Oxygen K-edge X-ray absorption near edge structures (XANES) of sublimated films of amino acids

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Oxygen K-edge X-ray absorption near edge structures (XANES) spectra of amino acids (glycine**,** L-α-alanine, β-alanine, L-serine, L-asparic acid and L-tyrosine) were measured. Several peaks of XANES spectra were successfully assigned on the basis of DV-Xα calculation.

Keywords: oxygen K-edge, XANES, amino acid.

1. Introduction

Amino acids are of great interest because those are fundamental building block of living matter. Those are known to be in the state of so-called "zwitter-ion" in solid phase (by X-ray diffraction of crystal (Dawson, 1960) and XPS (Clark *et al.*, 1976)), and its extremely low vapour pressure makes ultra high vacuum experiment such as the XANES experiment possible. Recently, C-K edge XANES spectra of aromatic amino acids were studied experimentally (Boese *et al.*, 1997) and theoretically (Carravetta *et al.*, 1998). Here we selected oxygen K-edge for XANES study of amino acids. From the view point of K-edge XANES study in amino acids, oxygen is interesting because its chemical states have the wealth of variety. A carboxylic group on the α carbon is in the state of carboxylic anion -COO^-, while carboxylic group in side-chain stays on -COOH, and some amino acids have -OH group in side-chain. Due to this variety of chemical environment, we can expect characteristic chemical shift for each oxygen atom in various amino acids.

In this paper, we report a result of oxygen K-edge XANES spectra of amino acids films. We discuss the details of spectra on the basis of DV-Xα calculation.

2. Material and Method

2.1 Sample preparation

Taking notice of chemical characteristic of oxygen, we selected following amino acids as samples in this experiment; glycine (Gly) ($H_3N^+CH_2COO^-$), L-α-alanine (L-Ala) ($H_3N^+CH(CH_3)COO^-$), β-alanine (β-Ala) ($H_3N^+CH_2CH_2COO^-$), L-serine(L-Ser) ($H_3N^+CH(CH_2OH)COO^-$), L-asparic acid (L-Asp) ($H_3N^+CH(CH_2COOH)COO^-$) and L-tyrosine (L-Tyr) ($H_3N^+CH(CH_2C_6H_4OH)COO^-$). These amino acids were purchased from Sigma Co. and Wako Chemical Co.. All samples were used without further purification.

Evaporated films of these amino acids were carefully prepared with vacuum sublimation technique. Powder sample was put on a heater and sublimated at about 370K on an Au-coated BeCu substrate in vacuum (~10^{-3} Pa). The thickness of the amino acid sublimated films was measured by a quartz oscillator to be about 0.5 μm.

2.2 XANES measurement

Measurements of XANES spectra were performed at the soft X-ray undulator beamline BL23SU (Yokoya *et al.*, 1998, Nakatani *et al.*, 1998) of SPring-8, Japan. Energy resolution was about 0.1eV. Scanning the photon energy (energy region; 525-550eV, energy step size; ~0.05eV), we measured sample drain current with a picoanmeter (Model TR 8652, Advantest Co.). The signal *I* was divided by incident photon intensity *Io* monitored as drain current on a post focus mirror. All measurements were performed at room temperature with normal incidence.

2.3 Theoretical analysis

For assignment of obtained XANES peaks, we performed Discrete Variational (DV)-Xα calculation (Adachi *et al,*. 1978). Atomic coordinates of amino acids were calculated by MOLDA and MOPAC programs.

3. Result and Discussion

XANES spectra obtained with *I/Io* are shown in Fig.1 and Fig.2. In Fig.1 and Fig.2, all spectra of 6 amino acids are shown with normalizing the magnitude of the 533eV peak.This peak was already ascribed to be O1s→π* excitation of carboxylic group COO^- (Plashkevych *et al.*, 1998). It should be noted that this normalization at 533eV peak should cause the under estimation of spectrum intensity for L-Asp, because L-Asp has two carboxylic groups.

As seen from Fig.1, spectrum intensity of around 540eV becomes larger with increase of number of oxygen atoms in a molecule except for L-Asp. This region is known to be composed from σ* resonance and excitation to continuum (Stöhr, 1992a).

In only L-Asp spectrum, a clear peak at 534.5eV was found (see Fig.1). The energy difference of this peak from the largest peak at 533eV was 1.5eV. This means that this peak is originated from O1s→π* excitation of C–OH oxygen in COOH group, which was assigned by Clark et al. with XPS measurement (Clark *et al.*, 1976). This situation is also confirmed by our DV-Xα calculation, in which O1s→π* excitation of C–OH oxygen at COOH group is found to be at 1.5eV higher energy than that of other O1s→π* excitation of L-Asp carboxylic group.

