

Nitrogen *K*-edge soft X-ray natural circular dichroism of histidine thin film

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Abstract. We report preliminary natural circular dichroism (NCD) spectra of amino acids, L- and D-histidine, thin films in the nitrogen *K*-edge energy region. NCD peaks were observed at 399.9 eV and were assigned to the $1s \rightarrow \pi^*$ transition of the nitrogen atoms in imidazole ring. Imidazole ring is positioned at the side chain distant from the asymmetric carbon. This result suggests that NCD spectroscopy in the soft X-ray region can selectively pick up the information on local structure around specific atoms, regardless of the position of atoms in molecules. It is interesting in view of application of NCD spectroscopy in the soft X-ray region to investigation of protein structures.

1. Introductions

Natural circular dichroism (NCD) is defined as the difference in the magnitudes of the optical absorption cross-section for left circularly polarized light (LCPL) and that for right circularly polarized light (RCPL). This phenomenon is exhibited in absorption bands of chiral molecules. NCD spectra are typically measured in the ultraviolet or infrared region and used in many different fields. For example, NCD spectroscopy in the ultraviolet region is one of the tools to investigate the molecular structures of chiral materials such as proteins. It is expected that the expansion of NCD measuring region extends applications of NCD.

Our group has been trying to measure NCD spectra in the soft X-ray region [1–4]. Soft X-rays are able to excite *K*-shell electrons of carbon, nitrogen, and oxygen atoms. It is expected that the NCD signals from transitions between core and valence levels can provide information about molecular structure in the vicinity of selected atoms because such transitions are an internal atomic process. Indeed, we measured NCD spectra in the soft X-ray (oxygen *K*-edge energy) region and succeeded to observe the differences reflecting in the local structure around excited oxygen atom which cannot be distinguished using ordinary soft X-ray absorption spectroscopy [4]. However, it has not been cleared yet whether NCD signals originating from core-absorption of atoms located at various sites, as well as next to the asymmetric carbon, in the molecules can be observed selectively.



In this study, we measured NCD spectra of an amino acid, histidine (His; Fig. 1) which has three nitrogen atoms, thin film in the nitrogen *K*-edge energy region, and confirmed that we can pick up the NCD signals originating from each nitrogen atom throughout the molecules.

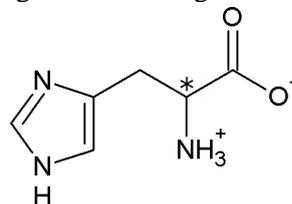


Figure 1. Structural formula of His zwitterion. The asymmetric carbon is marked with an asterisk.

2. Experimental

The detail experimental and analytical procedures have been reported in our previous paper [4]. Thin films of His were prepared by vacuum sublimation. The L- and D-enantiomers of His were purchased from Tokyo Chemical Industries (Japan) and used as amino acid reagents without further purification. The powder of each reagent was sublimated on SiC membranes (membrane size: square with sides of 3.0 mm, thickness 300 nm; MEM-C03003/10M, NTT Advanced Technology Corporation) by heating up to 370 K with a nickel-chrome heater. The base pressure of the vacuum sublimation chamber was about 10^{-3} Pa. The thickness of the films was estimated to be about 400 nm by a quartz crystal deposition controller (CRTM-500, ULVAC, Inc.).

Absorption and NCD measurements in the nitrogen *K*-edge were carried out at beamline BL25SU of SPring-8, Japan [5–7]. Photon energies were calibrated by measuring absorption spectrum of nitrogen gas and comparing it to literature data [8]. Absorption spectra were obtained by using transmission method. Incident light intensity was monitored by measuring the drain current from another SiC membrane positioned in front of the sample. The light through the SiC membrane and the sample reached a gold film sublimated on a stainless steel plate which was positioned at the back of the sample. The drain current from the gold film was recorded as the transmitted light intensity. Absorbance of each His film for LCPL and RCPL were determined. Absorption spectrum for RCPL was subtracted from that for LCPL to give ΔA spectrum. The ΔA spectrum is proportional to the NCD spectrum in ideal case. However, ΔA spectra often include contamination from linear dichroism (LD) and linear birefringence (LB) components in the case of oriented samples. To evaluate the magnitude of contamination, the ΔA spectra were obtained for 8 sets of rotation angles $(\theta, \phi) = (0^\circ, 0^\circ), (0^\circ, 180^\circ), (90^\circ, 0^\circ), (90^\circ, 180^\circ), (45^\circ, 0^\circ), (45^\circ, 180^\circ), (-45^\circ, 0^\circ),$ and $(-45^\circ, 180^\circ)$ for L- and D-samples, where θ and ϕ are rotation angles around axes parallel and vertical to the incident light, respectively. Since contamination of LD and LB signals were found by comparing ΔA spectra, we averaged those spectra for each sample and canceled out LD and LB components. Those averaged spectra are described as ΔA_{av} spectra, hereafter.

