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Optical Absorption Cross-Section of DNA Bases—Thymine and Guanine—in the Energy Region from 3.1 to 250 eV (5–400 nm)

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Abstract: (1) Background: Optical absorption cross-section—the absolute absorption intensity specific to each molecule—of nucleic acid bases enables us to estimate the reaction yields of DNA lesions induced by the exposure to not only photons but also ionizing radiations. However, it was unknown in the energy region exceeding ~10 eV (wavelength < ~120 nm). (2) Methods: Thin films of DNA bases—thymine and guanine—were prepared using a vacuum sublimation technique. Absorption spectra of these films were measured in the energy region from 3.1 to 250 eV (5–400 nm) at the synchrotron radiation facility UVSOR. (3) Results: The absorption spectra of both bases exhibited prominent absorption peaks around 20 eV and smaller peaks in the energy region below 10 eV. The determined optical oscillator strength distribution was verified to be reasonable based on the Thomas–Reiche–Kuhn oscillator strength sum rule. (4) Conclusion: Most of the oscillator strength distribution was positioned in the measured energy region, and therefore the absorption spectra significantly contributed to the quantitative study for the photo and radiation-chemical reactions of DNA.

Keywords: DNA bases; absorption spectroscopy; oscillator strength; Thomas–Reiche–Kuhn oscillator strength sum rule

1. Introduction

Exposure of organisms to ultraviolet (UV) [1] and ionizing radiation (IR) [2–4] is possible to induce DNA lesions that may lead to mutagenesis and carcinogenesis. There has been extensive research both in vivo and in vitro to understand the interaction of DNA and organisms with UV and IRs. Knowledge of the photo- and radiation-chemical reaction processes and yields of DNA damage induction is critical to understanding these phenomena and the treatment of cancer.

It is well known that the quantum yields of photochemical reactions depend on the optical absorption cross-sections, namely, the molecule-specific absolute absorption intensities of target molecules at the incident photon energy. Additionally, according to the "optical approximation" proposed by Platzman [5], radiation-chemical yields can also be estimated from the optical absorption cross-section over a wide energy range, as reviewed by Hatano et al. [6]. Therefore, the optical absorption cross-section is important for considering the interaction of molecules with both photons and IRs quantitatively.

Absorption spectra of nucleic acid bases, the building blocks of DNA, have been measured up to approximately 6.5 eV (wavelength > ~190 nm) for vapor [7–9] and solution [10–12] samples and to 10 eV (wavelength > ~120 nm) for thin film samples [13], though the absorption intensities of vapor and film samples were relative. The relative absorption spectra of DNA bases have also been measured in the carbon (280–320 eV; 3.9–4.4 nm), nitrogen (395–430 eV; 2.9–3.1 nm), and oxygen (525–565 eV; 2.2–2.4 nm) K-edge energy region, in which K-shell electrons are excited, using powder samples [14]. However, no absorption spectrum of DNA bases covering the complete energy region between the UV and carbon K-edge energy (denoted as the intermediate region hereafter) has, to the authors' knowledge, been reported, presumably owning to the lack of appropriate window materials.

In general, the absorption intensity in the intermediate region is much higher than that in other energy regions [6]. Therefore, the optical absorption cross-section of DNA bases in the intermediate region is essential to consider the reaction yields of bases irradiated with IRs and, of course, such photons. Our group developed a novel technique for the vacuum sublimation of amino acids onto collodion films, through which intermediate region photons are trasnmitted, and determined the optical absorption cross-section of four amino acids in the energy region from 3.1 to 250 eV (5–400 nm) [15]. A similar method is applicable to the absorption spectroscopy of DNA bases. Thus, in the present paper, we report an experimental method for the absorption spectroscopy of DNA bases in the intermediate region, and the optical absorption cross-section of thin films of thymine and guanine (pyrimidine and purine DNA bases, respectively) in the photon energy range from 3.1 to 250 eV.

