Vibrational circular dichroism (VCD) studies on disaccharides in the CH region: toward discrimination of the glycosidic linkage position

Tohru Taniguchi and Kenji Monde*

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The structural features of carbohydrates are a combination of 1) sequence and types of mono-sugars, 2) stereochemistry of their glycosidic linkages, and 3) their glycosidic linkage sites. We performed the first systematic VCD study on glycoside linking site discrimination. VCD spectra, in the CH stretching region from 2000 to 4000 cm^{-1}, of eleven glucobioses (trehalose (α1-α1), neotrehalose (α1-β1), isotrehalose (β1-β1), kojibiose (α1-2), nigerose (α1-3), maltose (α1-4), isomaltose (α1-6), sophorose (β1-2), laminaribiose (β1-3), cellobiose (β1-4), gentiobiose (β1-6)) suggested a possible new discrimination method for glyco analysis, while VCD spectra in the mid-IR region distinguished the stereochemistry (α or β) of the glycosidic linkage. Both reducing and nonreducing glucobioses showed different VCD spectral features compared to their constituent D-glucose and the anomer-fixed model compounds. Interresidue interaction such as hydrogen bonding was suggested to cause these spectral differences. Interplay between residues is a common phenomenon and thus VCD analysis could be applicable to other di-, oligo- or poly-saccharides. Several isotropic labeled compounds were also measured to support spectral assignment and interpretation.

Introduction

The diversity of carbohydrates comes from a combination of 1) the sequence of the monosaccharide residues, 2) the stereochemistry of their glycosidic linkages (α or β), and 3) their glycosidic linkage sites. The resultant complex structures relate to the biological roles of glycoproteins or glycolipids, through interactions with various receptor proteins on the cell surface and interior.[1] For these reasons, the development of a technique to characterize these complex carbohydrate structures is critical in the postgenomic era. However, the structural analysis of carbohydrates is complicated and needs to utilize several analytical methods in a complementary way. X-Ray crystallographic study requires suitable crystals for diffraction, and it is generally difficult for carbohydrates to form good crystals. On the other hand, NMR study is a widely used and powerful method,[2] however, for oligosaccharides, its measurement and interpretation could be time-consuming work. In recent decades, mass spectrometry analysis has successfully played an important role in glycobiology by means of tandem MS spectrometry (MS^n) and its fragmentation pattern.[3] Since MS analysis is based on an empirical method, stereochemical analyses of uncommon biological carbohydrates not in the library are still difficult. These methods are founded on achiral technologies which would not extract their chiral structural information, although carbohydrates are considered chiral cumulated devices. Recently, the advent of technical improvement of chiroptical techniques, such as vibrational circular dichroism (VCD),[4,5] vibrational Raman optical activity (VROA)[6] and vacuum-ultraviolet circular dichroism (VUV-CD),[7] opened a new field of carbohydrate analysis. An advantage for these chiral techniques over achiral ones is the availability of information on chiral properties, which are inherent in carbohydrates.

VCD measures the differential absorption of left *versus* right circularly polarized IR radiation associated with all the $3N$-6 fundamental molecular vibrational transitions, where N is the number of atoms in the molecule. Supported by the commercial availability of several Fourier Transform (FT)-VCD instruments with developed sensitivity, VCD is becoming one of the standard chiroptical techniques for absolute configuration determinations and conformation studies of all chiral molecules. For small molecules, these studies are reliably conducted with the aid of *ab initio* density functional theory (DFT) calculations.[8] On the other hand, for flexible medium-sized molecules and larger ones such as peptides, proteins, nucleic acids and even viruses, an empirical analysis based on comparison with VCD spectra of similar compounds or a database has been effectively applied.[9] For carbohydrate structure elucidation, because of the difficulties in managing their flexible structures and solvation effects, theoretical calculation studies have only been launched recently.[10] Meanwhile, based on a carbohydrate VCD database, we recently reported the discrimination of 1) monosaccharide residues and the 2) stereochemistry of their glycosidic linkage.[5,11,12] To resolve the remaining problem of 3) their glycosidic link sites, VCD spectra of eleven glucobioses were systematically measured in the CH stretching region. Current VCD studies are mainly focused on the mid-IR region from 800 to 2000 cm^{-1} where commercial FT-VCD spectrometers have operated, although the earliest ones, using dispersive instruments, were on the hydrogen and deuterium stretching region from 2000 to 4000 cm^{-1}.[13] Very recently, instrumental progress has made the CH stretching region available by using an FT-VCD instrument.[14] So far, there are

