alcohols are bound by Hb^+ in the concentration range of our study. Steinhardt, *et al.*,[25] have noted that in the presence of trace amounts of alcohol the rate of acid denaturation is increased but their evidence also suggests no direct binding between alcohols and ferrihemoglobin.

Kotani, *et al.*,[26,27] have shown that they could generate a compensation line by consideration of the high spin–low spin equilibria as a function of ligand using both ferrimyoglobin and ferrihemoglobin. Studies here[24] also suggest that the pH dependence of ΔH° and thus of the compensation behavior is closely associated with the change in spin state. In each case the implication is that there is a part process of the total ligand-binding process which generates the compensation behavior and which can be modified to alter α but not T_c (eq 6) by the formation of different derivatives.

The aggregate of these studies suggests that the binding of ligands is significantly influenced by the nature of the environment outside the heme and very likely as much in the peripheral water as in the protein itself. In previous studies[5,6] the thermodynamic results have been explained by changes in the hydration sheath which accompanies changes in pH. It is likely that modifications in the hydration sheath tend to change the low spin–high spin equilibrium. The involvement of the low spin–high spin equilibrium in the binding of ligands to Hb^+ is suggested by our results. It is interesting to note that independent results from infrared spectroscopy of N_3^- binding to ferrimyoglobin and ferrihemoglobin[28] have been interpreted as showing that in the high spin complex the iron–N_3^- bond is "ionic" and in the low spin complex it is "covalent."

Possibly the ability of *tert*-butyl alcohol to change the magnetic susceptibility is a result of its influence on the structure of the hydration sheath. Studies to test this hypothesis are in progress.

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Calcium Binding to Carbohydrates. Crystal Structure of a Hydrated Calcium Bromide Complex of Lactose

Charles E. Bugg

Contribution from the Institute of Dental Research and Department of Biochemistry, University of Alabama in Birmingham, Birmingham, Alabama 35233.
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Abstract: The crystal structure of a hydrated calcium bromide complex of lactose (4-*O*-β-D-galactopyranosyl-D-glucopyranose) was determined by use of three-dimensional X-ray diffractometer data. Crystals of $C_{12}H_{22}O_{11} \cdot 7H_2O \cdot CaBr_2$ are orthorhombic, space group $P2_12_12_1$, with $a = 21.952$ (3), $b = 13.705$ (3), and $c = 8.792$ (4) Å. The structure was solved by the heavy-atom method and was refined by least squares to $R = 0.043$. The absolute configuration was confirmed by anomalous dispersion effects. The crystal structure contains a mixture of the α and β anomers of lactose, with an α/β ratio of about 88/12. An outstanding feature of the crystal packing is the interaction of lactose molecules with calcium ions. The calcium ion binds two lactose molecules and four water molecules. One lactose molecule is coordinated to the calcium ion through O(3) and O(4) of its galactose moiety, and the second is coordinated through O(2′) and O(3′) of its glucose moiety. Similar interactions probably account for the chelation of calcium ions by lactose in aqueous solution, and may be involved in the mechanism by which lactose, as well as other carbohydrates and polyols, increase intestinal absorption of calcium.

Interactions of calcium ions with carbohydrates have been implicated in such biological processes as calcium transport,[1,2] calcification,[3–7] cell–cell adhesion,[8,9] and binding of glycoproteins to cell surfaces.[10] It has been demonstrated that calcium ions complex with both uncharged[11] and anionic[6,12] carbohydrates in aqueous solution, but little is known about either the factors involved in calcium–carbohydrate interactions, or the stereochemistry of the resultant complexes. We are currently examining the crystal structures of calcium–carbohydrate complexes[13] to obtain information concerning the structural factors that govern calcium–carbohydrate interactions in biological systems. This paper describes the crystal structure of a hydrated calcium bromide complex of lactose (4-*O*-β-D-galactopyranosyl-D-glucopyranose).

It is established that lactose, a component of milk, can increase the rate at which calcium is absorbed from

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the gastrointestinal tracts of rats and humans.[14-21] Despite numerous investigations of lactose physiology,[14-21] the mechanism by which this sugar promotes calcium absorption remains obscure. However, since lactose and calcium must be ingested simultaneously to influence calcium metabolism, it is generally accepted that lactose exerts a local effect on calcium transport through intestinal membranes. Charley and Saltman demonstrated that lactose binds calcium ions in aqueous systems, and presented data to support the possibility that lactose–calcium complexes contribute to the mechanism by which lactose promotes calcium transport;[1] therefore, the calcium binding properties of this simple disaccharide are of particular significance.