2. Materials and Methods

2.1. Sample Preparation

Tungsten meshes (wire diameter: 0.02 mm, 80 meshes/inch; Nilaco Corporation, Tokyo, Japan) supported on stainless steel plates with a hole (diameter: 12 mm) were placed on a sheet of perforated metal and submerged in a distilled water bath (diameter: 150 mm) equipped with a cock at the bottom. The water level was adjusted to be approximately 5 mm above the meshes. Ten microliters of a 3% solution of collodion in isoamyl acetate was dropped onto the surface of the water using a micropipette and formed a large film. After vaporization of the solvent (~20 min), the water was drained from the cock slowly (~1.5 mL/s). The meshes covered with the large collodion film were dried in a vacuum desiccator. In this way, collodion films with an effective diameter of approximately 12 mm were prepared on each tungsten mesh.

Thin films of thymine and guanine were prepared using the vacuum sublimation technique. The reagents (purity > 99%) were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan, and used without further purification. Each reagent was ground into a powder with a mortar and pestle before use. The powder was placed on a Kapton sheet and sublimated onto half of the collodion films by heating to 370 K with a nickel-chrome heater. The base pressure of the vacuum sublimation chamber was approximately 10^{-3} Pa. The films of different thicknesses were prepared to use suitable films depending on the measured energy region, because it was expected that the absorption intensities, which also depend on the thickness of the films, largely changed in the intermediate region [15]. The thickness of the films was roughly estimated to be tens of nanometers using a thin-film thickness monitor (CRTM-5000, ULVAC, Inc., Kanagawa, Japan). Uncertainty of the thickness was 10–20% depending on the positions of the substrates on our experience [15,16].

2.2. Absorption Spectroscopy

Absorption spectroscopy was performed at beamlines BL5B [17] and BL7B [18] of the synchrotron radiation facility UVSOR, at the Institute for Molecular Science, Japan. The beamline BL5B was used for the measurement of the absorption spectrum from 30 to 250 eV. Different combinations of gratings and mirrors were employed to obtain the desire energy ranges as follows: Grating 2 and Mirror 4 (G2M4) for 30–80 eV, G2M2 for 80–180 eV, and G2M1 for 180–250 eV. The beamline BL7B was used for

the measurement of the absorption spectrum from 3.1 to 30 eV. Two gratings of G1 (1200 lines/mm) and G2 (600 lines/mm) were used. To eliminate the effects of second and higher-order light on the spectra, an aluminum film (Nilaco Corporation, Tokyo, Japan) and a lithium fluoride window were placed in front of the samples in the case of the measurements for 36–72 eV and 3.1 to ~10 eV, respectively. The light intensity transmitted through the film of the DNA base on collodion (I (E)) and that of the collodion film (I_0 (E)) were measured for each incident photon energy E.

2.3. Determination of the Optical Absorption Cross-Section

The measured absorption intensities, absorbance (A_1 (E) = $\log_{10}$ (I_0/I)), are relative values, that is, depend on the number density and thickness of the sample films. Hence, we converted the absorbance to the absolute values, namely, optical absorption cross-section σ (E). According to the Beer–Lambert law, the absorbance can be converted to optical absorption cross-section as follows:

$$\sigma(E) = \frac{A_1(E)}{n\, l \log_{10} e}, \tag{1}$$

where n and l are the number density (in cm^{-3}) and thickness (in centimeters) of the films of thymine and guanine sublimated onto the collodion films, respectively. However, the number density of the films was unknown and the thickness estimated using the thickness-monitor was inaccurately as mentioned above [15,16]. Dissolving the DNA-base films and measuring the concentration of the solutions, the values of the product of n and l can be determined [16]. However, it was difficult to dissolve the DNA-bases selectively owing to the elution of the collodion films. Therefore, we adopted the following method to determine σ (E).