Laboratory of Advanced Chemical Biology, Graduate School of Advanced Life Science, Frontier Research Center for Post-Genome Science and Technology, Hokkaido University, Kita-ku, Sapporo 001-0021, Japan. E-mail: kmonde@glyco.sci.hokudai.ac.jp; Fax: +81-11-706-9042; Tel: +81-11-706-9041

only a few studies on carbohydrate VCD in the CH-stretching region,[12,15,16] which were dedicated exclusively to monosaccharides, and CH stretch VCD studies on di- or oligosaccharides remain to be investigated. By focusing on the CH region, we report (i) our findings about the first discrimination of linking positions by VCD and (ii) deuteration studies to gain understanding of the origin of the differences in their VCD patterns.

Results and discussion

Choice of the experimental system

The eleven D-glucobioses studied are shown in Fig. 1. The *O*-glycosidic bond can connect the hydroxyl group at the anomeric carbon (C-1) of a residue to any hydroxyl group of another. A simple monosaccharide, such as D-glucose, has five hydroxyls at the 1, 2, 3, 4 and 6 positions, which can give rise to a branched oligosaccharide. Hence, the analysis of glycosidic linkage is the most significant matter in carbohydrate structural studies and is not as straightforward as that of linear proteins and nucleic acids. If a residue links at the anomeric carbon, it is classified as a nonreducing residue and its anomeric configuration is fixed to either α or β. On the other hand, if it links at the other hydroxyl, it is described as a reducing residue and its anomeric configuration is an equilibrium mixture of α and β. Consequently, the eleven D-glucobioses shown in Fig. 1 cover all the possible *O*-glycosidic linking patterns of disaccharides. The selected set of glucobioses are a good starting point for the linkage position study, since all of them are made up solely from two D-glucose residues, which enables us to investigate the influence of the glycoside linkage on the spectra. Although previous VROA and VUV-CD studies reported the discrimination of a few disaccharides,[6a,7] this study in the CH stretching region and our recent one in the mid-IR region[5] are the first systematic chiroptical spectroscopic studies on disaccharides.

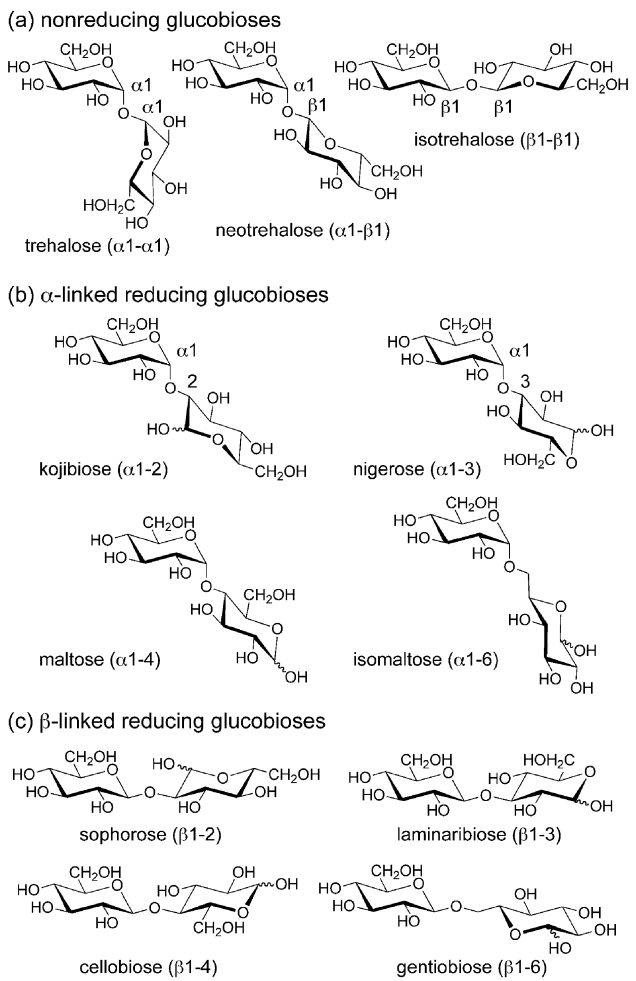

Fig. 1 The eleven D-glucobioses measured in this study.

