Carbohydrate Research, 76 (1979) 239–244

Note

Laser-Raman spectroscopic study of cyclohexaamylose and related compounds: spectral analysis and structural implications

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(Received March 26th, 1979; accepted for publication, April 17th, 1979)

Like proteins, macromolecular carbohydrates have complex conformations that partake in determining their physical, chemical, and biological activities[1]. Unfortunately, the conformations of carbohydrates have been less studied than those of other biological macromolecules. Recently, laser-Raman spectroscopy has been applied to the structural study of carbohydrates[2–10], but in contrast to the relatively few studies by Raman spectroscopy, carbohydrates have been extensively studied by infrared spectroscopy[11]. As Raman and infrared spectroscopy are complementary, infrared data are valuable in Raman spectroscopy.

Vibrational modes of carbohydrates are complex, and the interpretation of spectra is often difficult. In view of this complexity, it is advisable to examine the vibrational modes of the simpler carbohydrates first. For this study, we selected cyclohexaamylose (Schardinger α-dextrin), a cyclic compound composed of 6 α-D-glucopyranosyl residues. As it is cyclic, there is no free, hemi-acetal hydroxyl group; and all of the D-glucosidic linkages involved are α-D. We have compared the Raman spectrum of cyclohexaamylose with those of the related compounds maltotriose and maltose. Structural implications of the Raman spectra for these compounds are discussed.

EXPERIMENTAL

Materials. — Maltose hydrate, maltotriose, and cyclohexaamylose were purchased from Sigma Chemical Company.

Laser-Raman spectroscopy. — Laser-Raman spectra were obtained by excitation with the 514.5-nm green or 488.0-nm blue light of an argon-ion laser (Spectrophysics, model Sp-164) having a green or blue interference filter, and were recorded with a Spex Ramalog 5 instrument. Samples were examined in the solid phase (in powder form). For deuteration, a sample was dissolved in deuterium oxide. For complete deuteration, the solution was allowed to stand for 2 h or more, and then lyophilized, and the lyophilized sample was redissolved in deuterium oxide. Finally, the relyophilized sample was compressed into pellets. For Raman spectroscopy, this sample was placed in a specially made glass chamber, saturated with deuterium oxide vapor in order to prevent proton re-exchange.

RESULTS AND DISCUSSION

Glycosidic linkages. — Both infrared and Raman spectroscopy can be used to identify the types of glycosidic linkage. The equatorial C–H deformation vibration (bending vibration) for α-D anomers in the $^4C_1$(D) conformation appears at 865–837 cm^{-1}, and that of β-D anomers at 905–887 cm^{-1} in Raman spectra[10]. Similar results are obtained by infrared spectroscopy[12]. Therefore, it was of interest to examine the glycosidic linkages of the compounds investigated.

