X-ray absorption near-edge structure (XANES) spectral changes of 2-deoxy-*D*-ribose by irradiation within the energy region around the oxygen *K*-shell absorption edge

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The physicochemical characteristics of 2-deoxy-*D*-ribose moieties in DNA strands are important to understand biological radiation stress. So, the X-ray absorption near edge structure (XANES) of 2-deoxy-*D*-ribose within the energy region around the oxygen *K*-shell absorption edge was measured. 2-deoxy-*D*-ribose was exposed to 3 energies of X-rays, i.e., 526.3 eV (below O 1s→π*), 537.8 eV (at the absorption peak of O 1s→σ*) and 552.6 eV (above O 1s→σ*) for given periods. Slight differences in spectral changes were seen in the each irradiation energy, suggesting in fact that the chemical state and following rearranged chemical structure of 2-deoxy-*D*-ribose may be different between the 3 irradiation energies.

Keywords : XANES, 2-deoxy-D-ribose, chemical change

1. Introduction

Chemical changes in biological molecules such as DNA by γ- or X-rays are classified into two categories, 'indirect' and 'direct' effects. The 'indirect' effect is caused by the hydroxyl radicals (•OH) or atomic hydrogens (•H) due to radiolysis of water (Breen & Murphy, 1995). The 'direct' effect is caused by energy deposition to the atoms in DNA from photons or secondary electrons ejected from the photo-absorbed atoms. Particularly, chemical changes of bases or nucleosides by radicals such as •OH have been investigated in detail to clarify the radical and molecular structures of the components changed in DNA (Hüttermann *et al*., 1978 ; Burrows & Muller, 1998 ; Pogozelski & Tullius, 1998). However, knowledge of the 'direct' effect has been hardly developed probably because of the multiplicity in the radiation chemical chain reactions initiated by the excitation. Moreover, light sources such as white X-rays from an X-ray tube are also partly responsible for the multiplicity.

One of the successful methods to clarify the 'direct' effect is the use of synchrotron radiation. It has high photon flux over a wide energy range (from infrared to γ region), enough to give sufficient high-density monochromatic photons, which are essential to get high-resolution X-ray absorption near edge structure (XANES). With synchrotron radiation, it is possible to study the selective excitation of an inner shell electron to a specific transition state. In the last 10 years, several studies of radiation biological effects using synchrotron radiation have been reported (Kobayashi *et al*., 1991). Watanabe *et at*., 1992 ; Hieda *et al*., 1996). These studies demonstrated that some cellular effects, *e.g.* cell inactivation, induction of gene conversion, cell membrane impairment, mutation, transformation and DNA strand breaks frequently occur at the *K*-edge resonance absorption peak energy of phosphorus in DNA (2.153 keV), which was determined by XANES around its *K*-edge. Several Auger electrons ejected from phosphorus and the resulting multiple-charged phosphorus cation could be responsible for these phenomena. In addition, a computational study indicated that low-energy secondary electrons with less than 1 keV such as photo- or Auger electrons ejected from photo-absorbed atoms was most destructive for DNA (Nikjoo *et al*., 1997).

In this study, we selected 2-deoxy-*D*-ribose for the target of irradiation because the chemical change should directly lead to DNA strand breaks.

2. Materials and Methods

XANES studies were performed using a soft X-ray beamline with a variably polarizing undulator (BL23SU) in SPring-8 (Yokoya *et al*., 1998). The electron energy in the storage ring was 8 GeV, and the beam current was between 70 - 100 mA. A high-resolution-type monochromator equipped with a plane grating having 600 lines/mm was used to scan the proper energy range. The energy resolution (E/ΔE) was over 5,000. The pressure in the sample chamber was of the order of 10^{-6} Pa. 2-deoxy-*D*-ribose was purchased from Tokyo Kasei Ltd. The sample solution (10 mg/mL) was prepared in distilled water, and the aqueous solution was spread on a gold-coated plate to obtain a film sample by drying at room temperature. 2-deoxy-*D*-ribose was exposed to 3 energies of X-rays, i.e., 526.3 eV (below O 1s→π*), 537.8 eV (at the absorption peak of O 1s→σ*) and 552.6 eV (above O 1s→σ*) for given periods (from 0 to 8.0 h) before measuring each XANES spectrum. The beam spot size on the sample was approximately 4 mm². The photon flux was estimated using an ion chamber for soft X-rays (Saito & Suzuki, 1998 ; Saito & Suzuki, 1999). All the XANES spectra were obtained by measuring the photo- or Auger electron current on the sample plate.

3. Results and Discussion

Figure 1 (A - C) show the change of relative intensity of XANES spectra of 2-deoxy-*D*-ribose by interacting with monochromatic soft X-rays with the energies of 526.3 eV (below O 1s→π*), 537.8 eV (at the absorption peak of O 1s→σ*) and 552.6 eV (above O 1s→σ*) for the periods stated above and the figure caption. After irradiation, interestingly, a couple of overlapped peaks appeared (barely in series A) and gradually increased in all irradiation energies. Moreover, the rates of the increase of these peaks are different : the lower peak increased more rapidly than the other. These two peaks were both assigned to O 1s→π* transition resonance peaks (Wurth & Stöhr, 1990), which indicated the production of more than two kinds of C = O bonds during de-excitation processes after the energy deposition. In particular, the lowest peak at *ca.* 531 eV was really fresh, suggesting the production of 2-deoxy-*D*-ribose derivatives by the irradiation. On the other hand, the rate of reduction of the O 1s→σ* intensity were apparently different between series A and BC : that of series A was slower than the others. This tendency indicates that the energy of 526.3 eV (below 1s→π*) cannot excite O 1s→σ* intensity, although it is capable of exciting C 1s and all valence electrons in the molecule. The reduction of the peak intensity would mean the decrease of mass of the carbon-oxygen single bonds in the molecule.