3. Results and Discussion

Figure 2(a) shows an absorption spectrum of L-His at the nitrogen *K*-edge energy region. In the case of D-His, similar spectrum was observed. Those spectral shapes is similar to that of reported spectrum [9]. Two sharp peaks observed at 399.9 and 401.5 eV are assigned to the $1s \rightarrow \pi^*$ transition of the nitrogen atoms in C-NH-C and C-N-C of imidazole ring, respectively [9]. Two broad peaks observed at 406.7 and 412.2 eV are mainly assigned to the $1s \rightarrow \sigma^*$ transition of the nitrogen atoms in amino group (NH_3^+) and imidazole ring, respectively [9].

Figure 2(b) shows ΔA_{av} spectra of L- and D-His. The ΔA spectrum of SiN membrane (thickness 100 nm; MEM-N03001/7.5M, NTT Advanced Technology Corporation) measured for $(\theta, \phi) = (0^\circ, 0^\circ)$ is also shown for comparison. In the case of SiN membrane, no significant structure was observed as expected, because SiN is achiral and amorphous, that is, it has neither NCD, LD nor LB. This result shows that no pseudo NCD signal originates from beamline optics and so on.

In the case of L-His (Fig. 2(b)), a positive peak was observed at 399.9 eV, in contrast, a negative peak was observed at the same energy in the case of D-His. Since the NCD spectra of L- and D-type molecules possessed opposite signs each other, these peaks originate from the NCD component. The ratio of ΔA_{av} to absorption intensity was the order to 10^{-3} which is typical value in soft X-ray NCD spectroscopy. At around 401–415 eV, the intensity difference between L- and D-His was observed, while the NCD signs were not opposite. This result indicates that obtained ΔA_{av} spectra in this energy region involve the background (BG) component as well as the true NCD component.

The BG component should originate from His films, because BG component was not observed in the case of SiN membrane. To consider the origin of BG component, the BG component was analytically estimated. Since BG component was observed, the ΔA_{av} spectra of both samples are described as

$$\Delta A_{av}^x(E) = CD^x(E) + BG^x(E) \quad (x = L, D), \quad (1)$$

where CD is true NCD component and superposition of L and D represent L- and D-His films. Using equation (1), summation of BG^L and BG^D is described as

$$\Delta A_{av}^L(E) + \Delta A_{av}^D(E) = BG^L(E) + BG^D(E), \quad (2)$$

where it is assumed that NCD spectrum of D-His should be opposite of that of L-His in signs; that is, $CD^L(E) = -CD^D(E)$. In Fig. 3, the BG component determined by using equation (2) is shown. The ΔA spectrum of D-His measured for $(\theta, \phi) = (-45^\circ, 0^\circ)$, which shows the largest LD and LB components among obtained ΔA spectra, is also shown for comparison. The spectral shapes below ~ 410 eV seem similar. It implies that the BG component mainly originated from the LD and LB components which were not perfectly removed in this work. It is supported by the result that BG component was not observed in the case of amorphous SiN membrane. Thus, the ΔA_{av} difference between L- and D-His observed at around 401–415 eV (Fig. 2(b)) might not only originate from NCD but also LD and LB. In order to confirm whether NCD peaks exist at around 401–415 eV or not, remeasurements using randomly oriented His films, which show no LD and LB, are necessary.

