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Vacuum-ultraviolet circular dichroism study of saccharides by synchrotron radiation spectrophotometry

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Abstract—Vacuum-ultraviolet circular dichroism (VUVCD) spectra of five monosaccharides (D-glucose, D-mannose, D-galactose, D-xylose, and D-lyxose) and five disaccharides (maltose, isomaltose, cellobiose, gentiobiose, and lactose) were measured to 160 nm using a synchrotron-radiation VUVCD spectrophotometer in aqueous solution under high vacuum at 25 °C. Most of the saccharides show a positive peak with some shoulders at around 170 nm, except for D-galactose and lactose, which show two distinct negative peaks at around 165 and 177 nm. These spectra are influenced by such structural factors as α and β anomers at C-1, axial and equatorial hydroxyl groups at C-2 and C-4, trans (T) and gauche (G) conformations of the hydroxymethyl group at C-5, and the type of glycosidic linkage. Deconvolution of the VUVCD spectra of D-glucose, D-mannose, and D-galactose into six independent Gaussian components for α-GG, α-GT, α-TG, β-GG, β-GT, and β-TG conformations suggests that the α anomer has red-shifted spectra relative to the β anomer, and that GG and GT conformations have positive and negative circular dichroism signs, respectively, while the sign for TG conformation is anomer dependent. These speculations from the deconvolution analyses are also supported by the VUVCD spectra of disaccharides. These results give new insight into the equilibrium conformations of saccharides, demonstrating the usefulness of synchrotron-radiation VUVCD spectroscopy.

Keywords: Vacuum-ultraviolet circular dichroism; Saccharides; Synchrotron-radiation spectrophotometry

1. Introduction

Vacuum-ultraviolet circular dichroism (VUVCD) spectroscopy is a useful technique for the structural analysis of biomaterials since it provides detailed and new information on high-energy transitions of such chromophores as hydroxyl and acetal groups. This technique is especially advantageous for unsubstituted saccharides that show absorbance only below 190 nm. VUVCD data have been accumulated for many types of saccharides, including polysaccharides.[1–7] However, these spectra have not been explicitly assigned because saccharides can adopt many conformations in aqueous solution. Monosaccharides exist in a complex equilibrium involving α and β anomers at C-1, and staggered conformations of the hydroxymethyl group at C-5. Furthermore, the circular dichroism (CD) spectra of disaccharides are affected by the linkage between the constituent monosaccharides.

Nelson and Johnson measured the CD spectra for monosaccharides and methyl aldopyranosides to 165 nm in water and D_2O.[1–3] They found that the anomeric form has a decisive effect on CD, and contributions of axial and equatorial hydroxyl groups at C-2 and C-4 and of the hydroxymethyl group at C-5 have also been suggested. Arndt and Stevens extended the CD measurements of methyl monosaccharide glycosides to 140 nm, using film samples.[4] Deconvolution of the

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150–190 nm spectra into four components indicated that the signs of these components are correlated with the anomeric configuration, and that predominant contributions to CD arise from electronic transitions of the ring oxygen atom, as proposed by Listowsky and Englard.[8] However, the contribution of three staggered conformations of the hydroxymethyl group at C-5—gauche–gauche (GG), trans–gauche (TG), and gauche–trans (GT)—remains unclear. The VUVCD spectra of disaccharides in D_2O and as films have features qualitatively resembling those of the monomer components, but they are not necessarily additive combinations.[4,7] Thus, the VUVCD spectra of mono- and di-saccharides in solution cannot be interpreted simply in terms of their crystal structures. It is a matter of concern as to how the VUVCD spectra of such small saccharides can be explained based on the principle of pairwise interactions between the chromophore groups.

