DNA oxidation by reactive oxygen species produced by atmospheric pressure microplasmas

J.S. Sousa[1,2], G. Bauville[1], B. Lacour[1], P.M. Girard[3], E. Sage[3], J.L. Ravanat[4], V. Puech[1]
[1]Laboratoire de Physique des Gaz et des Plasmas, CNRS & Univ. Paris-Sud, Orsay, France
[2]Instituto de Plasmas e Fusão Nuclear - Laboratório Associado, Instituto Superior Técnico, Lisboa, Portugal
[3]Laboratoire de Biologie des Radiations, CNRS & Institut Curie, Orsay, France
[4]Laboratoire des Lésions des Acides Nucléiques, CEA & Univ. Joseph Fourier, Grenoble, France
e-mail: joao.santos-sousa@u-psud.fr

Résumé

Arrays of microcathode sustained discharges (MCSD) have been developed for the production of high fluxes of singlet delta oxygen (SDO) and ozone (O_3) at atmospheric pressure. SDO and O_3 densities higher than, respectively, 10^{17} and 10^{16} cm^{-3} have been efficiently produced and transported over distances longer than 50 cm. These arrays of MCSD have been optimized to supply well-quantified and tunable fluxes of either SDO or O_3. This plasma source has been found to be very useful for examining the reactivity of these reactive oxygen species with biological components. Experiments were performed strongly indicating that both SDO and O_3 are able to oxidize DNA, originating great damages in DNA such as double-strand breaks and base oxidation. It has been observed that while all bases of DNA are almost indifferently and quite effectively oxidized by O_3, SDO reacts mainly with guanine.

Introduction

Reactive oxygen species (ROS) are well known to play an important role in several biological systems, and generate oxidative damage to a variety of cellular components [1]. Among others, deoxyribonucleic acid (DNA) is of particular importance, due to its key role in cell survival and reproduction. Fundamental studies examining the cellular components targeted by different ROS generated in low-temperature plasmas, and the modifications induced by those interactions, are, thus, quite interesting and very promising for biomedical applications. In this context, we have developed arrays of microcathode sustained discharges (MCSD) for the production of ROS at atmospheric pressure. The remarkable stability of MCSD has allowed us to operate DC glow discharges in He/O_2 mixtures, free from the glow-to-arc transition, at high gas pressure, with low values of the reduced electric field (5–10 Td) and of the gas temperature (300–400 K) [2]. As a result, large amounts of singlet delta oxygen (SDO) and ozone (O_3) have been obtained at atmospheric pressure. In fact, SDO densities higher than 10^{17} cm^{-3} have been efficiently produced and transported over distances longer than 50 cm, providing SDO fluxes greater than 100 mmol/h. Furthermore, O_3 densities up to 10^{16} cm^{-3} have also been obtained. Besides that, the density ratio of SDO to O_3 can be finely and easily tuned in the range 10^{-3}–10^{+5} [3]. As so, these arrays of MCSD, by allowing a controlled production of either SDO or O_3 at atmospheric pressure, are ideal tools for studying in details the reactivity of these ROS towards biological components, notably DNA.

In order to study the reactivity of SDO and O_3 towards DNA, we have made interact aqueous solutions of DNA with a gas flow of either SDO or O_3, as previously described in details in [4]. Oxidation of DNA has been performed for different ROS flows and times of interaction. While the damages to the DNA backbone were analyzed by agarose gel electrophoresis [5], the products of oxidation were detected and quantified using the accurate and sensitive high performance liquid chromatography tandem mass spectrometry method (HPLC-EIS-MS/MS) [6].

Results and discussion

In the present work, preliminary results of experiments concerning the use of arrays of MCSD as a plasma source for biomedical applications are presented and discussed. As illustrated in Figures 1 and 2, the experiments that have been conducted strongly indicate that SDO and O_3 are able to oxidize DNA, originating great damages in DNA such as double-strand breaks and base oxidation. It has been observed that while all bases of DNA are almost indifferently and quite effectively oxidized by O_3, SDO reacts mainly with guanine. Moreover, O_3 seems to be much more effective on oxidizing DNA. Indeed, as one can see in Figure 1, double-strand breaks only occurred when using gas flows of O_3. Besides that, as exemplified in Figure 2, the amount of modified bases is also much higher when making O_3 molecules

interact with the DNA solutions, compared to the use of gas flows of SDO. The enhancing effect of heavy water (D_2O) has been used to confirm the SDO-mediated DNA oxidation. When using heavy water, not only the same trends have been observed as when using H_2O, but also from 5 to 30 times more damages were induced (cf. Figure 2), correlating, therefore, the oxidized nucleosides formation to the presence of SDO. In fact, the SDO lifetime in D_2O is about 10 times longer than in H_2O [7].

