Communication

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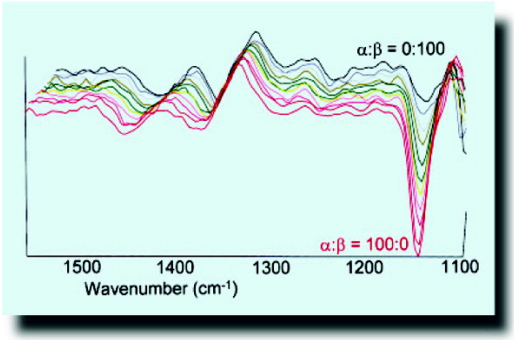

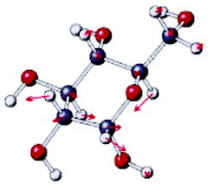

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Specific Band Observed in VCD Predicts the Anomeric Configuration of Carbohydrates

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The multiple roles of carbohydrates in the biological system have been found to include cell−cell interactions, pathogen−host recognitions, toxin−receptor interactions, and others.[1] The structural information of carbohydrates is not directly encoded in the DNA sequences but is determined by genes in order to generate glycosyl transferases that transfer sugar nucleotides to glycosyl acceptors in a step-by-step manner. In this reaction, the resulting stereochemical information, namely, glycosidic linkages α or β, gives the significant features of carbohydrates and the glycosidation position of the glycosyl acceptor. These structural features apparently relate to their functions, which, recently, have been extensively investigated. It has been found that the glycoside bond is fundamentally important in many aspects of chemistry and biology and forms the basis of carbohydrate chemistry.[2] In addition, stereochemical studies of glycoside bond solvolysis have been carried out in great detail because of the agricultural importance of food digestion.[3] For these reasons, much effort was made for the synthesis and analysis of the glycoside bond.

Conventionally, optical rotation was used to make the stereochemistry determination of the glycoside bond. Recently, routine analysis has been successfully carried out by ^{1}H NMR, but it was limited to up to oligosaccharides. To date, no other practical methods have been developed besides these. In continuous chiroptical analysis studies,[4] we applied the novel vibrational circular dichroism (VCD) technique to the carbohydrates. VCD measures the differential absorption of left versus right circularly polarized IR radiation by a molecular vibrational transition,[5] and the first VCD measurements were achieved in the early 1970s.[6] In a carbohydrate molecule, multiple chiral centers are assembled. Most carbohydrate analyses have been performed using achiral methods such as NMR or MS spectroscopic techniques. Only a few approaches, including electronic circular dichroism (ECD),[7] are known to target chiral properties. All carbohydrates show IR absorption, and thus the stereochemical information can be extracted from VCD without derivatization. Only a few VCD studies on carbohydrates have been reported until now.[7] We applied the novel chiroptical technique to carbohydrates and found a glycoside characteristic VCD band, named the "glycoside band." Here, we report our findings on this glycoside band and its application.

IR and VCD spectra were measured at 4 or 8 cm^{-1} resolutions with a Bomem/BioTools Chiral*ir* spectrometer. To avoid overlapping sample signals with strong water absorptions at ∼1650 cm^{-1}, all of the spectra were obtained in DMSO-d_6 with a BaF_2 cell of a 72 μm path length at a concentration of approximately 0.1 M. First, to construct an extensive carbohydrate database, VCD spectra of various monosaccharides and disaccharides were measured. The VCD spectra of naturally existing monosaccharides, which include amino sugars, showed distinct signs and frequency patterns, indicating the possibility of their difference.[9] Although some monosaccharide VCD spectra and their derivatives have been documented,[8] the present study is the first one providing an extensive VCD database of naturally occurring monosaccharides of higher organisms effective for simple carbohydrate identification.

In general, the VCD pattern of each monosaccharide was weakly and broadly shown in the mid-IR region (1600−1100 cm^{-1}), which was probably due to the effect of the intra- and/or intermolecular hydrogen bonds or the flexibility of their conformations. To neglect the equilibrium effect of the hemiacetal portion, a series of methyl and phenyl glycoside derivatives were prepared and compared with their VCD spectra. Figure 1 shows the VCD spectra of the α- and β-anomeric isomers of these compounds. The VCD spectra of the D- and L-glucose derivatives showed complete mirror images, as expected in both the α and β cases, respectively. Careful consideration of these carbohydrate VCD spectra led to the realization that only the monosaccharides having α-glycosidic linkages in the case of the D-series of sugars showed an intense, sharp, and negative band around 1145 cm^{-1}.