For maltose, the band at 854–850 cm^{-1} is definitely an indication of the pre-

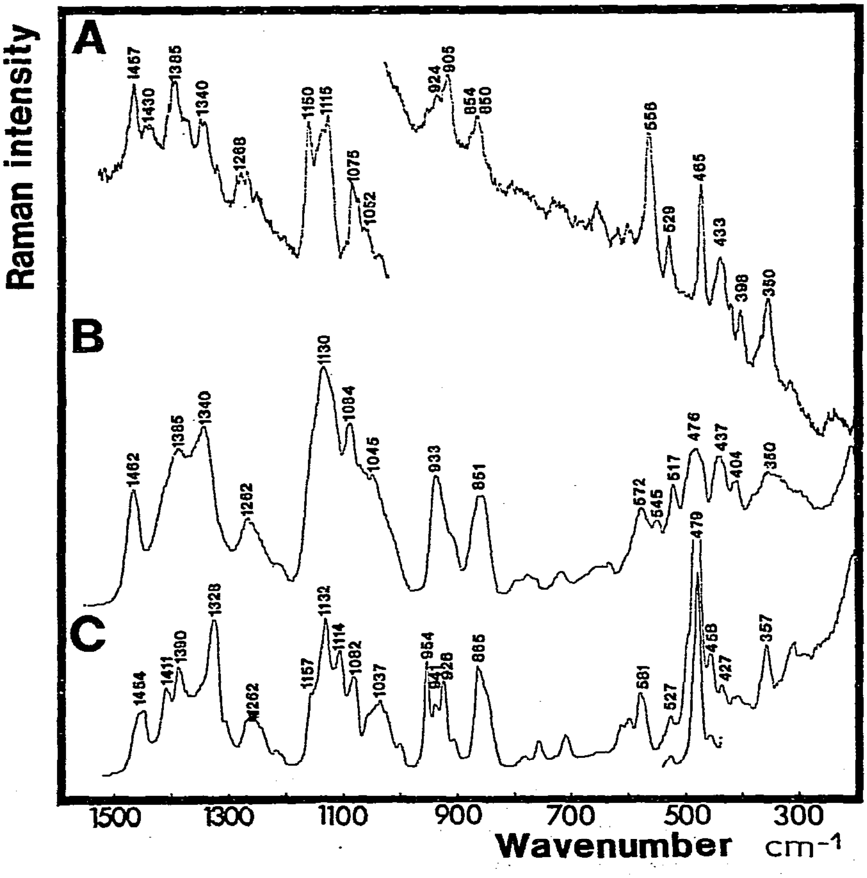

Fig. 1. Laser-Raman spectra of A, maltose; B, maltotriose; C, cyclohexaamylose in the range of 2000 to 1700 cm^{-1} (radiant power, A 220, B 100, and C 100 mW; slit width, A 500, B 300, and C 500 μm; integration time, 0.2 s; scanning speed 0.2 cm^{-1}/s).

sence of an α-anomeric linkage. Repeated scans indicated that this is actually one peak rather than the doublet shown in Fig. 1,A; the apparent doublet of 854 and 850 cm^{-1} is due to a pen-noise artifact of the spectrometer recorder. The band at 905 cm^{-1} indicates the β anomer of maltose. For maltotriose, a prominent band at 860–851 cm^{-1} (see Fig. 1,B) is a clear indication of the α anomer, and the lack of a clear band in the region of 900–890 cm^{-1} supports this conclusion. The spectrum of cyclohexaamylose (see Fig. 1,C), a cyclic compound that contains α-D-linkages exclusively, has a prominent band at 865 cm^{-1}. Again, the present investigation confirms the usefulness of Raman spectroscopy for the determination of glycosidic linkages.

Analysis of spectra below 1500 cm^{-1}. — In the spectrum of any carbohydrate, there are numerous bands below 1500 cm^{-1} that are due to highly coupled vibrations, rather than to characteristic group-frequencies.

For all three compounds investigated, prominent bands were observed at 1457 cm^{-1} (for maltose), 1462 cm^{-1} (for maltotriose), and 1454 cm^{-1} (for cyclohexaamylose.) The band arises from C–H bending vibrations.

The spectra in the region of 1440–1320 cm^{-1} are mixtures of C–C stretching vibrations, C–O–H bending vibrations, and CH_2 deformations. For maltose, 6 major bands; for maltotriose, 2 bands; and for cyclohexaamylose, 3 bands are observed. The bands for maltotriose are more similar to those of cyclohexaamylose than to those of maltose.

In the region of 1200–1000 cm^{-1}, the spectrum of maltotriose is very similar to that of cyclohexaamylose. This region originates from the C–O–H stretching vibrations and OH deformations. The spectrum of maltose differs from those of the other two compounds.

The C–O–C vibration involving α-D-(1→4)-linkages appears in the vicinity of 960–920 cm^{-1}. In this region, cyclohexaamylose exhibits marked differences from maltose and maltotriose. In the spectrum of cyclohexaamylose, three distinct bands, at 954, 941, and 926 cm^{-1}, are observed, whereas the other two compounds show only one band in this region. This seems reasonable as cyclohexaamylose contains 6 such linkages. Multiplicity of the band in this region may indicate that not all of the C–O–C bonds in cyclohexaamylose are equivalent. The spectrum of α-D-glucopyranose shows not a single band in this region[6]; this is reasonable, as this monomer contains only a ring C–O–C, and no C–O–C of a (1→4)-linkage.