We have recently constructed a VUVCD spectrophotometer at the Hiroshima Synchrotron Radiation Center (HSRC) that is capable of measuring the CD spectra to 140 nm for aqueous solution by keeping all the optical devices under a high vacuum.[9–12] In the present study, we measured the VUVCD spectra of five monosaccharides (D-glucose, D-galactose, D-mannose, D-xylose, and D-lyxose) and five disaccharides (maltose, isomaltose, cellobiose, gentiobiose, and lactose) down to 160 nm in aqueous solution using this spectrophotometer. The chemical structures of these saccharides are shown in Figure 1. The VUVCD data obtained are discussed here in terms of anomeric (α and β) and staggered (GG, GT, and TG) equilibrium conformations, and the type of glycoside linkage, using deconvolution analysis. To our knowledge, this is the first systematic VUVCD study of saccharides in aqueous solution using a synchrotron-radiation spectrophotometer.

2. Experimental

2.1. Materials

D-Glucose, D-mannose, and maltose were purchased from Katayama Chemical; D-galactose, D-xylose, D-lyxose, and cellobiose were products from Nacalai Tesque; gentiobiose and isomaltose were purchased from Tokyo Kasei Kogyo; and lactose was supplied by Wako Pure Chemicals. All saccharides were of high purity (>98%) and were used without further purification. The sample solutions were freshly prepared by dissolving the saccharides in double-distilled water at concentrations of 3.0–10.0 (w/v%), followed by incubation for a day to attain anomeric equilibrium. The concentration of saccharides was determined by a dry-weight method (gravimetric technique).

2.2. VUVCD measurements

The VUVCD spectra of saccharides were measured in the wavelength range from 200 to 160 nm under high vacuum (10^{-4} Pa) at 25 °C, using the VUVCD spectrophotometer constructed at the HSRC. The CD signals were detected using a Hamamatsu R6836 photomultiplier tube. The a.c. signals were rectified, amplified by a dual lock-in amplifier (Jasco), and finally recorded on a personal computer. Details of the optical devices of the spectrophotometer are provided in previous papers.[9,10] The performance of the spectrophotometer was confirmed by monitoring the CD spectrum of an aqueous solution of ammonium *d*-camphor-10-sulfonate, which exhibits positive and negative peaks at 291 and 192 nm, respectively, with an intensity ratio of 1:2.[12] All spectra were recorded with a 1.0-mm slit, 16-s time constant, 4-nm/min scan speed, 0.067-nm data interval, and using 4–16 accumulations. A commercial spectrophotometer

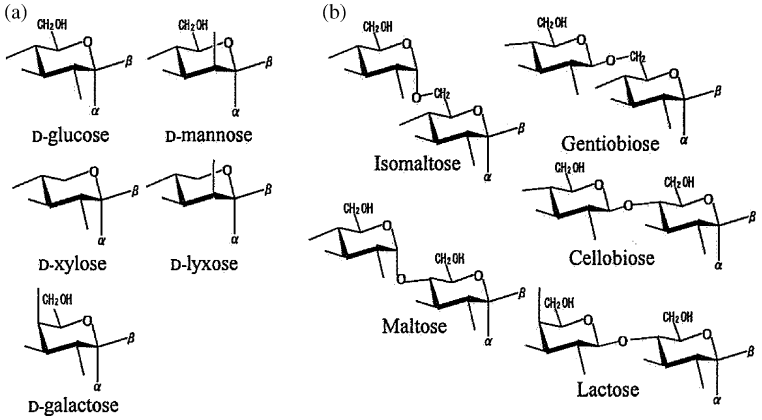

Figure 1. The chemical structures of the monosaccharides (a) and disaccharides (b) examined in this study.

(J-720W, Jasco) was also used for comparison of the CD spectra in the far UV region.

