Escherichia coli* B saturated with oxygen, argon and nitrous oxide gases and reported a higher radiosensitivity for nitrous oxide-saturated bacterial cells relative to others. Their result showed that in cells saturated with either argon or nitrogen, the reactive free radical species are $OH^\bullet$, e^-_{aq} and $H^\bullet$, while in the nitrous oxide-saturated cells, the reactive species are only $H^\bullet$ and $OH^\bullet$, with the concentration of the latter species twice that of the argon- or nitrogen-saturated cells. Therefore, a sensitizing action of N_2O was interpreted by a complete conversion of e^-_{aq} to $OH^\bullet$. Brustad and Wold (1976) found a small enhancement in the radiosensitivity of N_2O-saturated bacterial cells with respect to N_2-saturated cells. In a series of experiments, then Watanabe et al. (1981, 1982) reported a significant sensitizing effect of N_2O toward a radioresistant bacterium of *Pseudomonas radiora O*-1 in a dilute aqueous suspension. The sensitizing effect of N_2O anoxia with respect to N_2 anoxia was much higher in this bacterial system than the extent of sensitization found for mammalian cells in vitro in work of Roots et al. (1982).

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However, attempts to generalize N_2O sensitization to several types of bacteria, has met with limited success. Radiosensitization by N_2O has not been found in vegetative cells (Watanabe et al., 1982) except for *E. coli* B and results appeared to conflict with those from other laboratories. For instance, Tilby et al. (1982) observed not more than a slight radiosensitizing effect by N_2O on anoxic or oxic survival at high radiation doses. Michaels et al. (1983) and Ewing et al. (1986, 1991) did not find any direct evidence of nitrous oxide sensitization of Chinese hamster ovarian cells. These results could not discern why several vegetative cells are not sensitized in the presence of N_2O, and differences in the effect of N_2O among several bacteria suggest that sensitization by N_2O was governed by some parameters such as, initial cell concentration of suspension, different test system, dose rate, irradiation temperature, and growth phase. Thus, despite numerous studies on radiation response of cells systems to nitrous oxide, its radiosentization effect has yet to be resolved (Conere et al., 1991). It must be noted that in all studies, the role of N_2O was considered only with regard to its reaction with solvated electrons and the subsequent increase of $OH^{\bullet}$ radicals in aqueous suspension. Obviously, the presence of N_2O within the densely packed structure of the chromosomes in the nucleus can engender other reactions.

Reactions near the DNA of cells are first initiated by highly reactive intermediate species produced along radiation tracks. These species include radicals, ions and secondary electrons (Sonntag, 2006; O'Neill and Fielden, 1993). The latter are the most abundant of the secondary species and most of them have low energies (i.e., below 30 eV) (Sanche, 2009; Pimblott and LaVerne, 2007). Numerous studies on phenomena involving low energy electrons (LEEs) have shown that these particles play a central role in determining the effects of ionizing radiation. Thus, in recent years much research interest has been focused on the fragmentation and modification of DNA by LEEs (Sanche, 2002, 2005). The initial experiments on LEE-DNA were performed on DNA molecules under dry high vacuum conditions (Sanche, 2009; Boudaffa et al., 2000; Cai et al., 2005). However, the damaging effects of LEEs on the DNA molecule are known to be dependent on its environment (Sanche, 2002; Lehnert, 2008; Ptasińska and Sanche, 2007; Barilla and Lokajíček, 2000). Thus, to better understand the damaging effects of LEE on DNA, attempts have been made to perform LEE-irradiation experiments under conditions more closely approximating those found in the cell. For this reason, the effect of water and oxygen molecules on radiation-induced damage in biological systems has been extensively studied (Yokoya et al., 2002, 2009; Quintiliani, 1986; Alizadeh et al., 2011). Also in our laboratory some experiments have been completed under well-controlled environmental conditions at standard atmospheric temperature and pressure (STP) to show the induction of DNA damage by LEEs in presence of gases such as, air, pure N_2 and O_2. In preliminary experiments, Cai et al. (2005, 2006) studied the damage to DNA in vacuum resulting from the emission of secondary LEEs from a metal surface exposed to X-rays. Then, by improving the technique of Cai et al. (2006), Brun et al. (2009) and Alizadeh et al. (2011) were able to investigate dry plasmid DNA films irradiated at STP by LEEs (0–30 eV) and soft X-rays (1.5 keV) under air, N_2 and O_2. Due to these advances in the technique it is now possible to study the influence of various gaseous atmospheres at STP on LEEs induced DNA damage.

