Chemical Shift Images of Organelles in Leydig cells of Mice Testes

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Abstract. Soft X-ray transmission images of Leydig cells of mice testes changing incident wavelength were observed with the use of a contact microscope. After normalization of transmission images, absorbance images were obtained and compared with a visible differential interference image. Some organelles were identified by the image comparison, and absorption spectra of the organelles were obtained from the absorbance images. The absorption spectra show that peak structures are different depending on the observed organelles. The structures and the positions of organelles were clearly identified at C-K absorption.

1. Introduction

Metabolism in a bio-cell is a sequence process: a. an organic material ingested from outside of a bio-cell is transformed to the new one/ones changing its chemical-bonding state/states. b. Through the material transformation, energy is simultaneously produced to keep the vital activity of the bio-cell. c. The energy is consumed in/out of the bio-cell and used for the other transformations [1]. This process indicates all component materials in a bio-cell are the combination of some low-molecular-weight organic materials. For example, DNA and RNA are composed of 5 kinds of nucleotides, and the proteins in bio-cells are made of 20 kinds of amino acids [1]. These organic materials have a tendency that some of them concentrate in specific organelles. In cell nuclei, they are composed of DNA, RNA and specific proteins for keeping their functions [2]. In mitochondria, enzymes and proteins to produce the energy for metabolism concentrate in them. [3]. A membrane is composed of phosphorylated lipid. Necessary proteins and organic materials penetrate into and unnecessary substances are blocked out of the membrane.

These organic materials have different chemical bonding states, which can be identified by binding energy at an absorption edge of the component atom. The binding energies can be measured by X-ray absorption spectroscopy (XAS) [5]. Absorption spectrum of an organic material has a specific peak
position with a spectral shape depending on its chemical bonding states. Therefore, interpreting the spectral shape is inseparable from understanding the chemical bonding state of the material.

Based on the chemical bonding states of nucleotides and amino acids, the XAS spectra measured at C-K edges were roughly classified into 3 groups [5-7]: 1. Acyclic compounds composed of major atoms, such as H, C, N, and O. These compounds have a sharp peak originated from C $\pi^*$ orbital observed at around 289 eV with a broad peak originated from C $\sigma^*$ orbital at the higher binding energy side of the C $\pi^*$ peak. 2. Acyclic compounds including all component atoms except the group 1. These compounds show C $\pi^*$ peak at 289 eV with C $\sigma^*$ peaks. The C $\sigma^*$ peaks are often observed at the lower binding energy side of the C $\pi^*$ peak. 3. Cyclic compounds. The peak of C $\pi^*$ orbital in cyclic structure is observed at around 285 eV. Peaks originated from the side chain compounds are in almost the same energy positions as those in group 1 and 2.

According to this classification, XAS spectra of organelles can be interpreted as the combination of spectra in these 3 groups [8]. When a XAS spectrum of an organelle is measured, the spectral shape will be interpreted by the component organic materials or the chemical-bonding state of the organelle. In this study, we choose contact microscopy method to obtain absorption spectra of organelles through absorption images and select Leydig cells of mice testes as a measurement sample. The results of absorption measurements will be reported in this paper.

2. Experimental Conditions

Leydig cells were cultured on a SiN membrane and fixed by 4% paraformaldehyde. The Leydig cells preserved in the cultural fluid was packed into a cell holder. The cell holder can keep the liquid sample between the SiN membrane and a scintillator plate in a vacuum chamber. The scintillator plate used in the measurements was Ce:LYSO, which converts a shadow image under the sample to a visible fluorescent image. The converted image can be observed by a visible microscope. Thus, the Leydig cells can be observed changing the incident wavelength [9]. Observation was made at BL27SU, SPring-8, Japan [10]. Range of incident wavelength was from 4.77 to 2.07 nm (260 to 600 eV), and wavelength resolution $\lambda/\Delta\lambda$ of the incident light was 2000. Exposure time was 0.8 - 180 sec, which changed depending on the light intensity. The spatial resolution was estimated from the edge response. The edge was the boundary of the SiN membrane between the frame of the membrane and the SiN membrane (inset of Fig. 1). The value of the spatial resolution, 1.1 $\mu$m, was estimated from the peak width, which was obtained by a numerical differentiation of the edge response at line A-A’ in Fig. 1. After the soft X-ray observation, visible transmittance- and visible differential-interference- images were taken at the same position and compared with the soft X-ray ones.

3. Results

3.1. Soft X-ray Images and Spectra

At first, the background images were subtracted from the measured soft X-ray images, and the images were normalized by both the incident light images, from which the background images were also subtracted, and the exposure time to obtain transmittance images. Absorbance images were calculated from the obtained transmittance images under the assumption that there is no interference in the sample. Thus the absorbance images were compared with the visible differential interference image (Fig. 2(a)). Detailed comparison between these images indicates that absorption intensity in each organelle is different. According to the different values of the absorption intensity in organelles,
regions of interest (ROIs) in each bio-cell were divided into three parts as designated in Fig. 2(d): the central region should correspond to cell nucleus (ROI A), surrounding regions around the central area are expected to be small and different organelles (ROI B), and the external part of ROI B is considered to be cell membrane under the cultural conditions (ROI C). The area outside each bio-cell is filled with the cultural fluid (ROI D).

