Vibrational Circular Dichroism Is a Sensitive Probe of the Glycosidic Linkage: Oligosaccharides of Glucose

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Vibrational circular dichroism (VCD) measures[1] the differential absorption of left versus right circularly polarized infrared light originating from vibrational transitions of chiral molecules. This differential absorption is typically 5 orders of magnitude smaller than the vibrational absorption. Despite this weak nature of the signal, all $(3N - 6)$ vibrational modes of a chiral molecule (N being the number of atoms in the molecule) can give rise to circular dichroism, so that VCD can lead to a much more detailed stereochemical information on the molecule of interest. This may be compared to electronic circular dichroism (ECD),[2] where only a limited number of electronic transitions are usually accessible. This becomes all the more important for unsubstituted carbohydrates, which do not have electronic transitions in the visible range, and the ultraviolet region is more difficult to access.

Vibrational Raman optical activity (VROA),[3] which is the Raman counterpart of VCD, has been shown[4] to be a useful tool for studying carbohydrates. The complicated nature of vibrational modes and overlapping vibrational bands generally pose problems in the interpretation of vibrational optical activity spectra. Nevertheless, qualitative trends in the observed VROA spectra of carbohydrates provided useful applications.[4]

There were no prior VCD studies on disaccharides. The smaller VCD signals seen for carbohydrates, in general, compared to those seen for molecules with rigid structures, limited the prior VCD investigations to monosaccharides and that too in a limited number of studies.[5] Improvements in VCD instrumentation provided better quality VCD measurements with enhanced signal-to-noise ratio, providing an opportunity to investigate difficult samples. In this report we present, for the first time, the VCD spectra obtained for aqueous solutions of selected disaccharides in the ∼1200−900 cm^{-1} region, using state-of-the-art instrumentation, and demonstrate that VCD is a sensitive probe of the glycosidic linkage.

The infrared and VCD spectra were recorded at 4 cm^{-1} resolution on a commercial Fourier transform VCD spectrometer, Chiralir (Bomem-BioTools, Canada), with a ZnSe beam splitter, BaF_2 polarizer, optical filter (transmitting below 2000 cm^{-1}) and a 2 × 2 mm HgCdTe detector. All samples were purchased from Sigma Chemical Co., and doubly distilled water was used for preparing the aqueous solutions. Due to the strong absorption of water at ∼1650 cm^{-1}, a shorter path length and higher sample concentrations were necessary. All spectra were recorded for aqueous solutions in a fixed path length (6 μm) cell with BaF_2 windows. For all of the spectra presented here, solvent absorption and VCD have been subtracted out; the absorption and VCD spectra were scaled to give a maximum absorbance of 1.0 in the region shown. The transmission properties of optical filter and BaF_2 substrates used in the instrument restrict the range of measurements to 2000−900 cm^{-1}. There are no fundamental vibrational absorption bands for the saccharides studied here in the 2000−1500 cm^{-1} region. At the concentrations and path length used, the absorbance in the 1500−1200 cm^{-1} region is not high enough for VCD measurements with adequate signal-to-noise ratio. For these reasons, the presented spectra are limited to the ∼1200−900 cm^{-1} region. The vibrations appearing in this region arise from exo- and endo-cyclic C−C and C−O stretches with some contribution from C−O−H bending vibrations. However, coupling between different C−C and C−O stretches makes it difficult to assign the bands to specific bonds.[6] Such a detailed assignment, however, is not necessary for the present analysis.

