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Measurement and comparison of absolute value of soft X-ray natural circular dichroism of serine and alanine

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Abstract. Soft X-ray natural circular dichroism (SXNCD) spectra of serine (Ser) and alanine (Ala) thin films, were measured in absolute value with the focus at the oxygen 1s → π* transition of COO −. In the case of L-Ser, a positive and negative SXNCD peaks were observed around 531.5 and 532.5 eV, respectively. In the case of L-Ala, a negative SXNCD peak was observed around 532.8 eV. The SXNCD intensity of L-Ala was about ten times as large as that of L-Ser. Those differences would reflect the chiral environment of the core hole oxygen atoms of COO −.

1. Introduction

Natural circular dichroism (NCD) is a difference in the magnitude of absorption cross section for left circularly polarized light (LCPL) and right circularly polarized light (RCPL). NCD in ultraviolet region is well known to reflect the chiral structure of molecules and thus is commonly used as a powerful tool to study chiral structures such as secondary structures of proteins [1]. Now NCD measurement for biomolecule is being extended to soft X-ray region, in which NCD due to inner-shell transitions is expected to provide information on chiral structure at the vicinity of the concerned atom. We carried out the first observation of NCD spectra in soft X-ray region (SXNCD spectra) of two amino acids in nitrogen and oxygen K-edge region [2] and explored SXNCD spectra of other amino acid molecules [3] especially focusing the oxygen 1s −→ π* transitions of COO − [4]. It should be noted that, although nitrogen and oxygen are not chiral center, SXNCD of these molecules was clearly
observed. Our experimental results have some theoretical support based on the electric-dipole-magnetic-dipole interaction mechanism (E1M1 mechanism) [5-8]. There is no calculation based on the electric-dipole-electric-quadrupole interaction mechanism (E1E2 mechanism) for amino acids. Because our previous results were not absolute spectra, measurement of the absolute values of SXNCD spectra is necessary to study the detailed nature of SXNCD of biomolecules.

In this work, we report the absolute value of SXNCD spectra of serine (Ser) and alanine (Ala) thin films with the focus at the oxygen 1s → π* transition of COO− and comparison of the absolute value with theoretical calculations [5, 8].

2. Experimental procedure
Amino acids, L-Ser (HOCH2CH(NH3+)COO−; purity > 98%), D-Ser (purity > 98%), L-Ala (CH3CH(NH3+)COO−; purity > 98%) and D-Ala (purity > 98%), were purchased from Sigma-Aldrich Corporation and used without further purification. Thin films were prepared by vacuum sublimation technique [9] on a SiN membrane (3 mm × 3mm, thickness 100 nm; ATN/MEM-N03001/7.5M, NTT Advanced Technology). Thickness of films was estimated to be about 300 nm by using a quartz-crystal-oscillator thickness monitor. According to AFM observation studies, evaporated films of amino acid are aggregate of randomly oriented microcrystallites [10].

Measurement of absorption spectra (XANES) and SXNCD of Ser and Ala was carried out at the SPring-8 BL25SU, Japan [11]. LCPL and RCPL were switched with 1 Hz using the twin helical undulators and kicker magnets. Incident light intensity and transmitted light intensity were obtained by measuring the drain current from a gold film evaporated onto SiC membrane positioned at the upstream side of samples and from a gold film evaporated onto stainless steel plate positioned at the downstream side of samples, respectively. Optical axes of the twin helical undulators were carefully adjusted each other not to produce any signal for the oxygen K-edge SXNCD measurement of oxygen gas sample. All measurements were carried out at room temperature.

Measurement of absorption spectra was carried out in the both cases of LCPL and RCPL in the photon energy region 525 ≤ E ≤ 560 eV and optical density (OD( E) = log10(I0/I)) was determined, where I0 is incident light intensity and I is transmitted light intensity through the samples at photon energy E. Absolute absorption cross section σ(E) was determined by assuming that the experimental values of OD at pre-edge and post-edge region should be equal to the summation of atomic absorption cross section of its component atoms [12]. We subtracted σ for RCPL (σRCPL) from σ for LCPL (σLCPL) and obtained SXNCD of L-type ( ΔσL-type (E) = σLCPL L-type (E) − σRCPL L-type (E) ) and of D-type ( ΔσD-type (E) = σLCPL D-type (E) − σRCPL D-type (E) ). We carefully examined ΔσL-type (E) and ΔσD-type (E) as a function of rotational angles of samples around both parallel and perpendicular axes to the direction of incident light beam, since linear dichroism (LD) and linear birefringence (LB) might be observed as pseudo NCD signals which depend on rotational angles [13, 14]. We confirmed that SXNCD was independent from rotation angles, thus we concluded that our samples had no LD and LB.

It is established that SXNCD of R- and S-type enantiomers should have the same modulus and opposite sign [15, 16]. But obtained spectra did not have the same modulus. Assuming that observed SXNCD is summation of true SXNCD and some background component originated from the optical system, we cancelled out the background using similar way reported by Turchini et al. [16] and obtained true SXNCD of L-type; ΔσL-typeTrue (E) = (ΔσL-type (E) − ΔσD-type (E))/2 . Hereafter, true SXNCD is simply represented as Δσ(E).

3. Results and Discussion
3.1. XANES and SXNCD of L-Ser film
Figure 1-(a) shows XANES spectra σ(E) of L-Ser. Experimental error was less than 5 %. The spectrum by theoretical calculation was reproduced from Flashkevych et al. [5], but its photon energy was
shifted by about 1.5 eV to lower energy side in order to compare with our experimental data around 532.1 eV. In the theoretical spectrum, two peaks were predicted around 532.1 and 533.6 eV. A peak was observed around 532.1 eV. But no peak was found around 533.6 eV. Absorption cross section $\sigma(E)$ of experimental and theoretical XANES at around 532.1 eV was $3.5 \times 10^{-18}$ cm$^2$ and $5.0 \times 10^{-18}$ cm$^2$, respectively. Theoretical edge jump was twice as large as experimental edge jump.