VUVCD measurements were carried out for two different concentrations of each saccharide using an assembled-type optical cell with MgF_2 windows that can tolerate a high vacuum (10^{-4} Pa) and which is attached to the temperature-control unit.[12] The path length of the cell could be altered by using various Teflon spacers. A 50-μm path length was used for the measurements from 200 to 180 nm. For the measurements below 180 nm, no spacer was used, to reduce the effect of light absorption by water. The spectra measured without a spacer were calibrated by normalizing the ellipticities with the spectra measured with a 50-μm spacer in the overlapped wavelength region of 200–180 nm. The reproducibility in path length of the no-spacer cell thus estimated was within ±0.05 μm for the identical MgF_2 disc, although the path length changed slightly (in most case by 1.3–2.0 μm), depending on the MgF_2 disc used. The molar ellipticity, [θ], was calculated using the molecular weight of monosaccharides and the averaged molecular weight per monomer unit for disaccharides. The spectra were reproducible within an error of ±5%, which was mainly attributable to noise in the signals and inaccuracies in the light-path length.

2.3. Deconvolution analysis of VUVCD spectra

The VUVCD spectra of monosaccharides were deconvoluted into contributions from the coexisting different conformations by nonlinear least-squares regression, assuming that each component had a Gaussian distribution.

3. Results

Figures 2 and 3 show the VUVCD spectra of the five monosaccharides and the five disaccharides investigated. None of these spectra exhibit any concentration dependence, suggesting no intermolecular interaction between the solutes in the concentration range examined (3–10 w/v%). These VUVCD spectra were superposed upon those measured by a commercial spectropolarimeter down to 190 nm, which indicated that the constructed VUVCD spectrophotometer and the optical cell performed well.

The VUVCD spectra of D-glucose and D-mannose show a positive peak, with some shoulders centered at 168 and 170 nm, respectively. Similar spectra with positive signs are observed for D-xylose and D-lyxose at around 167 and 172 nm, respectively. In contrast, D-galactose shows two negative CD peaks centered at 177 and 162 nm. The molar ellipticities, [θ], at the peak wavelengths for these monosaccharides are listed in Table 1. The spectra for D-glucose, D-xylose, and

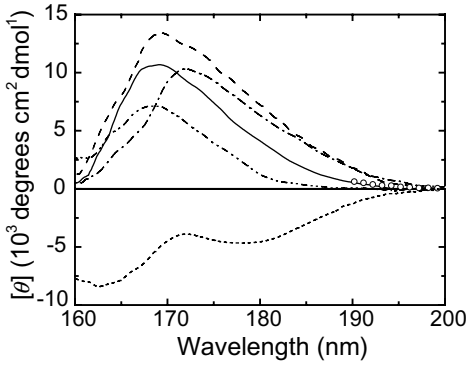

Figure 2. VUVCD spectra of D-glucose (—, concn 10%), D-mannose (– – –, concn 5%), D-galactose (- - -, concn 5%), D-xylose (– - – -, concn 10%), and D-lyxose (– - – -, concn 3%) at 25 °C in aqueous solution. A cell with a 50-μm path length was used for the measurements from 200 to 180 nm, and no spacer was used below 180 nm. All spectra were recorded with a 1.0-mm slit, 16-s time constant, 4-nm/min scan speed, and using 4–16 accumulations. Circles indicate the spectrum of the D-glucose solution obtained with a 50-μm spacer using a commercial spectropolarimeter (J-720W, Jasco).

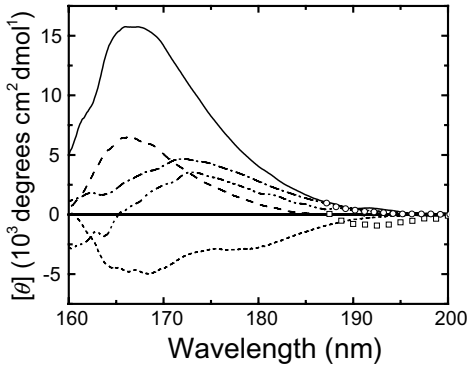

Figure 3. VUVCD spectra of maltose (– – –, concn 8%), isomaltose (—, concn 8%), cellobiose (– - – -, concn 8%), gentiobiose (– – –, concn 8%), and lactose (- - -, concn 3%) in aqueous solution at 25 °C. Squares indicate the 20-fold spectrum of maltose. All spectra were recorded under the same conditions as described for Figure 2. Circles indicate the spectrum of an isomaltose solution with obtained with a 50-μm spacer using a commercial spectropolarimeter (J-720W, Jasco).