VCD spectra of glucobioses

Figs. 2, 3 and 4 show the IR and VCD spectra of D-glucose and three nonreducing glucobioses, four α-linked reducing glucobioses, and four β-linked glucobioses, respectively, in the range of 3050–2750 cm^{-1}. In this region, there are only a few vibrational transitions mainly contributed to by stretch motions of each CH bond among the 3*N*-6 normal modes, in addition to overtones. This makes interpretation of VCD signals in this region easier with the assistance of deuterium substitutions. In fact, all three

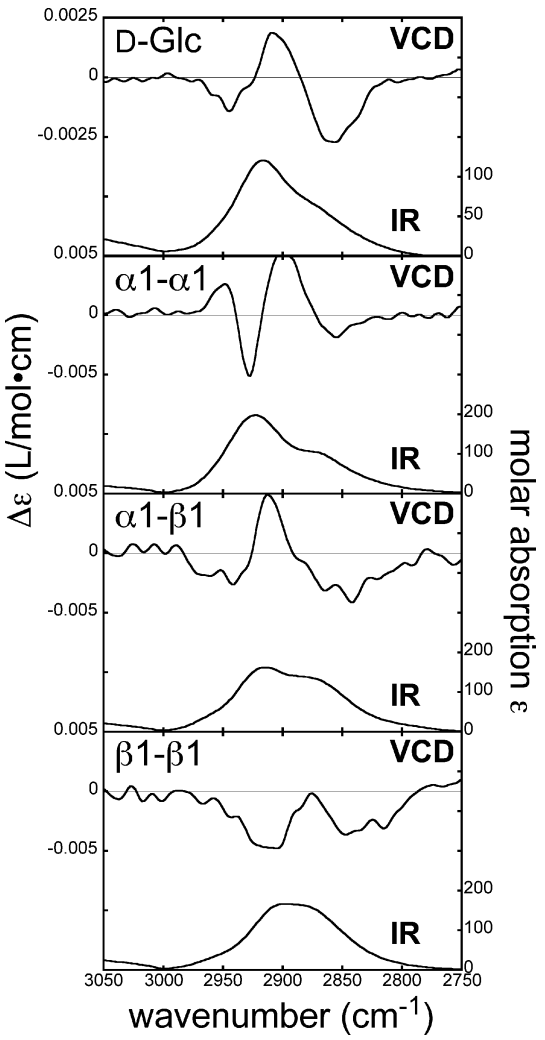

Fig. 2 IR and VCD spectra of D-glucose and three nonreducing D-glucobioses: α1-α1 (trehalose), α1-β1 (neotrehalose), β1-β1 (isotrehalose).

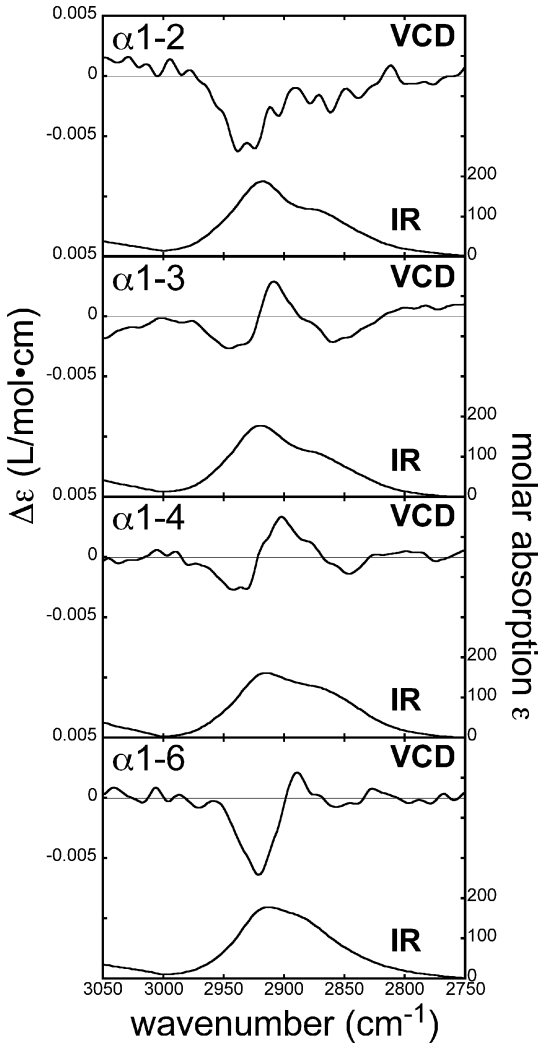

Fig. 3 IR and VCD spectra of four α-linked reducing D-glucobioses: α1-2 (kojibiose), α1-3 (nigerose), α1-4 (maltose), α1-6 (isomaltose).

