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XANES Spectral Changes of Hydrated Deoxyribose Induced by *K*-Shell Ionization of Oxygen

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Abstract. We investigated the initial process of DNA strand breakage induced by ionizing radiation by recording XANES spectra of hydrated deoxyribose (dR) films before and after *K*-shell ionization of oxygen. The experiments were performed on beamline BL23SU at SPring-8, Japan. The hydrated dR films were prepared by exposing the cooled dR film surface to water vapor. The XANES spectra were then obtained by measuring the sample drain current. The soft X-ray irradiation treatment led to an increase in the intensity of the π*(C=O) peak (around 532 eV) and a decrease in the intensity of the σ*(C–O) peak (around 538 eV). Furthermore, a characteristic peak was observed at around 534–536 eV. in the hydrated dR film after irradiation. This peak, which was ascribed to a newly formed carboxyl group, was not observed in the XANES spectrum of the dry dR film after irradiation. This carboxyl group was considered to originate from the reaction of a nearby water molecule with the carbonyl group produced by the direct ionization of dR. This structure may be formed at the strand-break termini of DNA after radiation damage. Therefore, waters of hydration may play a protective role against irreparable strand breakage.

INTRODUCTION

XANES spectroscopy has been applied extensively in the study of biological molecules [1-10]. In particular, this analytical technique has proved valuable for investigating radiation damage to amino acids and DNA or its building blocks [11-17], which is important for evaluating fundamental processes of radiation biology and damage to X-ray crystallography samples. Such analyses are typically performed using dry solid films in an ultrahigh-vacuum chamber, because the *K*-shell ionization energies of all of the non-hydrogen atoms that comprise DNA, namely, carbon, nitrogen, oxygen, and phosphorus, occur in the soft X-ray region. Recently, through a combination of molecular dynamics simulations and soft X-ray spectroscopic experiments, we revealed that the degradation of deoxyribose (dR) after *K*-shell ionization of oxygen is suppressed by proton transfer from the dR molecule to a water of hydration, which occurs on a timescale of ~10 fs [18]. Waters of hydration play a crucial role in the radiation damage of biological molecules. Recent developments in vacuum pumping apparatuses and spectroscopic methods have enabled experiments to be performed using liquid samples [19-23]. However, these highly developed techniques are not suitable for evaluating radiological effects in DNA, because the products of radiation damage are unstable in the solution phase. Little is known regarding the chemical structures of these products in hydrated samples. Elucidating the nature of these structures is crucial for understanding the subsequent repair processes, because the repairability of damaged DNA strongly depends on the chemical structure of the terminal sites after cleavage owing to the specificity of the repair enzymes [24]. In our previous paper, we demonstrated that oxygen *K*-shell ionization significantly contributes to DNA strand breakage and the formation of nucleobase lesions as the yield of these lesions was clearly increased above the *K*-shell ionization energy of oxygen [25, 26]. In this study, we used XANES spectroscopy to investigate the changes in chemical structure that occur in hydrated dR molecules upon irradiation with monochromatic soft X-rays to induce *K*-shell ionization of oxygen.

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MATERIALS AND METHODS

2-Deoxy-D-ribose (dR) powder was purchased from Sigma-Aldrich Co. Ltd. (Tokyo, Japan) and used without further purification. To form the film samples, a 100 μL aliquot of a 10 mg/mL solution of dR in distilled water was spread onto a Si(100) plate (20 mm × 20 mm; KN Platz Co. Ltd, Aira-gun, Kagoshima, Japan), and allowed to dry at room temperature under atmospheric pressure. The film sample was placed in a low-vacuum ($<10^{-5}$ Pa) chamber and then transferred to a high-vacuum ($<10^{-7}$ Pa) chamber installed at the beamline. The cooled (~150 K) dR film was exposed to water vapor (1×10^{-6} Pa) for 100 s to form one layer of hydration water on the dR film.