Table 1. Peak wavelengths, λ, and molar ellipticities, [θ], of the VUVCD spectra of saccharides in water at 25 °C

Saccharides	λ (nm)	[θ] (deg cm^2 dmol^{-1})
D-Glucose	168	10,700
D-Mannose	170	13,500
D-Xylose	167	7100
D-Lyxose	172	10,300
D-Galactose	177	−4600
	162	−8400
Isomaltose	167	15,500
Maltose	166	6500
	192	−50
Gentiobiose	172	4600
Cellobiose	173	3500
Lactose	177	−3000
	168	−5000

D-galactose are consistent, within experimental error, with those reported by Nelson and Johnson.[1]

Maltose exhibits a small negative peak at 192 nm and a positive one at 166 nm. On the other hand, isomaltose has a large positive peak centered at 167 nm. Gentiobiose shows a positive peak at around 172 nm. Cellobiose shows a similar positive peak at around 173 nm, which turns negative below 165 nm. The [θ] values for these disaccharides are listed in Table 1. The [θ] values of maltose and cellobiose in water at 25 °C are considerably smaller than those in D_2O at 10 °C reported by Lewis and Johnson.[7] This discrepancy may be attributable to the different temperature and solvent conditions, since these affect the conformational equilibrium of the saccharides.[13] In contrast to other disaccharides, lactose—a conjugate of glucose and galactose—shows two successive negative CD peaks centered at 177 and 168 nm with [θ] equal to −3000 and −5000 deg cm^2 dmol^{-1}, respectively. These peak wavelengths are in good agreement with those in films observed by Arndt and Stevens,[4] although the CD intensity cannot be directly compared because their data were not normalized by the molecular weight.

4. Discussion

Figures 2 and 3 reveal that unsubstituted saccharides have characteristic VUVCD spectra below 190 nm that are dependent on their structures and conformations. This is the first time that CD spectra have been measured to 160 nm for various saccharides in aqueous solution using a synchrotron-radiation spectrophotometer. A theoretical assignment of the spectra is outside the scope of this study, but it can be speculated that the 160–180 nm spectra originate from the n–σ^* transition of lone-pair oxygen electrons of hydroxyl groups and acetal bonds, based on simple quantum mechanical considerations at the molecular orbital level, since valence electrons in the CC, CO, and CH groups give rise to σ–σ^* transitions of higher energy.[6] It is of interest to determine how these VUVCD spectra are related to the configuration and conformation of the saccharides.

4.1. Relationships between VUVCD spectra and the structure of monosaccharides

The optical properties of monosaccharides in aqueous solution are determined by many equilibria, such as the anomeric configuration (α and β) of the hydroxyl group at C-1 and the staggered (GG, GT, and TG) orientations of the hydroxymethyl group at C-5, in addition to the axial or equatorial configurations of the hydroxyl groups at other carbon atoms. In the present work, we selected five structurally correlative monosaccharides to compare their VUVCD spectra. The three possible

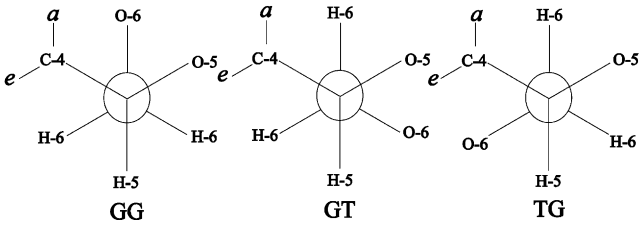

Figure 4. Three possible staggered conformations (GG, GT, and TG) of the hydroxymethyl group at C-5. The C-4 hydroxyl is axial (*a*) for D-galactose and equatorial (*e*) for D-glucose. This is a view looking down the bond from C-5 to C-6.

