Immunochemical Studies on Blood Groups. XXXIX. Optical Rotatory Dispersion and Circular Dichroism Spectra of Oligosaccharides from Blood-Group Lewis[a] Substance*

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ABSTRACT: Optical rotatory dispersion and circular dichroism spectra of some oligosaccharides from blood-group Lewis[a] substance have supported structural studies (Lloyd, K. O., Kabat, E. A., and Licerio, E. (1968b), *Biochemistry 7*, 2976) and have provided information on the anomeric configuration of the sugars in these oligosaccharides. The spectra also provide additional support for the conclusions reached earlier (Beychok, S., and Kabat, E. A. (1965), *Biochemistry 4*, 2565; Lloyd, K. O., Beychok, S., and Kabat, E. A. (1967a), *Biochemistry 6*, 1448) on the effects of substitution on optical activity of 2-acetamido sugars: (i) compounds with unsubstituted β-D-GNAc residues have relatively small Cotton effect troughs and ellipticity band, (ii) their magnitude increases with substitution on the 2-acetamido sugar by D-galactose, and (iii) the largest Cotton effect troughs and circular dichroism bands are given by oligosaccharides on which β-D-GNAc is disubstituted, *i.e.*, on C-3 and C-4 by D-Gal and L-Fuc.

One of the Le[a] oligosaccharides contains a 2-acetamido-2-deoxy-D-galactitol residue and the optical rotatory dispersion and circular dichroism spectra of 2-acetamido-2-deoxy derivatives of D-galactitol and D-glucitol have been determined to estimate the contribution of this residue.

N-Acetyl-D-glucosamine and *N*-acetyl-D-galactosamine and their glycosides show characteristic Cotton effect troughs at about 220 mμ in their optical rotatory dispersion spectra (Beychok and Kabat, 1965) and ellipticity bands at about 210 mμ in their circular dichroism spectra (Lloyd *et al.*, 1967a,b; S. Beychok and E. A. Kabat, paper in preparation) due to an n–π* transition of the 2-acetamido chromophore. Recently another stronger and positive Cotton effect peak near 200 mμ has been found (Drs. I. Listowsky, G. Avigad, and S. Englard, personal communication, 1968); this Cotton effect was attributed to a π–π* transition of the acetamido group. Optical rotatory dispersion and circular dichroism spectra have been used to study oligosaccharides from milk containing 2-acetamido sugars (Beychok and Kabat, 1965) and immunologically active oligosaccharides from blood-group A, B, and H substances (Lloyd *et al.*, 1967a). In these studies it was found that the sign and size of the Cotton effects and ellipticity bands could be correlated not only with the anomeric configuration of the sugars but also, to a certain extent, with the positions and extent of substitutions on the 2-acetamido sugar residues. Optical rotatory dispersion and circular dichroism spectra of some reduced oligosaccharides formed by the treatment of blood-group Le[a] substance with NaOH–$NaBH_4$ (Lloyd *et al.*, 1968a,b) have been examined in an effort to extend these findings to other oligosaccharides.

Materials

Isolation and structures of oligosaccharides from blood-group Le[a] substance have been described in a previous paper (Lloyd *et al.*, 1968b). 2-Acetamido-2-deoxy-D-glucitol (mp 151–152°) and 2-acetamido-2-deoxy-D-galactitol (mp 170–172°) were prepared by reduction of the corresponding sugars with sodium borohydride (Wolfrom and Thompson, 1963). α-L-Fuc-(1→2)-β-D-Gal-(1→4)-2-acetamido-2-deoxy-D-galactitol was a gift from Drs. E. H. Eylar and R. Katzman (Katzman and Eylar, 1966).

Methods

Circular dichroism spectra were measured on a Jouan dichrograph modified for tenfold increased sensitivity (Beychok, 1965). The instrument measures directly the difference in optical density for left and right circularly polarized light. This difference is converted into a difference in extinction coefficient, $\epsilon_l - \epsilon_r = OD/lm$, in which l is the path length in centimeters and m is molarity. In this paper, circular dichroism intensity is expressed in terms of molecular ellipticity, which is proportional to $\epsilon_l - \epsilon_r$. $\theta = 3300(\epsilon_l - \epsilon_r)$. The units are (deg cm^2)/dmole.

