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Circular dichroism of the O-specific polysaccharide of *Vibrio cholerae* O1 and some related derivatives

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Abstract

The O-specific polysaccharide (O-SP) of *Vibrio cholerae* O1 is a homopolymer of α-(1 → 2)-linked 4-amino-4,6-dideoxy-D-mannopyranose whose amino group is acylated with 3-deoxy-L-*glycero*-tetronic acid [*N*-(3-deoxy-L-*glycero*-tetronyl)-α-D-perosamine]. The circular dichroism (CD) of the O-SP as well as of a number of *N*-acyl (formyl, acetyl, 4-hydroxybutyl, 3-deoxy-L- and D-*glycero*-tetronyl) derivatives of methyl α-glycosides of 4-amino-4,6-dideoxy-D-manno-pyranose (methyl α-D-perosaminide) has been studied for solutions in water, acetonitrile and 1,1,1-trifluoroethanol. The strong solvent dependence of the sign and intensity of the CD observed for the monosaccharide amides bearing achiral acyl groups is explained by solvent-mediated change of the orientation of the amido group relative to the proximal hydroxyl group at C-3. A change in the population of the nonplanar conformers with a pyramidal arrangement of bonds at the amido nitrogen has also been considered. The effect of solvents upon the CD spectra of compounds bearing chiral *N*-acyl substituents is less pronounced than that of their counterparts bearing achiral *N*-acyl substituents. The sign of the CD for the O-SP was found negative in all solvents used. This result is in agreement with the negative sign of the CD of the $n \rightarrow \pi^*$

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electron transition observed, independent of the solvent, for the monosaccharide derivative containing the L-*glycero*-3-deoxytetronamido group, and the positive sign found for its D-*glycero* -counterpart.

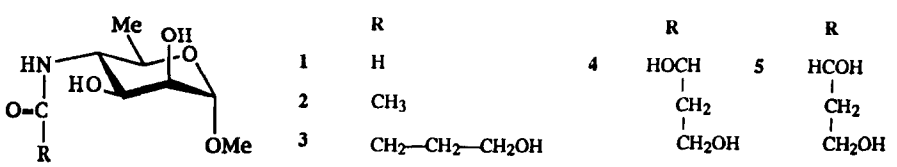
Keywords: O-Specific polysaccharide; *Vibrio cholerae*

1. Introduction

The surface polysaccharides of many pathogenic bacteria are both essential virulence factors and protective antigens. One type of these polysaccharides, the lipopolysaccharides (LPS), are composed of three domains. The domain external to the bacteria and most in contact with the host is the O-specific polysaccharide (O-SP). The two main serotypes of *Vibrio cholerae* O1, denoted Inaba and Ogawa, have two different but closely related O-SPs. Their constituent sugar is 4-amino-4,6-dideoxy-α-D-mannopyranose (α-D-perosamine), the amino group of which is acylated with 3-deoxy-L-*glycero*-tetronic acid. *N*-Acyl groups occur frequently in bacterial polysaccharides and LPS [1], and they often participate in epitopes that elicit immunological responses. Variation of acyl groups in homopolysaccharides composed of the same aminosugar often distinguishes LPS of one bacterial species from another. For example, *N*-formylated α-(1 → 2)-linked D-perosamine occurs in the O-specific chain of the LPS of *Yersinia enterocolitica* [2] serotype 0:9 and *Brucella abortus* [3]. Polysaccharide composed of the same monosaccharide but *N*-acylated with 3-deoxy-L-*glycero*-tetronic acid forms the O-antigen of *V. cholerae* O1 [4]. We have proposed [5] that serum antibodies to the latter structure confer protective immunity to cholera.

Structural and conformational properties of glycosaminoglycans and *N*-glycopeptides containing the *N*-acetamido group have been studied by circular dichroism (CD) (e.g. ref. [6,7]). The strong influence of solvent upon the conformation of molecules studied [8,9] has been explained on the basis of theoretical calculations [10,11]. It has been proposed that the solvent dependence of the CD spectra of substances containing *N*-acetamido groups is caused by the changed conformation of this group and the varying distribution of rotamers of the neighboring hydroxyl groups. A different explanation, based on the nonplanar amido group as the main source of overall chirality, was supported by molecular calculations [12–14] and CD measurements with 3-acetamido-hexopyranosides [15]. The studies indicate that the CD of acetamido group-containing substances is sensitive to the influence of the environment.