The absorption and VCD spectra of D-maltose, α-D-cyclodextrin, α,α-D-trehalose, D-glucose, D-gentiobiose, and D-cellobiose are shown in Figure 1. The VCD spectrum of maltose (Figure 1a) shows VCD features in the 1200−900 cm^{-1} region, with a negative VCD ($\Delta A/A = -1.8 \times 10^{-4}$) at 1148 cm^{-1}, positive VCD ($\Delta A/A = +1.2 \times 10^{-4}$) at 1101 cm^{-1}, weak positive VCD ($\Delta A/A = +0.5 \times 10^{-4}$) at 1076 cm^{-1}, and negative VCD ($\Delta A/A = -0.6 \times 10^{-4}$) at 1053 cm^{-1}. A weak positive VCD ($\Delta A/A = +0.6 \times 10^{-4}$) at 1011 cm^{-1} is also seen that is associated with a shoulder to the 1039 cm^{-1} absorption band. The VCD spectrum of maltotriose (not shown) is very similar to that of maltose. For isomaltose (not shown) the VCD spectrum has a negative band ($\Delta A/A = -1.0 \times 10^{-4}$) at 1151 cm^{-1}, a broad positive band ($\Delta A/A = +0.9 \times 10^{-4}$) at 1080 cm^{-1} and a negative band ($\Delta A/A = -0.3 \times 10^{-4}$) at 1030 cm^{-1}. For α-cyclodextrin (Figure 1b), a negative VCD band ($\Delta A/A = -2.5 \times 10^{-4}$) at ∼1149 cm^{-1}, positive VCD band ($\Delta A/A = +2.4 \times 10^{-4}$) at ∼1080 cm^{-1}, positive VCD band ($\Delta A/A = +2.8 \times 10^{-4}$) at ∼1060 cm^{-1} and a negative VCD band ($\Delta A/A = -2.6 \times 10^{-4}$) at ∼1027 cm^{-1} are seen. The negative VCD at ∼1027 cm^{-1} originates from an absorption band that is overlapped by stronger absorption band at ∼1036 cm^{-1}. The VCD spectrum of α,α-D-trehalose (Figure 1c) shows VCD signals with negative VCD ($\Delta A/A = -2.9 \times 10^{-4}$) at 1148 cm^{-1}, positive VCD ($\Delta A/A = +2.6 \times 10^{-4}$) at 1105 cm^{-1}, negative VCD ($\Delta A/A = -2.6 \times 10^{-4}$) at 1057 cm^{-1}, and positive VCD ($\Delta A/A = +1.1 \times 10^{-4}$) at 995 cm^{-1}. In the case of glucose (Figure 1d) some weak VCD signals are present

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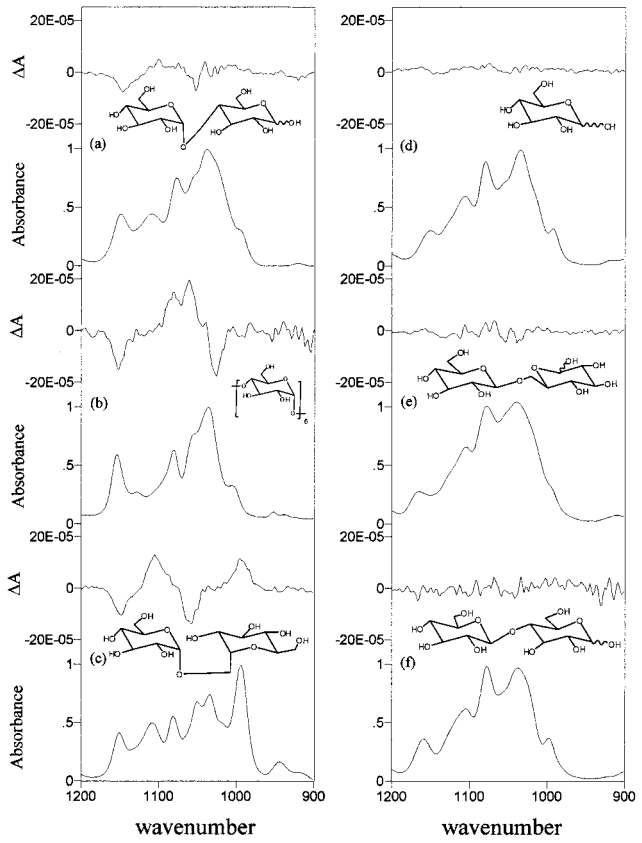