Optical rotatory dispersion measurements were carried out on a Bendix Ericcsen spectropolarimeter,

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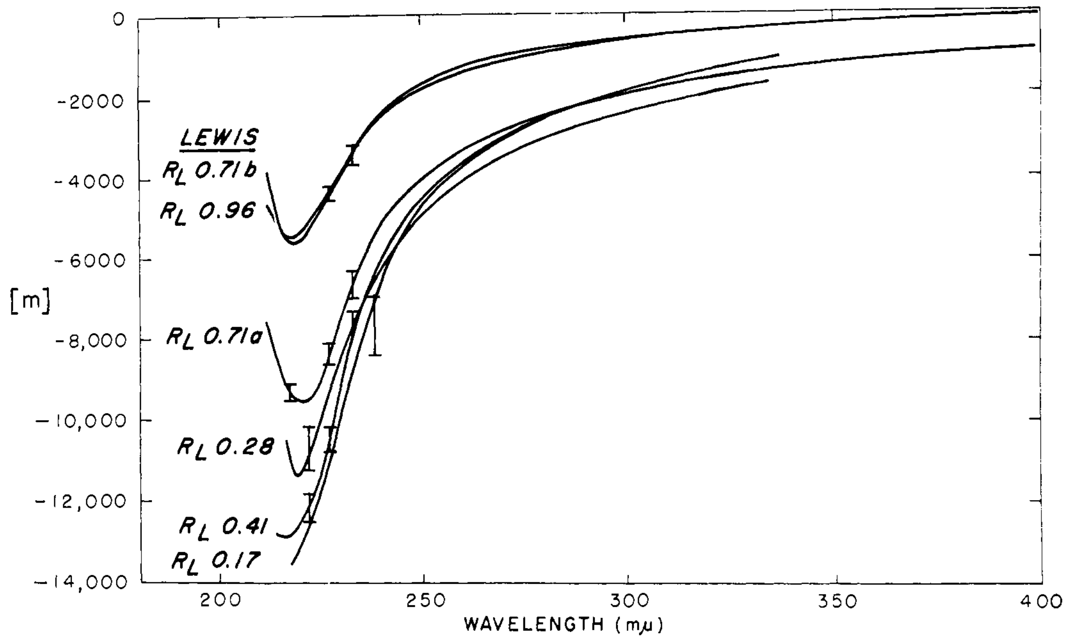

FIGURE 1: Optical rotatory dispersion spectra of oligosaccharides from Le[a] substance.

Model Polarmatic 62 as described previously (Beychok and Kabat, 1965). Each spectrum in Figure 1 is the average for at least two separate determinations run in duplicate. All of the optical rotatory dispersion spectra were checked with a Cary Model 60 spectropolarimeter and results agreed within experimental error although aberrant runs are occasionally obtained in the Cary probably due to difficulty in positioning the cells reproducibly and in the Bendix for unknown reasons.

Results

Figure 1 shows the optical rotatory dispersion spectra of the oligosaccharides. Table I gives the $[m]_{\text{trough}} - [m]_{300}$ values for the Cotton effect troughs; this procedure of subtracting the molar rotation at 300 mμ from the molar rotation at the trough of the Cotton effect is an attempt to separate the 2-acetamido Cotton effect from contributions of the other chromophores in the molecule (Beychok and Kabat, 1965). Oligosaccharide Lewis R_L 0.41 contains a 2-acetamido-2-deoxy-D-galactitol residue and Figure 2 shows the spectra of 2-acetamido-2-deoxy-D-glucitol and -D-galactitol as well as that of an oligosaccharide from hog submaxillary gland containing the latter alcohol.

Figure 3 shows the circular dichroism spectra of the oligosaccharides from Lewis substance. $[\theta]_{\max}$ values are given in Table I as well as those for a related com-

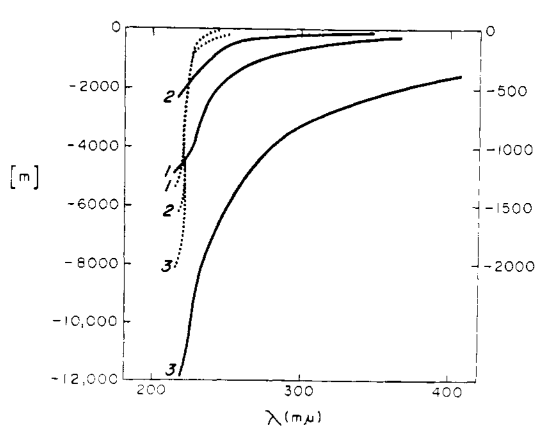

FIGURE 2: Optical rotatory dispersion (solid lines) and circular dichroism (dotted lines) spectra of 2-acetamido-2-deoxy-D-galactitol (**1**), 2-acetamido-2-deoxy-D-glucitol (**2**), and α-L-Fuc-(1→2)-β-D-Gal-(1→4)-2-acetamido-2-deoxy-D-galactitol (**3**).

