Study of Soft X-ray-Stimulated Free Radicals Inducing DNA Base Damage

Studies employing synchrotron soft X-rays as probes to investigate genetic changes have highlighted the biological effects, such as mutation or carcinogenesis, related to the molecular process in DNA damage [1]. Site-selective photoabsorption in a DNA molecule is one of the most powerful techniques for understanding the physicochemical mechanism of DNA damage, which induces the radiobiological effects. We have developed an EPR (Electron Paramagnetic Resonance) spectrometer (Fig. 1) installed in a synchrotron soft X-ray beamline at SPring-8 to observe free radical species as intermediates leading the complicated DNA damage [2]. This system enables us to investigate the radical process *in situ* in DNA molecules by core-excitation with irradiation by the soft X-rays [3].

The experiments were performed at the Biological Application Station in the JAERI soft X-ray beamline **BL23SU**. The measured sample was a pellet of guanine and thymine bases, which were chosen as typical purine and pyrimidine bases in a DNA molecule. The oxygen in the bases was selected as a target atom for *K*-shell excitation in the molecules. The sample pellet was irradiated with soft X-ray photons in a microwave cavity mounted in a vacuum chamber (< 10^{-6} Pa). The sample temperature was controlled from 15 to 300 K, and relatively low microwave power ranging 0.1 - 7 mW was used to avoid power saturation of EPR signals. Monochromatic soft X-ray photons were provided using a grazing incidence monochromator equipped with varied-line-spacing-plane-gratings (VLSPGM). The resolution power E/ΔE is ~ 10,000 in the 0.5 keV region, and a photon flux of the order of 10^{11} (photons/sec/100 mA ring current/0.02% band width) was realized.

Obtained EPR spectra show the induction of short-lived unstable radicals clearly distinguished from stable ones which still exist after exposing to soft X-rays. The signal intensity of a short-lived doublet signal observed in the guanine spectrum at 77 K (Fig. 2(a)) linearly increases with an increase of the photon flux density. The signal immediately

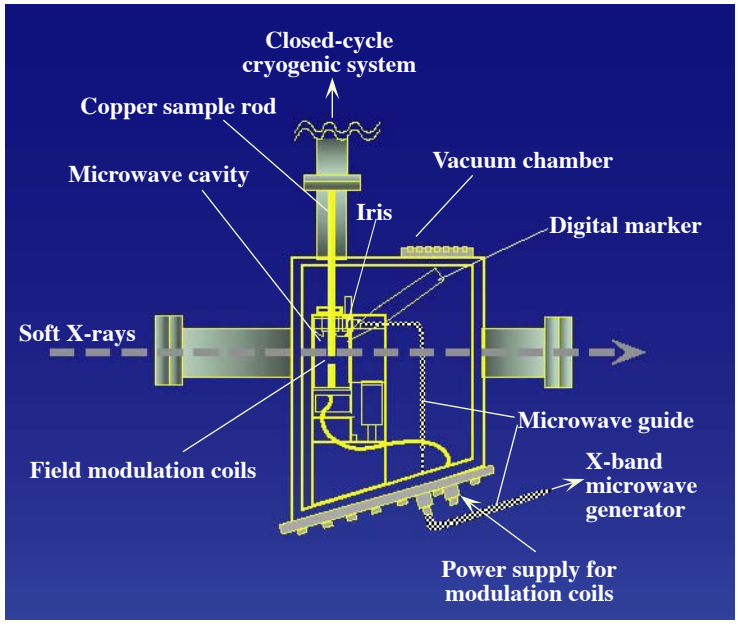

Fig. 1. The microwave cavity of the EPR apparatus mounted in the high vacuum chamber. The sample is set on a copper rod and installed in the narrow gap between field modulation coils. X-band microwaves are guided into the chamber via copper wave-guides connected with a vacuum-sealed junction.

disappeared by beam-off. Only a singlet signal (Fig. 2(b)), which is consistent with previous data on a guanine cation (one electron oxidation) [4] remained on the spectrum. It is inferred that the lifetime of the short-lived radical should be longer than 10 μsec due to the magnetic field modulation of 100 kHz for the EPR spectrometer. Considering the experimental time response limit, the presence of a doublet signal would indicate a transient radical species following the Auger final state that promptly decays within a psec. Thus, we propose a pathway for forming a final guanine lesion in DNA, such as 7,8-dihydro-8-oxo-guanine (8-oxo-G), shown in Fig. 2.

In the case of thymine, a strong singlet signal appeared during irradiation by soft X-rays (Fig. 3(a)). In contrast to guanine, however, over 10^{16} photons were needed obtain the detectable signal of stable radical at room temperature. The stable doublet signal with an additional minor structure (Fig. 3(b)) is similar to thymine anion radical previously reported [5]. Based on these aspects, we propose a possible pathway for forming a final thymine lesion as shown in Fig. 3. The anion radical would be induced by a secondary reaction with photo or Auger electrons to thymine in bulk condition.