staggered conformations for the hydroxymethyl group are shown in Figure 4, and the average contents of each anomeric and staggered conformations taken from the literature are listed in Table 2.[14–17]

As shown in Figure 2, D-glucose has a slightly blue-shifted CD band with a smaller [θ] value as compared to D-mannose. D-Glucose and D-mannose differ only in the configuration of the hydroxyl group at C-2: equatorial in D-glucose and axial in D-mannose. However, D-glucose favors the β anomer (62%) over the α anomer (38%), whereas D-mannose favors the α anomer (65%), probably due to steric exclusion from the hydroxyl group at C-2. The sum of GG contents in the α and β anomers is 55% and that of the GT content is 45%, in both D-glucose and D-mannose (Table 2). Therefore, it can be speculated that the hydroxymethyl group contributes similarly to the CD of both monosaccharides, and that the difference in their CD spectra is mainly attributable to the different anomeric contents of the two saccharides. This is essentially consistent with the prediction by Nelson and Johnson.[2]

D-Galactose and D-glucose differ only in the configuration of hydroxyl group at C-4: equatorial in D-glucose and axial in D-galactose. As shown in Figure 2, however, the CD spectrum of D-galactose has a negative sign and hence is qualitatively different from that of D-glucose. The content of α anomer is only slightly different between D-galactose (32%) and D-glucose (38%), but the GG, GT, and TG contents are very different: the GG content decreases from 55% to 20% and the TG conformation (20%) is present in D-galactose. According to Lemieux and Brewer,[16] the equilibrium between the three staggered conformations (GG, GT, and TG) is influenced by the configuration of the hydroxyl group at C-4. The hydroxymethyl group of D-glucose favors GG and GT conformations over TG, because the TG conformation involves unfavorable periplanar interactions between the equatorial hydroxyl group at C-4 and the hydroxyl group at C-6 (see Fig. 4). On the other hand, the hydroxymethyl group of D-galactose favors the TG conformation, because the interaction between the hydroxyl groups at C-4 (axial) and C-6 are not unfavorable stereochemically (Fig. 4). Therefore, it can be speculated that the difference in CD spectra between D-glucose and

Table 2. Population (%) of anomeric (α and β and staggered (GG, GT, and TG) conformations of monosaccharides in aqueous solution[14–17]

Monosaccharides	α:β	GG:GT:TG	α-GG:α-GT:α-TG	β-GG:β-GT:β-TG
D-Glucose	38:62	55:45:0	20.90:17.10:0.00	34.10:27.90:0.00
D-Mannose	65:35	55:45:0	35.75:29.25:0.00	19.25:15.75:0.00
D-Galactose	32:68	20:60:20	6.40:19.20:6.40	13.60:40.80:13.60
D-Xylose	37:63			
D-Lyxose	71:29			

D-galactose is mainly due to a conformational change of the hydroxymethyl group at C-5 induced by the hydroxyl group at C-4. This is essentially consistent with the proposal by Listowsky and Englard[8] and Nelson and Johnson.[2]

The CD spectra of D-xylose and D-lyxose, which have no hydroxymethyl group at C-5, resemble those of the structurally correlative D-glucose and D-mannose. The smaller [θ] values for D-xylose and D-lyxose than for D-glucose and D-mannose could be attributed mainly to the absence of a hydroxymethyl group at C-5, because the configuration of the hydroxyl group at C-2 is the same in the respective corresponding monosaccharides (equatorial in D-xylose and D-glucose, and axial in D-lyxose and D-mannose), and because the α-anomer contents are similar in D-xylose (37%) and D-glucose (38%) and in D-lyxose (71%) and D-mannose (65%) (Table 1).

