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From the gas phase to aqueous solution: Vibrational spectroscopy, Raman optical activity and conformational structure of carbohydrates

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Abstract

Structural investigations of isolated and hydrated glucose, galactose and lactose structures in the gas phase based upon infra-red ion dip (IRID) spectroscopy conducted at low temperatures, are linked with parallel investigations conducted in aqueous solution at 298 K based upon measurements of their vibrational Raman and Raman optical activity (ROA) spectra. 'Basis sets' of computed (gas phase) Raman and ROA spectra associated with their low-lying conformational structures are used to construct 'weighted sums' which can approximate their experimental Raman and ROA spectra recorded in solution and provide estimates of their conformational population distributions in aqueous solution at 298 K; they are compared with estimates based upon analysis of NMR measurements and molecular mechanics and molecular dynamics calculations. The altered conformational preferences in the singly hydrated complexes of glucose and galactose isolated in the gas phase, appear to be sustained in aqueous solution, supporting the view that explicit hydration provides a key influence on their conformational preferences in solution. On the other hand, the conformational preference of the isolated β(1 → 4) disaccharide, lactose which resists conformational alteration when singly hydrated, is altered when it is transferred to aqueous solution at 298 K. The computational evidence suggests the control is exerted by entropic effects associated with a loosening of the structure around the glycosidic linkage.

Keywords: Raman; Carbohydrate; Conformation; Hydration

1. Introduction

There are many approaches to exploring biomolecular structures and the forces which control them: through investigations from the 'top down' – in situations approximating the natural environment; through measurements conducted in vitro in condensed phase environments; or through measurements from the 'bottom up' – in the gas phase, isolated from the environment. The differing strategies, environments and degrees of molecular size and complexity, tend to define individual scientific communities addressing related issues but evolving along complementary paths (often with different sets of technical terms and tacit assumptions).

The gas phase communities include:

- optical spectroscopists, using vibrational or rotational spectroscopy coupled with density functional theory or ab initio computation, to explore the structures and non-bonded interactions in mainly small, neutral biomolecules and their hydrated complexes, see for example [1,2],
- mass spectrometrists, using ion mobility studies coupled with gas kinetic theory to characterize the shape and 'size' of larger polymeric biomolecular ions and their molecular complexes, see for example [3,4].

The condensed, solution phase communities include, primarily:

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- NMR spectroscopists, using nuclear Overhauser or residual dipolar coupling methods, see for example [5,6], to determine the solvated structures of both neutral and ionic molecules, again aided by computation, particularly molecular mechanics/dynamics simulations [6–8],
- chiroptical spectroscopists, exploiting the chirality of virtually all molecules of biological importance and using their electronic or vibrational circular dichroism [9,10] or vibrational Raman optical activity (ROA) [9–12], to explore their preferred conformational structures.

Each community depends very strongly upon:

- computational chemists, employing quantum mechanics, molecular mechanics or molecular dynamics methods to provide the menu of structural possibilities from which Nature, and the experimentalists make their choice.

The challenge facing physical scientists, who adopt the reductionist, 'bottom up' approach is to establish links between the investigation of structure and molecular interactions in individual biomolecules and within their molecular, particularly hydrated, complexes isolated in the gas phase; their solvated structures in the condensed phase, particularly in aqueous solution; and ultimately, their architecture and function in biomolecular assemblies in the organised 'bio-phase'.

The present article describes an experimental strategy designed to address the first stage of this challenge—building a bridge linking the computational and spectroscopic investigation of isolated biomolecular structures in the gas phase, based upon infra-red ion dip (IRID) spectroscopy conducted at low temperatures, with parallel investigations conducted in aqueous solution at ambient temperature based upon measurements of their vibrational Raman and ROA spectra. A recent trial step across this bridge [13] compared the experimental vibrational ROA spectrum of 1-(*R*)-phenyl ethanol, both pure and in an aqueous solution, with its 'gas phase' ROA spectrum computed ab initio, and the molecular structures of the isolated molecule and its singly and doubly hydrated complexes, determined through IRID spectroscopy [14]. Many features in the experimental ROA spectrum were reproduced in the 'gas phase' spectrum, indicating retention of the essential core molecular conformation in the condensed phase. The absence or very low intensity of a small group of bands in the experimental spectrum of the 'neat' alcohol, all associated with large amplitude coupled C_α–H and O–H deformation of the hydroxyl-ethyl group, also implicated an influence of inter-molecular hydrogen bonding on its ROA spectrum. This trial investigation has now been developed to address the influence of the aqueous environment, and the temperature, on the conformational preferences of carbohydrate molecules.