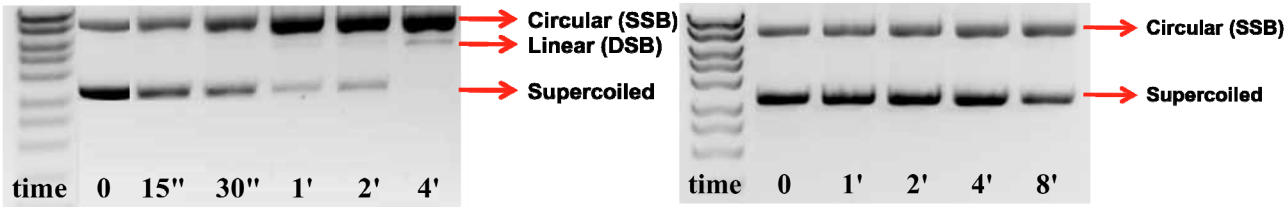

Fig. 1: Digital photographs of the agarose gel showing typical separation of DNA populations of different conformation subsequent to different times of interaction between the aqueous solution of plasmid DNA and an afterglow gas flow of O_3 (left) and SDO (right).

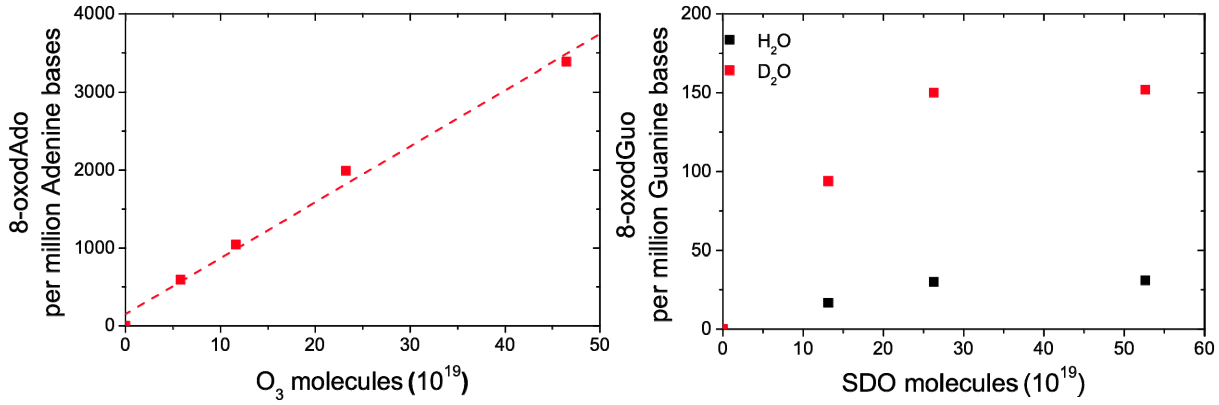

Fig. 2: Evolution, versus the O_3 (left) and the SDO (right) molecules reaching the DNA solution during given times of oxidation, of the quantity per million bases of the products of oxidation of adenine (left) and guanine (right) resulting from the degradation of different aqueous solutions of DNA: black squares = H_2O, and red squares = D_2O.

The results that have been obtained, even if preliminary, are very significant and demonstrate that arrays of MCSD are a quite promising plasma source, being very suitable and useful tools for biological studies, and are, thus, likely to lead to new biomedical applications. In fact, in the context of the new field of Plasma Medicine, our plasma source is unique. Indeed, contrary to the other available sources of reactive oxygen species, our arrays of MCSD are able to supply well-quantified and tunable fluxes of either SDO or O_3. Nevertheless, there are still many open questions on the reactivity of ROS with DNA. For a better understanding of the mechanism of ROS-mediated oxidation of DNA, efforts are to be made to gain further insights into the chemistry of the liquid phase. A more detailed study on this subject is currently in progress.

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