XANES spectra shown in Fig.1 and Fig.2 were analysed in detail by a DV-Xα calculation. At first, we subtracted an excitation to continuum from the observed spectrum. Value of ionization threshold of L-Ala were taken from the STEX ab initio calculation by Plashkevych *et al* (Plashkevych *et al.*, 1998) and values of those of other amino acids were estimated by the difference between O1s energy level resulted from DV-Xα calculation and that of L-Ala. Through this procedure, we deconvoluted observed spectrum into Gaussian functions and Gaussian error function in the same manner as Stöhr (Stöhr, 1992b). Obtained results are shown in Fig.1. To relate observed peaks with result of the DV-Xα calculation, we adjusted the value of O1s→π* excitation energy resulted from the DV-Xα calculation to 533eV which had already assigned to O1s→π* resonance.

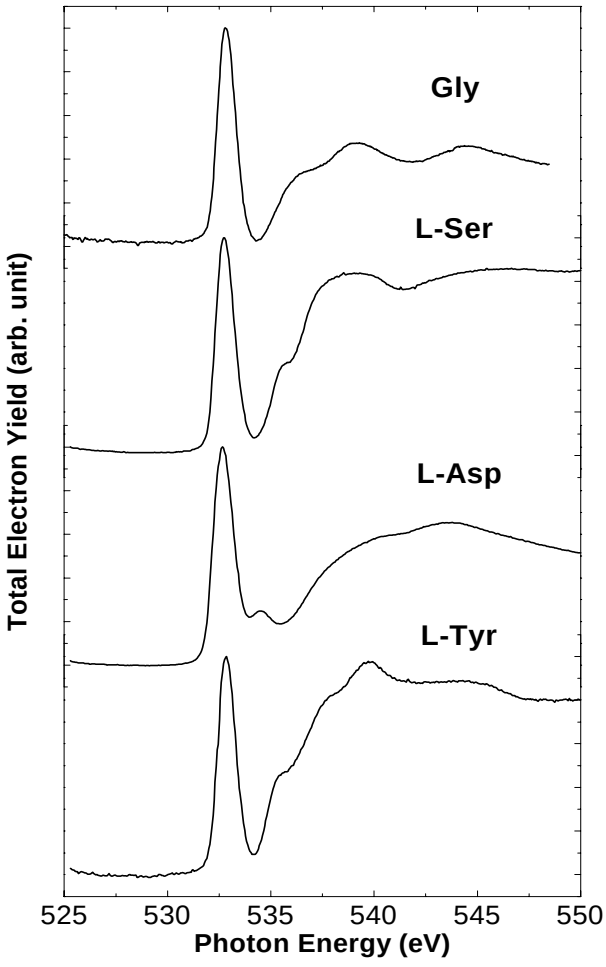

Figure 1
Normalized O-K edge XANES spectra of oxygen characteristic amino acids.

For Gly spectrum, we deconvoluted 4 peaks and 1 step components, which were called to be π_1*, π_2*, σ_1*(σ_3*), σ_2* and continuum (see Fig.2-a). Assignment of π_1* peak was already discussed above. According to result of DV-Xα calculation, the 540eV peak corresponds to oxygen contribution to the C-C σ* resonance (σ_1* resonance) and C-H σ* resonance (σ_3* resonance) (relatively weak), and the 545eV peak corresponds to C=O σ* resonance (σ_2* resonance). Our assignment was in good agreement with the result of XANES study for the monolayer Gly adsorbed on Cu (110) (Hasselstörm *et al.*, 1998). The 536eV peak was fairly small. This peak was reported by Hasselstörm *et al.* (Hasselstörm *et al.*, 1998) to have the same polarization nature with the strongest π* resonance (~533eV). Our DV-Xα calculation also assigned this peak to the weak COO^- π* resonance (π_2* resonance) which seemed to be formed by bond-bond interaction of COO^- π* resonance and other resonance (probably C-N σ* and N-H σ* resonances) (Stöhr, 1992c). This result indicated that our assignment of π_2* is reasonable.

For L-Ala and β-Ala spectrum, we could deconvolute 4 peaks and 1 step components (see Fig.2-b and Fig.2-c). Those were assigned to be π_1* (~533eV), σ_1* (~536eV), σ_2* (~545eV), σ_3* (~538eV at L-Ala, ~540eV at β-Ala) and continuum based on the following discussion.

At first, the 533eV and 545eV peaks appeared at the same energy position for these three amino acids. These peaks correspond to the same resonance of Gly in our calculation, namely 535eV peak corresponds to the π_1* resonance and 545eV peak the σ_2* resonance.

It seemed to be that the σ_1*(σ_3*) peak of Gly split into the σ_1* and σ_3* peak for the case of L-Ala and β-Ala due to substitution of methyl group. According to result of DV-Xα calculation, lower energy shift of C-C σ* resonances (σ_1* resonance) were found to be about 2.0~2.5eV than that of Gly. Therefore, 536eV peaks of L-Ala and β-Ala which shift to lower energy (~3.5eV) side than that of Gly σ_1* peak are assigned to be mainly σ_1* resonance. In addition to this resonance, 536eV peaks may also contain π_2* resonance. Our DV-Xα calculation shows that oxygen population of π_2* resonance for L-Ala and β-Ala was larger than that of Gly. In spite of large population of π_2*, this resonance was not observed apparently for these spectra. This reason is not clear at the present time.

