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Absorption Spectroscopy of Biological Specimens Near X-ray Absorption Edges of Constituent Elements

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Absorption spectra of biological specimens in the soft X-ray region have been presented with special reference to the XANES (X-ray absorption Near Edge Structure) of constituent elements. Absorption spectrum in this wavelength region is characterized by the absorption edges from which elemental content could be derived. In addition, XANES has a characteristic profile for chemical environment around the element such as chemical bond. Using the specific absorption peak we can assign not only the chemical bond but also molecules having such a chemical bond. In the present paper, absorption spectrum of DNA was measured in the wavelength range from 1.5nm to 5nm. Spectrum of Chinese Hamster Ovary (CHO) cells was compared with the DNA spectrum. XANES were distinct at the K absorption edges of major elements, C, N and O. In the spectrum of the cells prominent peaks at the L absorption edge of minor element Ca were also detectable. XANES profiles in small local areas in a cell could also be measured in combination with X-ray microscopy. These give information about local chemical environment in a cell. XANES at the phosphorus K absorption edge in a human HeLa cell was successfully obtained corresponding to a sharp and intensive XANES peak of DNA.

Key words: absorption spectrum, soft X-rays, XANES, biomolecules, X-ray microscopy, synchrotron radiation

INTRODUCTION

Absorption spectrum provides essential information for the interpretation of mechanisms of wavelength dependence of photo-induced damage. Quantum yield spectrum of a photo-product can be elucidated from a set of absorption spectrum and action spectrum, which eventually gives a clue to mechanisms of photo-product formation.

However, measurement of absolute values of absorption coefficient has not been achieved in the soft X-ray region ranging from 0.1nm to 10nm, because of the difficulty of specimen preparation with thin layer of defined thickness. Alternatively, Hieda and Ito [1] calculated an absorption coefficient of DNA molecule from atomic absorption coefficients of constituent elements. But fine absorption structure observed near the absorption edge of elements that results from the transition of inner shell electron to an excited state of a molecular orbital can not be simulated by such a calculation. The fine structure known as XANES (X-ray Absorption Near Edge Structure) reflects chemical environment of an element, and particularly sharp and prominent resonance peaks have been known to be useful for chemical bond or molecular mapping [2] or photo-effects on a specific element [1].

In the present study, we measured absorption spectrum of DNA and mammalian cells over the wavelength range of 1.5 nm to 5 nm in the relative absorption scale. Both spectra exhibited absorption jump at the absorption edges of major elements, C, N and O with less content of N. In the spectrum of the cells XANES were also detected at these absorption edges with significant XANES peaks at the L absorption edge of Ca in the cell spectrum.

XANES measurement could be achieved even for local regions in a cell with the aid of X-ray microscopy. The spectrum would give us information about chemical environment of intracellular small areas around constituent elements, which leads to chemical mapping of a cell. We obtained XANES of the phosphorus K absorption edge in several small areas in a human HeLa cell. This method may be unique in high resolution molecular mapping.

MATERIALS AND METHODS

Light source. Soft X-rays generated from synchrotron radiation were obtained at the Photon Factory, Tsukuba, Japan. Beamline BL-11A and 11B were used for the photon energy

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region from 1.5 to 5 nm and from 0.496 to 0.590 nm, respectively. The resolving power $\lambda/\Delta\lambda$ is 1500 to 2000.

Specimen preparation. DNA was prepared in dry state on a collodion film spread on a 150-mesh EM grid. To obtain cell specimens Chinese Hamster Ovary (CHO) cells were suspended in phosphate buffer saline solution and dropped onto the collodion film, and then dried in the air. For the specimen of X-ray microscopy, human HeLa cells were cultured on a SiN membrane, fixed with glutaraldehyde, and then subjected to the critical point drying.

Absorption spectra and XANES measurement. Spectral measurements were performed using the transmission method, i.e. transmitted X-rays through a specimen were detected by a silicon photodiode (AXUV-100, International Radiation Detectors Inc., U.S.A.).