The vibrational ROA spectra of carbohydrates present rich and informative band structure over a wide spectral range, containing information on the ring conformation of monosaccharides, the relative disposition of OH groups around the ring, the absolute configuration and axial or equatorial orientation of groups attached to the anomeric carbon, and the conformation of the exocyclic CH_2OH group [15]. ROA spectra of disaccharides contain, in addition, information on the type and conformation of the glycosidic link [16]. In the present study, comparisons are made between the conformational preferences of the disaccharide lactose and its two monosaccharide units, glucose and galactose, (1) determined from their IRID spectra, both free [17–19] and in hydrated complexes [20], measured at low temperature in the gas phase; (2) their relative energies (0 K) and free energies (at 300 K) computed ab initio; (3) their Raman and ROA spectra, measured in the aqueous phase at 300 K; (4) 'basis sets' of computed (gas phase) Raman and ROA spectra associated with the low-lying conformational structures populated (or potentially populated) in the free and hydrated carbohydrates; (5) their 'weighted sum' and experimental Raman and ROA spectra, which provide estimates of their conformational population distributions in aqueous solution at 300 K; (6) estimates based upon analysis of NMR measurements and molecular mechanics and molecular dynamics calculations [6,21].

2. Experimental and computational procedures

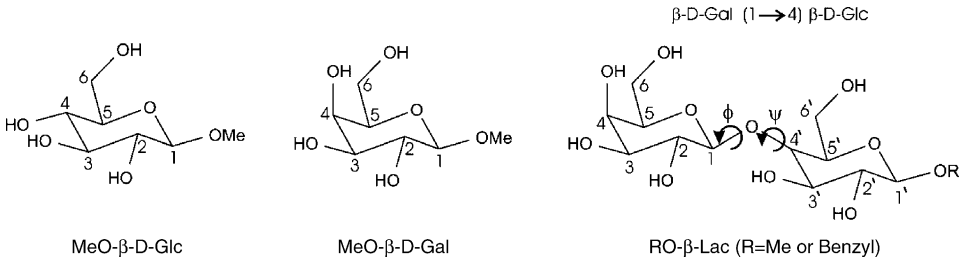
2.1. Raman optical activity

The Raman and ROA spectra were measured in Glasgow using the Chiral*RAMAN* scattered circular polarization (SCP) spectrometer (BioTools, Inc.) described previously [22]. They are displayed in analog-to-digital counter units as a function of the Stokes wavenumber shift with respect to the exciting laser wave number, and are presented as circular polarization intensity differences ($I_R - I_L$), where I_R and I_L denote the intensities of the right- and left-circularly polarized components, respectively, of the scattered light. The parent Raman spectra are presented as corresponding circular polarization intensity sums ($I_R + I_L$).

The carbohydrates were purchased from the Sigma–Aldrich Company Ltd. and used without purification. Samples were prepared by dissolving the compounds in de-ionized water at concentrations of 0.75 M (monosaccharides) and 0.5 M (lactoses) and left to equilibrate for 24 h before being filtered through 0.22 μm Millipore filters into quartz micro-fluorescence cells and then centrifuged for 10 min. Visible fluorescence from traces of impurities, which can contribute to the background in Raman spectra, was quenched by leaving the samples to equilibrate in the laser beam before acquiring ROA data. The experimental conditions were as follows: laser wavelength 532 nm; laser power at the sample, ~500 mW; spectral resolution, ~10 cm^{-1}; acquisition times 3–4 h.

2.2. Ab initio and DFT calculations

Quantum chemical calculations were performed using the Gaussian 03 package [23]. Initial structures were based on previously determined structures of phenyl or benzyl tagged carbohydrates [17–20]. Geometry optimisations and calculations of harmonic vibrational frequencies, Raman and ROA intensities (at 532 nm) were carried out using density functional theory (the B3LYP functional) with a 6-31+G^* basis set. Single point MP2 calculations with a larger basis set (6-311++G^{**}) provided



Scheme 1. Mono- and di-saccharide structures.

relative energies. Zero-point and free energy corrections to the electronic energy were calculated using the harmonic frequencies. Computed Raman and ROA spectra were convoluted using bandwidths of 20–25 cm^{-1} to account for temperature broadening.

2.3. Notation

The structures of the mono- and disaccharides selected for detailed investigation, namely methyl β-D-gluco-pyranoside (MeO-β-Glc), methyl β-D-galacto-pyranoside (MeO-β-Gal), and methyl (and benzyl)[3] lactoside, *O*-4-methyl β-D-Gal-(1 → 4)-β-D-Glc, (MeO-β-Lac, BzO-β-Lac) are shown in Scheme 1. (In Glc all the peripheral OH groups are equatorial; in Gal, OH4 is axial.)