Examination of the structures and the immunological properties of LPS/PS [16–18] of pathogenic organisms prompted us to examine further, by CD, the properties of



Scheme 1.

sugars bearing *N*-acyl groups. *N*-Acyl analogues of methyl α-D-perosaminide, namely, *N*-formyl, *N*-acetyl, and *N*-(4-hydroxybutyryl), were studied by CD for solvent-induced conformational changes of their *N*-acylamido groups. The CD spectra of the constituent sugar of the O-PS of the *V. cholerae* O1 containing the chiral 3-deoxy-L-*glycero*-tetronyl group [4] and its D-analogue were also examined. Knowledge of the CD occurring with these *N*-acyl substituents can provide, inter alia, a means of differentiation between the D- and D-forms of optically active (α-hydroxyacyl)amido groups in other natural products, including bacterial antigens.

2. Experimental

Compounds **1** and **2** were prepared according to Bundle et al. [19]. The synthesis of **4** has been reported [20]. The amorphous D-*glycero* derivative **5** was prepared as described [20], and crystallization from acetone gave material melting at 119.5–120.5°C. Compound **3** was prepared [21] in a similar manner, using γ-butyrolactone (Aldrich Chemical Company). The isolation and purification of the O-PSs from *V. cholerae* O1, Ogawa and Inaba strains, has been described [22]. CD spectra were recorded at 25°C and 70°C, with a Jasco 720 Spectropolarimeter (Japan Spectroscopic Co.), using a Neslab temperature control unit. Solutions (0.1–0.4 mg/mL) of compounds **1–5** were prepared in deionized water, acetonitrile (spectrophotometric grade, Aldrich Chemical Company) and 2,2,2-trifluoroethanol (TFE, OmniSolv, MCB). CD measurements of polysaccharides were obtained on solutions in water and organic solvents containing 50% of water. The spectra were measured from 250 to 185 nm at a pathlength of 1 and 2 mm.

3. Results

CD spectra of derivatives of D-perosamine **1–5** in various solvents at 25°C and 70°C are shown in Fig. 1. The first dichroic band, corresponding to the $n \rightarrow \pi^*$ electrontransition, occurs at a wavelength above 200 nm. The second dichroic band, associated with the $\pi \rightarrow \pi^*$ electron transition [10], is located below 200 nm and is not discussed here.

Compounds with achiral acyl groups (**1–3**).—Compound **1** is a mixture of isomers with a formyl group in s-*cis*- and s-*trans*- orientation around the N–C(O) bond (s-*cis* : s-*trans* ~ 7:3, in D_2O) [19,23]. It showed different CD spectra in various solvents (Fig. 1, **1**). In aqueous solution, the first and second band have opposite signs. Reductions of ellipticities and the split of the band above 200 nm with the cross-over shifted to 218 nm are observed for solutions in acetonitrile. The CD spectrum for the TFE solution, in contrast, shows the opposite shift (cross-over at 195 nm) from that observed for the aqueous solution.

In compounds **2** and **3**, the amido group is in the energetically most stable s-*trans* conformation. The CD of compound **2** is shown in Fig. 1 (**2**, **3**). The sign of the ellipticity of the first band changes from positive in water to negative in acetonitrile or TFE. Stronger ellipticity was observed for the TFE solution than for the acetonitrile solution. Compound **3**, containing the 4-hydroxybutyramido group, shows similar CD