The difference in CD spectra between D-xylose and D-lyxose could be attributed mainly to the difference in anomer content of both saccharides, as already discussed in the comparison between D-glucose and D-mannose. It is noteworthy that the CD band of D-xylose begins at a shorter wavelength (by ~8 nm) than that of D-glucose, but no shift of the CD band is observed for D-lyxose relative to D-mannose. Nelson and Johnson[3] suggested that the addition of a hydroxymethyl group to C-5 in D-xylose shields the ring oxygen atom from the solvent (water), thereby inhibiting its hydrogen bonding. The distance between the ring oxygen and the C-6 hydroxy oxygen in GG and GT conformers is 2.7–2.9 Å,[18,19] which is similar to the distance (about 2.8 Å) between the ring oxygen and the axial hydroxyl oxygen at C-2 in D-lyxose.[20] Therefore, it is probable that the onset shift of the CD band will not occur for D-lyxose if its axial hydroxyl group at C-2 can shield the ring oxygen from the solvent as well as from the hydroxymethyl group at C-5.

4.2. Relationships between VUVCD spectra and the structure of disaccharides

Disaccharides have many conformations in aqueous solution, depending on the constituent monosaccharides, and the position and anomeric configuration of glycosidic linkages. The structures of the five disaccharides examined are shown in Figure 1. Maltose and isomaltose have α-(1→4)- and α-(1→6)-glycosidic linkages between two D-glucose molecules, respectively. Cellobiose and gentiobiose contain two D-glucose molecules joined by β-(1→4)- and β-(1→6)-glycosidic linkages, respectively. Lactose is a disaccharide of D-galactose and D-glucose joined by a β-(1→4)-glycoside linkage.

As shown in Figure 3, isomaltose and maltose exhibit similar CD spectra: the peak wavelength is similar, but the peak for isomaltose is higher than that for maltose. The CD spectra of gentibiose and cellobiose are also similar, except that gentiobiose shows a larger intensity than cellobiose. Therefore, compared to the (1→4)-glycosidic linkage, the (1→6)-glycosidic linkage increases CD intensity but affects the peak wavelength only slightly. The hydroxymethyl group at C-5 in the second glucose unit remains free in a (1→4)-glycosidic linkage, but it participates in the bonding of two glucose units in a (1→6)-glycosidic linkage. However, these changes in structure would not significantly affect the electronic states of an acetal bond C–O–C and hydroxyl groups, but instead affecting mainly the absorptivities of their circularly polarized components.

Evidently, the CD bands of gentiobiose and cellobiose are red shifted relative to those of isomaltose and maltose, respectively. This result suggests that the α-glycoside linkage is a higher energy bond than the β-glycoside linkage in both (1→4)- and (1→6)-bonds. X-ray analyses of maltose and cellobiose indicate that an intramolecular hydrogen bond between the constituent two glucose units is formed at O-2–H···O-3′ in maltose and at O-5···H–O-3′ in cellobiose.[21,22] Such hydrogen bonding may be partly responsible for the different CD properties of the two saccharides in solution, since the solvent accessibility of the ring oxygen (O-5) is an important factor, as already discussed for monosaccharides.

Lactose shows a very different CD spectrum from the other four disaccharides. This specificity may arise mainly from the constituent galactose unit, since D-galactose has negative CD bands (Fig. 2). The CD spectrum of lactose in solution resembles that in a film,[4] but the cross-over point shifts from 152 nm in the film to 160 nm in solution. A similar red shift in the CD band in solution is also observed for cellobiose and methyl saccharides glycosides.[4,7] These differences in solution and film CD spectra may be attributable to two factors affecting electronic energy of chromophores: hydration and conformational flexibility.