Ring vibrations. — It has been shown[6,7,10] that monosaccharides show many Raman bands in the region below 600 cm^{-1}. Similarly, maltose, maltotriose, and cyclohexaamylose also show complex bands in the regions of lower frequency. These bands arise from complex modes of skeletal and torsional vibrations, commonly called the ring vibrations. Most of these low-frequency bands in Raman or infrared spectra have not been extensively studied, because of difficulty in assigning vibrational modes. However, Hineno[13] recently made detailed assignments for low-frequency, infrared bands of α-D-glucopyranose; he analyzed the vibrational bands of the 500–72-cm^{-1} region by normal coordinate calculations, and found that the vibrational

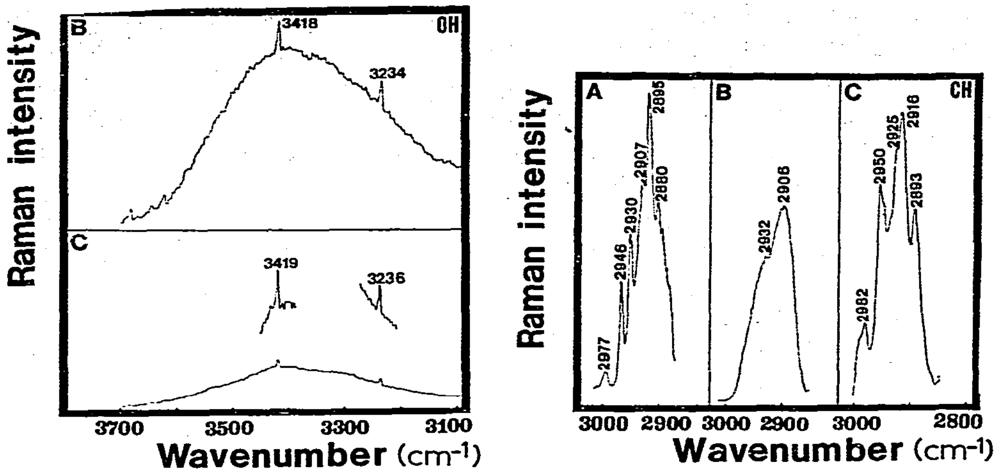

Fig. 2. Left: Laser-Raman spectra of -OH stretching vibration for B, maltotriose; C, cyclohexaamylose (radiant power, 100 mW; slit width, 300 μm; integration time, 0.2 s; scanning speed 0.2 cm^{-1}/s). Right: Laser-Raman spectra of -CH stretching vibration for A, maltose; B, maltotriose; C, cyclohexaamylose (radiant power, 100 mW; slit width, 300 μm; integration time, 0.2 s; scanning speed, 0.2 cm^{-1}/s).

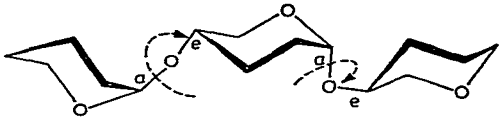

Fig. 3. Possible rotational isomers of maltotriose along the C-1–O–C-4 bonds [α-D-(1→4)-glucosidic linkage]. The symbol *a* denotes an axial bond, and *e* an equatorial bond.

frequencies calculated agreed well with the observed frequencies in the region above 250 cm^{-1}.

Hydroxyl group and C–H stretching vibrations. — Because maltose is hydrated, the -OH vibration in the region of 3700–3100 cm^{-1} is completely masked, and so this region of the spectrum could not be analyzed. Maltotriose and cyclohexaamylose have almost identical spectra in this region (see Fig. 2); they show a broad band from 3700 to 3100 cm^{-1}, with two small, but distinct, bands at 3418 and 3234 cm^{-1}. After deuteration, the broad band in the region of 3700 to 3100 cm^{-1} shifted to the 2700–2400-cm^{-1} region. The frequency ratio $\nu3400/\nu2500$ is 1.36, which is close to the predicted ratio of 1.41 (*i.e.*, $\sqrt{2}$). This indicates that the broad band originally observed is, indeed, that for the -OH vibration. Although the hydroxyl-group vibration is very similar for maltotriose and cyclohexaamylose, the -CH stretching vibration of maltotriose is not similar to that of cyclohexaamylose (see Fig. 2).