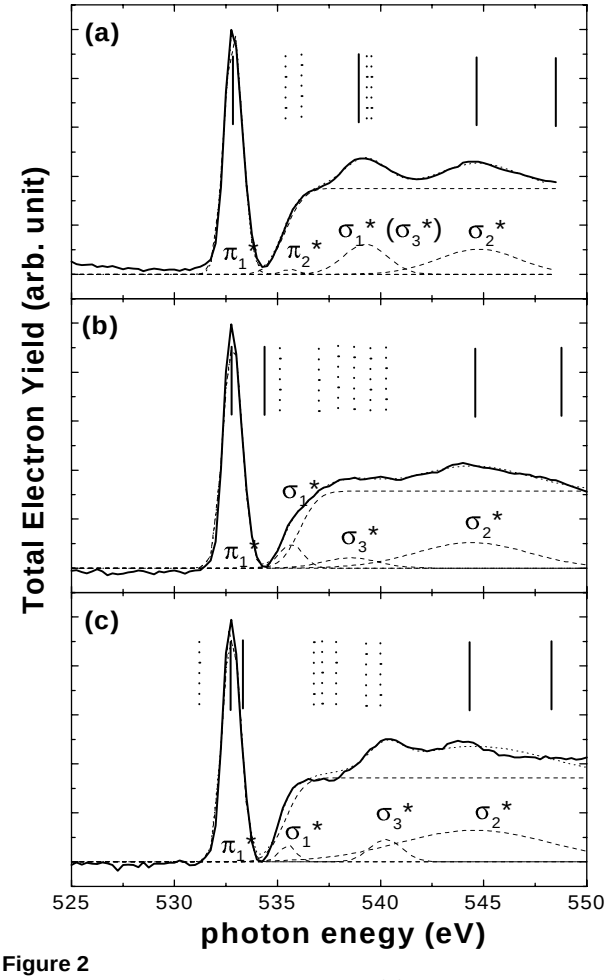

Figure 2
Normalized O-K edge XANES spectra of fundamental amino acids and results of curve fitting for (a) Gly, (b) L-Ala and (c) β-Ala O-K edge spectrum. : Solid line; measured XANES spectra, dotted line; results of curve fitting technique, and broken line; the individual components of dotted curves. Vertical lines show oxygen-contained energy level obtained DV-Xα calculation: Solid vertical line; large population of oxygen, dotted vertical line; small population of oxygen.

The 538eV peak for L-Ala and the 540eV peak for β-Ala were assigned via our DV-Xα calculation to be σ_3* resonance, which is oxygen contribution to the C-H σ* resonance. Since the calculation indicated the σ_3* resonance also for Gly at 540eV, 540eV peak of Gly was assigned to be σ_1*(σ_3*) resonance (see Fig.2-a).

According to result of our DV-Xα calculation, remarkable difference was not obtained between L-Ala and β-Ala. But in XANES spectra, we could found clear difference around 535~540eV region. It is seemed to be that oxygen spectra may be affected not only by change of close environment but also farther environment. For more detailed explanation, more sophisticated calculation is necessary.

For more complex molecules such as L-Ser, L-Asp and L-Tyr, it is not so easy to deconvolute the spectra, because such amino acids have 2 or 3 types of oxygen atoms and have the same number of ionization potentials and many oxygen-related resonances. In spite of these difficulties, we can extract some interesting features by DV-Xα calculation as follows.

Roughly speaking, 535-542eV region of L-Ser and L-Asp seems to correspond to ionization thresholds and oxygen contribution mainly to the C-C σ* resonance, and 543-548eV region corresponds to C=O σ* and C-O-H σ* resonance. About 534.5eV peak of L-Asp was assigned to π* resonance of C-OH of COOH group as discussed above.

We found that the L-Tyr XANES spectrum was similar to that of phenol (Francis and Hitchcock, 1992) above 535eV region. According to our DV-Xα calculation, 537eV peak may correspond to oxygen contribution of the delocalized C=C π*, 540eV peak corresponds to C-OH σ* resonance in phenol group. This assignment was in a good agreement with extended Hückel calculation (Francis and Hitchcock, 1992). The broad shape around 542-545 eV corresponds to a number of oxygen contribution of the delocalized C=C σ* of phenol group and C=O σ* resonance of carboxylic group.

In summary, we reported XANES study of some amino acids films and made a tentative assignment of observed peaks by DV-Xα calculation. But for large amino acids, assignment was not easy. Further experimental and theoretical studies are necessary.

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