Their flexibility can generate a huge range of alternative potential conformations (very few of which are populated in practice) and several alternative notations can be used to describe them. In the present work these are restricted for simplicity, to the following:

(a) the symbols 'c' and 'cc' are used to denote the conformation of the peripheral hydroxyl groups attached to the pyranoside ring, oriented through co-operative H-bonding into clockwise 'c' or counter-clockwise 'cc' chains, e.g., OH2 → OH3 → OH4 or its inverse.
(b) The symbols G, g and T, t denote the orientation of the exocyclic hydroxymethyl groups—represented by the dihedral angle O6–C6–C5–O5, which can adopt Gauche (G+ or G−) or Trans (T) orientations, and its terminal OH6 group—represented by the angle H6–O6–C6–C5, which can adopt g+, g− or t orientations.
(c) The relative disposition of the Gal and Glc units about the glycosidic linkage in lactose is described by the two dihedral angles, ϕ and ψ, defined as ϕ(H1Ga1–C1Gal–O1Gal–C4Glc) and ψ(C1Gal–O1Gal–C4Glc–H4Glc).

The $^4C_1$ chair conformation of the pyranose rings, shown in Scheme 1, is supported by a very wide range of experimental and computational data [24].

3. Results and discussion

3.1. Methyl β-D-glucoside and β-D-galactoside

Calculations conducted at the MP2/6-311++G**//B3LYP/6-31+G* level of theory, have been used to explore the conformational landscapes of the two methyl monosaccharides isolated in the gas phase. The calculated structures of their lowest-lying conformers are displayed in Fig. 1: they parallel those of their phenyl 'tagged' analogues, PhO-β-Glc and PhO-β-Gal,[4] the structures of which have been identified experimentally, through IRID spectroscopy measurements conducted at low temperature in the gas phase [17,18,20]. Their calculated relative energies (0 K) and free energies (298 K) are listed in Table 1, where they can be compared with the results of earlier DFT estimates, conducted at the B3LYP/6-311++G**//B3LYP/6-31G* level [21].

IRID measurements of the two phenyl glycosides have established the population of three low-lying structures in a jet-cooled gas phase environment at ∼10 K, associated with the conformations ccG+g−, ccG-g+(PhO-β-Glc) or cG-g+(PhO-β-Gal) and ccTg+ [17,18,20]. The conformation, ccG+g− is by far the most abundant, in broad agreement with the ab initio predictions for both the phenyl and methyl glycosides (but not the earlier DFT predictions [21]), and the relative populations of the minor conformers follow the ordering of their computed relative energies (at 0 K) and also their free energies (at 298 K).

IRID and computational investigations of the mono-hydrate structures of the two phenyl glycosides, stabilized at low temperature in the gas phase have revealed the strong influence that explicit water binding can have on their conformational preferences [20,24]. In PhO-β-Glc(H_2O) and PhO-β-Gal(H_2O), the water molecule inserts into the weakest links in the encircling H-bonded chain of OH groups and the hydroxymethyl group can be rotated away from its preferred G+ orientation to promote a 'sympathetic switch' in their orientation, from counter clockwise to clockwise. In PhO-β-Glc(H_2O) the cG−g+ conformation becomes the more strongly favoured, and global minimum structure; in PhO-β-Gal(H_2O) both the cG−g+ and ccG+g− conformations are strongly populated. At low temperature, their conformational preferences are driven by the need to maximize the length of the cooperatively linked chain of intra- and inter-molecular hydrogen bonds, see Fig. 2 [20].

[3] The conformational preference of the 'benzyl-tagged' disaccharide has already been determined in the gas phase at low temperatures, ∼10 K, through IRID measurements [19]. The tag, which provides the UV chromophore required in the IRID resonant two photon ionization detection scheme, has a negligible effect on the structure of the disaccharide to which it is attached.

[4] Again, the phenyl tag, which is needed to provide the UV chromophore for the IRID detection scheme, does not significantly alter the conformational structure of the mono-saccharide unit [17,18].