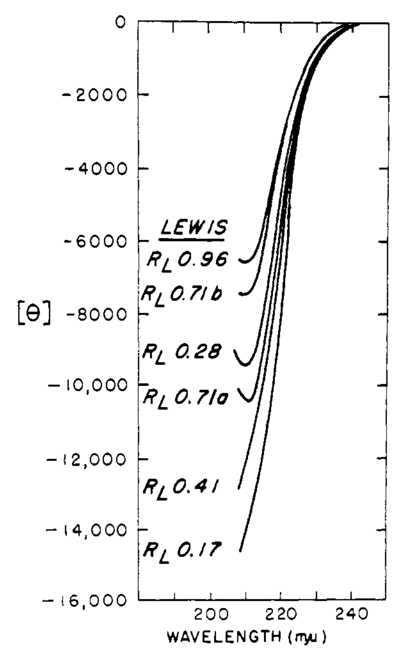

FIGURE 3: Circular dichroism spectra of oligosaccharides from Le[a] substance.

TABLE I: Optical Rotatory Dispersion and Circular Dichroism Characteristic of Oligosaccharides from Blood-Group Substances.

Oligosaccharide	Structure	Optical Rotatory Dispersion $[m]_{trough} - [m]_{300}$	Circular Dichroism $[\theta]_{max}$
Hog R_{Gal} 0.85	β-D-GNAc-hexenetetrols	−2,100[a]	
Lewis R_L 0.96	β-D-Gal-(1→4)-β-D-GNAc-hexenetetrols	−5,480	−6,500
Lewis R_L 0.71b	β-D-Gal-(1→4)-β-D-GNAc-hexanepentols	−5,630	−7,400
HR_L 0.75	α-L-Fuc 1 ↓ 2 β-D-Gal-(1→4)-β-D-GNAc-hexenetetrols	−8,250[b]	−7,250[b]
Lewis R_L 0.71a	α-L-Fuc 1 ↓ 3 β-D-Gal-(1→4)-β-D-GNAc-hexenetetrols	−9,500	−10,600
Lewis R_L 0.28	α-L-Fuc 1 ↓ 4 β-D-Gal-(1→4)-β-D-GNAc-D-galactitol	−11,400	−9,400
Lewis R_L 0.41	β-D-Gal-(1→3 or 4)-β-D-GNAc ↘(1→6) 2-acetamido-2-deoxy-D-galactitol ↗(1→3) β-D-Gal	−12,800	−13,100
Lewis R_L 0.17	β-D-Gal-(1→3)-β-D-GNAc ↘(1→3) D-galactitol ↗(1→6) β-D-Gal-(1→4)-β-D-GNAc	−13,600	−14,400

[a] Value taken from Lloyd and Kabat (1967). [b] Values taken from Lloyd *et al.* (1967a).

pound from blood-group H substance (Lloyd *et al.*, 1967a).

Discussion

Previous studies on the optical rotatory dispersion and circular dichroism spectra of 2-acetamido sugars and oligosaccharides (Beychok and Kabat, 1965; Lloyd *et al.*, 1967a,b) permitted the following conclusions. (a) α and β anomers of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine could easily be distinguished since the β isomers of both had larger negative Cotton effect troughs and circular dichroism bands than the α isomers. (b) β-D-GNAc residues substituted on C-3 (by D-galactose) showed larger optical rotatory dispersion troughs than those substituted on C-4. (c) Oligosaccharides containing disubstituted β-D-GNAc residues (*i.e.*, substituted on C-3 and C-4 by D-galactose and L-fucose) had the highest values for Cotton effect troughs and ellipticity bands.

Spectra of the oligosaccharides from Le[a] substance also show the large negative Cotton effect troughs and ellipticity bands characteristic of β-D-GNAc residues. The increased negative rotation accompanying substitution of an L-fucosyl residue on GNAc provides evidence for the α-L-fucopyranosyl linkage (Lloyd *et al.*, 1968b). Oligosaccharides Lewis R_L 0.96 and Lewis R_L 0.71b have $[m]_{trough} - [m]_{300}$ values of −5480 and −5630, respectively; these values fall within the range for other oligosaccharides substituted on C-3 or C-4 by D-galactose, *e.g.*, lacto-*N*-tetraose and lacto-*N*-neotetraose[1] (Beychok and Kabat, 1965) when allowance is made for the different residues present at the reducing ends. The values are considerably larger than the $[m]_{trough} - [m]_{300}$ value for a related oligosaccharide with an unsubstituted β-D-GNAc residue (Table I, hog R_{Gal} 0.85).