Experimental Section

Clear, prismatic crystals of the complex were grown by evaporating an aqueous solution that contained an approximately equimolar mixture of α-lactose monohydrate and calcium bromide. The complex appears to be identical with that isolated by Herrington[22] and by Jensen, *et al.*[23] Weissenberg and oscillation photographs showed that the crystals are orthorhombic; the space group is $P2_12_12_1$, as indicated by the systematic absence of reflections $h00$ with h odd, $0k0$ with k odd, and $00l$ with l odd. A crystal fragment with approximate dimensions of 0.25, 0.20, and 0.15 mm was mounted on a Picker FACS-1 diffractometer with its a axis slightly inclined to the Φ axis of the diffractometer. Approximate cell parameters for use in collecting intensity data were calculated by a least-squares analysis of the angular settings for six high-angle reflections (Cu $K\alpha_1$, λ = 1.54051 Å).

Intensity data were collected with the diffractometer, by use of nickel-filtered copper radiation, a scintillation counter, and a θ–2θ scanning technique. Measurements were made for the 2905 reflections with $2\theta < 128°$. Three strong, medium-angle reflections were chosen as standards and were monitored periodically. During data collection, a decrease of about 15% in the intensities of these standard reflections indicated that crystal decomposition had occurred. Immediately after data collection, accurate values for the cell parameters were determined by a least-squares analysis of 2θ values for 13 high-angle reflections (Cu $K\alpha_1$); these cell parameters were not significantly different from those obtained prior to the measurement of intensities. Crystal data are listed in Table I.

The intensity values were scaled by a least-squares procedure in which the intensities of the standard reflections were used to calculate scale factors as a function of crystal exposure time. The intensities were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus a correctional term $(0.03S)^2$, S being the scan counts. The intensities and their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied by using the computer program ORABS.[24] Finally, the data were scaled by means of a Wilson[25] plot.

Table I. Crystal Data[a]

Stoichiometry	$C_{12}H_{22}O_{11} \cdot CaBr_2 \cdot 7H_2O$
Z	4
Space group	$P2_12_12_1$
a	21.952 (3) Å
b	13.705 (3) Å
c	8.792 (4) Å
ρ (calcd)	1.678 g cm^{-3}
ρ (obsd)	1.68 g cm^{-3}
μ (Cu Kα)	66.2 cm^{-1}

[a] The unit cell parameters were measured at 25 ± 3°. The density was measured by flotation in a benzene–ethylene dibromide mixture.

A suitable trial structure was obtained by the heavy-atom method: coordinates for one bromide ion were determined from a sharpened Patterson map; coordinates for the second bromide ion and the calcium ion were determined from a sum-function superposition of sharpened Patterson maps translated to the first bromide ion position; and the remaining nonhydrogen atoms were located in a Fourier map that was calculated by using phase angles derived from the three ions. The trial structure was refined by using a modified version of the full-matrix least-squares program ORFLS.[26] The quantity minimized was $\Sigma w(F_o^2 - F_c^2/k^2)^2$, where k is a scale factor and weight w is equal to $1/\sigma^2(F_o^2)$. Scattering factors for the nonhydrogen atoms were from the International Tables for X-Ray Crystallography,[27] and hydrogen atom scattering factors were from Stewart, *et al.*[28] Coordinates for those hydrogen atoms bonded to carbon atoms were calculated by assuming tetrahedral coordination around the carbon atoms and C–H bond distances of 0.9 Å. All those hydrogen atoms bonded to oxygen atoms, except two belonging to a water molecule (W-5) that exhibited excessive thermal motion, were located in difference Fourier maps that were calculated during the latter stages of refinement. The hydrogen atoms were assigned the isotropic temperature factors of the heavy atoms to which they are bonded, and were included in the calculation of structure factors but not in the least-squares refinement. The heavy-atom positional parameters and anisotropic temperature factors, as well as Zachariasen's[29] isotropic extinction parameter g (as formulated by Coppens and Hamilton[30]), were included in the refinement. Because of the limited core storage capacity of the computer it was impracticable to refine all parameters simultaneously; consequently, the heavy atoms were refined in alternating cycles, with about half the atoms in each cycle. As the refinement proceeded, the coordinates of the hydrogen atoms that are attached to oxygen atoms were improved by the use of difference Fourier maps.