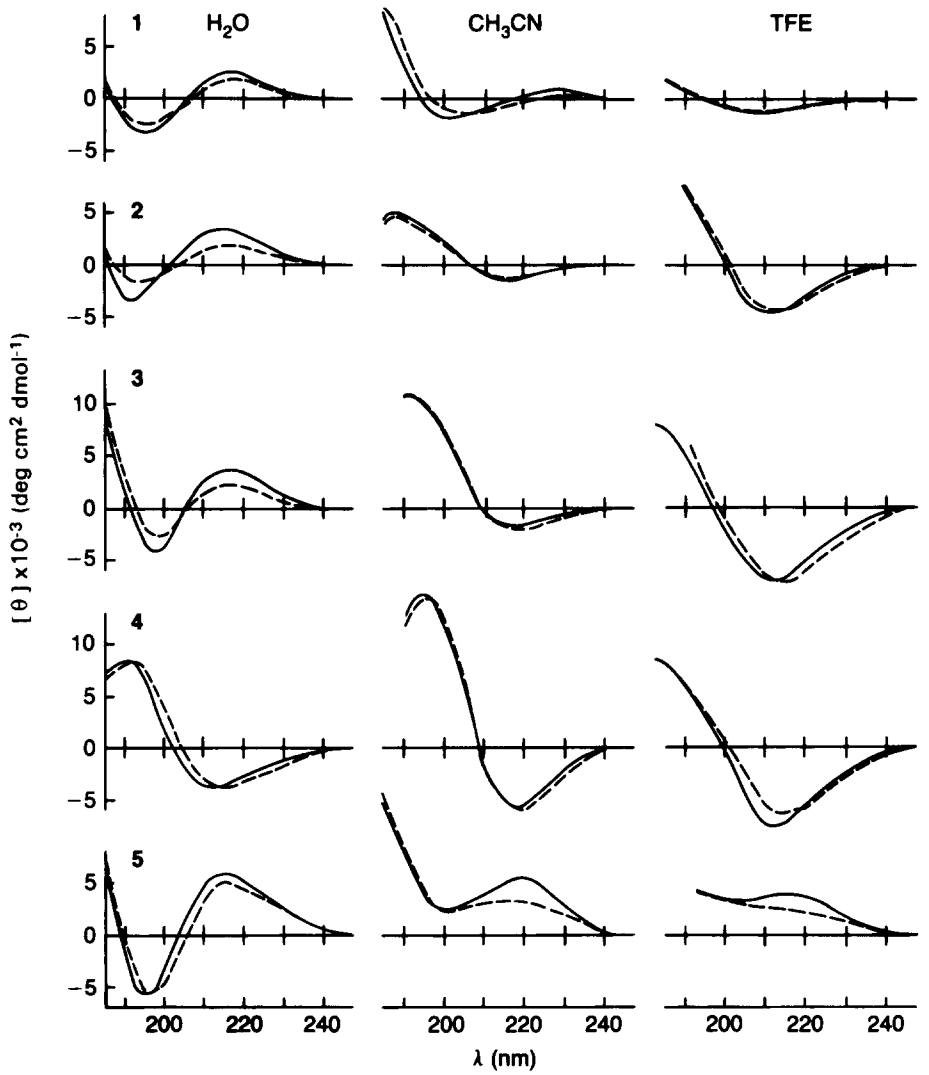


Fig. 1. The CD spectra of *N*-acyl derivatives of methyl α-D-perosaminides **1**–**5** at 20°C (solid line) and at 70°C (dashed line).

spectra (Fig. 1) as those observed for compound **2**, except for a slight increase in ellipticity.

Compounds with chiral acyl groups.—Compounds **4** and **5** each contain a chiral acyl group having the asymmetric center at C-2', adjacent to the carbonyl group of the amide chromophore. The CD originating from this new chiral center superimposed on that from the chirality of the pyranose ring. Fig. 1 (**4**, **5**) shows that the sign of the first dichroic band remains unchanged in different solvents. The CD spectra of isomers **4** and **5** are of opposite sign: the negative sign for **4**, having the L-*glycero* configuration, and

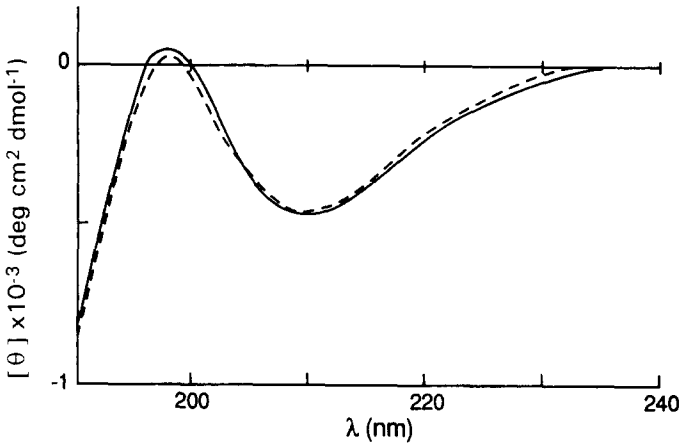


Fig. 2. The CD spectra for aqueous solutions of O-specific polysaccharides of *V. cholera* O1, serotype Inaba (solid line) and Ogawa (broken line) containing N-(3-deoxy-L-*glycero*-tetronyl)-D-perosamine.