The reduced pentasaccharide, Lewis R_L 0.17, has two residues of D-GNAc and shows a very large Cotton effect trough. Per mole of D-GNAc, however, the $[m]_{trough} - [m]_{300}$ value is −6800, in good agreement

[1] These compounds have a lactose residue replacing the alcohols present in the Lewis oligosaccharides. Since lactose has a $[m]_{220} - [m]_{300}$ value of +2000, this figure has been subtracted from the $[m]_{trough} - [m]_{300}$ values for the milk oligosaccharide when comparison between the two series of compounds is made.

with the values of the other two oligosaccharides; this indicates that Lewis R_L 0.17 has two β-D-GNAc residues substituted by D-galactose, a conclusion confirmed by chemical studies (Lloyd *et al.*, 1968b). A similar conclusion can be drawn from the circular dichroism data.

Lewis R_L 0.71a and Lewis R_L 0.28 are monofucosyl oligosaccharides each with a β-D-GNAc residue substituted on both C-3 and C-4. According to the findings of Beychok and Kabat (1965) discussed above, these compounds would be expected to have quite large $[m]_{trough} - [m]_{300}$ values. The values (Table I) are considerably larger than those given by the two trisaccharides Lewis R_L 0.96 and R_L 0.71b; however a considerable portion of this increase is due to the contribution of an additional α-L-fucopyranosyl residue (*ca.* −2400; Lloyd *et al.*, 1967a). Circular dichroism spectra also support the importance of disubstituted β-D-GNAc residues. Thus, whereas the two trisaccharides, Lewis R_L 0.96 and Lewis R_L 0.71b, have $[\theta]_{max}$ of −6500 and −7400, the two monofucosyl oligosaccharides give values of −10,600 and −9400. That this increase does not arise from direct contributions of the α-L-fucopyranosyl residues can be concluded from the low value (−7250; Table I) given by the isomeric monofucosyl oligosaccharide from H substance (HR_L 0.75; Lloyd *et al.*, 1967a) in which the α-L-fucopyranosyl residue is linked to D-galactose rather than to a D-GNAc residue. It is interesting that Lewis R_L 0.28 has the largest Cotton effect trough of the two monofucosyl oligosaccharides whereas Lewis R_L 0.71a has the largest ellipticity band in circular dichroism. In considering the effect of substituents linked to β-D-GNAc residues on the optical rotatory dispersion and circular dichroism characteristics of the oligosaccharides, it is important to remember that although in optical rotatory dispersion spectra the predominant contribution at 200 mμ may be from the 2-acetamido chromophore, the background rotation due to other chromophores in the molecule must be taken into account and is often considerable. Circular dichroism bands, however, have narrow half-band widths and, assuming that no other chromophores in the molecules occur above 200 mμ, any changes in $[\theta]_{max}$ for these oligosaccharides can be considered as reflecting changes in the orientation and assymetry of the 2-acetamido group alone.

Oligosaccharide Lewis R_L 0.41 differs from the others in being terminated by a reduced 2-acetamido sugar, *i.e.*, 2-acetamido-2-deoxy-D-galactitol. The large negative rotation and ellipticity band of this compound can be explained as being due to the contribution of both the acetamido alcohol and a β-D-GNAc residue. Figure 2 shows that both 2-acetamido-2-deoxy-D-glucitol and -D-galactitol have substantial negative rotations, although no trough was reached at the lowest wavelength studied; the spectra of the trisaccharide containing 2-acetamido-2-deoxy-D-galactitol shows that the optical rotatory dispersion and circular dichroism spectra are affected by substitution on the sugar alcohol residue.

A characteristic of the spectra of the acetamido sugar alcohols is that the maxima in the optical rotatory dispersion troughs and circular dichroism bands were not reached at 215 mμ (Figure 2). Most of the Lewis oligosaccharides showed troughs in the 217–220-mμ region and ellipticity bands at 210–212 mμ. However, the optical rotatory dispersion spectrum of Lewis R_L 0.17 did not exhibit a trough down to 217 mμ and maxima in the ellipticity bands for this oligosaccharide and for Lewis R_L 0.41 were also not observed down to 210 and 208 mμ, respectively. Variations in the wavelengths of the optical rotatory dispersion troughs and circular dichroism bands have been observed in earlier studies (Beychok and Kabat, 1965; Lloyd *et al.*, 1967a) and, although they are probably related to differences in the environment of the 2-acetamido group, their exact significance is uncertain.

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