The experiments were performed on beamline BL23SU at SPring-8, Japan [27, 28]. XANES spectra were obtained by measuring the sample drain current and were used to evaluate the deposited water layer as described in our previous paper [18]. A photon energy of 560 eV was chosen for the soft X-ray irradiation to ionize the oxygen *K*-shell electrons of dR. The horizontal and vertical dimensions of the soft X-ray beam were 1.4 mm and 0.5 mm, respectively. The incident photon flux was determined by measuring the photocurrent on a photodiode (AXUV-100, International Radiation Detectors Inc., Torrance, CA). The typical flux at 560 eV was 8.0×10^{11} photons/s for the irradiation (20 min) and 1.6×10^{11} photons/s for the XANES measurement (1 min). To examine the chemical changes that occurred in the dR molecules upon irradiation, oxygen *K*-edge XANES spectra were obtained for a specific region of the sample before and after the irradiation treatment.

RESULTS AND DISCUSSION

Figures 1(a) and 1(b) show the XANES spectra for the dry and hydrated films, respectively, before and after irradiation treatment. Figures 1(c) and 1(d) show subtraction spectra for the dry and hydrated films, respectively, to allow quantitative evaluation of the spectral differences before and after the irradiation treatment. The irradiation treatment resulted in an increase in the intensity of the π*(C=O) peak (around 532 eV) and a decrease in the intensity of the σ*(C–O) peak (around 538 eV), demonstrating that the soft X-ray irradiation induced C–O bond cleavage and C=O bond formation. These results are consistent with the previously observed XANES and ion desorption experiments [12, 29]. In addition to these spectral changes, a characteristic peak at approximately 534–536 eV was observed in the spectrum of the hydrated sample, as indicated by the arrow in Fig. 1(d). This peak, which was assigned to a newly formed carboxyl group [30], was not observed in the spectrum of the dry dR film after irradiation. The intensity of the oxygen *K*-edge jump was greater for the dry film than for the hydrated film as a result of abundant oxygen-containing small fragment species desorb from the sample surface [18]. The decrease in the intensity of the σ*(C–O) peak was smaller for the hydrated film than for the dry film, which indicates that the water of hydration suppressed the decomposition of dR upon irradiation with soft X-rays. This is consistent with the results described in our previous paper, where we revealed that the suppression of radiation damage by the water of hydration may originate from charge re-distribution after the ultrafast proton transfer from dR to a nearby water molecule [18]. Ultrafast dissociation reactions are induced on the time scale of ~5 fs, which is the lifetime of an inner-shell vacancy in an oxygen atom. This depends on the dissociative characteristics of the core vacancy. For the elements present in a DNA molecule, Auger processes occur with a probability of approximately 100% on a time scale of picoseconds following the ionization of an inner-shell electron. Covalent bonds are cleaved, resulting in the formation of doubly ionized states by two valence holes after the Auger process, ultimately producing positively charged ions [31]. An aldehyde moiety might be produced after these processes. This aldehyde could then react with a nearby water molecule to afford a carboxyl group, as depicted in Fig. 2. This structure might be formed at the strand-break termini of DNA due to radiation damage. When this structure is produced in a DNA molecule, a hydroxyl group remains at the strand-break terminus. This hydroxyl group can be easily repaired by a glycosylase enzyme [24]. In contrast, the direct effect of ionizing radiation on dry films could produce a β-δ-SSB terminal structure [29]. This structure is irreparable when an 8-oxo-7,8-dihydroguanine moiety is present in the vicinity of the strand breakage [24]. Consequently, waters of hydration may play a role in generating a repairable structure at the strand-break terminus rather than an irreparable structure upon *K*-shell ionization of oxygen.