In the present work, we apply this radically different experimental approach, to study other reactions than those of solvated electrons that may be associated with the radiosensitization induced by N_2O on irradiated DNA. The experiments are performed with dry DNA films deposited onto two different substrates, irradiated by 1.5 keV X-rays, under nitrous oxide-saturated atmosphere at STP. Total damage and single- and double-strand breaks are measured as a function of dose. The damage to DNA deposited on glass is due to the X-rays. From comparison of results obtained with DNA films deposited on glass and tantalum, the damage created by LEEs emitted from tantalum is deduced. More damage is induced in plasmid DNA under an N_2O atmosphere by LEEs than under N_2 and O_2 atmospheres, demonstrating strong radiosensitization by N_2O of plasmid DNA extracted from *E. coli* B, without the presence of H_2O and hence solvated electrons.

2. Materials and methods

2.1. Plasmid DNA preparation

pGEM-3Zf(−) bacterial plasmid DNA (3197 base pairs, ca. 1.97×10^6 amu, Promega) was obtained from *Escherichia coli* JM109 host, and followed by purification using the QIAfilter Plasmid Giga Kit (QIAprep Miniprep Handbook, 2003). TE buffer (Tris–EDTA: 10 mM/1 mM) was extracted from the plasmid with a home-made microcolumn of Sephadex G-50 resin on a bed of glass beads. Sephadex G-50 is highly efficient for the removal of the small molecules and thus purified DNA solution was considered containing a minimal amount of Tris–EDTA (Dumont et al., 2010). The DNA concentration was measured spectrophotometrically by measuring its absorbance at 260 nm, assuming a molar absorption coefficient of 5.3×10^7 L mol^{-1} cm^{-1} at pH 7.0 for DNA (Manchester, 1996). DNA solution was diluted in ddH_2O until a final concentration of 50 ng μL^{-1} was reached. In the present study, about 96% of the extracted plasmid was in the supercoiled form and the rest was in the relaxed circular (C > 3%) and concatemeric (CM < 1%) configurations.

Owing to the very short range of LEEs, DNA films were prepared as thin and uniform as possible. The method of preparation is the same as that used by Mirsaleh-Kohan et al. (2011). These authors showed that 5-monolayer-thick films of lyophilized DNA bombarded by LEEs gave the same products as the well-ordered self-assembled monolayer films of DNA bombarded by the same 2–20 eV electrons. To obtain a fairly uniform thickness of approximately 5 monolayers (5 ML) 10 μL of solution containing 500 ng of purified DNA in nanopure water without any salts, was deposited on the glass and tantalum substrates. These substrates were cleaned by rinsing in the ethanol and then ddH_2O and after dried under N_2 flow. Samples were frozen at −70 °C and then freeze-dried by pumping under the pressure of 1–3 mTorr for two hours. The film thickness has been estimated as previously described (Alizadeh et al., 2011; Goodhead, 1990) by taking 6.0 ± 0.2 mm as the diameter of the DNA film, a thickness of 2 nm for each ML of plasmid and the known density of 1.71 g/cm^3 of the plasmid extracted from *E. coli* (Adams et al., 1986). This procedure resulted in a dry film of 10 nm average thickness on the substrates, assuming minimal clustering of the plasmids.

2.2. Experimental setup and irradiation conditions

All the experimental data were recorded with the apparatus recently developed by Alizadeh et al. (2011), which has already been described in detail. Only a short survey is given here. The apparatus, shown schematically in Fig. 1, comprizes a chamber evacuated to pressure < 5 mTorr, connected to a pressure gage (*A*) and an adjustable leak valve (*B*) connected to a nitrogen gas source. A negative potential of 3.4 kV is applied to a concave aluminum cathode (*C*) through a high-voltage electrical feedthrough (D) fixed in a glass-ceramic (Macor) support (*E*) and placed as a cap on a long quartz tube (*F*). A nitrogen plasma