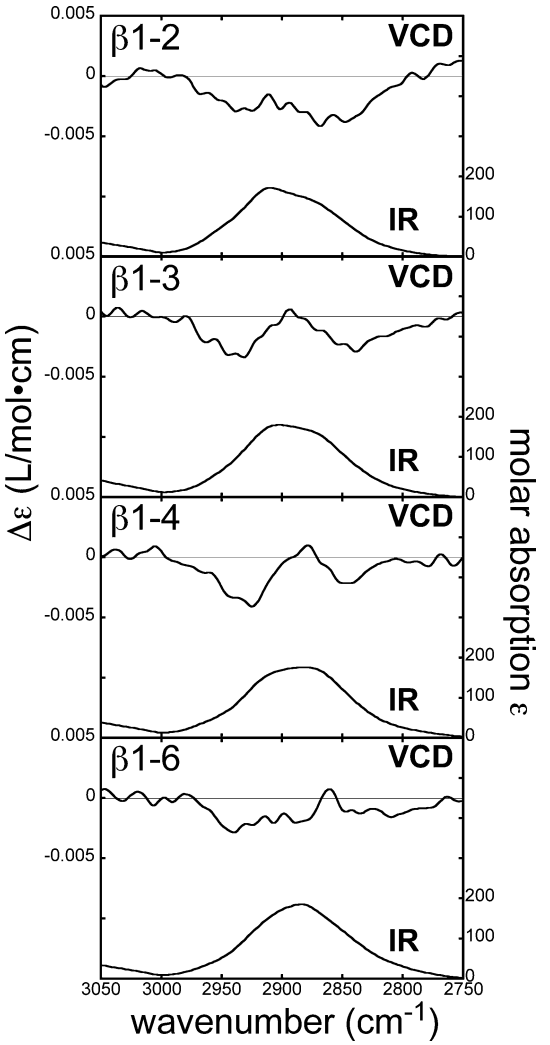

Fig. 4 IR and VCD spectra of four β-linked reducing D-glucobioses: β1-2 (sophorose), β1-3 (laminaribiose), β1-4 (cellobiose), β1-6 (gentiobiose).

reports on carbohydrate CH-stretching VCD so far successfully assigned the normal mode of characteristic signals mostly based on experimental evidence from deuteration.[12,15,16]

In this study, the IR and VCD spectra of anomer-fixed D-glucopyranosides and their C1-deuterated derivatives were compared at first to assign the anomeric methine stretching band (Fig. 5). A few IR and VCD characteristics in the C1-H monosaccharides were missing in the corresponding C1-D isotopomers, allowing their assignment. Namely, the positive VCD band at ~2890 cm^{-1} observed in methyl α-D-glucopyranoside and a part of the negative band ~2840 cm^{-1} in the β-anomeric isomer come from a C1-H stretching vibration in each anomeric configuration. These assignments are consistent with a previous conclusion based on D-glucose and 1-*d*-D-glucose except for a slight wavenumber shift.[16] The C(β)-H1 signal appeared at a lower frequency than C(α)-H, which is reasonable considering an electron donation from the lone pair of the ring oxygen to an antibonding σ* orbital of the C1-H bond.[17] This idea was confirmed by *ab initio* calculations.[18] While, recent density functional theory calculations indicated that the C1-H distance in the β-anomer is longer than that in the α-anomer, this again agrees with the current assignment.[19] Due to Fermi resonance and anharmonic effects in the CH region, the theoretical assignment of the whole spectra must await further studies.

As one would expect, the IR and VCD spectra of glucobioses in this region show much simpler features than those in the mid-IR region.[5,20] The VCD spectra in Figs. 2–4 share a similar feature represented by two valleys (roughly centered around 2940 and 2840 cm^{-1}) and an upward local maximal peak between them (around 2900 cm^{-1}) but all differed in their wavelengths, intensities and shapes (Table 1). As assigned for the monosaccharides (*vide supra*), the central strong positive VCD feature around 2900 cm^{-1} is dominated by a C1-H stretching vibration in the α-configuration. This positive band was observed in D-glucose, trehalose (α1-α1), neotrehalose (α1-β1), nigerose (α1-3), maltose (α1-4) and isomaltose (α1-6). In particular, this band was strong in trehalose, which has two α-anomeric centers. On the other hand, isotrehalose (β1-β1) showed no positive-signed peak due to lack of the α-anomeric configuration. Instead, a C(β)1-H negative broad feature around 2840 cm^{-1} is more obvious in isotrehalose (β1-β1) as well as in β-linked neotrehalose (α1-β1), sophorose (β1-2), laminaribiose (β1-3) and cellobiose (β1-4). It is noteworthy