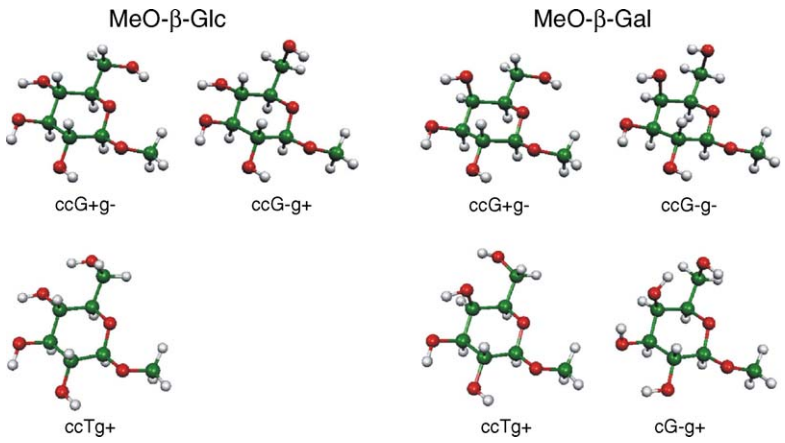


Fig. 1. Computed conformational structures of the lowest-lying conformers of methyl β-D-glucose (MeO-β-Glc) and methyl β-D-galactose (MeO-β-Gal); their relative energies are listed in Table 1.

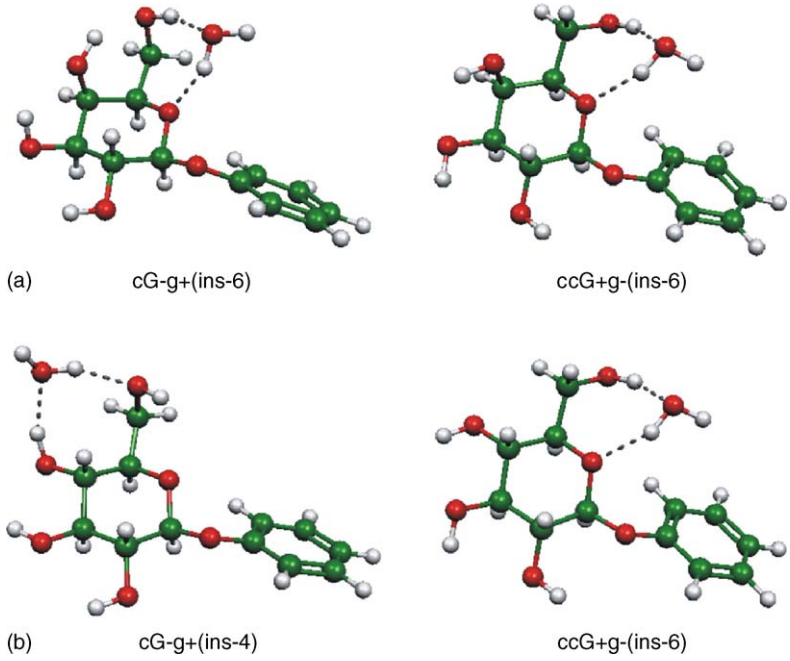


Fig. 2. Preferred structures for the singly hydrated complexes of (a) phenyl-β-galactopyranoside and (b) phenyl-β-glucopyranoside [20].

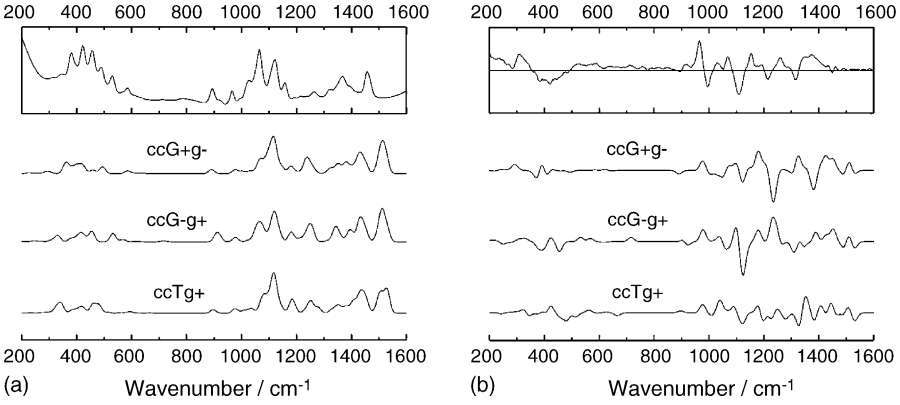


Fig. 3. Experimental (aqueous solution, 298 K) and computed ('gas phase') Raman (a) and ROA (b) spectra of methyl-β-D-glucopyranoside. Top row: experimental spectra. Bottom rows: unscaled computed spectra of individual low-lying conformers.