X-ray microscopy. Contact X-ray microscopy using an electronic zooming tube as a two dimensional X-ray detector [3] was applied to obtain intracellular XANES spectra at the P-K absorption edge, which was extended to the phosphorus mapping in a cell.

RESULTS AND DISCUSSION

Absorption spectrum of DNA

Figure 1 shows an absorption spectrum of calf thymus DNA in a dry state from 1.5 to 5 nm in a relative absorption scale. K absorption edges of C, N and O were clearly observed as expected. Near the absorption edges fine structure and prominent intensive peaks were detected. To our knowledge, this is the first measurement of an absorption spectrum of DNA over a wide wavelength range in the soft X-ray region although XANES at each absorption edge have been already reported [2, 4-6]. For comparison, calculated linear absorption coefficient based on mass absorption coefficients of atoms tabulated in the reference [7] was superimposed as filled circle symbols. In the calculated spectrum, there is neither information on the XANES nor the data around absorption edges. The absorption spectrum obtained here should be compared with action spectrum of DNA damage to understand mechanisms leading to such damage.

Absorption spectrum of CHO cells

Absorption spectrum of CHO cells was shown in Figure 2 in the same wavelength range as in Figure 1. The absorption edges of C, N and O were apparent as was the case of DNA spectrum, but the degree of absorption jump was different. Particularly it is noticed that nitrogen is not so abundant as DNA. The most remarkable characteristics of the cell spectrum is the XANES at the Ca-L absorption edge around 3.5 nm. Sharp resonance peaks were observed despite that absorption jump was not detectable. Since XANES is sensitive to chemical environment of elements, action spectra for cellular photo-effects should be compared with such an absorption spectrum.

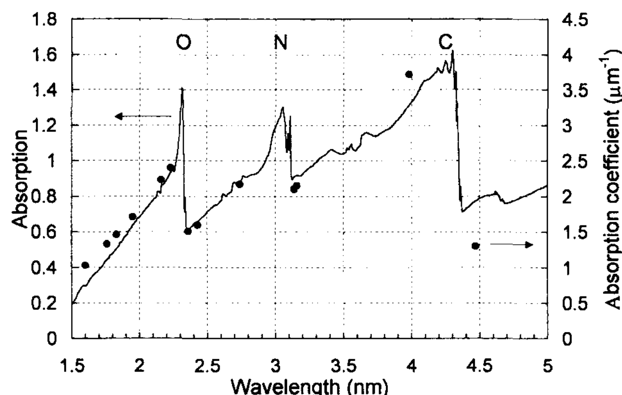

Figure 1. Absorption spectrum of calf thymus DNA.
The filled circles represent linear absorption coefficients (right axis) calculated from atomic absorption coefficients tabulated in [7].

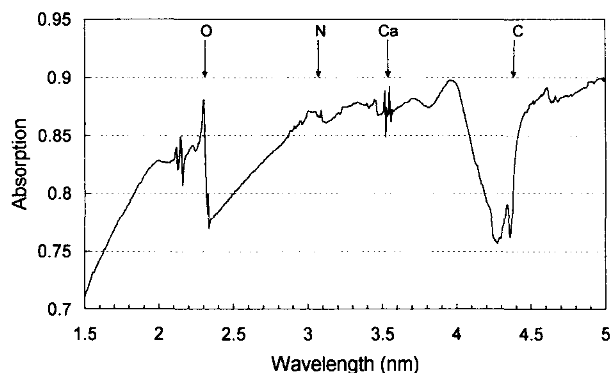

Figure 2. Absorption spectrum of CHO cells in a dry state.
The wavelengths of absorption edges of C, N, O and Ca are indicated as arrows.

