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Note

Conformational differences among mono- and oligosaccharide fragments of the O-specific polysaccharides of *Vibrio cholerae* O1 revealed by circular dichroism

Slavomír Bystrický [a,*], Shousun C. Szu [a], Jian Zhang [b], Pavol Kováč [b]

[a] *Laboratory of Developmental and Molecular Immunity, NICHD, National Institutes of Health, Bethesda, MD 20892-2720, USA*

[b] *Laboratory of Medicinal Chemistry, NIDDK, National Institutes of Health, Bethesda, MD 20892-0815, USA*

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Abstract

The circular dichroism (CD) of synthetic mono- and oligosaccharides that represent the terminal, non-reducing group of O-antigens of *Vibrio cholerae* O1 from the subtypes Ogawa and Inaba was measured in various solvents. We found differences in the CD of the monosaccharides of these subtypes that decrease with increasing chain lengths of the oligosaccharides. The differences can be explained by different orientations of the N-acyl side chain of the terminal monosaccharides. The linear relationship of ellipticity versus the number of residues in an oligosaccharide chain follows the principle of optical superposition. This, together with a similar contribution by internal units to the overall ellipticity, suggests an identical, regular conformation of oligosaccharide fragments of both Ogawa and Inaba series.

Keywords: *Vibrio cholerae*; Polysaccharides; O-antigens

1. Introduction

There are 139 serotypes of *Vibrio cholerae* based on their lipopolysaccharide structures. *V. cholerae* O1 and the emerging O139 cause most of the disease in humans. *V. cholerae* O1 consists of two subtypes, Inaba and Ogawa. The O-specific polysaccharides (O-SP, O-antigens) of these subtypes contain the same intracatenary monosaccharide repeating unit, α-(1→2) linked 4-amino-4,6-dideoxy-D-mannose (D-perosamine) whose amino groups are acylated with 3-deoxy-L-*glycero*-tetronic acid. The difference between Inaba and Ogawa is that the Ogawa has the O-2 of its terminal non-reducing perosamine methylated [1,2].

* Corresponding author. Permanent address: Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovak Republic.

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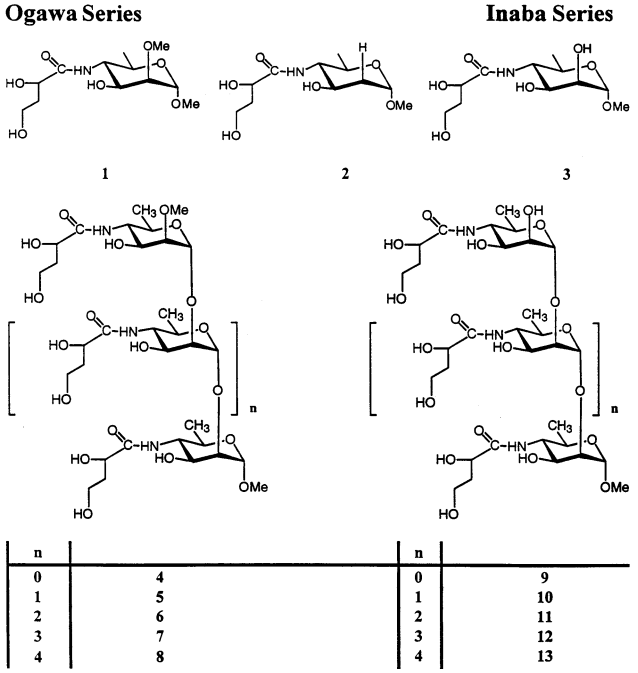

n		n	
0	4	0	9
1	5	1	10
2	6	2	11
3	7	3	12
4	8	4	13

In spite of the exceptionally small structural difference, the two subtypes are serologically distinct [3]. Binding of methyl glycosides of terminal oligosaccharide fragments of Inaba and Ogawa O-SPs with monoclonal antibodies specific for Ogawa O-SP did not show cross-reactivity [4]. This observation is indicative of the immunological difference being caused not only by structural difference (OH vs. OMe in the terminal perosamine moiety), but also by other, possibly conformational, differences.

