Vacuum-Ultraviolet Circular Dichroism of Amino Acids as Revealed by Synchrotron Radiation Spectrophotometer

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(Received April 30, 2002; CL-020376)

We succeeded in constructing a vacuum-ultraviolet circular dichroism (VUVCD) spectrophotometer with a small-scale synchrotron radiation source (0.7 GeV) at Hiroshima Synchrotron Radiation Center (HiSOR). This VUVCD system revealed for the first time the CD spectra of amino acids in aqueous media in the 310–140 nm region under high vacuum. These data, which cannot be observed by any types of existing CD spectrophotometers, now open a new field in the structural analysis of biomaterials on a basis of the higher energy transition of chromophores.

The extension of circular dichroism (CD) measurements to the vacuum-ultraviolet (VUV) region can provide more detailed and new information on the structure of biomaterials based on the higher energy transition of chromophores such as hydroxyl and acetal groups in solution. In order to achieve this goal, we constructed a VUVCD spectrophotometer, which can be operated to the short wavelength limit of 140 nm under high vacuum, using a small-scale SR source (HiSOR) at Hiroshima Synchrotron Radiation Center (HSRC).[1,2] The performance of the new system has been confirmed to be satisfactory in the 310–140 nm region under high vacuum (10^{-4} Pa), by monitoring the CD spectrum of an ammonium *d*–camphor-10-sulfonate aqueous solution, which shows the characteristic peaks at 291 and 192 nm at a 1 : 2 intensity ratio.[2] This paper reports the VUVCD spectra of eight typical amino acids of considerably high solubility and low light absorption (alanine, valine, leucine, isoleucine, proline, lysine, serine, and threonine). The results show that the newly constructed VUVCD spectrophotometer can be applied successfully to the structural analysis of biomaterials with isolated chromophores in VUV region.

Since the 1980's, a great deal of efforts has been made to construct the VUVCD spectrophotometer using synchrotron radiation (SR) as an intense light source at several facilities;[3–7] the Brookhaven National Laboratory (BNL) in the U.S.A., the Daresbury Synchrotron Radiation Source (SRS) in the U.K., the Aarhus Storage Ring (ASTRID) in Denmark, and the Beijing Synchrotron Radiation Facility (BRSF) in China. New VUVCD spectrophotometers are under construction at the University of Science and Technology of China (USTC, Hefei) in China, the Berlin Electron Storage Ring Company for Synchrotron Radiation (BESSY) in Germany, and the Campinas National Synchrotron Light Laboratory (LNLS) in Brazil. However, these CD spectrophotometers are available only under nitrogen gas atmosphere so that the short wavelength limit sofar remains at about 170 nm for aqueous solutions.

All amino acids are of reagent grade purchased from Katayama Chemical Co. and Wako Pure Chemical. They were dissolved in double-distilled water. The concentration of amino acids (w/v %) was determined by calibrating the moisture content. The ellipticities involve about 5% error, which is mainly caused by the noise of CD spectra and the inaccuracy of light path length.

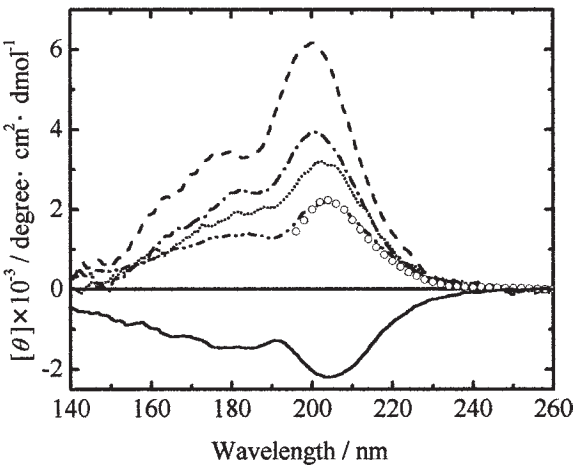

Figure 1. VUVCD spectra of L-alanine (–··–, pH 6.2, concn. 10%), D-alanine (—, pH 6.2, concn. 10%), L-valine (·····, pH 6.0, concn. 3%), L-isoleucine (–·–·–, pH 6.2, concn. 2.5%), and L-leucine (– – – –, pH 6.3, concn. 1.5%) at 25 °C. All spectra are recorded with 25- and 46-μm path langth MgF_2 cells, 1.0 mm slit, a 16-s time constant, 4 nm/min scan speed, and 4–16 accumulations. Circles indicate the spectrum of L-alanine solution using a JASCO J720 spectropolarimeter.