the positive sign for **5**, having the D-*glycero* configuration. The intensity of the CD is solvent dependent, as the dichroic band becomes more negative for compound **4** and less positive for **5**, when changing solvents from water → acetonitrile → TFE. This trend is more pronounced with compounds **1–3** where the change of solvent (water → acetonitrile → TFE) results in the change of the sign of CD (Fig. 1, **1–3**).

O-Specific polysaccharides.—The O-PS of *V. cholerae* O1, serotypes Inaba and Ogawa, show identical CD spectra. The two O-PSs differ only by the presence, in the Ogawa species, of the methyl group at O-2 of the terminal, nonreducing perosamine moiety [18]. The sign of the first dichroic band is negative, irrespective of the solvent, water (Fig. 2), acetonitrile + water (1:1) and TFE + water (1:1), similar to that observed for compound **4**.

4. Discussion

The O-SP of *V. cholerae* is soluble in water but insoluble in organic solvents. Here, the effect of solvent on the conformation of several model D-perosamine monosaccharide derivatives has been studied by CD. The intermolecular solvent–solute interactions vary with the nature of the solvent. We used acetonitrile which is a proton acceptor, trifluoroethanol, both a proton donor and a proton acceptor, and water for comparison.

Formation of solvent–solute hydrogen bonds could disrupt putative intramolecular hydrogen bonds, such as that linking the oxygen of a hydroxyl group and the hydrogen of the acylamido group in the α-anomers of 2-acetamido-2-deoxy-hexoses [8]. In the case of the α-anomer, N–H and C–O(H) bonds are parallel (Fig. 3a). Such an orientation is favorable for an effective H-bond formation. The occurrence of an intramolecular hydrogen bond stabilizing the overall conformation was experimentally confirmed in the case of the α-anomer, but not with the β-anomer [8]. For the β-anomer (Fig. 3a), which is analogous to the situation with our compounds **1–5** (Fig. 3b), to form

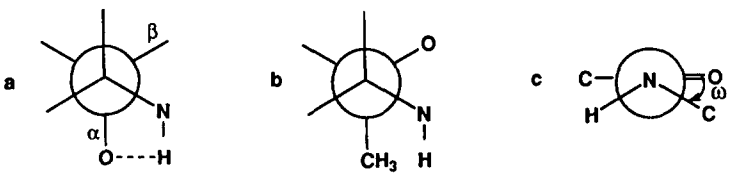


Fig. 3. The Newman projection of: (a) 2-acetamido derivatives, viewed along the C(2)–C(1) bond; (b) 4-acylamido derivatives, viewed along the C(4)–C(3) bond; (c) nonplanar amido group with *S*-chirality viewed along the N–C(1') bond.

such an intramolecular hydrogen bond would require an unfavorable rotation of the acetamido group. Our finding that the CD spectra of **1–5** show little temperature-dependence support the assumption that intramolecular H-bonds do not occur here.

The overall steric arrangement of acylamido sugars may be affected by several factors:

Orientation of the acylamido group.—It can be assumed that the amido group in compounds **2–5** takes the energetically most stable orientation angle, i.e. the double bond of the C = O group and the C(4)–H bond are eclipsed within the range of +20 to −20°. However, the *N*-formyl group in compound **1** can exist as an equilibrium of s-*cis* and s-*trans* isomers (with strong prevalence of the former), as a result of rotation about the N–C(O) bond. The split first dichroic band present in the CD spectrum of **1** taken in acetonitrile results from the combination of the effects of the individual isomers.