Spectra in all ROIs were obtained from the absorption images. Spectrum of each ROI was normalized by the spectrum of ROI D on the assumption that incident light passes through the cultural fluid in all ROIs. The normalized spectra at C-K absorption edge are represented in Fig. 3. In the spectra of ROI A and B, a broad peak is observed at around 283 eV and 286 eV, respectively. In the spectrum of ROI C, no peak is observed. At N- and O-K absorption edges, absorption spectra were obtained in the same manner as at C-K edge. At N-K edge, no peak structure was observed, because the absorption of SiN membrane was stronger than the signal of the bio-cells. At O-K edge, 4 peaks at 526, 529, 532, and 536 eV were observed in all ROIs, and the spectral shape of each ROI is similar to each other. Because the wavelength interval was too wide in the measurement, further details of O-K edge results will be discussed in the future study.

3.2. Chemical Shift Images

Spectral shapes of ROI A and B show a broad peak structure at C-K edge (Fig. 3), and are reproduced well by Lorentzian curves (solid curves in Fig. 3). Fitting results show that the peak positions of ROI A and B are 283.3 eV and 285.4 eV, respectively. Peak widths of ROI A and B are 8.7 eV and 6.6 eV, respectively, in FWHM. Peak intensity of ROI A doubles that of ROI B.

The images taken at 283 eV and 285 eV were selected as the peak position images, and the background image at 275 eV was subtracted from these peak images. These image results in Fig 2 (b) and Fig 2 (c) showed the strong absorption by cell nucleus and by small organelles, respectively.

4. Discussion

To interpret the broad peak observed at 283.3eV in ROI A, the spectral shapes of the organic materials, which were mentioned in Introduction, are assumed. Nucleotides have a cyclic structure and the XAS peak originated from this structure is observed at around 285 eV. Then the broad peak observed at 283.3eV in ROI A will be originated from nucleotides. Other organic materials with XAS peaks at the higher binding energy side are the following amino acids: Phenylalanine, Tyrosine, Tryptophan, and Histidine. Most of these amino acids have an acyclic structure and XAS peaks originated from this structure are observed at around 289 eV. Then the broad peak in ROI B observed at the higher energy side of the peak in ROI A will be originated from amino acids. This speculation coincides with component materials of the organelle, which are assigned as nuclei in ROI A, as mitochondria in ROI B, and as cell membrane in ROI C [5-7].

Peak width of the C $\pi^*$ peak in nucleotides is sharp and the value is less than 1 eV. If the broad peak structures observed in ROI A and B are originated from the C $\pi^*$ peak, each spectral shape is an overlap of the sharp peaks at the different energy positions. Judging from the observed broad peak

![Figure 2: a) Visible differential interference image, b) SX image at 283 eV, c) SX image at 285 eV, d) ROIs are identified by colour: red is ROI A, cyan, ROI B, green, ROI C, and blue, ROI D.](image-url)
structures, chemical bonding states in each organelle will be complex.

Peak positions in each ROI are about 2 eV lower than those in nucleotides and amino acids. This peak shift is caused by the sample conditions. The values in ROI A and B were measured using a liquid sample, whereas the values in these organic materials were measured in solid samples such as a thin film (in Ref. 5 and 6) and a crystalline powder (in Ref. 7). Therefore, chemical shift will occur depending on the electronic structure of the measured samples.

5. Summary

Normalized XAS spectra of organelles in Leydig cells of mice testes were obtained from the transmission images. The different peak positions observed at C-K edge are interpreted by XAS spectra of nucleotides and amino acids which are the component organic materials of bio-cells. And the background-subtracted images at the peak positions show both structures and positions of the organelles. Identification of a small organelle or a specific protein is usually made by fluorescence proteins with the use of visible light microscopes. Present results show the identification of the organelles will be made by the binding energy difference at an absorbance edge without any modification of the organelles. For this purpose, sample conditions should be clarified to observe small organelles in a bio-cell, and laboratory-type SX microscope should be developed to change incident wavelength without any restriction of the light source.

References
[1] for example, B. Alberts, et al., Molecular biology of the cell, (Garland Science, Taylor & Francis Group, New York, 2010) Chap. 2.
[2] for example, B. Alberts, et al., Molecular biology of the cell, (Garland Science, Taylor & Francis Group, New York, 2010) Chap. 4.
[3] for example, B. Alberts, et al., Molecular biology of the cell, (Garland Science, Taylor & Francis Group, New York, 2010) Chap. 6.
[4] for example, B. Alberts, et al., Molecular biology of the cell, (Garland Science, Taylor & Francis Group, New York, 2010) Chap. 10.
[5] J. Boese, A. Osanna, C. Jacobsen, and J. Kirz, J. Elec. Spectro. Rel. Phenom. 85, (1997) 9.
[6] K. Kaznacheyev, A. Osanna, C. Jacobsen, O. Plashkevych, O. Vahtras, H. Ågren, V. Carravetta, and A. P. Hitchcock, J. Phys. Chem. A 106, (2002) 3153.
[7] Y. Zubavichus, A. Shaporenko, V. Korolkov, M. Grunze, and M. Zharnikov, J. Phys. Chem. B 112, (2008) 13711.
[8] J. S-Ornstein, A. P. Hitchcock, D. H. Cruz, P. Henklein, J. Overhage, K. Hilpert, J. D. Hale, and R. E. W. Hancock, J. Phys. Chem. B 111, (2007) 7691.
[9] T. Ejima, et al., in preparation.
[10] http://www.spring8.or.jp/wkg/BL27SU/instrument/lang-en/INS-000000287