XANES of nucleotides at the N-K absorption edge measured by total photon yield

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Introduction

XANES spectra of biological specimens provide fundamental information to the analysis of electronic state of biomolecules, molecular imaging using resonance absorption peaks assigned to a specific chemical bond, etc. So far XANES spectra of biomolecules have been scarcely reported. Among them XANES at the N-K edges for DNA and related compounds were most accumulated. Kirtley et al. compared XANES of bases and nucleosides at the N-K absorption edge [1]. We have found unique structure in XANES of DNA compared with that of histone mixture, a nuclear protein, at the N-K absorption edge [2], suggesting possible DNA imaging in a nucleus using this DNA specific resonance peak. In the present study, we compared XANES of nucleotides with different phosphorus numbers at the N-K absorption edge using high energy-resolution apparatus.

Materials and Methods

XANES was obtained at BL-19B by measuring total photon yield and total electron yield. These yields can be measured simultaneously. Slit width of the monochromator was set to 20 μm.

Nucleotides used are as follows: ATP (adenosine triphosphate), ADP (adenosine diphosphate), and AMP (adenosine monophosphate). These reagents were purchased from Sigma Co. U. S. A., dissolved in pure water, deposited on a collodion coated EM grid, and then dried in the air.

Results and Discussion

Figure 1 shows a comparison between photon and electron yield for ATP. Although both spectra were very similar in shape, the electron yield spectrum was rather noisy, and in some cases, the spectra exhibited extensive broadening. These results could be interpreted that these biomolecules have insulation nature. Therefore we determined to adopt the photon yield spectrum for XANES measurement of biomolecules. The peak in the lower energy region split into two, probably assigned to N1s $\rightarrow$ π^* transition. The broader peak located at the higher energy region is probably attributable to N1s $\rightarrow$ σ^* transition. These peaks were also observed in our previous measurement of DNA at BL-11A [2]. Figure 2 shows XANES of adenine nucleotides with different number of phosphorus. The relative height of the split peak due to N1s $\rightarrow$ π^* transition appears to be changed depending on the number of phosphorus; The ratio of the peak at the lower energy side to that at the higher energy side was increased with decreasing number of phosphorus. The extension of these measurement at the other absorption edges, and nucleotides with other bases are being planned.

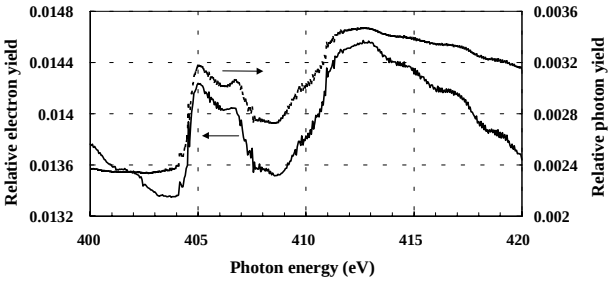

Fig. 1. Photon and electron yield spectra of ATP at the N-K absorption edge.

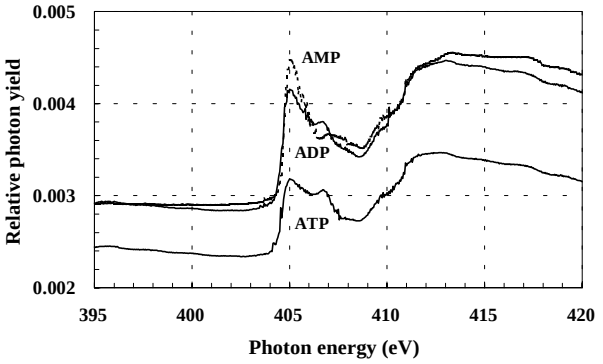

Fig. 2. Photon yield spectra of ATP, ADP and AMP at the N-K absorption edge.

References

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