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

Koichi Matsuo

Hiroshima Synchrotron Radiation Center, Hiroshima University
Circular dichroism

Circular Dichroism (CD) is observed when certain material absorbs left- and right-circular polarized light slightly differently. This is very sensitive to the structure of chiral molecules.

\[ \Delta \varepsilon = \Delta \varepsilon_L - \Delta \varepsilon_R \]
Circular dichroism of biomolecules

Polypeptide

Amino-acid sequence

Protein

α-helix

β-sheet

Turn

CD spectra of Poly-L-Lysine

Peptide bond

$\text{H}_2\text{O}$

$\text{C} = \text{O}$

$\text{C} - \text{N}$

$\text{C} - \text{C}$

$\text{R}_1$

$\text{H}$

$\text{R}_2$

$[\psi] \times 10^{-3}$ deg cm$^{-1}$ dmol$^{-1}$

Wavelength / nm

α-helix

β-sheet

Coil
Circular dichroism of biomolecules

Saccharide (sugar)

DNA


Riazance and Johnson 1992, Biopolymers 32, 271-276
Synchrotron-radiation vacuum-ultraviolet circular dichroism (VUVCD)

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>120</th>
<th>140</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
<th>320</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum-ultraviolet (VUV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Far-UV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near-UV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conventional CD spectrophotometer

Synchrotron-Radiation VUVCD spectrophotometer

Amino acid in thin film

Polypeptide in water

VUVCD spectrophotometer

SR : Synchrotron Radiation   PM : Photomultiplier
AMP : Amplifier    M : Mirror    POL : Polarizer
PEM : Photo Elastic Modulator   S : Shutter
HV : High Voltage Supply    ANA : Analyzer

Sample chamber

Top view of sample chamber

Temperature control unit


Temperature range from –30 to 70 °C
Assembled-type optical cell

Spacer: 100, 50, 25, 10, and 5 μm
No spacer: 1.4 - 1.8 μm

1, Cylindrical screw; 2, Stainless-steel cap; 3, Stainless-steel cover; 4, 8, and 9, Fluoride-rubber O-ring; 5, and 7, MgF₂ disc; 6, Spacer (aluminum or Teflon); 10, Stainless-steel bottom block; A, Needle; B, Disk stopper

VUVCD spectra of five mono-saccharides

\[ n-\sigma^* \text{ transitions (hydroxy group and acetal bond)} \]

Conc.: 10%, Path length: 1.4μm

\[ [\theta] \times 10^{-3} / \text{deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1} \]

Wavelength / nm

Six conformers of D-glucose in aqueous solution

Two anomers

α-anomer

β-anomer

Three rotamers

Gauche-Gauche (GG)  Gauche-Trans (GT)  Trans-Gauche (TG)

α-GG, α-GT, α-TG, β-GG, β-GT, and β-TG conformers
Theoretical calculation of CD spectra of small biomolecule using a time-dependent density functional theory (TDDFT)

- **L-Alanine with nine water molecules**  

- **D-Lactic acid with six water molecules**  
  Fukuyama *et al.* (2011) *Chirarity* **23**, E52

- **Di- and tri-Peptides**  

- **DNA**  
VUVCD spectrum and chemical structure of methyl $\alpha$-D-glucopyranoside (methyl $\alpha$-D-Glc)

Methyl $\alpha$-D-Glc is composed of $\alpha$-GT, $\alpha$-GG, $\alpha$-TG rotamers

Relative population = $\alpha$-GT : $\alpha$-GG : $\alpha$-TG = 38 : 58 : 4

$J. \text{Phys. Chem. A} \ (2012) \ 116, \ 9996$
Initial structures of three rotamers of methyl α-D-Glc

**α-GT**
(X-ray crystal structure)

**α-GG**

**α-TG**

ϕ = O-5–C-5–C-6–O-6

ϕ = 60°

ϕ = -60°

ϕ = 180°
VUVCD spectra of methyl α-D-glucopyranoside in water (Onsager model)

Schemes of CD calculations

**Initial structures**

α-GT, α-GG, and α-TG rotamers

**Optimizations**

DFT method (Onsager model)
at the CAM-B3LYP/6-311++G** level

**CD calculations**

TDDFT method
at the CAM-B3LYP/6-311++G** level (PCM)
MD simulation of methyl α-D-Glc in aqueous solution

Initial structures in water

α-GT, α-GG, and α-TG rotamers

TIP3P water molecules

= MD simulation

The initial structures in a periodic box water molecules were simulated for 20-ns under the AMBER/GLYCAM force field at 298 K and 1 atm.