Within our efforts to develop a polysaccharide-based vaccine for cholera [5], we investigated the basis for the effect of the single methyl group at O-2 in the terminal perosamine on the antigenicity of the O-SP. The aim of this work was to unravel differences in the conformation of the terminal oligosaccharide fragments in the two O-SPs, if there are any, and to verify if the conformation found for oligosaccharides correlate with that of the polysaccharide. Circular dichroism (CD) of monosaccharides [6] and various derivatives of the Inaba O-SP [7] showed solvent mediated changes in the orientation of the amide chromophore, relative to the proximal hydroxyl groups. Also, the L- and D-forms in the tetronamido moiety could be distinguished by the sign of CD [7]. Here the synthetic mono- and oligosaccharides, up to hexamers, reflecting the upstream (non-reduc-

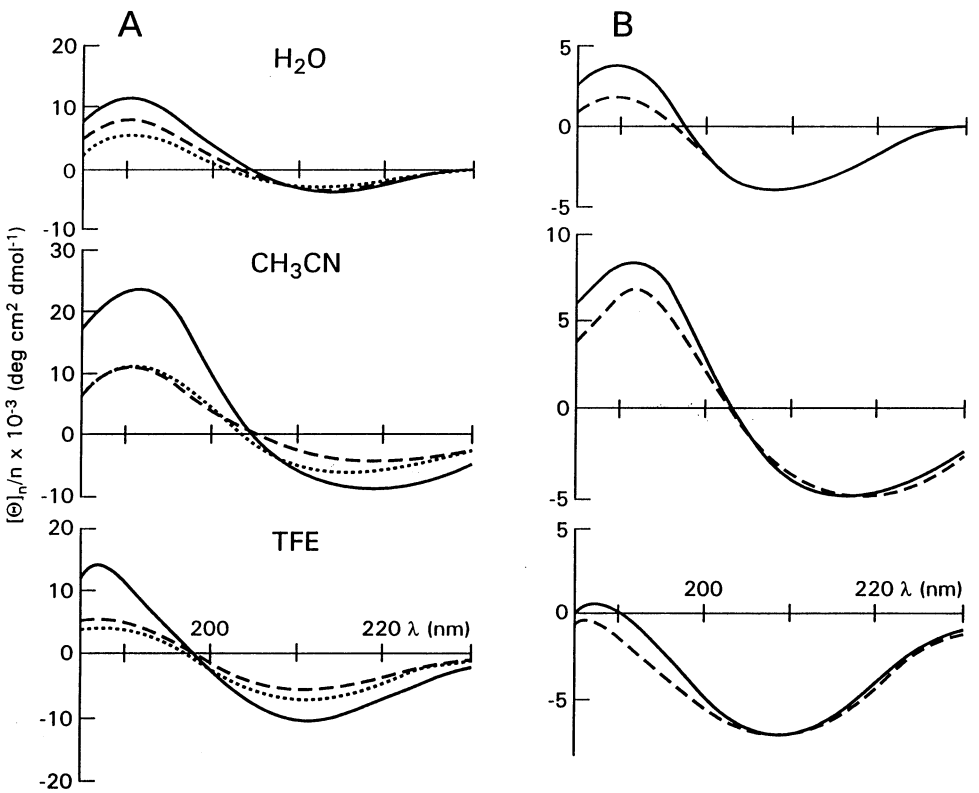

Fig. 1. The CD spectra of (A) monosaccharides (**1**, solid line; **2**, dotted line; **3**, broken line), and (B) disaccharides (**4**, solid line; **9**, broken line).

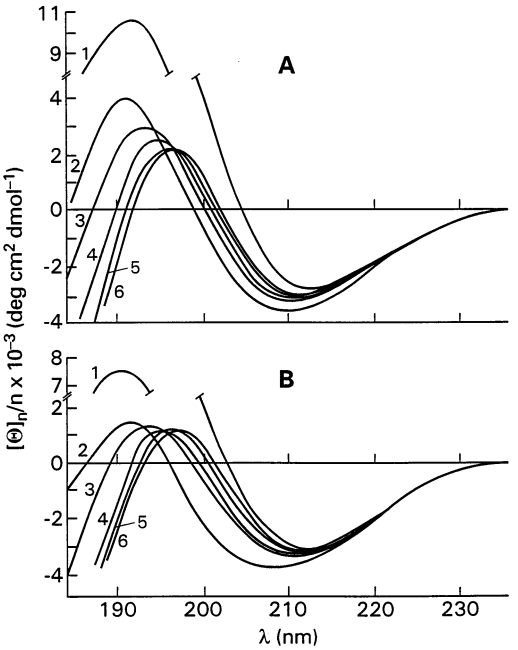

Fig. 2. The CD spectra of the studied mono- and oligosaccharides, $n = 1$–6: (A) Ogawa series; (B) Inaba series in water.

ing) termini of the Ogawa or Inaba O-SP, are used as models to study the conformational differences by CD spectrometry.