Table 1 Observed frequencies and intensities in the CH stretching IR and VCD spectra of D-glucose, D-glucobioses and methyl-d_3 α- and β-D-glucobioses. For VCD, the local minima in two valleys and the local maximum between them are shown. In some D-glucobioses, these peak tops are less clear due to their broadness or weakness, so the extrema around there are written in parentheses

	IR		VCD				IR		VCD		
	λ/cm^{-1} ($\varepsilon \times 10^{-2}$)		λ_{min}, λ_{max}, λ_{min}/cm^{-1} ($\Delta\varepsilon \times 10^3$)				λ/cm^{-1} ($\varepsilon \times 10^{-2}$)		λ_{min}, λ_{max}, λ_{min}/cm^{-1} ($\Delta\varepsilon \times 10^3$)		
D-Glc	2916	2875 (sh)	2945	2908	2856	α1-β1	2914	2875 (sh)	(2943)	2912	(2843)
	(1.2)	(0.7)	(−1.4)	(+1.9)	(−2.7)		(1.6)	(1.4)	(−2.6)	(+4.9)	(−4.1)
α1-α1	2924	2871 (sh)	[a]2927	2902	2856	β1-β1	2899	2881 (sh)	2904	2875	2847
	(2.0)	(1.1)	(−5.1)	(+5.3)	(−1.9)		(1.7)	(1.6)	(−4.7)	(−0.1)	(−3.6)
α1-2	2918	2875 (sh)	(2939)	(2891)	(2862)	β1-2	2910	2879 (sh)	(2939)	(2912)	(2870)
	(1.9)	(1.2)	(−6.2)	(−1.0)	(−3.0)		(1.7)	(1.5)	(−2.9)	(−1.5)	(−4.1)
α1-3	2920	2875 (sh)	2947	2908	2860	β1-3	2902	2877 (sh)	(2931)	2895	(2839)
	(1.8)	(1.1)	(−2.6)	(+2.9)	(−2.1)		(1.8)	(1.7)	(−3.4)	(+0.6)	(−2.9)
α1-4	2916	2875 (sh)	(2943)	2902	2846	β1-4	2908 (sh)	2881	2925	2879	2844
	(1.6)	(1.3)	(−2.7)	(+3.4)	(−1.4)		(1.6)	(1.8)	(−4.1)	(+1.0)	(−2.1)
α1-6	2914	2889 (sh)	2922	2889	2858[b]	β1-6	2902 (sh)	2885	(2941)	2862	(2810)
	(1.8)	(1.6)	(−6.4)	(+2.1)	(−0.8)		(1.7)	(1.9)	(−2.8)	(+0.7)	(−1.6)
α-D-CD_3	2911	2898 (sh)	2915	2885	2851	β-D-CD_3	2901	2875	(2941)	2897	(2838)
	(1.1)	(1.0)	(−3.4)	(+3.6)	(−1.1)		(0.8)	(0.8)	(−1.5)	(−0.6)	(−2.2)

[a] An additional positive VCD peak at 2949 (+2.6). [b] An additional positive peak at 2827 (+0.8).

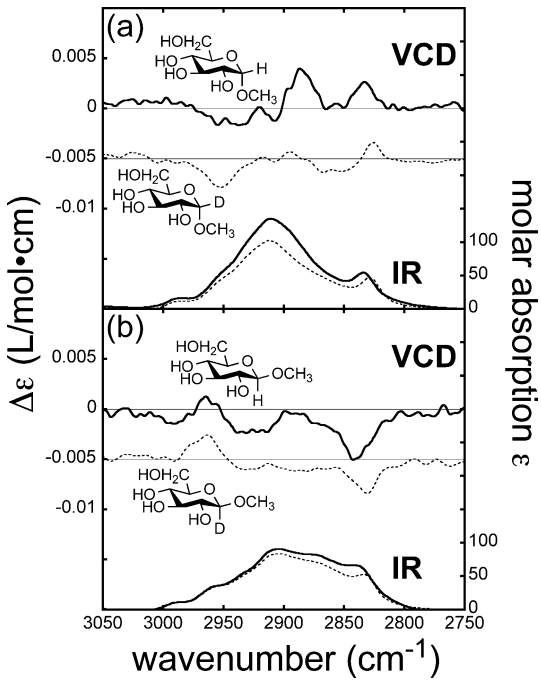

Fig. 5 IR and VCD spectra of (a) methyl α-D-glucopyranoside (solid line) and methyl 1-*d*-α-D-glucopyranoside (dotted line) and (b) methyl β-D-glucopyranoside (solid line) and methyl 1-*d*-β-D-glucopyranoside (dotted line).