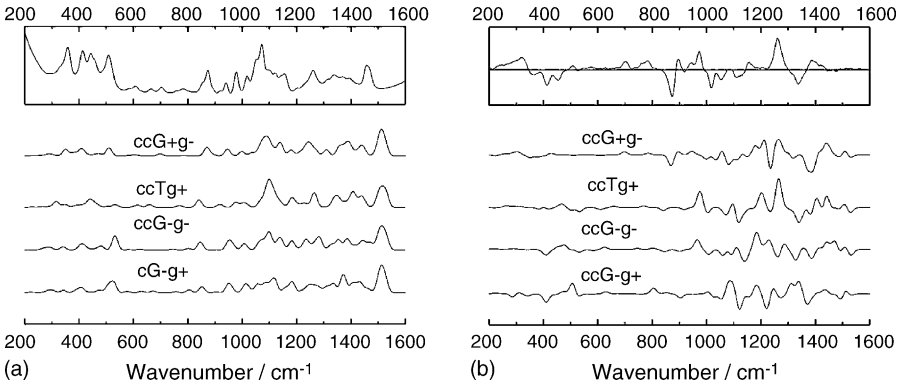


Fig. 4. Experimental (aqueous solution, 298 K) and computed (gas phase) spectra of methyl-β-D-galactopyranoside: (a) Raman and (b) ROA. Top row: experimental spectra. Bottom rows: unscaled computed spectra of individual low-lying conformers.

The Raman and ROA spectra of the two methyl glycosides, MeO-β-Glc and MeO-β-Gal, recorded in aqueous solution at 300 K are shown in Figs. 3 and 4, together with the computed individual ('gas phase') Raman and ROA spectra associated with their lowest-lying conformations, calculated through a combination of ab initio and DFT calculations and including the conformers identified in the analogous gas phase experiments conducted at low temperatures. The computed spectra, particularly the ROA spectra, are clearly sensitive to conformational change though none of them separately, is able to provide a good match with the ROA or Raman spectra recorded experimentally, in aqueous solution at 298 K. Is it possible however, that they might provide basis sets for appropriately weighted combinations that would generate a convincing match with the experimental Raman and ROA spectra? The assumption would be that, apart from an increase in their spectral bandwidth in solution, the elements of the basis set, but not their weights were unaltered by the changed environment, gas phase to aqueous solvent, and temperature, ~10 to 298 K.

Comparisons between the computed and experimental Raman and ROA spectra, guided by the results of the IRID and computational investigations of the phenyl tagged glycoside mono-hydrates in the gas phase [20], are shown in Figs. 5 and 6. The 'summed basis set' spectra, generated assuming the relative weightings, cG−g+ (0.5) and ccG+g− (0.5) for MeO-β-Glc, and ccG+g− (0.5), ccTg+ (0.2), cG−g+ (0.15) and ccG−g- (0.15) for MeO-β-Gal, provide a much better match between the experimental and the computed spectra (particularly for MeO-β-Glc) and the assumed weights cannot be too far from a 'best fit' choice. They can be compared with the range of estimates that have been based upon NMR measurements in aqueous solution, summarized in Ref. [21], which provide relative populations associated with the three hydroxymethyl rotamers, G−, G+ and T. The NMR estimates lie within a broad range but those for MeO-β-Glc, which are centred around the values G− (~0.5), G+ (~0.5), T (~0), are in good agreement with the present estimate; for MeO-β-Gal however, the NMR estimates, G− (~0.6), G+ (~0.2), T (~0.2), are in poorer agreement with those based upon ROA measurements, G− (~0.3), G+ (~0.5), T (~0.2). In both cases, the relative populations based upon ROA measurements in aqueous solution at 298 K are comparable with those of the (explicitly hydrated) G+g− and G−g+ mono-hydrate structures (of the phenyl analogues shown in Fig. 2) in the gas phase at low temperature. In the absence of explicit hydration the G− conformation is barely detectable [20].

Table 1
Relative energies, ΔE_0 (0 K) and free energies, ΔG (300 K)/kJ mol^{-1} of the lowest-lying conformers of methyl and phenyl β-D-glucose and galactose calculated ab initio at the MP2/6-311++G**//B3LYP/6-31+G* level

Conformer	ΔE_0 (0 K)			ΔG (298 K)	
	MeO-β-Glc	Ref. [21][a]	PheO-β-Glc	MeO-β-Glc	Ref. [21][a]
ccG+g−	0.4	0.0	0.0	0.0	0.0
ccG−g+	0.0	8.8	0.3	0.3	5.2
ccTg+	2.9	0.0	4.5	3.7	5.6
Conformer	ΔE_0 (0 K)			ΔG (298 K)	
	MeO-β-Gal	Ref. [21][a]	PheO-β-Gal	MeO-β-Gal	Ref. [21][a]
ccG+g−	0.0	2.8	0.0	0.0	4.2
ccTg+	2.9	0.0	4.8	3.1	2.9
ccG−g−	5.0	–	6.0	6.6	–
cG−g+	8.2	18.9	6.6	9.3	16.8

Relative energies include zero point energy correction; free energies calculated using harmonic vibrational frequencies.
[a] Reported in this reference on the basis of DFT calculations.