CD calculations

The spectra of 40 structures extracted at 500-ps intervals were calculated by TDDFT method at the CAM-B3LYP/6-311++G** level (PCM) and averaged
Hydrations of the $\alpha$-GT rotamer during the simulation

The configuration of the methyl $\alpha$-Glc would fluctuate accompanying the change of hydration to reflect the VUVCD spectra.

Theoretical and experimental CD spectra of methyl α-D-Glc

MD simulations

Optimizations

Pairwise relationships between CD and configurations of the α-GT, α-GG, and α-TG rotamers

The differences in hydrogen bonds should be responsible for the characteristic CD spectra of the α-GT, α-GG, and α-TG rotamers.

Summary in the VUVCD of saccharides

1. VUVCD spectra are sensitive to the structural characteristics such as $\alpha$- and $\beta$-anomers of hydroxy group, and trans and gauche configurations of hydroxymethylene group.

2. Theoretical calculations clarify the pairwise relationships between configurations and VUVCD of saccharides.

3. Combining VUVCD spectroscopy, MD, and TDDFT could provide important information about the conformations, interactions, and hydrations of saccharides.
Protein structure analysis by VUVCD

VUVCD spectra of reference proteins

Component spectra of secondary structures

VUVCD spectrum of unknown protein

Sequence-based prediction by Neural Network

VUVCD-NN

Secondary-structure contents and segment numbers

Amino-acid sequence

Predicted secondary structure

H : α-Helix
E : β-Strand
C : Coil

Proteins (2008) 73, 104
Protein structure analysis using VUVCD Spectroscopy

X-ray crystallography and NMR
- 3D Structure at atomic resolution
- Limited to crystallized or small protein

VUVCD Spectroscopy
- Estimation of secondary-structure contents
- Available for any proteins
- Useful for various solvent conditions

VUVCD spectroscopy is becoming useful in the technique for structure analysis of protein
VUVCD provides the structural characteristics of proteins at denatured states

VUVCD spectra of six proteins in TFE-denatured state

Secondary structures of six proteins at 50% TFE concentration

Proteins (2012) 80, 281
VUVCD characterizes the roles of disulfide bridges in the structural formation of protein

VUVCD spectra of thirteen disulfide-deficient variants of lysozyme

Proteins (2009) 77, 191
VUVCD gives new insights for the membrane-induced conformations of $\alpha_1$-Acid Glycoprotein (AGP)

Sequence of secondary structures of AGP

Speculated AGP conformations at native and membrane-binding states
VUVCD evaluates the tertiary structure from a homology modeling

<table>
<thead>
<tr>
<th>State</th>
<th>α-Helix</th>
<th></th>
<th>β-Strand</th>
<th></th>
<th></th>
<th>Unordered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Content (%)</td>
<td>Number</td>
<td>Content (%)</td>
<td>Number</td>
<td>Turn (%)</td>
<td></td>
</tr>
<tr>
<td>Native</td>
<td>VUVCD</td>
<td>14.4</td>
<td>3</td>
<td>37.7</td>
<td>10</td>
<td>19.3</td>
</tr>
<tr>
<td>Modeler</td>
<td></td>
<td>11.5</td>
<td>3</td>
<td>36.6</td>
<td>10</td>
<td>23.5</td>
</tr>
</tbody>
</table>

*Biochemistry (2009) 48, 9103*
Summary in the VUVCD of proteins

1. VUVCD analysis can estimate the contents, numbers of segments, and sequences of secondary structures of proteins.

2. VUVCD can be applied to the structural analysis of denatured proteins, disulfide deficient proteins, membrane-binding proteins, and amyloid fibrils.
Summary

1. The VUVCD spectra are very sensitive to the conformations of saccharides and proteins in aqueous solution.

2. The VUVCD spectroscopy coupled with MD and TDDFT methods could provide important information about the conformation, interaction, and hydration of saccharides.

3. The VUVCD spectroscopy combined with bioinformatics technique has a great advantage for the structure analysis of not only native proteins but also non-native proteins.

Further accumulations of VUVCD data and developments of VUVCD analysis should open a new field in the structural biology.