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Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Exploration of Biomolecularly Transparent IR Region for Structural Identification of Biomolecules Using VCD
（生体分子の構造同定を目指した生体分子非吸収スペクトル領域における VCD 研究）

Biomolecules such as amino acid, sugars, lipids, and nucleic acid make up the fundamental building block of biological system. Their stereostructure significantly influences their roles in biological processes. Thus, a comprehensive knowledge of their structural properties is the key to fully understand and regulate their functions. However, stereostructural elucidation of biomolecules is rather challenging because of the limitation of conventional analytical methods. In order to characterize the structure of biomolecules and their synthetic derivatives, one of the major bottlenecks is that they possess multiple chiral centers. Conventional NMR spectroscopy and mass spectrometry is not always reliable in representing stereostructural informations. As a result, multiple analytical methods (e.g., Mosher–Kusumi method, NMR DP4 analysis, electronic circular dichroism spectroscopy, vibrational circular dichroism (VCD) spectroscopy, Raman optical activity (ROA) spectroscopy, and chemical correlation) are applied to elucidate the stereochemistry of biomolecules with many chiral centers. Vibrational circular dichroism (VCD) spectroscopy has proven to be a precise and powerful tool for molecular stereostructural characterization. Interactions of two chromophores such as carbonyl groups yield a strong VCD couplet that reflects the molecular structures. The use of VCD couplets for biomacromolecular structural studies limited due to the low signal intensity and severe signal overlap caused by numerous carbonyl functional groups that originally exist in biomolecules. Stereochemical elucidation of molecules with multiple chiral centers is difficult. Even with VCD spectroscopy, excluding all but one diastereomeric structural candidate is challenging because the stereochemical inversion of one chiral center among many centers does not always result in noticeable differences in their VCD spectra. VCD spectroscopy has become one of the most used methods to assign the absolute configuration of biomolecules and their synthetic derivatives. The VCD couplet approach should also serve as a new analysis tool with less physicochemical perturbation. Due to the nature of biomacromolecules possessing many carbonyl groups, extraction of their structural information needs introduction of IR chromophores with less overlap with their intrinsic IR absorption. This study demonstrated that introduction of chromophores in the 2300–1900 cm^{-1} region yields strong VCD signals that reflects the molecular structures. Nitrile, isonitrile, alkyne, azido and C-Deutereium groups show characteristic IR absorption in the 2300-1900 cm^{-1} region, where biomolecules do not strongly absorb. We herein examined the usefulness of these functional groups as chromophores to observe a strong VCD couplet that can be readily interpreted using theoretical calculations. This work demonstrates that the introduction of a suitable VCD chromophore with absorption in the 2300–1900 cm^{-1} region can be used for extracting local stereochemical information. Here we deliberate the work in two parts, where part 1 describes on binapthyls with two chromophore groups and part 2 focuses specifically on sugar moiety with a single deuterated chromophore as a VCD probe in the biomolecularly transparent region.

In part 1, we focuses on a chiral binaphthyl scaffold as a chiral model system where two identical chromophores were installed, i.e., nitrile, isonitrile, alkyne and azide groups. Space interactions of two suitable chromophores managed to yield an exciton circular dichroism couplet, i.e., a pair of positive and negative Cotton effects. The sign and intensity of the couplet reflects on the absolute arrangement of the electric dipole transition moments (EDTMs) associated with the chromophores. This phenomenon has provided a basis for a structural analysis method using electronic circular dichroism (ECD) spectroscopy, named the exciton chirality method. The nature of their anharmonic VCD patterns is discussed by comparison with the VCD spectrum of a mono-chromophoric molecule and by anharmonic DFT calculations. An expected obstacle to this goal is that anharmonic vibrational contribution may be significant in the 2300–1900 cm^{-1} region and that its influence on the VCD spectra of chosen chromophores has not been studied. Here, by using harmonic and anharmonic DFT calculations, we discuss the usefulness of these chromophores as well as their anharmonic