Disc-shaped thin films of thymine and guanine, of 10-mm diameter, were newly sublimated onto quartz plates (Ohyo Koken Kogyo Co., Ltd., Tokyo, Japan), of which cutoff wavelength was ~165 nm (~7.5 eV), similarly to the manner already described. The absorbance of those films in the energy region of 3.1–7.5 eV (165–400 nm), A_2 (3.1–7.5), was measured using a commercial vacuum monochromator system (VM-504, Action Research Corporation, Greer, SC, USA). The optical absorption cross-section σ (3.1–7.5) is also described as follows obeying the Beer–Lambert law:

$$\sigma(3.1-7.5) = \frac{A_2(3.1-7.5)}{n'\, l'\, \log_{10} e}, \tag{2}$$

where n' and l' are the number density (in cm^{-3}) and thickness (in centimeters) of the films of thymine and guanine sublimated onto the quartz plates, respectively. After the absorption measurements, we dissolved the films on the quartz plates in ethanol and collected the solutions independently to determine the values of the product of n' and l'. The concentration of the obtained solution C can be determined using the Beer–Lambert law as follows:

$$C = \frac{A_3}{A_s} C_s \tag{3}$$

where A_3 and A_s are, respectively, the absorbance of the dissolved film and standard sample, the concentration C_s of which is known, at the prominent peaks. These measurements were performed in the energy region of 3.1–6.2 eV (200–400 nm) using a spectrophotometer (UV1240; Shimadzu Corporation, Kyoto, Japan). Using the values of C and the volume of sample solution V, we can determine the values of the product of n' and l' as follows:

$$n'l' = \frac{N_A\, C\, V}{\pi\, r^2} \tag{4}$$

where N_A and r are Avogadro's number and the radius of the film (5 mm), respectively. Hence, σ (3.1–7.5) was determined using Equations (2)–(4). Assuming that the films of DNA bases exhibit

the same values of optical absorption cross-section regardless of the substrate, namely, collodion and quartz plates, Equation (2) can be rewritten as

$$\sigma(3.1-7.5) = \frac{A_1(3.1-7.5)}{n\,l\,\log_{10} e} \tag{5}$$

Therefore, by substitution, Equation (1) can be rearranged as

$$\sigma(E) = \frac{\sigma(3.1-7.5)}{A_1(3.1-7.5)} A_1(E) \tag{6}$$

Thus, σ (E) was determined for entire measured region, namely, 3.1–250 eV.

3. Results and Discussion

Figure 1 shows the obtained absorption spectra of the thymine and guanine thin films. The observed peak energies corresponded to those in the literature for energies lower than 10 eV [13]. Those peaks are mainly assigned to $\pi \rightarrow \pi^*$ transitions [12,19]. The largest peaks assigned to mainly $\sigma \rightarrow \sigma^*$ transitions [20,21] were observed at around 17.7 eV for thymine and 21.0 eV for guanine.