These O 1s→π* transition resonance peaks would not only be responsible for the excitation of oxygen 1s electrons, but also that of oxygen nonbonding, carbon 1s and C-O valence electrons as shown in Figure 1A. On the other hand, a well-developed isosbestic point at *ca.* 525 eV was shown in every series in Figure 1. This suggests that the decrease of mass of 2-deoxy-*D*-ribose on the sample plate by the irradiation could proportionally be responsible for the production of its derivatives having similar XANES spectrum .

2-deoxy-*D*-ribose was considered to have five distinct oxygens with regard to the electron environment around each nucleus of the oxygen, taking into account α- and β- anomers of 2-deoxy-*D*-ribose, corresponding to the existence of five different O 1s→σ* transitions of these distinct C–O bonds. However, these absorption peaks were indistinguishable. Therefore, irradiation with the X-rays tested here resulted in excitation of every C–O bond in the molecule.
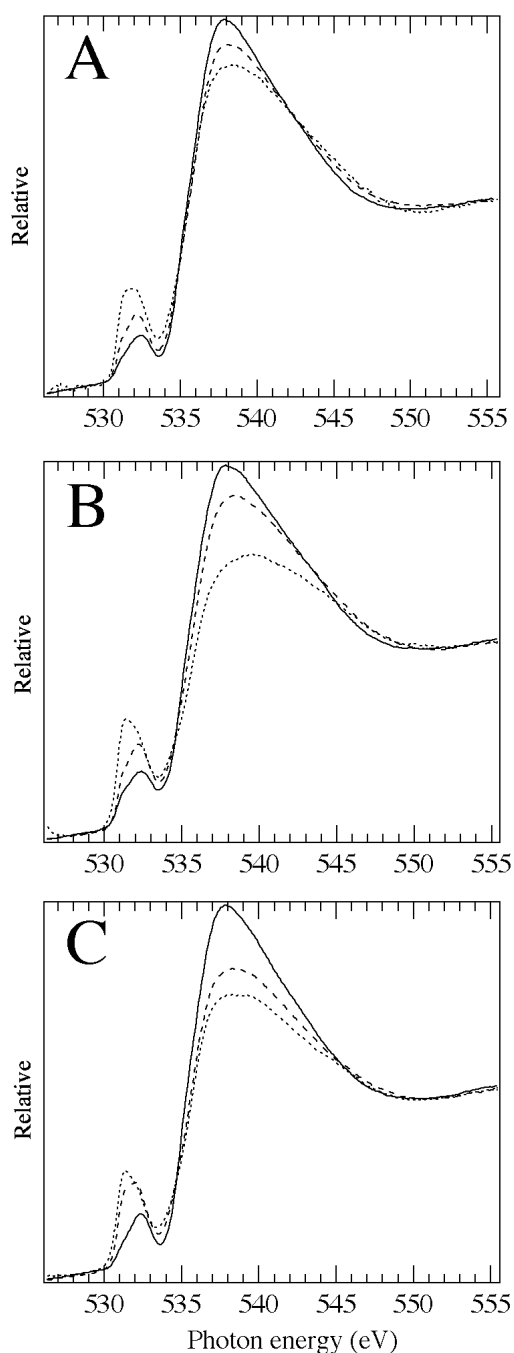
Figure 1
XANES spectral changes of 2-deoxy-*D*-ribose after irradiation of monochromatic X-ray photons (**A** : 526.3 eV, **B** : 537.8 eV, **C** : 552.6 eV) for given periods. Total photon numbers used for the irradiation were estimated using an ionizing chamber for soft X-rays (Saito & Suzuki, 1998 ; Saito & Suzuki, 1999) : **A** (solid line : before irradiation, broken line : 3×10^{15} (2.0 h), dotted line : 1×10^{16} (4.5 h)), **B** (solid line : before irradiation, broken line : 2×10^{15} (0.5 h), dotted line : 1.6×10^{16} (6.0 h)), **C** (solid line : before irradiation, broken line : 7×10^{15} (2.0 h), dotted line : 3×10^{16} (8.0 h)). Vertical axes are based on the sample current (in arbitrary units).

In general, the terminal states after these de-excitation processes are chemically unstable, which may lead to cleavage of the covalent bond to produce a neutral radical fragment and a cationic one. In the case of C-O-H bonding, if ion-fragmentation occurs, four cleavage patterns may be proposed: H• + $^+$O–; H$^+$ + •O–; –O• + $^+$C–; –O$^+$ +•C–. The frequency of each cleavage pattern depends on the environment around H-O-C- such as its whole chemical structure and the other small molecules or ions near to the H-O-C-, *i.e.* H_2O, H$^+$ and OH in aqueous solvent. These cations and radicals generally rearrange by inter- or intramolecular processes to chemically stabilized molecules. The intermolecular process causes crosslinking or hydrogen abstraction from neighboring molecules. The intramolecular process produces double bond formation such as C=O in aldehydes, carboxylates or ketones. Indeed, electron-impact excitation of alcohols or ethers using a 70 eV electron beam is generally known to produce the corresponding carbonyl compounds (Silverstein *et al.*, 1983 ; Davis & Frearson, 1987). Then, the emergence of the new peaks in Figure 1 would reveal these carbonyl compounds.

In conclusion, XANES studies of biological molecules are important to understand physicochemical changes in the molecules irradiated. Particularly, the production of π orbital is important to understand the DNA strand break patterns by the 'direct' energy deposition of radiations. These approaches will play a crucial role in biological signal transduction and environmental science studies.

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