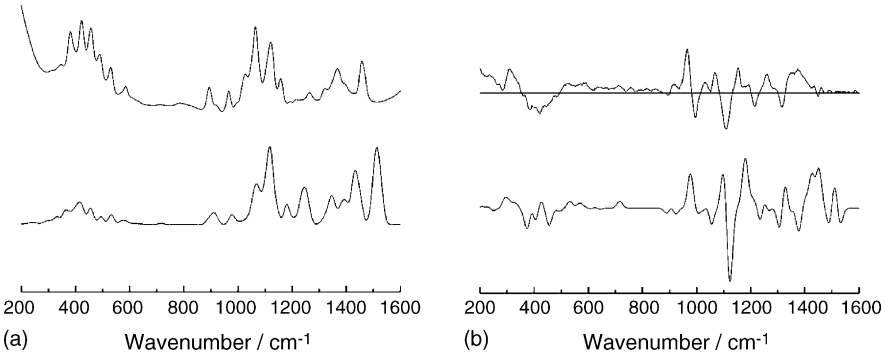


Fig. 5. Comparison between the experimental (upper trace) Raman (a) and ROA (b) spectra of methyl-β-D-glucopyranoside recorded in aqueous solution (298 K), and the computed spectra (lower trace) generated by summing the 'gas phase' basis set components associated with the conformers, ccG+g− and cG−g+. 'Gas phase' line widths, 25 cm^{-1}; relative weightings, ccG+g−:ccG−g+ = 0.5:0.5. Computed frequencies not scaled for anharmonicity.

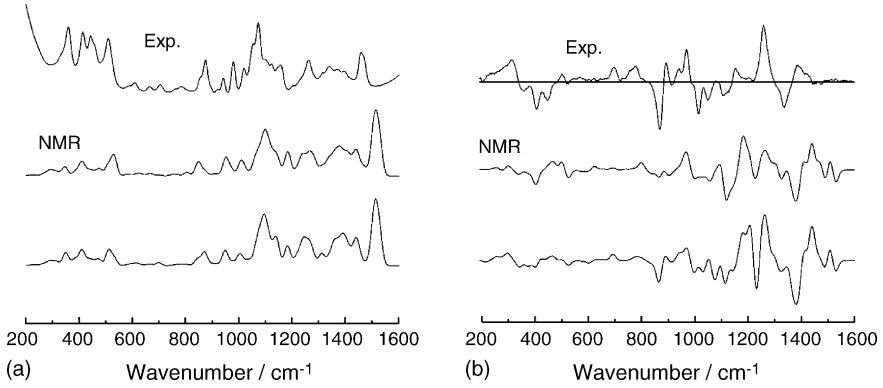


Fig. 6. Comparison between the experimental Raman (a) and ROA (b) spectra of methyl-β-D-galactopyranoside recorded in aqueous solution (298 K), and the computed spectra generated by summing the 'gas phase' basis set components associated with the conformers, ccG+g−, ccTg+, ccG−g− and cG−g+. 'Gas phase' line widths, 25 cm^{-1}; relative weightings, ccG+g−:ccTg+:ccG−g−:cG−g+ = 0.5:0.2:0.15:0.15, cf. 'NMR', G+:G−:T ∼ 0.2:0.6:0.2. (Note the negative feature at 870 cm^{-1} which signals an enhanced contribution from the G+ conformer in the ROA spectrum.) Computed frequencies not scaled for anharmonicity.

4. Lactose

An IRID determination of the conformational structure of the benzyl-tagged lactoside, *O*-4-benzyl β-D-Gal-(1 → 4)-β-D-Glc (BzLac), has established the predominant population of a single conformer only, in the gas phase at low temperature in a free jet expansion. Comparisons between its near and mid-IR spectra and the IR spectra of its lowest-lying conformers computed ab initio, identified it as the global minimum energy structure **1** shown in Fig. 7, which also shows the computed structures of