Figure 1. Vibrational absorption (bottom) and VCD (top) spectra in aqueous solution of: (a) maltose (*c* 1.8 M); (b) α-cyclodextrin (c 0.14 M); (c) α,α-trehalose (*c* 1.9 M); (d) D-glucose (*c* 3.8 M); (e) gentiobiose (*c* 2.7 M); (f) cellobiose (*c* 0.5 M). The weak positive VCD band seen in maltose at 1042 cm^{-1} is due to noise. The absorption and VCD spectra were scaled to give maximum absorbance of 1.0 in the region shown. The actual peak absorbances used in the measurements were: 0.88 for maltose; 0.35 for α-cyclodextrin; 0.76 for α,α-trehalose; 0.79 for glucose; 1.04 for gentiobiose; 0.28 for cellobiose. Water solvent absorption and VCD were subtracted out. The data collection time was 3 h for glucose and 1 h for other samples.

in the 1200−900 cm^{-1} region, but they are significantly smaller than those seen for maltose, maltotriose, isomaltose, α-cyclodextrin, and α,α-trehalose and are not noticeable on the VCD scale used in Figure 1. For gentiobiose (Figure 1e) and cellobiose (Figure 1f) no VCD signals are seen in the 1200−900 cm^{-1} region.

All of the oligosaccharides considered here are made up of two or more D-glucose units, connected by *O*-glycosidic linkages. In maltose two glucose units are connected[7] through an α(1→4) linkage, so one glucose unit is locked in the α-anomeric orientation, while the second one is present as a equilibrium mixture of α- and *β*-anomers. In maltotriose three glucose units are connected[7] through two α(1→4) linkages, so that two glucose units are locked in α-anomeric orientation, and one is present as a equilibrium mixture of α- and *β*-anomers. In isomaltose two glucose units are connected[7] through an α(1→6) linkage, so that one glucose unit is locked in α-anomeric orientation, while the second one is present as a equilibrium mixture of α- and *β*-anomers. α-cyclodextrin is a cyclic oligomer made up of six D-glucose units connected[7] via an α(1→4) linkage, so that all glucose units are locked in an α-anomeric orientation. In α,α-trehalose, since the two D-glucose units are connected[7] through an α(1→1) linkage, both glucose units are locked in the α-anomeric orientations. In cellobiose the two D-glucose units are connected[7] via a *β*(1→4) linkage, so that one glucose unit is locked in the *β*-anomeric orientation, while the second one is present as an equilibrium mixture of α- and *β*-anomers. In gentiobiose also[7] one glucose unit is locked in the *β*-anomeric orientation, and the second one is present as a equilibrium mixture of α- and *β*-anomers, but the glucose units are connected via a *β*(1→6) linkage.

Since D-glucose itself does not exhibit significant VCD in the 1200−900 cm^{-1} region, the strong VCD features seen for α-linked disaccharides must be arising from a favorable (for VCD) coupling between the two glucose units. The VCD magnitudes are larger in the order α,α-trehalose ≈ α-cyclodextrin > maltose ≈ maltotriose ≈ isomaltose, which is expected to be the order of the coupling between the glucose units, that results in larger VCD. In maltose, maltotriose, and isomaltose, where one of the glucose units is present as equilibrium mixture of α- and *β*-anomers, this coupling influence and hence VCD decreases. The fact that *β*-anomer orientation of glucopyranose is not favorable for VCD was also observed in D-glucose; in DMSO-d_6 solutions, VCD intensities in the 1500−1200 cm^{-1} region for *β*-D-glucose were found to be significantly smaller[5e] than those for α-D-glucose. As a result, the equilibrium composition[7] (∼64% *β*-anomer and ∼36% α-anomer) of glucopyranose results in lower VCD magnitudes. The *β*-linkage also results in unfavorable coupling between the glucose units since no significant VCD is seen for *β*-linked disaccharides cellobiose and gentiobiose.

These observations suggest that VCD in the 1200−900 cm^{-1} region serves as a sensitive probe to distinguish between α- and *β*- glycosidic linkages in oligosaccharides of glucose. Furthermore, VCD may be used to distinguish between α(1→1), α(1→4), and α(1→6) linkages, because they give different VCD sign patterns and intensities. However, it is not possible to differentiate between *β*(1→4) and *β*(1→6) linkages using VCD, as both linkages give no significant VCD. These observations are complimentary to VROA observations[4f] where *β*-linked species exhibit more changes than the α-linked species in the 1200−900 cm^{-1} region.

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