that neotrehalose which possesses only one α-anomeric center exhibited a stronger C(α)1-H positive band than the other α-linked reducing glucobioses, considering that they should have more α-anomeric contribution from their reducing residue. Kojibiose (α1-2) lacks a C(α)1-H band probably due to suppression by a strong negative broad tendency by other vibrations. This negative tendency, which is observed also in sophorose (β1-2), makes the central local maximum less clear for both 1-2 linked disaccharides. For 1-6 linked isomaltose (α1-6) and gentiobiose (β1-6), the valley around 2840 cm^{-1} was shallower than the others. It is known that a Fermi resonance diad of D-glucose involving the symmetric CH_2 stretch and the overtone of the CH_2 scissors mode renders negative features around here as well as around 2940 cm^{-1}.[16] Therefore participation of C6 in a glycoside bond, through perturbation of CH_2 vibrational modes of a reducing residue, can dramatically change overall spectral features. The VCD spectra of α1-3 and β1-3 glucobioses seemed similar to those of α1-4 and β1-4 glucobioses, respectively (Figs. 3,4). However, for 1-4 linked glucobioses, a negative peak around 2940 cm^{-1} and a positive one around 2900 cm^{-1} are shifted to a lower frequency and the negative band around 2840 cm^{-1} is smaller, showing potential for their discrimination. Table 1 summarizes these spectral features indicating that all eleven linking patterns were distinguishable. This is the first finding of discrimination of linkage positions for disaccharides by VCD. In our recent disaccharide study in the mid-IR region, VCD spectra were able to distinguish the anomeric configuration but were less sensitive for the linking position.[5] In this way, the CH region and the mid-IR region provided complementary information about the carbohydrate structure. This speculation may promote carbohydrate VROA investigation into the CH stretching region.

Comparison of VCD spectra between nonreducing glucobioses and nonreducing residue models

As discussed in 1-6 linked sugars, the reducing residue in a disaccharide can exhibit a distinct spectral pattern from that of a corresponding free monosaccharide *via* a vibrational perturbation through the glycosidic covalent bond. But this explanation is not applicable for the three nonreducing glucobioses, which are devoid of a reducing unit but show thoroughly different spectra from that of D-glucose. To interpret this discrepancy, the VCD spectra of methyl-d_3 α- and β-D-glucopyranosides were compared with those of nonreducing disaccharides. They are appropriate models for nonreducing α- and β-D-glucose residues since their anomeric configurations are fixed but the methyl-d_3 group does

not have any VCD signal in this region. Their VCD spectra are shown in Fig. 6. Although the VCD spectrum of the methyl-d_3 β-D-glucopyranoside was almost flat, both VCD spectra showed two negative features and an upward one in the center, as did the other glucobioses (Table 1). Surprisingly, the nonreducing glucobiose spectra were completely different from either of the deuterated compounds. As an example, a positive peak at 2949 cm^{-1} was observed only in trehalose (α1-α1). It is possible that a C1-H stretching VCD was perturbed by a glycosidic linkage: however this 2949 cm^{-1} band would not be assigned to an anomeric methine stretch, since a C(α)1-H signal is suggested to appear around 2900 cm^{-1} as discussed earlier, and such a large shift from ~2900 to 2949 cm^{-1} is unlikely to happen in this system. Moreover, a C(α)1-H feature is also observable in this trehalose VCD spectrum with reasonable intensity at 2902 cm^{-1}, indicating the 2949 cm^{-1} band originates from other CH vibrations not related to the glycosidic linkage. The comparison between the VCD spectra of these nonreducing residue models and nonreducing glucobioses revealed that disaccharide spectra were not just additive of the two residues.