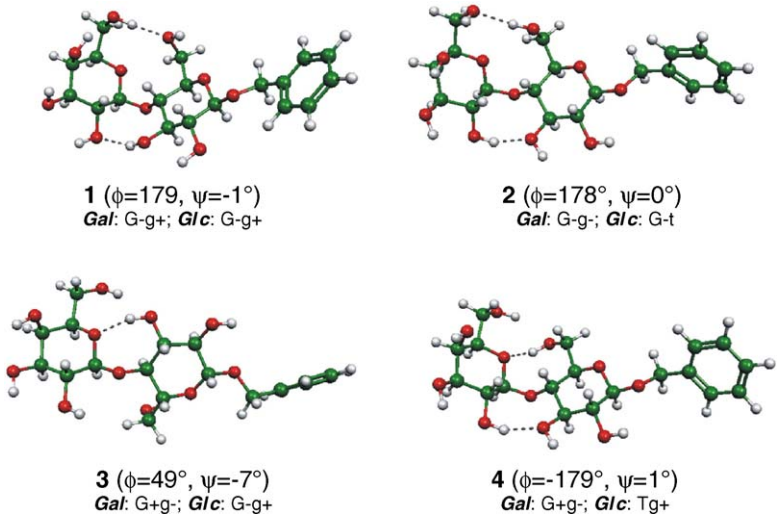


Fig. 7. Conformational structures of the four lowest lying conformers of benzyl lactoside, and of the hydroxymethyl groups associated with its component monosaccharides [19] (relative energies and free energies listed in Table 2).

Table 2
Calculated relative energies, ΔE_0 (0 K), enthalpies, ΔH (298 K) and free energies, ΔG (298 K)/kJ mol^{-1} of benzyl lactoside [19]

Conformer[a]	ΔE_0 (0 K)	ΔH (298 K)	ΔG (298 K)
1	0.0	0.0	2.0
2	2.7	3.4	3.2
3	8.4	12.6	0.0
4	10.4	11.8	9.6

Relative energies: MP2/6-311++G** single point, calculated at B3LYP/6-31+G* geometry including B3LYP zero point energy; relative enthalpies and free energies using MP2 energies and B3LYP harmonic frequencies.
[a] Conformational structures shown in Fig. 7.

its energetically nearest neighbours [19]; their relative energies (0 K) and free energies (298 K) are listed in Table 2.

In contrast to the two mono-saccharides, an increase in temperature is predicted to influence the relative conformational populations: at 298 K the minimum free energy structure is predicted to be the relatively flexible conformer **3** ($\phi = 49°$, $\psi = -7°$), rather than conformer **1** ($\phi = 179°$, $\psi = -1°$), which presents a more rigid structure involving two inter-ring H-bonds across the glycosidic linkage.

The rotation about the glycosidic dihedral angle ϕ, between conformer **1** and **3**, allows the hydroxymethyl group in the Gal residue to relax from the G−g+ (**1**) to the G+g− (**3**) conformation but the G−g+ conformation is retained in the Glc residue. At elevated temperatures, the calculations predict compensation of the increased enthalpy of conformer **1** by the entropic gain associated with the more flexible conformation, **3**. A new NMR investigation of the conformational structure of lactose in aqueous solution at 298 K [6], based upon measurements of residual dipolar couplings combined with molecular dynamics calculations, has established the almost exclusive population of a conformation centred around the dihedral angles, $\phi \sim 49°$, $\psi \sim 13°$ (termed *syn* ϕ/*syn* ψ). This structure is closely akin to the calculated structure of conformer **3** ($\phi \sim 49°$, $\psi \sim -7°$) but nothing like that of conformer **1** ($\phi \sim 180°$, $\psi \sim 0°$) for which, in aqueous solution at 298 K, there was no experimental evidence [6].

The Raman and ROA spectra of methyl lactoside, recorded in aqueous solution at 298 K, are shown in Fig. 8, together with the experimental ROA spectra of benzyl lactose and lactose itself. The ROA spectra are all very similar: neither the phenyl nor the methyl tag has any significant effect on the lactose disaccharide conformation in aqueous solution which offers some relief to the (future) computationally demanding ab initio calculation of a conformational basis set of its individual ROA spectra. If the Gal(G+g−): Glc(G−g+) structure associated with conformer **3** of the lactoside predominates in aqueous solution at 298 K, the experimental ROA spectrum of methyl lactoside might be approximated by an equally weighted sum of the (appropriately broadened) computed spectra associated with the same pair of conformations, Gal(G+g−): Glc(G−g+), in its two component residues. Fig. 8 displays the comparison; the agreement between the two is striking. Fig. 8 also shows an ROA spectrum constructed by simply summing the experimental spectra recorded for the two methyl glycosides (each of which includes a spread of hydroxymethyl conformations). The level of agreement is noticeably degraded, particular in the low frequency region, below 600 cm^{-1} and also in the region ∼1000–1100 cm^{-1}.