These results strongly indicate that the *in situ*

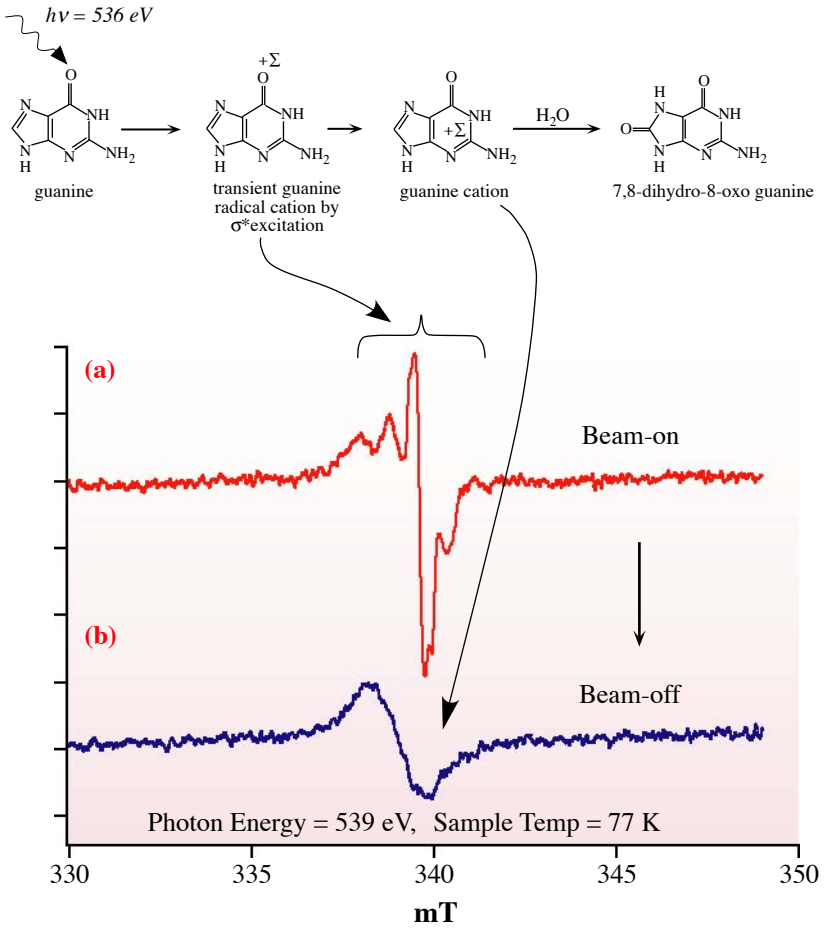

*Fig. 2. EPR spectra of guanine during irradiation by soft X-rays (536 eV) at 77 K (**a**), and just after exposure (**b**). The microwave power was 200 μW and the width of the magnetic field modulation of 100 kHz was 0.5 mT.*

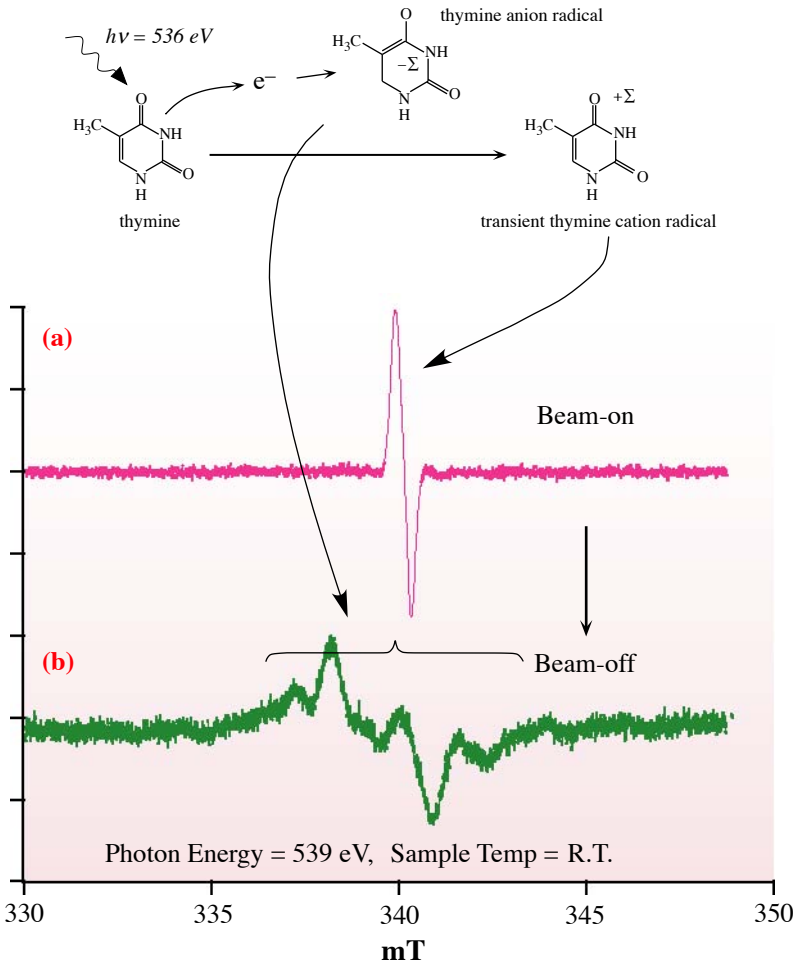

Fig. 3. ***(a)*** *EPR spectrum of thymine during irradiation of soft X-rays (536 eV) at room temperature. The microwave power was 1 mW and width of the magnetic field modulation of 100 kHz was 0.5 mT.* ***(b)*** *EPR spectrum of thymine after exposure of a high flux of about 1016 photons. The microwave power was 7 mW and width of the magnetic field modulation of 100 kHz was 0.5 mT. The gain was two times larger than that for* ***(a)****.*

EPR measurement provides evidence of the transient radical species induced, presumably, as single-electron oxidation by core level excitation. These are inferred to be precursors of chemically stable DNA base lesions. Our findings will open discussions on physicochemical processes of DNA damage induction by ionizing radiation.

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