behaviours. In the case of that nitrile and isonitrile groups, they generate moderately-strong but complex VCD signals due to anharmonic contributions. This work revealed that the interplays between two chromophores enhanced anharmonic VCD intensities. We also demonstrated a possibility that complex anharmonic VCD signals can be interpreted by means of anharmonic DFT calculations. On the other hand, through studies on diazido binaphthyl, we demonstrated that the azido group is more promising and advantageous for structural analysis of biomolecules due to its simple, strong VCD couplet whose spectral patterns are readily predicted by harmonic DFT calculations. In combination with other techniques (e.g., 2D IR, FRET and ESR), VCD spectroscopy using these chromophores should facilitate future structural studies of biomacromolecules in the solution state. While this study focuses on small model molecules, the insight obtained here can be transferred to study larger molecules.

In continuation to the study on VCD chromophores in the biomolecular transparent IR region, the necessity of introducing two azido groups to suitable positions limits its application to multi-functionalized middle-sized biomolecules. Stereochemical elucidation of multi-functionalized middle- sized molecules is difficult even with the use of several analysis methods. If one chromophore, not two, in the 2300–1900 cm^{-1} region is found sufficient to extract local stereochemical information and if the resultant VCD pattern is predictable by theoretical calculations, these should pave the way for developing new methods to elucidate the selected stereochemistry of the target molecules. To prove this concept, one needs to prepare a series of diastereomeric pairs with a suitable VCD chromophore and confirm that their VCD spectra in the 2300–1900 cm^{-1} region exhibit drastically different, ideally mirror-image patterns irrespective of the other chiral centers. To provide a proof of concept, in this work we studied a methoxy-d_3 group (–OCD_3) installed at the C-1 position of a series of mono- and disaccharides and their derivatives. Sugars are chosen as a model system because of their diverse stereochemistry and functional groups, but we hope to apply the concept proven here to other types of molecules in future studies. Herein, we show the feasibility of the concept to determine the absolute configuration of the chiral center, the C-1 stereochemistry of sugars in this case study, in the vicinity of an introduced chromophore by VCD spectroscopy in the 2300–1900 cm^{-1} region. Through studies on a series of epimeric pairs of monosaccharides and their derivatives, we found that the introduction of one –OCD_3 group to each C-1 position produced almost mirror-image VCD patterns in the 2300–1900 cm^{-1} region, depending on the C-1 stereochemistry and irrespective of the other molecular moieties. This work also shows that comparison of the observed VCD signals and the calculated ones enables the stereochemical assignment of a chiral center in the vicinity of the chromophore. To showcase the wide applicability of the concept of extraction of local stereochemical information using the –OCD_3 group, we studied the VCD spectra of the C-1 epimeric pairs of D-gentiobiose (β1,6-linked glucose dimer, D-Gen), 5-membered D-glucofuranoside (D-fGlc), and 2,3,4,6-*O*-tetrabenzoylated D-glucopyranoside (D-Bz4G). All the α epimers (1*S* series) showed a positive ν_s CD_3 signal and a negative ν_{as} CD_3 one, whereas the β (1*R*) counterparts showed the opposite pattern. Thus, their OCD_3 VCD signals well reflected the stereochemistry of interest even for a disaccharide with 10 chiral centers, a sugar with a different ring size, a sugar with long side chain and a multi-chromophoric larger molecule. These results highlighted the potential of the concept of the stereochemical assignment of the of the selected site for larger, complex molecular systems. Isotopically labelled molecules are often processed by enzymes in the same way as their non-labelled counterparts. This permits the biochemical utility of deuterium-containing chromophores for sample preparation and for tracking the stereochemical outcome of reactions. Such applications were demonstrated in a study on an epimeric pair of 6-O-lauroylated D-glucose (6LG). Deuterium-containing VCD chromophores should also be versatile when biochemical studies are involved. Although this work provided a proof of concept through studies on sugar molecules and the –OCD_3 group, further studies on this concept should lead to the development of a method useful for the structural elucidation of other types of complex molecules. This study provides a proof of concept that the use of a VCD chromophore in the 2300–1900 cm^{-1} region enables the analysis of selected stereochemistry of suitable molecular systems. Further studies on this concept should lead to the development of a method useful for the structural elucidation of other types of complex molecules.