Turchini et al. reported that SXNCD spectrum of randomly oriented sample (methylxirane gas) agreed with theoretical calculation based on the E1M1 mechanism [16]. We also compared our spectrum and theoretical calculations based on E1M1 mechanism [5]. Figure 1-(b) shows experimental SXNCD spectrum $\Delta \sigma (E)$ of L-Ser and rotatory strengths of L-Ser calculated on the basis of the E1M1 mechanism in length gauge [5]. The relation between rotatory strength $R$ [esu$^2$ cm$^2$] and SXNCD $\Delta \sigma$ [cm$^2$] was given by the equation $R = 6.0 \times 10^{-19} \int_{E_1}^{E_2} (\Delta \sigma / E) dE$ (1). In the experimental spectrum, a negative peak was observed around 532.5 eV ($\Delta \sigma = -1.7 \times 10^{-21}$ cm$^2$). It would correspond to the summation of two negative rotatory strengths predicted at 532.05 ($R = -1.8 \times 10^{-43}$ esu$^2$ cm$^2$) and 532.15 eV ($R = -1.5 \times 10^{-43}$ esu$^2$ cm$^2$). In order to compare the experimental SXNCD with theoretical rotatory strengths, we obtained the rotatory strength using equation (1). The experimental rotatory strength was $-1.4 \times 10^{-42}$ esu$^2$ cm$^2$ and about four times as large as sum of theoretical rotatory strengths. Thus, the photon energy of SXNCD peaks of L-Ser were partly reproduced by calculation, but the intensity of absorption cross section and SXNCD were not.

It should be noted that the positive rotatory strengths predicted at 532.02 eV and 533.39 eV. A positive peak predicted at 532.02 eV may correspond to the positive SXNCD signals found around 531.5 eV of which S/N ratio was not so good. Another positive peak was also predicted at 533.39 eV. Although two XANES peaks were predicted around 532.6 and 533.0 eV, single XANES peak was observed at 532.1 eV. Two negative SXNCD peaks were predicted around 532.5 eV ($\Delta \sigma = -1.7 \times 10^{-21}$ cm$^2$), but the intensity of absorption cross section and SXNCD were not.

3.2. XANES and SXNCD of L-Ala film

Figure 2-(a) shows XANES spectra $\sigma(E)$ of L-Ala. The spectrum of theoretical calculation was reproduced from Jiemchooroj et al. [8], but its photon energy was shifted by about 14.4 eV lower energy side in order to compare with our experimental data at the peak found around 532.8 eV. Although two XANES peaks were predicted around 532.6 and 533.0 eV, single XANES peak was observed at 532.8 eV. Probably the experimental resolution was not able to resolve the energy difference of 0.2 eV. Experimental absorption cross section was $4.1 \times 10^{-18}$ cm$^2$. Experimental error was less than 5 %. Since theoretical absorption spectrum was relative value, we could not compare the absorption cross section.

Figure 2-(b) shows theoretical SXNCD spectrum of L-Ala on the basis of E1M1 mechanism [8] and experimental SXNCD spectrum $\Delta \sigma(E)$ of L-Ala. Two negative SXNCD peaks were predicted around 532.6 and 533.0 eV and a negative SXNCD peak was observed around 532.8 eV. The intensity of SXNCD was $-9.3 \times 10^{-21}$ cm$^2$, $-6.2 \times 10^{-21}$ cm$^2$ and $-1.4 \times 10^{-20}$ cm$^2$, respectively. Therefore we concluded that the intensity of experimental SXNCD of L-Ala was reproduced by this theoretical calculation within spectral resolution although we had to carry out large energy shift (about 14.4 eV).

3.3. Comparison of experimental SXNCD

In order to compare two experimental SXNCD spectra, we obtained the ratio $g$ of SXNCD to absorption cross section ($g = \Delta \sigma / \sigma$). The $g$ value of L-Ser around 532.5 eV and L-Ala around 532.8 eV were $5.5 \times 10^{-3}$ and $3.0 \times 10^{-3}$, respectively. Although both SXNCD peaks were assigned to oxygen 1s $\rightarrow \pi^*$ transition of COO$^-$, the $g$ value of L-Ala was about five times as large as that of L-Ser. The reason why L-Ala shows stronger SXNCD than L-Ser is open question at the present time, which is very interesting in view of experimental and theoretical studies including E1E2 calculations.
Figure 1. (a) XANES spectra of L-serine (Ser), black: experiment; red: calculation [5].
(b) Experimental SXNCD spectrum of L-Ser (black; left axis) and calculated rotatory strengths of L-Ser (red; right axis) [5].

4. Conclusion
A negative SXNCD peak assigned to oxygen 1s → π* transition of COO\(^-\) was observed in both cases of L-Ser and L-Ala. The intensity was approximately \(-1.7 \times 10^{-21}\) cm\(^2\) and \(-1.4 \times 10^{-20}\) cm\(^2\), respectively. For L-Ser, a positive SXNCD peak was observed around 531.5 eV. Difference between the chiral environment of the core hole oxygen atoms of COO\(^-\). Theoretical calculations based on E1M1 mechanism [5-8] can reproduce our SXNCD spectra qualitatively. It is important to accumulate more experimental and theoretical studies to establish the understanding of SXNCD of amino acid molecules.

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