2. Experimental

Syntheses of compounds **1–13** were described previously [8–14]. CD spectra (240–184 nm) were recorded at room temperature with a Jasco 720 Spectropolarimeter (Japan Spectroscopic) using 0.2 cm pathlength cells under nitrogen purge. Solutions (0.15–0.4 mg/mL) of mono- and disaccharides of the Ogawa and Inaba series were prepared in deionized water, acetonitrile (spectroscopic grade, Aldrich Chemical) and 2,2,2-trifluoroethanol (TFE, OmniSolv, MCB). The CD spectra of higher oligosaccharides were measured in deionized water. The spectra were averaged over six scans and smoothed by a built-in data filtration method. The resultant CDs are expressed in molar residual ellipticity. The effect of chain length on the conformation of oligosaccharides was analyzed according to the principle of optical superposition [15,16]. The ellipticity of an n-member polymer $[\theta]_n$ can be expressed as the sum of ellipticities of two terminal units $[\theta]_2$ and central units $[\theta]$:

$$[\theta]_n = [\theta]_2 + (n-2)[\theta]$$

$$[\theta]_n/n = [\theta]_2/2 + \{[\theta] - [\theta]_2/2\}(n-2)/n \qquad (1)$$

The molar residual ellipticity ($[\theta]_n/n$) was plotted against $(n-2)/n$ of the positive dichroic band and analyzed.

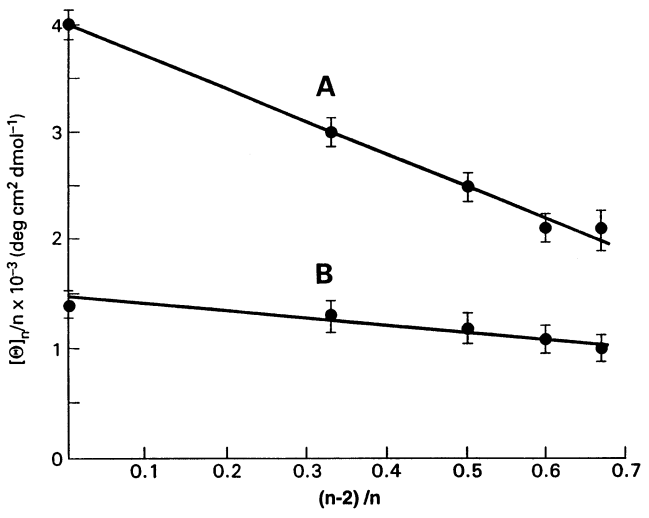

Fig. 3. Plot of molar ellipticity $[\theta]_n/n$ vs. $(n-2)/n$ for the oligosaccharides: (A) Ogawa series; (B) Inaba series.

3. Results

CD spectra of mono- (**1–3**) and disaccharides (**4** and **9**) in water, acetonitrile and 2,2,2-trifluoroethanol are presented in Fig. 1. There is an overlap of the first (n–π*) and second (π–π*) dichroic bands of the opposite signs. The negative sign of the first dichroic band is characteristic [7] of the L-*glycero* configuration of the chiral N-acyl group. The CD spectra of the Ogawa and Inaba structural types differ considerably. Higher values of ellipticity are observed with Ogawa mono- and disaccharides in all solvents. The difference between the two series of oligosaccharides is more pronounced in solvents which can interact with the amide chromophore. The maxima of the spectra taken in acetonitrile shift to higher wavelengths in both. The intensity of the positive ellipticity is much lower in disaccharides than in monosaccharides. The CD spectra of the 2-deoxy derivative **2** resemble those of **3** (Inaba series) more than those of **1** (Ogawa series)

The CD spectra of saccharides **1–13** in deionized water are shown in Fig. 2. The

spectra of the oligosaccharides differ little with respect to the first negative dichroic band. The intensity of the second (positive) band in the Ogawa series decreases noticeably with increasing chain-length. The values of ellipticity at positive maxima were used to correlate the change of optical activity versus chain-length. According to the principle of optical superposition, the optical activity of polymers obeys the additivity rule [15–17]. For the linear Eq. (1), the intersect on the ordinate represents the mean value of ellipticity of both terminal units. The slope expresses the difference between the molar residual ellipticity of the central unit and the mean value of terminal units. With the Ogawa oligosaccharides, the values of positive ellipticity decrease with increasing chain-length. The plot $[\theta]_n/n$ versus $(n-2)/n$ is shown in Fig. 3. There is a negative value for the slope of oligosaccharides in the Ogawa series (**4**–**8**), and a similar trend in the Inaba oligosaccharides (**9**–**13**). The values of ellipticity in the Inaba series do not decrease as much as they do in their Ogawa counterparts, and the value of the slope is lower. The constant slope in both series within the whole range of $(n-2)/n$ indicates that the oligosaccharides form a regular structure. The CD data indicate that the internal chains of the oligomers in both have the same conformation. Values of parameters, obtained from Fig. 3, for Ogawa oligosaccharides are: $[\theta]_2/2 = 4 \times 10^3$; $[\theta] - [\theta]_2/2 = 3.1 \times 10^3$. Molar residual ellipticity of the internal units is then calculated to be $[\theta] = 0.9 \times 10^3$. The parameters for Inaba oligosaccharides are: $[\theta]_2/2 = 1.45 \times 10^3$; $[\theta] - [\theta]_2/2 = 0.6 \times 10^3$; the molar residual ellipticity of the internal unit is $[\theta] = 0.85 \times 10^3$.

