

## Synchrotron Radiation Chiral Spectroscopy and Biomolecules

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Circular dichroism (CD), which is defined as the difference in absorbance between left- and right-circularly polarized light (LCPL and RCPL), is the established technique to monitor the steric structures of chiral molecules such as biomolecules (amino acid, natural product, protein, DNA, and polysaccharide). The usage of synchrotron radiation (SR) as a light source has further enhanced the usefulness of this spectroscopy through the extension of the wavelength region of CD spectrum into vacuum-ultraviolet (VUV) region (down to 140 nm), the developments of new VUV-CD analytical methods combined with computational science<sup>[1]</sup>, and the installation of the linear dichroism (LD)<sup>[2]</sup> and spatial- and time-resolved measurement systems. In this study, we introduce the CD techniques using SR and its applications to the characterization of the structures of chiral molecules.

The relationship between chromophores of steric configurations and their substituents in organic compounds can be analyzed from the sign of CD (positive or negative), and when targeting the chromophores that has an absorbance only in the VUV region (~200 nm), the SR-CD becomes effective tool. For example, the absorption of allene exhibits around 180 nm and hence the absolute configuration of its substituents is determined from the CD sign around 180 nm, not in the wavelength region above 200 nm<sup>[3]</sup>. The acetal bonds and hydroxyl groups of sugars also have the CD peak around 170 nm, reflecting their configurations. Since, even for mono-saccharide, sugar in solution takes an equilibrium state of two anomers and three rotamers, it is difficult to determine the configurations from the CD sign. However molecular dynamics (MD) simulation and time-dependent density functional theory can disclose the unique CD of each isomer and the situations of their intramolecular hydrogen bonding<sup>[4]</sup>, and further can reveal the relationships between isotope effect and hydration<sup>[5]</sup>. Thus, it is possible to discuss the steric configuration and the hydrogen bonding network of monosaccharide at the isomer level.

The CD analysis of globular proteins combined with bioinformatics and LD allowed us to estimate the contents, numbers of segments, sequences, and orientations of secondary structures of proteins. The combination method was applied to the elucidation of the mechanism of protein-membrane interaction related to drug transportation into cell, myelin formation around neuron cell, antimicrobial activity in immune system, and amyloid fibril formation<sup>[6,7]</sup>. The antimicrobial peptide magainin 2 interacts with the bacterial membrane, causing damage to its structure. The CD analysis revealed that the M2 peptides assembled in the

membrane and turned into oligomers with a  $\beta$ -strand structure. LD and fluorescence anisotropy suggested that the oligomers are inserted into the hydrophobic core of the membrane, disrupting the bacterial membrane. Thus, VUVCD and its combination with other polarization experimental methods pave the way for unraveling the molecular mechanisms of biological phenomena related to protein-membrane interactions. The time-resolved system installed into the CD instrument realized the kinetics analysis of conformation change of protein under the interactions with other biomolecules. Micro beam using the schwarzschild objective made it possible to measure the CD with microvolume sample and the position-dependent CD<sup>[8]</sup>. This system was used for revealing a part of mechanism of DNA damage response in X-ray irradiated human HeLa cells<sup>[9]</sup> and for measuring the spatial-resolved CD of solid sample.

Origin of homochirality in terrestrial biomolecules (L-amino acids and D-sugars dominant) remains an unresolved problem. The CD spectroscopy can easily detect the emergence of optical anisotropy due to the asymmetric reaction. The thin solid films of racemic alanine ( $L/D = 1$ ) were prepared by the vacuum evaporation system and irradiated by the LCPL and RCPL generated from SR undulator, and the emergence of amino acid homochirality were investigated by the CD measurements. The SR-CD spectroscopy was also used for the study of biochemical effects by loss of homochirality<sup>[10]</sup>.

The CD technique with SR light source would further enhance the performance of chiral spectroscopy and open a pathway of next-generation of molecular chirality research.

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