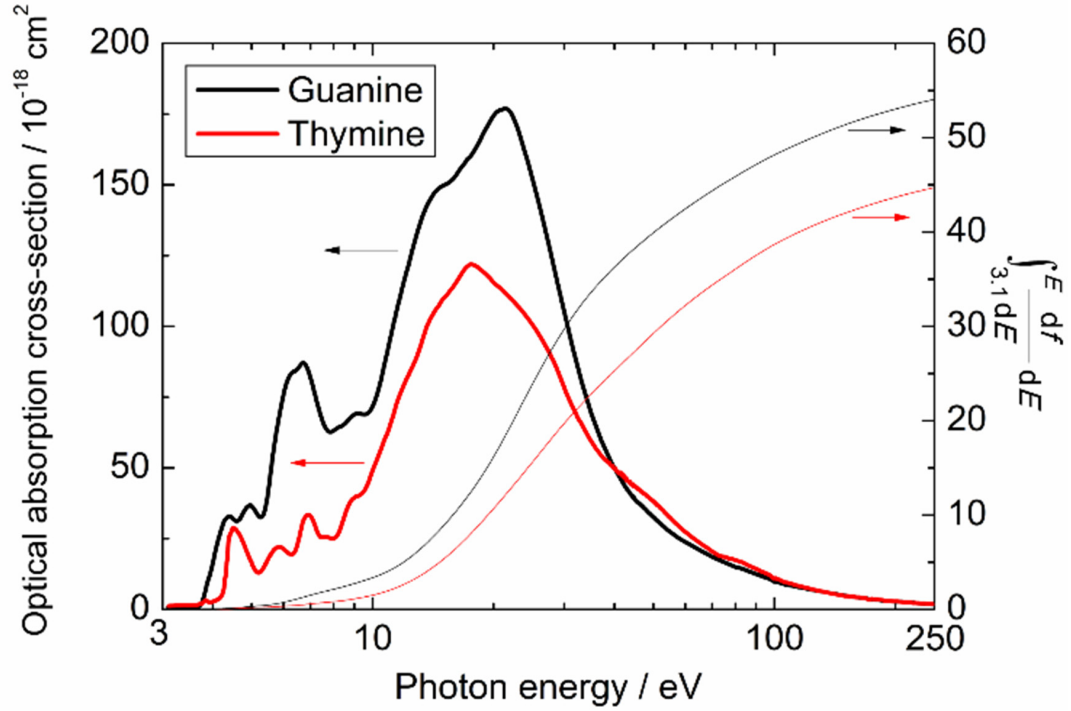

Figure 1. Absorption spectra (thick lines) and integrated values of optical oscillator strength distribution df/dE from 3.1 eV to the photon energy E (thin lines) of thymine (black) and guanine (red) thin films.

Since the values of the optical absorption cross-section from 3.1 to 250 eV were calibrated only in the region from 3.1 to 7.5 eV as described in the previous section, we confirmed the accuracy of the optical absorption cross-section by using the Thomas–Reiche–Kuhn oscillator strength sum rule [22], per the literature [6,15,23,24].

According to the sum rule, the sum of the oscillator strength distribution df/dE, including discreet and continuum spectra, is equal to the number of electrons (N_e) associated with the optical transition within the given energy range (E_1 to E_2) of the molecule,

$$f_{total} = \int_{E1}^{E2} \frac{\mathrm{d}f}{\mathrm{d}E}\mathrm{d}E = N_e \tag{7}$$

In the present work, N_e corresponds to the number of electrons of the samples excluding the K-shell electrons because the measured absorption spectra did not include the K-edge energy region. The oscillator strength distribution df/dE can be obtained from the optical absorption cross-section σ (E) as follows:

$$\sigma(E) = \frac{\pi e^2 h}{m_e c} \frac{df}{dE} \quad (8)$$

where e, h, m_e, and c are the elementary electric charge, Planck constant, electron rest mass, and speed of light, respectively. The value of the constant term of Equation (8) is 109.8×10^{-18} cm^2 eV when the units of df/dE and σ (E) are eV^{-1} and cm^2, respectively.

The values of f_{total} in the energy range from 3.1 to 250 eV of thymine and guanine were 44.7 and 54.0, respectively (Figure 1), and agreed well with the values of N_e (48 for thymine and 56 for guanine) within experimental and analytical errors [15]. Therefore, it was concluded that the values of the optical absorption cross-section determined in the present work are reasonable.

As shown in Figure 1, the main part of the oscillator strength distribution was placed in the intermediate region, namely, the region from 10 to 250 eV, and therefore the absorption spectra obtained in this work would substantially contribute to estimations of the interaction between DNA bases and IRs using the optical approximation. The optical absorption cross-section of the remaining DNA bases—adenine and cytosine—and of the RNA base—uracil—should also be determined in the future. The optical absorption cross-section of thymine and guanine in the K-edge energy region has not been determined yet. Since the oscillator strength distributions are uncharacterized in this region, it is also an important future work to determine them experimentally although they can be roughly estimated from the number of atoms comprising the molecules [25]. In such experiments, the method described in this work is applicable by replacing the collodion films for silicon nitride [26,27] or silicon carbide films [28]. By combining such future works, more precise estimations of the interaction between DNA bases and IRs or photons can be achieved.

4. Conclusions

The optical absorption cross-section of thymine and guanine thin films was determined in the energy region from 3.1 to 250 eV for the first time. Most of the oscillator strength distribution was positioned in the measurement region. Therefore, the presented spectra would substantially enable estimations of the interaction between DNA bases and IRs or photons, and as a result, contribute to quantitatively understanding reaction processes and yields of DNA-damage-inductions.

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