Comparisons between molecular mechanics/molecular dynamics simulations of oligosaccharides in aqueous solution at 300 K and experimental NMR data have identified explicit hydration as a key determinant of oligosaccharide conformational preferences [7,8]. Almond however, has also identified a key distinction between α- and β-linked residues [7]: in the latter case, inter-residue hydrogen-bonding across the linkage remains persistent and is not disrupted by the aqueous environment. A similar conclusion has been reached in the case of the (computed) gas phase mono-hydrate of the (β-linked) methyl lactoside conformer **1** [19,24]; rather than disrupting the much stronger inter-residue linkages, the water molecule preferentially inserts into the weakest link in the H-bonded chain of OH groups, localized on the Glc residue. When this result is taken together with the relative free energies of (benzyl) lactoside at 298 K, it would suggest the change in the preferred disaccharide conformation is driven by entropic effects rather than by explicit hydration.

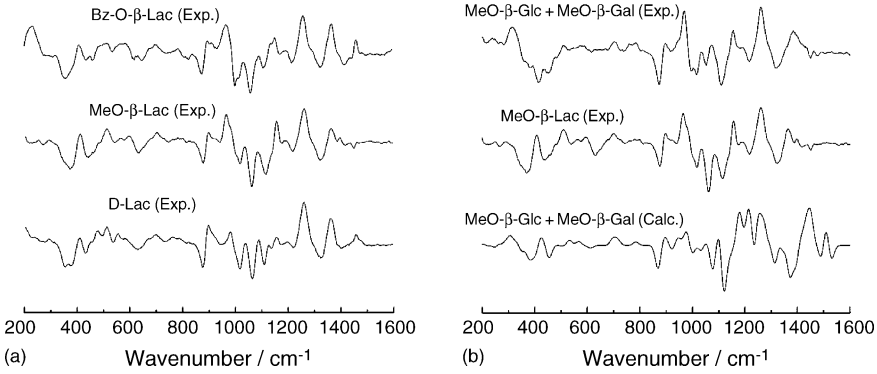


Fig. 8. (a) Raman and ROA spectra of lactose, and methyl and benzyl lactoside recorded in aqueous solution at 298 K. (b) A comparison between the 'summed' ROA spectra of MeO-β-Gal and MeO-β-Glc recorded in aqueous solution at 298 K; the ROA spectrum of methyl lactoside recorded under the same conditions; and an ROA spectrum generated by an equally weighted sum of the computed 'gas phase' components, MeOβ-Gal (ccG+g−) and MeO-β-Glc (ccG−g+), i.e., the monosaccharide unit structures corresponding to conformer **3** of the lactoside.

5. Conclusions

Many of the roles played by oligosaccharides and glycoconjugates in molecular cell biology are mediated through their inter-molecular interactions and these are controlled in part, by their dynamical three-dimensional structures. Determination of their conformational preferences is a demanding task, even when they are isolated in the gas phase at low temperature; determination of their hydrated structures under the same conditions is more demanding still [20]; and NMR determination of their structures in aqueous solutions is the most demanding task of all [7]—not least because of the long time scale and the consequent averaging of NMR measurements, and the need for reliable molecular dynamics simulations involving explicit hydration for their analysis and interpretation. Measurements of Raman spectra and Raman optical activity in contrast, provide a 'short-time snapshot' of the population of individual conformational structures but their interpretation has been constrained by limitations in the development of ab initio methods of sufficient accuracy. The recent lifting of this constraint however, [23,25] signals the 'coming of age' [11] of the ROA approach and it has allowed comparisons to be made between the conformational structures of carbohydrate molecules (and their mono-hydrates) isolated in the gas phase at low temperature and their structures in aqueous solution at 298 K.

A reductionist approach, which employs a basis set of computed ROA spectra based upon known conformational preferences in the gas phase, to model the summed ROA spectra measured experimentally in aqueous solution has proved surprisingly successful. The altered conformational preferences in the singly hydrated complexes of glucose and galactose isolated in the gas phase, appear to be sustained in aqueous solution, supporting the view that explicit hydration provides a key influence on their conformational preferences in solution. On the other hand, the conformational preference of the isolated $\beta(1 \rightarrow 4)$ disaccharide, lactose which resists conformational alteration when singly hydrated [20], is altered when it is transferred to aqueous solution at 298 K. The computational evidence suggests the control is exerted by entropic effects associated with a loosening of the structure around the glycosidic linkage [21].

There is much still to do. More efficient ways of computing the basis sets of ROA spectra need to be developed. The basis sets could be extended to include more low-lying and explicitly hydrated conformers to widen the structural library. One day perhaps, a way might be found of recording ROA spectra in the gas phase.

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