Orientation of the HO-3, vicinal to the acylamido group, and its interaction with the solvent.—Each of compounds **1–3** contains an achiral acyl side chain. The CD spectra reflect the overall chirality of the pyranose ring. Of the individual segments in the pyranose ring, the HO-3 has the strongest influence on the CD. Varying the length of the side chain does not significantly affect the CD: all compounds bearing achiral acyl side-chains produced similar CD spectra. However, the CD for solutions in acetonitrile and TFE were of opposite sign to that in aqueous solution. This could be caused by the small size and high polarity of water molecules resulting in the formation of more stable solvent–solute hydrogen bonds.

The mutual orientation of the amido and the HO-3 group can be deduced from the CD spectra applying data for $n \rightarrow \pi^*$ rotational strength calculated [11] for 2-acetamido-2-deoxy-D-glucose. The structural segment RHNC(4)–C(3)OH in compounds **1–3** can be looked upon as a mirror image of the system RHNC(2)–C(3)OH in 2-acetamido-2-deoxy-D-glucose derivatives. The interaction of the solvent with *HO*-3 and H*O*-3 controls the orientation of the HO-3. Following the above rationale [11], the positive sign of the CD observed for **1–3** in water can be explained by the orientation of HO-3 within the range of 60–180° [corresponding to the clockwise rotation from the reference position having HO-3 eclipsing C(3)–H]. Here, the lone electron pairs of the hydroxyl oxygen are oriented towards the acetamido group. In contrast, the negative sign of the CD observed for the solution of **1–3** in acetonitrile and TFE may arise from other possible orientations of the proximal HO-3 which accomodate the less polar and bulkier solvent.

Non-planarity of the amido group.—The bonds around the nitrogen can form a nonplanar pyramidal arrangement and give the amide chromophore its inherent chirality. This possibility is supported by the significant deviation from the planarity [$\omega = -12.50°$ (Fig. 3c)] found for the amido group in compound **4** by X-ray structural analysis [20]. In solution, as a result of the solvent–solute interactions, the proportion of one of the enantiomers (R/S) can vary and cause a sign change in the CD. Water, with its hydrogen bonds, can act as a bridge between the amide oxygen and a hydroxyl group. Thus, the attraction of the carbonyl group towards the hydroxyl group can lead to a prevalence of the *S*-enantiomer of the amido group (Fig. 3c). TFE forms hydrogen bonds through its OH group mainly with the carbonyl oxygen. The trifluoromethyl group can interact with the hydrogen atom on the nitrogen atom and the HO-3. Due to the solvating interaction by the bulky TFE molecules, compared to the size of molecules of water, the amido group and the vicinal hydroxyl groups may be repelled. This effect could favor *R*-chirality of the amido group and, consequently, result in the opposite sign of the CD, compared to that found for the aqueous solution. Acetonitrile interacts with the solute only by its nitrogen, which is capable of hydrogen bonding. Acetonitrile, also space demanding, can cause the *R*-chirality of the amido group to prevail. The observed negative and positive signs of CD for *R* and *S* chirality, respectively, hold for all three compounds **1–3** and are in agreement with those found and calculated for some 3-acetamido compounds [12,13].

The CD characteristics of compounds containing chiral *N*-acyl groups have not been described previously. Compounds **4** and **5** belong to such a category. We have found that the sign of the first dichroic band in their spectra is solvent independent and reflect the chirality at C-2 of the *N*-acyl substituent. Overall, the CD contribution from the asymmetric carbon atom vicinal to the carbonyl group of the amido chromophore predominates over other sources of chirality. The negative sign observed for **4** having the L-*glycero* configuration and the positive one for **5** having the D-*glycero* configuration is solvent independent. However, a certain small effect of solvent on the CD intensity can be observed. The dichroic band becomes more negative in the case of compound **4** and less positive with compound **5**, in the series water → acetonitrile → TFE. This solvent effect was found to be more pronounced with compounds **1–3** (Fig. 1).

The O-SP of serotypes Ogawa and Inaba showed identical CD spectra. The negative sign of the first dichroic band is present in the spectra of the O-SPs as well as in that of the monosaccharide **4**. Thus, the negative sign of the CD appears to be characteristic of compounds having the L-*glycero* configuration of the (α-hydroxyacyl)amido substituent. Our observation that the chirality of an *N*-acyl group affects the overall CD characteristics more than does the solvent-induced conformation of the chiral arrangement may be used to distinquish between these types of chiral compounds.

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