4. Discussion

The CD of the mono- and disaccharides representing the terminal units of *V. cholerae* O-SP subtypes Ogawa and Inaba were measured in water and in organic solvents (Fig. 1). Oligosaccharides greater than dimers are insoluble in organic solvents. The CD reflect the stereochemical situation near the amide chromophore. The first (negative) and the second (positive) dichroic bands are characteristic of the L-*glycero* configuration in the chiral acyl chain [7]. In our previous work [7], a strong influence of solvent on the interaction of the amide chromophore with the neighboring hydroxyl group at C-3 was found. In the present work we made a similar observation regarding the influence of the substitution at C-2. The difference between the CD of Ogawa (**1**) and Inaba (**3**) monosaccharides indicates a different conformational arrangement of the N-acyl chromophore in these two terminal monosaccharides that differ only by C-2 substituents. Compounds belonging to the Ogawa series showed higher ellipticity than the Inaba series. This suggests that the intramolecular interactions of protons in the Ogawa and the Inaba structures are different, resulting in different Cotton effects. In the case of the 2-deoxyderivative **2**, the position of the first maximum in the CD spectrum taken in acetonitrile appears at a lower wavelength than in the derivatives having oxygen at C-2. This may indicate that there is a hydrogen bond between HO-3 and the carbonyl oxygen, which is weaker in compounds having an oxygen atom at C-2. Generally, the positions of maxima of the dichroic bands are similar for Ogawa and Inaba structures. The types of intramolecular interactions involving O-2, HO-3 and the carbonyl group are probably the same. These interactions (hydrogen bonding) are strongly affected by intermolecular interactions with the solvent. The difference in values of ellipticity can be caused by variably intense contacts of different protons and oxygens in the above structural segment. As a result, the tetronamido side chain can adopt a conformation which differs considerably from the ideal, interaction-free conformation. It includes, for example, a deviation from the normal zig-zag carbon arrangement, rotations of vicinal hydroxyls, and a significant variation of the common planarity of the amido group. At this stage we cannot exclude any of these. The intrinsic difference between the conformation of Ogawa and Inaba monosaccharides is evident from the CD data in different solvents.

The CD spectra of the two disaccharides (**4** and **9**) differ somewhat; the ellipticity of the positive dichroic band is smaller than in the

monosaccharides. The CD spectra of the two series of oligosaccharides show a gradual change with increasing chain-length (Fig. 2). Eq. (1) allows a correlation between the ellipticity of the chain and the number of residues. For oligosaccharides having regular three-dimensional structure, the slope should be constant [17]. In contrast, for example, CD spectra of oligosaccharides composed of α-(2→8)-linked sialic acid [18] showed no linear correlation, suggesting that the flexible chain does not adopt a regular structure. In the present case, a linear relation of residual molar ellipticity versus $(n-2)/n$ values was observed, starting with the dimers **4** and **9** (Fig. 3). Parameters for the linear Eq. (1) were extracted from the graphical plot (Fig. 2). The α-(1→2) diaxial glycosidic bond is one of the most rigid structures in polysaccharides. The oligosaccharides studied herein belong to this category. The difference in the conformation of the terminal units is evident here as a strong end-effect. Contribution of internal units to the total ellipticity was calculated from graphical parameters (Fig. 3). The data indicate that the spatial arrangements of every internal monomeric unit are identical. The values for molar residual ellipticity of internal units, $[\theta] = 0.9 \times 10^3$ for Ogawa and $[\theta] = 0.85 \times 10^3$ for Inaba oligosaccharides, are the same within experimental error. Such a remarkable accord suggests a similar arrangement within both polysaccharide chains, which can be realized only by an identical, regular conformation.

The structural and conformational differences of the non-reducing terminal units in Ogawa and Inaba oligosaccharides are not reflected in the conformation of the polysaccharide chains. This study suggests that the immunological difference between the two subtypes [4] is not related to different conformations of the polysaccharides but to the immunodominant role of the terminal saccharide of each.

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