XANES of local areas in mammalian cells at the P-K absorption edge

XANES of DNA at the phosphorus K absorption edge has been known to have a very prominent single resonance peak [1]. XANES of cell pellet resembles that of DNA with a slight peak shift [8]. We obtained XANES of intracellular local areas in a cell using contact X-ray microscopy. Figure 3 shows a group of HeLa cells taken at 0.579 nm. Absorption spectra of small white areas of 0.5μm square were presented in Figure 4. Images at the 10 wavelengths around the P-K absorption edge were used to obtain XANES. XANES in the areas 2, 3 and 5 exhibited the similar profile to that of DNA shown by the dotted line. In contrast, since area 1 and 4 locate outside the cells, the spectra exhibited broad structure. Any significant

shift in the peak energy was not detected among spectra of area 2, 3 and 5. Increase of a number of wavelengths used for imaging would give fine spectral feature that may result in different XANES profiles among local areas.

The ratio of absorption at the peak wavelength and the wavelength just below the peak was calculated throughout all the pixels of the image. In this way DNA mapping was completed as shown in Figure 5. This image indicates that DNA distributes throughout the cells, suggesting that the group of cells situate near the mitotic phase in the cell cycle stage. It should be noted that this image probably includes phosphorus-containing biomolecules because XANES profiles of nucleotides are very similar to that of DNA [8].

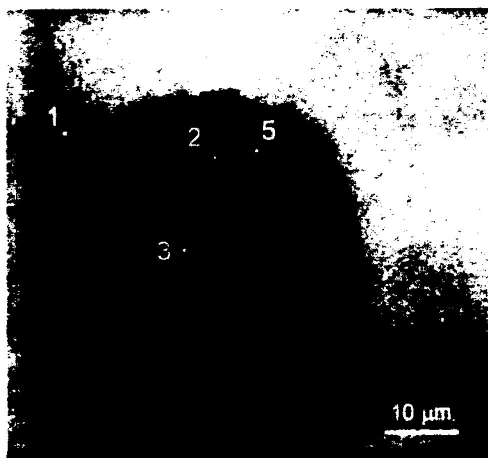

Figure 3. Soft X-ray image of HeLa cells at the wavelength of 0.579 nm.

XANES were measured in the areas of 1 to 5 the size of which is 0.5 µm square.

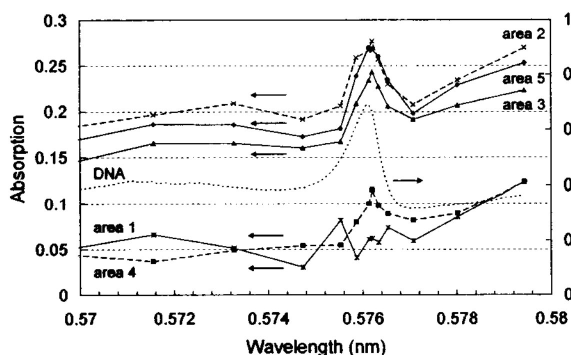

Figure 4. XANES spectra of local areas in a HeLa cell at the P-K absorption edge.

Dotted line shows a spectrum of calf thymus DNA.

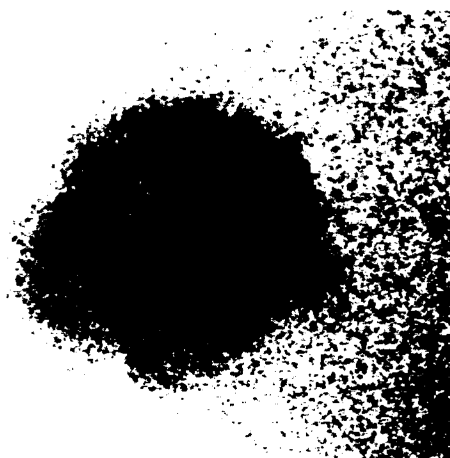

Figure 5. DNA image in HeLa cells.

CONCLUSION

Absorption spectroscopy of biological specimens in the soft X-ray region gives not only element information but also molecular (chemical bond) information. Sharp and intense XANES resonance peaks can be used for molecular or element mapping at high resolution with X-ray microscopy. Such mapping would be useful for the studies on molecular or element arrangements during physiological process and photo-damage targeted to a specific molecule or element.

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