Novel mechanism of DNA damage induced by soft X-ray irradiation as observed by an electron paramagnetic resonance spectroscopy

During ionizing irradiation of a cell, especially by high-energy radiation such as ion beams applied to cancer therapy, energy of various magnitudes is supplied to DNA molecules leading to multiple radiation damage of DNA molecules in a cell nucleus. Because of technical difficulties in observing physicochemical processes of damage induction, the entire mechanism of DNA damage has not yet been clarified. Monochromatic soft X-rays with specific energies less than 1 keV enable us to investigate the process of DNA damage due to photoelectric effects at carbon, nitrogen, and oxygen atoms, which are the constituent atoms of DNA molecules. Using soft X-rays, we study intermediate species of DNA damage, focusing particularly on unstable unpaired electron species.

Ionization of an electron from a molecule leaves an unpaired electron in a valence orbital. The unpaired electron in DNA is thought to play an important role in the reaction pathways leading to final damage. In general, the unpaired electron species have very short lifetimes because of their high reactivity. Electron paramagnetic resonance (EPR) is a powerful probe for the study of the unpaired electron species. Previous studies using a conventional EPR technique have provided useful knowledge about stable DNA radicals that can exist for more than a few seconds after termination of the irradiation at a liquid helium temperature. In those studies, samples must be kept at liquid nitrogen or helium temperatures during irradiation, transfer from an irradiator to an EPR spectrometer, and EPR measurement. Therefore, the very fast radical processes that are expected to be induced just after the occurrence of photoelectric effect initiated from inner shell photoionization in DNA are rarely observed because the unstable unpaired electron species might decay during the irradiation, sample transfer and EPR measurement.

To overcome the above problem, we have developed a unique EPR system at the synchrotron soft X-ray beamline **BL23SU** (Fig. 1) [1]. This system enables us to examine the EPR "*in situ*" signal of an unstable short-lived unpaired electron species of DNA and DNA-related molecules during soft X-ray irradiation in an EPR microwave cavity inside a high-vacuum chamber [2,3]. In this work, we measured the yield of the unpaired electron species of calf thymus DNA thin film around the nitrogen and oxygen *K*-edge, and discussed the origin of the unpaired electron species [4].

Figure 2 shows the variation in EPR intensity (spin concentration of the unpaired electron species) for DNA thin film as a function of soft X-ray energy. The right-hand axis of the figure shows soft X-ray absorption. As shown in Fig. 2, the EPR intensity changes along with the soft X-ray absorbance. One can see that the EPR intensities were significantly enhanced at energies slightly above the *K*-shell thresholds of nitrogen and oxygen.

After the creation of a *K*-shell hole of a light atom or molecule by soft X-ray absorption, Auger electron emission follows, which normally results in the production of a doubly charged parent cation (Auger effect). However, through the absorption of energy slightly higher than the *K*-shell ionization potential, the charge state of the produced cation is somewhat altered owing to the post collision interaction (PCI), i.e., the first emitted slow photoelectron is decelerated by the appearance of dication core upon the Auger effect. The significant deceleration of slow photoelectrons can lead to their recapture in an unoccupied orbital or a high-lying Rydberg orbital, which then results in unpaired electron species. In order to evaluate the PCI effect on the enhancement of the present EPR intensity, the recapture cross section after the Auger effect in DNA film was calculated using a semiclassical approximation. The calculated result of recapture probability (for the production of a singly charged parent cation) is almost unity at the *K*-shell ionization threshold and steeply decreases to less than 10% at 7 eV above the threshold. The cross section for the recapture, which is the product of the recapture probability and the *K*-shell ionization cross section, shows a sharp increase and a subsequent gradual decrease with increasing soft X-ray energy above the ionizing threshold and reproduces the significant

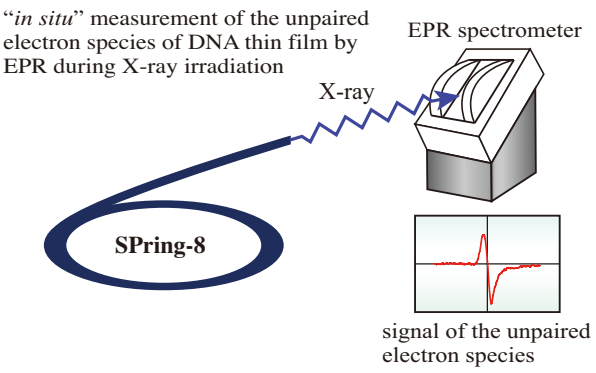

Fig. 1. Schematic of EPR system installed.