4.3. Deconvolution analysis of VUVCD spectra

As already shown, the VUVCD spectra of monosaccharides are mainly affected by their anomeric forms at C-1 and the conformations of the hydroxymethyl group at C-5. Therefore, to evaluate the contributions of α-GG, α-GT, α-TG, β-GG, β-GT, and β-TG conformers, the VUVCD spectra of D-glucose, D-mannose, and D-galactose were deconvoluted into six independent components by a nonlinear least-squares method, assuming a Gaussian distribution for each conformer as follows:

$$[\theta]_{\mathrm{m,obs}} = X_{\mathrm{m},\alpha\text{-GG}}[\theta]_{\mathrm{m},\alpha\text{-GG}} + X_{\mathrm{m},\alpha\text{-GT}}[\theta]_{\mathrm{m},\alpha\text{-GT}} + X_{\mathrm{m},\alpha\text{-TG}}[\theta]_{\mathrm{m},\alpha\text{-TG}} + X_{\mathrm{m},\beta\text{-GG}}[\theta]_{\mathrm{m},\beta\text{-GG}} + X_{\mathrm{m},\beta\text{-GT}}[\theta]_{\mathrm{m},\beta\text{-GT}} + X_{\mathrm{m},\beta\text{-TG}}[\theta]_{\mathrm{m},\beta\text{-TG}} \quad (1)$$

$$[\theta]_{\mathrm{m}} = [\theta_0] \times \exp\left(-\left(\lambda_0 - \lambda\right)^2 / w^2\right) \quad (2)$$

where $[\theta]_{\mathrm{m}}$ and X_{m} are the molar ellipticities and contents, respectively, of the conformers indicated by the subscripts (α-GG, α-GT, α-TG, β-GG, β-GT, and β-TG) in monosaccharide, m (D-glucose, D-mannose, and D-galactose); $[\theta]_{\mathrm{m,obs}}$ is the observed molar ellipticity of the monosaccharide; and $[\theta_0]$ is the molar ellipticity at the peak wavelength, λ_0 of each Gaussian CD component with a half bandwidth of w. These deconvolutions were commenced with initially obtained Gaussian components, $[\theta_0]$, λ_0 and w, for six conformers (α-GG, α-GT, α-TG, β-GG, β-GT, and β-TG), which were determined by nonlinear least-squares method using Eq. 1, assuming that each CD spectrum of six conformers is identical in D-glucose, D-mannose, and D-galactose. These calculations allow us to assign analytically α-GG, α-GT, β-GG, and β-GT spectra, but α-TG and β-TG conformers of D-galactose cannot be assigned because each Gaussian spectrum of the two conformers has two possibilities of α-TG and β-TG. Therefore, we assigned the spectra of α-TG and β-TG conformers by taking into consideration the spectrum of each conformer measured by Nelson and Johnson.[2]

The results of the deconvolution analysis are shown in Figure 5. The spectra of D-glucose and D-mannose are divided into four spectra and the spectrum of D-galactose into six, corresponding to the existing conformers (see Table 2). The CD spectra reconstituted from these component spectra are in good agreement with the experimentally observed ones, indicating the usefulness of the deconvolution analysis. Evidently, GG and GT conformers exhibit, respectively, positive and negative bands in any monosaccharide. Therefore, it can be concluded that the positive CD bands of D-glucose and D-mannose are predominantly caused by the GG conformation of the hydroxymethyl group at C-5. On the other hand, the CD band of the TG conformer in D-galactose is positive and negative for α and β anomers, respectively. This suggests that the negative peaks at around 180 and 165 nm in the spectrum of D-galactose arise mainly from β-TG and β-GT conformers, respectively. Another notable finding is that α anomers have lower energy CD bands than β anomers for a given GG or GT conformation.