To clarify the characteristic band, 11 disaccharides composed of only D-glucose units were submitted to the VCD study (Figure 2). The disaccharide VCD spectra, whose glycosidic linkage was α, showed a strong, sharp, negative band around 1145 cm^{-1} [α,α-trehalose (α1 → α1, 1146 cm^{-1}, $g = \Delta A/A = -2.4 \times 10^{-4}$), kojibiose (α1 → 2, 1142 cm^{-1}, $g = -1.0 \times 10^{-4}$), nigerose (α1 → 3, 1146 cm^{-1}, $g = -1.3 \times 10^{-4}$), maltose (α1 → 4, 1146 cm^{-1}, $g = -1.7 \times 10^{-4}$), isomaltose (α1 → 6, 1149 cm^{-1}, $g = -1.3 \times 10^{-4}$)], while the β glycosidic disaccharides did not exhibit such characteristic bands [isotrehalose (β1 → β1), sophorose (β1 → 2), laminaribiose (β1 → 3), cellobiose (β1 → 4), gentiobiose (β1 → 6)]. Not surprisingly, α,α-trehalose, which has two α-glycosides, showed a very strong negative glycoside band.

To confirm the assignment of this band, a stable isotope experiment was conducted. Methyl 1-^{18}O-α-D-glucopyranoside was prepared by Fischer glycosidation using ^{18}O-methanol to compare each VCD spectrum. Both spectra of the labeled and nonlabeled compounds exhibited almost the same pattern and wavenumber, but the glycoside band of the labeled compound was apparently shifted to a lower wavenumber by 4 cm^{-1}. This result strongly supports the idea that an anomeric moiety is related to this vibration, causing the glycoside band. A preliminary computational approach was also performed, and two of the stable conformers showed VCD bands similar to that of the observed spectrum, supporting the experimental results.[9]

As an application of the glycoside band for quality control in agricultural fields as well as in glycoconjugate medicines, a VCD study of a maltose and cellobiose mixture was examined. Figure 3a shows the VCD spectra of the maltose−cellobiose mixture

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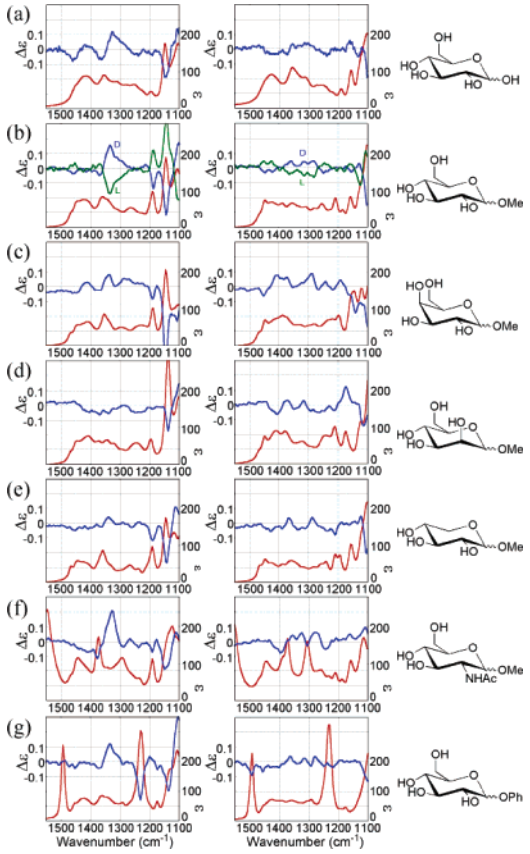

Figure 1. IR (red) and VCD (blue or green) spectra in DMSO-d_6 (c = 0.16 M, l = 72 μm) of (a) D-glucose, (b) methyl D- and L-glucopyranoside, (c) methyl D-galactopyranoside, (d) methyl D-mannopyranoside, (e) methyl D-xylopyranoside, (f) methyl 2-acetamide-2-deoxy-D-glucopyranoside, and (g) phenyl D-glucopyranoside. Left column for α-anomers and right column for β-anomers. Data collection time and resolution were 1 h and 4 cm^{-1}, respectively, for spectra in (a) and 3 h and 8 cm^{-1}, respectively, for others.