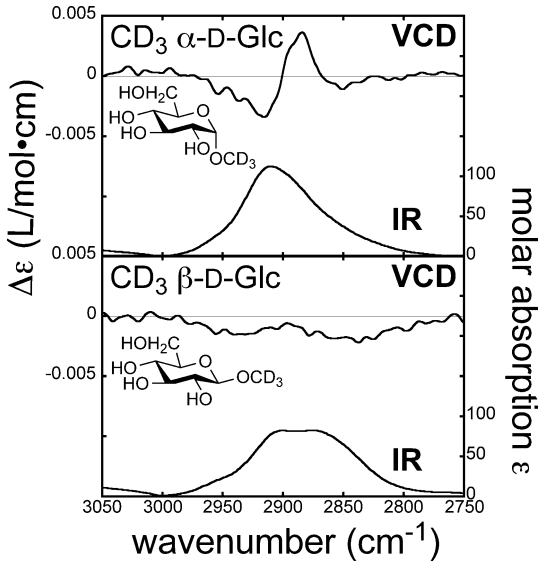

Fig. 6 IR and VCD spectra of methyl-d_3 α- and β-D-glucopyranosides.

VCD studies on the non-hydrogen bonding saccharides

The VCD data presented above indicate that there are other factors to induce spectral change than through covalent bond interresidue perturbation. X-Ray and NMR studies on glucobioses have suggested an intramolecular hydrogen bond between two glucose residues.[21] To examine the effect of hydrogen bonding, we prepared permethyl-d_3 α-D-glucopyranoside and permethyl-d_3 trehalose. These molecules would not form hydrogen bonding due to the capping of all hydroxyls by the methyl-d_3 groups, which again leave the hydrogen stretching signal observable. Both are composed of an α-D-glucoside unit as a monomer or a dimer, hence they would be reasonable models for a mono- and disaccharide with no hydrogen bond. Fig. 7 presents a direct comparison of their spectra and wavenumbers for each VCD peak. The central positive C(α)1-H stretching band is shifted to a higher wavenumber in per CD_3-capped D-trehalose, which would result from the aforementioned

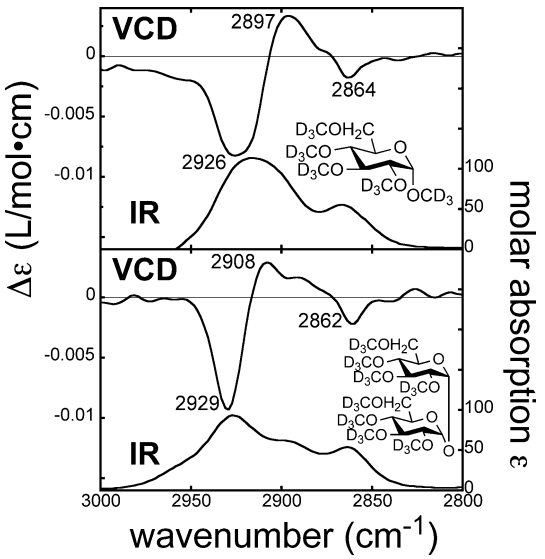

Fig. 7 IR and VCD spectra of permethyl-d_3 α-D-glucopyranoside and permethyl-d_3 D-trehalose. The wavenumbers of VCD extrema are indicated.

perturbation from the glycosidic covalent bond. The similarity of the relative VCD intensities and frequencies, except for the center absorption, indicated that CH stretching VCD of the disaccharides becomes similar in the absence of a hydrogen bond. In other words, hydrogen bonds affect VCD spectral features. VCD intensity is known to be proportional to an imaginary part of the scalar product of the electric and magnetic dipole transition moments.[22] Both intramolecular and through-solvent hydrogen bonds that may affect a local electronic environment will dramatically modify VCD signals attributed to CH bonds nearby. Moreover, it was proposed that the vibration of each CH bond can generate a ring current around the adjacent ring system containing the hydrogen bond, which would strongly change the VCD features.[23] By any means, these indications explain that, not only a reducing residue, but also a nonreducing one in an oligosaccharide system can show various VCD signals depending on the glycoside linking patterns therein. Therefore VCD spectra in the CH region could be sensitive to glycosidic linkage position as shown in this glucobiose study. Recently, interresidue hydrogen bonds are beginning to be simulated at the DFT level.[24] Further computer and theoretical development will assist carbohydrate VCD analysis in the future.

Conclusions

We performed the first systematic VCD study on glycoside linking site discrimination. Both reducing and nonreducing glucobioses showed different spectral features compared to their constituent D-glucose and the anomer-fixed model compounds. From the per CD_3-protected sugar study, it was indicated that interaction between two components such as intramolecular hydrogen bonding causes these spectral changes. Interresidue interaction is a common phenomenon in glycoscience, hence spectral differences will also be observed in di-, oligo- and poly-saccharides containing other sugar species which are often difficult to analyze. This is the first discrimination of the linkage position of carbohydrates using VCD. With recent insights into discrimination of monosaccharide

residues and anomeric configurations, this study will encourage further carbohydrate chiroptical studies to establish a new field of carbohydrate structural analysis. In cooperation with achiral tools such as NMR and MS, chiral spectroscopic analyses will allow the rapid progress of glycobiology.