Structural implications. — All three compounds investigated consist of D-glucopyranose units, and so, it is reasonable that many of their spectral features should be similar. Yet structural differences exist among these compounds. For instance, there is the possibility of a number of rotamers along the C-1–O–C-4 axis for maltose, but not for cyclohexaamylose (as all of the D-glucopyranosyl residues are locked into a

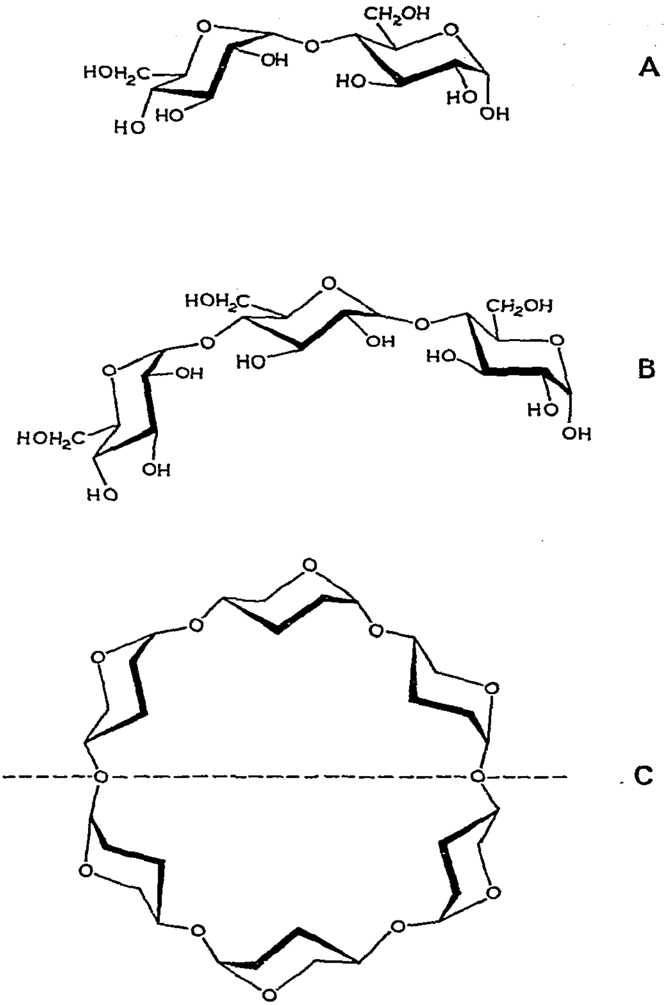

Fig. 4. Structure of A, maltose; B, maltotriose; C, cyclohexaamylose. (Note that a maltotriose molecule is exactly half the size of that of cyclohexaamylose, and it is very likely that it has a conformation similar to that of half of cyclohexaamylose.)

ring structure); therefore, it is not surprising that there are some differences in their spectra.

If there are free rotations along the glycosidic-linkage axes, a great number of rotamers are possible for maltotriose (see Fig. 3), and it would be expected to give a Raman spectrum more complicated than that of cyclohexaamylose. For the latter, there is probably only one conformation (due to the restriction of rotation because of its cyclic nature). However, the Raman spectrum of maltotriose is no more complex than that of cyclohexaamylose. By careful examination of the three carbohydrate spectra, it was found that the spectrum of maltotriose is, overall, more similar to that of cyclohexaamylose than to that of maltose, suggesting that the trisaccharide probably exists in a conformation very similar to that of cyclohexaamylose. Instead of a D-glucose unit rotating freely around the C-1–O–C-4 axis, maltotriose probably has a somewhat fixed conformation, like that of half of the conformation of cyclohexaamylose (see Fig. 4).

ACKNOWLEDGMENTS

This research was supported by NIH grant GM 19172. Thanks are extended to Dr. Y. C. Lee for his valuable suggestions and advice in many phases of this research.

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