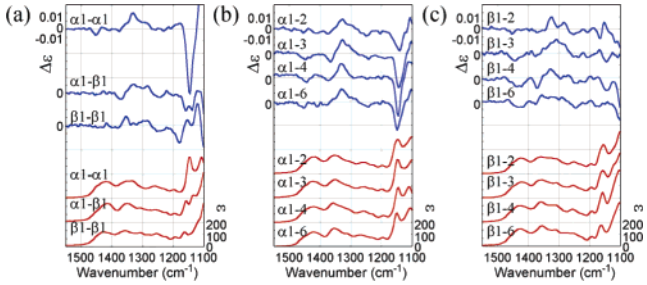

Figure 2. IR (red) and VCD (blue) spectra in DMSO-d_6 (c = 0.08 M, l = 72 μm) of (a) three nonreducing glucobioses, (b) four α-linked reducing glucobioses, and (c) four β-linked reducing glucobioses. Data collection time and resolution were 3 h and 8 cm^{-1}, respectively.

solution at various ratios. As expected, the peak at 1145 cm^{-1} became larger in proportion to the quantity of maltose (α-glycosidic linkage), indicating that the prediction reliabilities of the maltose−cellobiose ratios are based on the intensities of their glycoside bands. The same result was obtained in the case of the hexasaccharide (maltohexaose−cellohexaose). It demonstrated that this glycosidic band could be used in determining a dietary fiber ratio, which is extremely important for agricultural matter.

Versatility, an advantage of the glycoside band, was demonstrated by the monitoring of an enzymatic reaction. Figure 3b shows the VCD peak intensity at 1145 cm^{-1} of the glycoside band in a buffer solution with maltohexaose and amyloglucosidase. As amyloglucosidase hydrolyzes maltohexaose (α-glycosidic linkage) to a simple D-glucose, the VCD intensity at 1145 cm^{-1} gradually decreases,

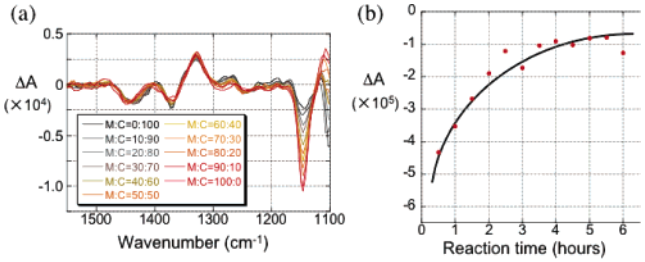

Figure 3. (a) VCD spectra of a maltose (M)−cellobiose (C) mixture at various ratios (DMSO-d_6). Data collection time and resolution were 1 h and 8 cm^{-1}. (b) VCD monitoring of maltohexaose degradation by amyloglucosidase in a sodium acetate buffer solution. The intensities of the VCD peaks at 1149 cm^{-1} (resolution 8 cm^{-1}) are plotted.

becoming almost flat after 5 h. The real-time monitoring technique for the enzymatic reaction by VCD could be a new practical tool for its kinetic study. It has the great advantage of not needing a special reagent or enzyme and can also check its specificity and product. To the best of our knowledge, this is the first example of an enzyme reaction being monitored by VCD.

In this study, we found the glycoside band and confirmed that this vibration was related to the α-configurations of D-carbohydrates by isotope substitution. The glycoside band is defined as a negative, sharp, and intense VCD band at around 1145 cm^{-1} and is exhibited by D-sugars with an axial α-glycosidic linkage in the $^4C_1$ conformation. The powerful features of the glycoside band give this approach some advantages over use of other spectroscopies such as NMR or optical rotation.

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Supporting Information Available: Experimental procedures, spectral data for a new compound, VCD spectra, and computation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) See Supporting Information for experimental details.

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