Nevertheless, it is quite reliable that the peaks observed at 399.9 eV (Fig. 2(b)) originate from NCD, because they were opposite in signs each other as mentioned above. Those are assigned to the $1s \rightarrow \pi^*$ transition of the nitrogen atoms in C-NH-C of imidazole ring which positioned at the side chain. Thus, we concluded that core absorption NCD originating from nitrogen atoms in the side chain distant from the asymmetric carbon is also detectable. This result suggests that NCD spectroscopy in the soft X-ray region can selectively pick up the information in the vicinity of specific atoms, regardless of the position of atoms in molecules. It is interesting in view of application of NCD spectroscopy in the soft X-ray region to investigation of protein structures.

4. Conclusion

We measured NCD spectra of thin films of L- and D-His in the nitrogen K -edge energy region. NCD peaks assigned to $1s \rightarrow \pi^*$ transition of the nitrogen atoms in C-NH-C of imidazole ring were observed. We concluded that NCD signals originating from core-absorption of atoms located at various sites in the molecules can be site-selectively observed. This result is expected that structural information at the vicinity of excited atoms will be obtained by using NCD spectroscopy in the soft X-ray region. It is interesting in view of investigation of protein structures.

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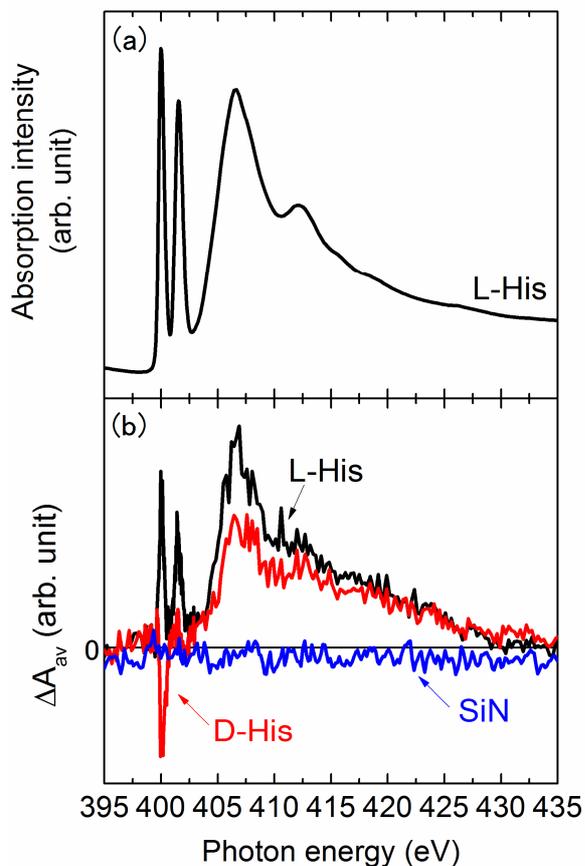


Figure 2. (a) Absorption spectrum of L-His, (b) ΔA_{av} spectra of L- and D-His (black and red lines), and ΔA spectrum of SiN membrane measured for $(\theta, \phi) = (0^\circ, 0^\circ)$ (blue line).

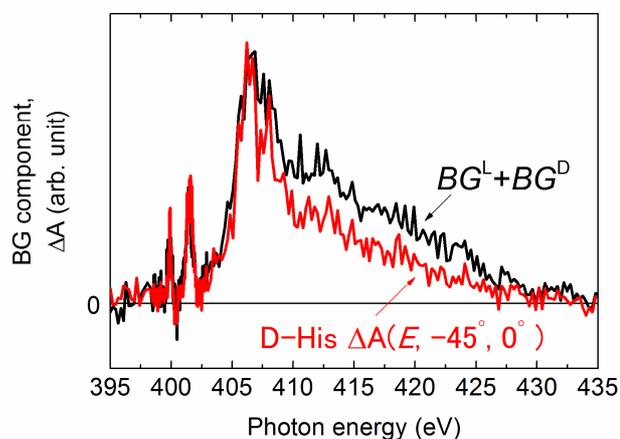


Figure 3. BG component estimated using equation (2) (black line) and ΔA spectrum of D-His measured for $(\theta, \phi) = (-45^\circ, 0^\circ)$ (red line).

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