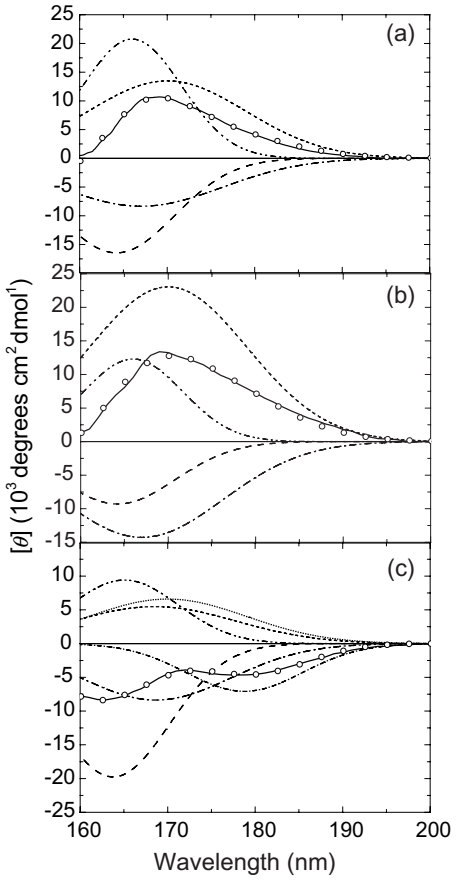

Figure 5. Deconvolution of the VUVCD spectra of D-glucose (a), D-mannose (b), and D-galactose (c) into the existing conformers α-GG (- - -), α-GT (– – –), β-GG (– - – -), β-GT (– – –), α-TG (· · ·), and β-TG (– · – · –). Solid lines show the observed spectra, and circles represent the spectra reconstituted from the spectrum for each conformer.

Figure 6 shows the spectra of α and β anomers estimated for D-glucose and D-mannose at GG:GT = 55:45 and D-galactose at GG:GT:TG = 20:60:20. The spectra of α and β anomers for D-glucose are very similar to those measured by Nelson and Johnson,[2] although their CD spectra were of slightly lower intensity than the present spectra, probably because the fast equilibrium inevitable between α and β anomers occurred in the solutions they used. The spectra for α and β anomers of D-glucose are nearly consistent with those of D-mannose, supporting the concept that the difference in the CD spectra between D-glucose and D-mannose is mainly due to the different anomer content.

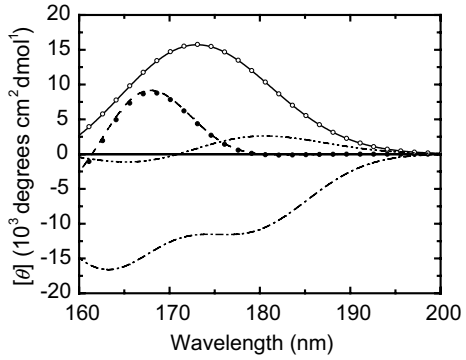

Figure 6. VUVCD spectra for α and β anomers of D-glucose (GG:GT = 55:45), D-mannose (GG:GT = 55:45), and D-galactose (GG:GT:TG = 20:60:20): α anomer (—) and β anomer (– – –) of D-glucose; α anomer (○ ○ ○) and β anomer (● ● ●) of D-mannose; and α anomer (– - – -) and β anomer (– – - –) of D-galactose.

The results of the deconvolution analysis for monosaccharides are not inconsistent with the CD spectra of disaccharides. As expected from the CD intensity of the α anomer being larger than that of β anomer (Fig. 6), maltose and isomaltose (consisting of an α-glycosidic linkage) exhibit larger CD intensities than cellobiose and gentiobiose (containing a β-glycosidic linkage), respectively (Fig. 3). The β-TG conformation exhibits a negative band at around 180 nm, as found for D-galactose (Fig. 5), indicating that the small negative band at around 190 nm exhibited by maltose (Fig. 3) may be attributed to the β-TG conformation. In fact, it is known from NMR investigations[23] and molecular dynamics simulations[24] that 60% of β anomer and a small proportion of TG conformation (6%) exist in maltose solution.

5. Concluding remarks

The present study constitutes the first successful measurement of the VUVCD spectra of mono- and di-saccharides down to 160 nm in aqueous solution using a synchrotron-radiation spectrophotometer. Although the detailed assignment of these spectra must await further theoretical analysis using molecular orbital theory and molecular dynamics, it is possible to evaluate the contributions of various equilibrium conformations of saccharides to VUVCD spectra using a comparative study and deconvolution analysis. The accumulation of VUVCD data should open a new field in the structural analysis of saccharides and other biomaterials, based on the higher-energy transitions of chromophores.

Acknowledgements

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