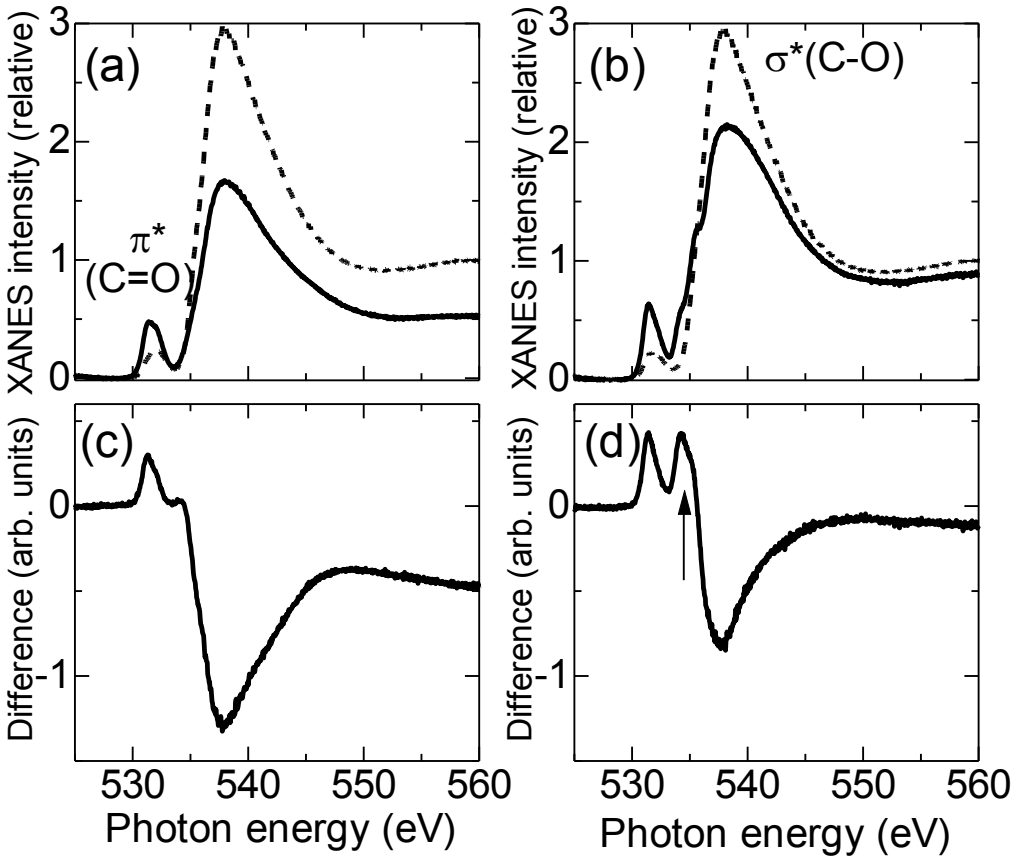

FIGURE 1. Oxygen *K*-edge XANES spectra of (a) dry and (b) hydrated dR films before (dashed lines) and after (solid lines) the *K*-shell ionization of oxygen. The subtraction spectra for the (c) dry and (d) hydrated films are also shown.

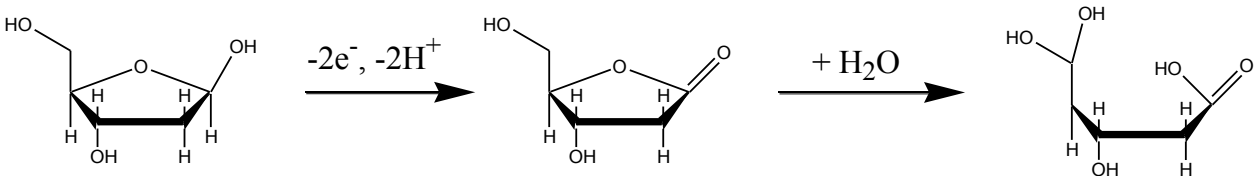

FIGURE 2. Proposed reaction scheme.

CONCLUSION

Waters of hydration contribute not only to suppressing the destruction of the furanose ring structure of dR but also to producing carboxyl groups that can be enzymatically repaired. Water molecules in the hydration layer of DNA may therefore play a protective role against the occurrence of irreparable strand breakage.

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