Figure 1 shows the VUVCD spectra of nonpolar amino acids; L-alanine, D-alanine, L-valine, L-isoleucine, and L-leucine at 25 °C. These spectra are difficult to obtain by any commercially available instruments. To our knowledge, they are revealed for the first time by a SR spectrophotometer. The spectra obtained by the present apparatus were quite superimposable upon those observed by a commercial JASCO J720 spectropolarimeter in the wavelength region down to 200 nm. The D- and L-isomers of alanine and also proline give rise to the symmetrically inverted spectra with positive and negative ellipticities, indicating a good performance of the present instrument. The spectra of L-alanine, L-valine, L-isoleucine, and L-leucine (Figure 1) show two successive positive peaks at around 200 and 180 nm. The peak wavelengths and molar ellipticities are summarized in Table 1. These two peaks may be assigned, respectively, to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the carboxyl group, as suggested by Inagaki[8] and Snyder et al.[9] Evidently, these peaks shift to the shorter wavelength side with the increase in intensity as the side chain alkyl group becomes bulky. Isoleucine with an asymmetric β-carbon atom has $[\theta]$ values smaller than those of leucine,

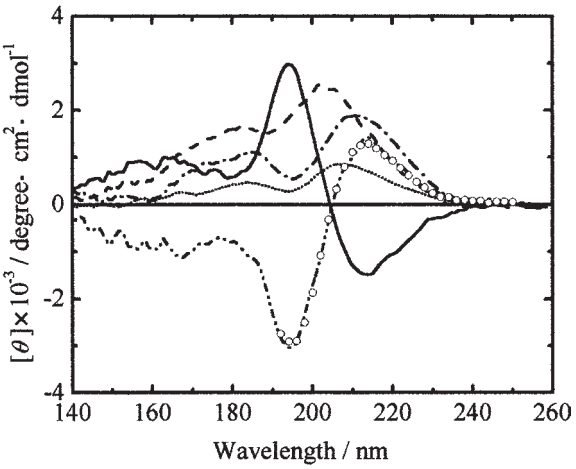

Figure 2. VUVCD spectra of L-proline (–··–, pH 6.2, concn. 4%), D-proline (—, pH 6.2, concn. 4%), L-threonine (· · · · · ·, pH 5.6, concn. 4%), L-lysine (–·–·–, pH 10.3, concn. 3%), and L-serine (– – – –, pH 5.7, concn. 3%) at 25 °C. All spectra were recorded under the same conditions as stated in Figure 1. Circles indicate the spectrum of L-proline measured by a JASCO J720 spectropolarimeter.

Table 1. Peak wavelengths and molar ellipticties, $[\theta]$, of the VUVCD spectra of L-amino acids in water at 25 °C

Amino acids	λ /nm	$[\theta]$ /degree·cm^2·dmol^{-1}	pH
L-Alanine	203.3	2200	6.2
	184.1	1400	
L-Valine	202.3	3200	6.0
	181.4	1900	
L-Isoleucine	200.3	4000	6.2
	182.3	2500	
L-Leucine	200.1	6200	6.3
	179.4	3500	
L-Proline	213.6	1500	6.2
	194.1	−3000	
	166.0	1000	
L-Threonine	207.5	900	5.6
	186.0	500	
L-Lysine	210.9	1900	10.3
	184.4	1100	
L-Serine	201.7	2600	5.7
	183.7	1600	

although both the amino acids have the same number of alky groups. The electronic transitions of carboxyl group are apparently sensitive to the size and asymmetry of the side chain. In addition to the 200 and 180 nm peaks, at least another small positive peak appears to exist at around 170–160 nm. For the definite confirmation of these higher-energy CD transitions, the level of signal-to-noise ratios and spectral resolution must be improved by increasing the intensity of HiSOR light sourse to 200 mA and by adopting a better-blazed grating mirror in the VUV region.

Figure 2 shows the VUVCD spectra of amino acids; L-proline, D-proline, L-lysine, L-serine, and L-threonine. The VUVCD spectra of L-serine and L-threonine show two positive peaks at around 200 and 180 nm, which are probably ascribed to the electronic transitions of carboxyl group, as similarly observed for the spectra of nonpolar amino acids. For threonine with an asymmetric β-carbon atom, the peaks shift towards the longer wavelength side with much lowered ellipticities (Table 1). Some peaks or shoulders are also observed in the wavelength region lower than 170 nm, which may be due to the side chain hydroxyl group and/or the main chain amino group. For L-lysine two peaks are separated more clearly and shift further to the longer wavelength side at 210 and 185 nm. Some shoulders in the wavelength region lower than 180 nm may be associated with the transition from the side chain amino group. The VUVCD spectrum of L-proline (and also D-isomer) is most characteristic, i.e., a positive peak appears at 213 nm and two successive negative peaks at 194 and 166 nm. This is obviously due to its cyclic five-membered ring structure being different from other amino acids.

As shown above, we have succeeded for the first time in measuring the VUVCD spectra of amino acids in water in the VUV region down to 140 nm using a SR source. At present, the ab initio assignment of these spectra is in progress using molecular orbital calculations (Mopac and Gaussian 98) in our laboratory. The assignment of VUVCD spectra still remains unsolved because of limited numbers of theoretical and experimental studies,[10] although an assignment has been made for the vibrational CD in the infrared region.[11] Accumulation of the VUVCD data should open the new field in the structural analysis of biomaterials based on the higher energy transitions of the chromophores, which are so far unavailable by any types of existing CD spectrophotometers.

We thank Professors K. Yamaoka and R. W. Woody for their helpful discussion. This work was financially supported by HSRC, Japan Atomic Energy Institute, and by a Grant-in-Aid for Scientific Research (No. 12559007) from the Ministry of Education, Science, Sports and Culture of Japan.

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