Experimental

IR spectra were collected for 20–30 scans on an FT/IR-470 spectrometer (JASCO, Co. Japan). VCD spectra were measured for 20 000 scans on a JV-2001 spectrometer (JASCO, Co. Japan), equipped with an InSb detector. An optical filter to cut off incident light lower than 2650 cm^{-1} was situated directly before the polarizer to increase sensitivity in the region of interest. DMSO-d_6 was selected as the solvent for its strong solubility of the all unprotected glucobioses including the β-linked series, and non-absorption in the CH stretching region. Permethyl-d_3 sugars were measured in $CHCl_3$ or CCl_4, which does not have absorption in this region. The sample solutions were prepared at a concentration of 0.64 M for monosaccharides and 0.32 M for glucobioses, except for permethyl-d_3 α-D-glucopyranoside at 0.75 M. All spectra were recorded in a CaF_2 cell with a 50 μm pathlength at 4 or 8 cm^{-1} resolution under ambient temperature. The raw IR and VCD spectra were corrected by a solvent spectrum obtained under the same experimental conditions and presented in molar absorptivity ε (L mol^{-1} cm^{-1}). Except for minor noise, these results were reproducible. Maltose and cellobiose were also measured in 0.16 M and 0.64 M, showing virtually the same VCD as 0.32 M (data not shown).

D-Glucose and the eleven D-glucobioses were purchased from Wako Pure Chemicals (D-glucose, kojibiose, nigerose and maltose), Aldrich (trehalose), Sigma (neotrehalose, isotrehalose, sophorose, laminaribiose and isomaltose), Seikagaku Corporation (cellobiose) and Kanto Kagaku (gentiobiose), and used without further purification. The preparation of methyl-d_3 glucopyranosides were reported previously.[12] Starting from 1-*d*-glucose (Cambridge Isotope Laboratories, Inc.), methyl 1-*d*-glucopyranosides were prepared in a similar procedure. Permethyl-d_3 α-D-glucopyranoside was prepared from methyl-d_3 α-D-glucopyranoside and iodomethane-d_3 (C/D/N Isotopes Inc.) in the presence of potassium *tert*-butoxide. Permethyl-d_3 D-trehalose was prepared from D-trehalose and iodomethane-d_3 in the presence of sodium hydride.†

† **Permethyl-d_3 α-D-glucopyranoside.** ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.81 (1H, d, J = 3.6 Hz, H-1), 3.61–3.54 (3H, m, H-5, H-6a, H-6b), 3.49 (1H, dd, J = 9.3, 9.3 Hz, H-3), 3.20 (1H, dd, J = 3.6, 9.6 Hz, H-2), 3.18 (1H, dd, J = 9.3, 9.3 Hz, H-4). ^{13}C NMR (150 MHz, $CDCl_3$): δ 97.6 (C-1), 83.4 (C-3), 81.7 (C-2), 79.4 (C-4), 71.0 (C-6), 69.9 (C-5). HRMS (EI) m/z for $C_{11}H_7D_{15}O_6$ (M^+), calcd 265.2358, found 265.2355. $[\alpha]_D$ +107° ($CHCl_3$, c 1.0). **Permethyl-d_3 D-trehalose.** ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.17 (1H, d, J = 3.6 Hz, H-1), 3.98 (1H, ddd, J = 2.3, 2.9, 10.1 Hz, H-5), 3.60 (1H, dd, J = 3.5, 10.6 Hz, H-6a), 3.52 (1H, dd, J = 2.0, 10.6 Hz, H-6b), 3.48 (1H, dd, J = 9.3, 9.3 Hz, H-3), 3.22 (1H, dd, J = 9.5, 9.7 Hz, H-4), 3.18 (1H, dd, J = 3.6, 9.6 Hz, H-2). ^{13}C NMR (150 MHz, $CDCl_3$): δ 93.7 (C-1), 82.8 (C-3), 81.4 (C-2), 79.2 (C-4), 70.9 (C-6), 70.4 (C-5). HRMS (FAB) m/z for $C_{20}H_{15}D_{24}O_{11}$ ([M + H]$^+$), calcd 479.3999, found 479.3995. $[\alpha]_D$ +157° ($CHCl_3$, c 1.0).

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