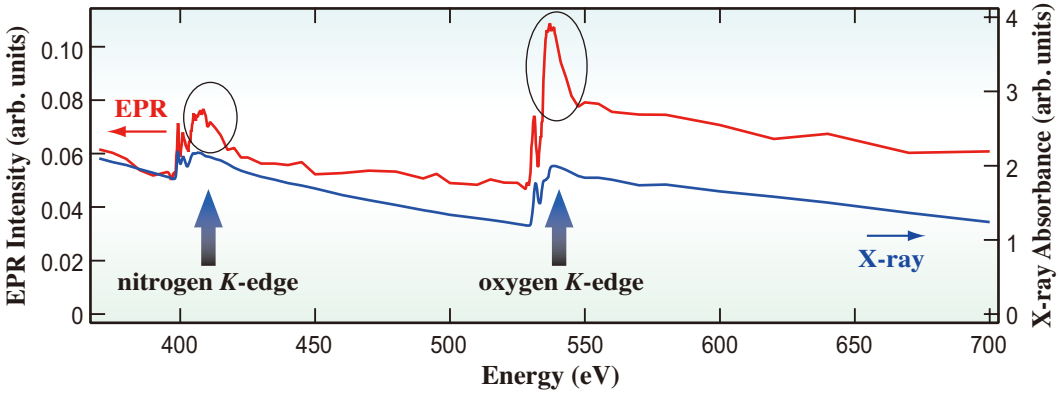

Fig. 2. Variation in EPR intensity for DNA thin film and X-ray absorbance as a function of X-ray energy. The EPR intensity is significantly enhanced around both the nitrogen and oxygen *K*-edges, as indicated by circles in the figure.

enhancement of the EPR intensities. This indicates that the enhanced EPR intensity at the *K*-shell thresholds can be attributed to the recapture of a photoelectron as a result of the PCI effect. The result suggests that the DNA film forms unpaired electron species through the excitation of enhanced electron recapture by the PCI effect (Fig. 3). Our result is the first observation of the PCI effect measured in condensed matter of DNA film. Different from the well-known low photoelectron attachment to an electronegative site in DNA followed by dissociation [5] or the recombination of slow electrons with ion holes, the observed ionization process induced by soft X-ray absorption is a novel mechanism of DNA damage.

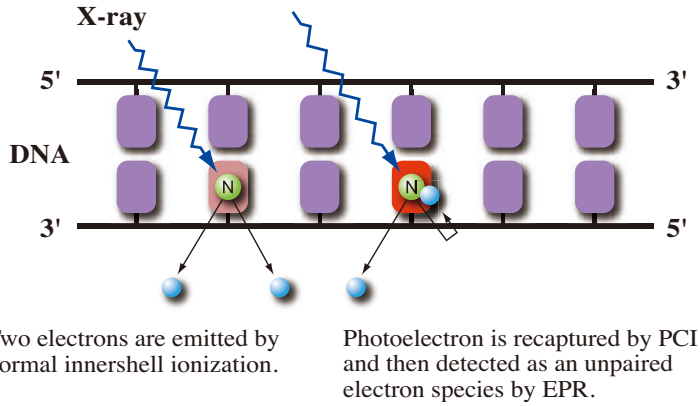

Fig. 3. X-ray absorption processes at the *K*-shell of a specific atom in DNA. Photoelectrons are ejected from the DNA upon irradiation with X-rays of slightly higher energy than the ionization threshold, decelerated and recaptured by PCI, and then detected as unpaired electron species.

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