Structural Analysis of Biomolecules Using Synchrotron-Radiation Vacuum-Ultraviolet Circular Dichroism Spectroscopy

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Abstract

Circular dichroism (CD) spectroscopy is a powerful tool for the structural analysis of biomolecules, because the CD spectra are measurable at a low concentration under various solvent conditions. However, no commercial CD spectrophotometer is capable of measuring the CD spectra of aqueous solution in the vacuum ultraviolet (VUV) region below 190 nm, which makes it difficult to obtain detailed and accurate structural information of biomolecules. We have constructed a vacuum-ultraviolet circular dichroism (VUVCD) spectrophotometer that can measure the CD spectra down to 140 nm under a high vacuum, using a synchrotron radiation (SR) of BL-15 at HiSOR. The path length of the optical cell (CaF₂) of this spectrophotometer is adjustable to 1.3–50 μm with a Teflon spacer, and the temperature can be controlled in the range from −20 to 70°C with Peltier element. We have applied this spectrophotometer to measure the CD spectra of various saccharides, amino and hydroxy acids, and proteins in aqueous solution. Mono-, di-, and oligo-saccharides exhibited characteristic CD spectra in the wavelength region from 180 to 160 nm, depending on anameric and axial/equatorial configurations of hydroxyl groups, trans/gauche configurations of hydroxymethyl groups, and types and numbers of glycosidic linkages. The trans/gauche configurations of hydroxymethyl groups were confirmed to make important contributions to the VUVCD spectra by a time-dependent density functional theory (TDDFT) and a molecular dynamics simulation. The VUVCD spectra of glycosaminoglycans sensitively reflected the characteristic contributions of constituent functional groups in the VUV region. L-amino acids and L-hydroxy acids showed the unique CD spectra below 210 nm depending on the types of side chains. The VUVCD spectra of alanine and lactic acid theoretically calculated by TDDFT method revealed the important role of hydration in stabilizing their structures. The VUVCD spectra of globular proteins down to 160 nm allowed us to more accurately estimate the contents and numbers of segments of α-helix and β-strand using an analytical program SELCON3. The positions of α-helices and β-strands on the amino-acid sequence were also predictable with about 75% accuracy by combining VUVCD data with a neural-network algorithm. These secondary-structure analyses were also successfully applied to various types of non-native proteins such as acid-, cold-, heat-, and alcohol-denatured proteins, amyloid fibrils, disulphide-deficient variants, and membrane-bound protein. The obtained results demonstrate that SR-VUVCD spectroscopy is a powerful technique for the structural analysis of biomolecules in aqueous solution, and hence could open a new field in structural biology.

Keywords – Biomolecule, Circular dichroism, Structural analysis, Synchrotron radiation, Time-dependent density functional theory

References