Assuming that all of the lactose sites were occupied by the α anomer, I refined the structure to an R index $(\Sigma||F_o| - |F_c||/\Sigma|F_o|)$ of 0.045 and goodness-of-fit $\{[\Sigma w(F_o^2 - F_c^2/k^2)^2/(m - s)]^{1/2}$, where m is the number of reflections used and s is the number of parameters refined$\}$ of 2.66. At this stage, a difference Fourier map showed a residual peak of 0.7 e/Å^3 in the vicinity of atom H(Cl′); there were no other peaks in excess of 0.35 e/Å^3 except in the immediate vicinities of the calcium and bromide ions. The peak near H(Cl′) indicated that, like the crystal structure of lactose monohydrate,[31] this structure contains a mixture of the α and β anomers of lactose, with the residual electron density resulting from the O(1′) oxygen atom of the β anomer. Therefore, two positions that corresponded to αO(1′) and βO(1′) were assigned, and the population parameters of these two sites were included as parameters in the final refinement. The population parameters, which were refined independently and were not constrained to a total value of 1.0, converged to values of 0.866 (13) for the αO(1′) position and 0.110 (12) for the βO(1′) position, thereby indicating that 86–90% of the lactose molecules are in the α form. The final R index, including all reflections, is 0.043. The final goodness-of-fit is 2.55. During the final cycle of refinement, no parameter shifted more than one-fifth of its estimated standard deviation, except the z coordinate of βO(1′), which showed a shift equal to one-third of its standard

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Table II. Final Heavy-Atom Parameters and Their Standard Deviations[a]

ATOM	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Ca	92239(5)	54545(8)	96775(14)	00118(2)	00342(6)	01044(15)	-00002(3)	-00028(5)	-00085(8)
Br(1)	93349(3)	91391(6)	94642(11)	00159(2)	00571(5)	02163(16)	-00020(2)	00086(4)	00318(7)
Br(2)	69080(3)	57121(5)	101720(8)	00173(1)	00423(4)	01328(10)	-00008(2)	00077(3)	-00094(5)
LACTOSE									
C(1)	3631(2)	6901(4)	5990(7)	0013(1)	0025(3)	0093(8)	-0003(2)	0001(3)	0004(4)
C(2)	3376(2)	7477(4)	7319(6)	0013(1)	0031(3)	0077(7)	0003(2)	0006(2)	-0000(4)
C(3)	3519(3)	8544(4)	7111(7)	0015(1)	0028(3)	0076(7)	0004(2)	-0003(3)	-0001(4)
C(4)	4178(3)	8762(4)	6712(7)	0018(1)	0021(3)	0091(8)	0000(2)	-0001(3)	0003(4)
C(5)	4372(3)	8109(4)	5420(7)	0018(1)	0029(3)	0088(8)	-0004(2)	0005(3)	0001(4)
C(6)	5042(3)	8198(4)	5038(8)	0019(1)	0033(3)	0130(10)	-0004(2)	0016(3)	0006(5)
O(1)	3568(2)	5908(3)	6282(5)	0013(1)	0024(2)	0092(5)	-0002(1)	0002(2)	-0008(3)
O(2)	2742(2)	7362(3)	7423(5)	0012(1)	0039(2)	0144(7)	-0004(1)	0009(2)	-0006(4)
O(3)	3387(2)	9082(3)	8468(5)	0013(1)	0036(2)	0116(6)	0002(1)	0002(2)	-0018(3)
O(4)	4550(2)	8671(3)	8032(5)	0014(1)	0037(2)	0100(6)	0003(1)	-0006(2)	-0007(3)
O(5)	4265(2)	7099(3)	5836(5)	0012(1)	0024(2)	0094(5)	-0001(1)	0006(2)	-0001(3)
O(6)	5189(2)	7588(3)	3745(5)	0023(1)	0047(3)	0109(6)	-0001(2)	0015(2)	-0001(4)
C(1')	3756(3)	3300(4)	3862(8)	0012(1)	0037(3)	0154(11)	0001(2)	-0003(3)	-0022(5)
C(2')	4286(2)	3999(4)	3827(6)	0011(1)	0035(3)	0083(7)	0001(2)	-0000(2)	-0006(4)
C(3')	4244(2)	4725(4)	5129(7)	0011(1)	0022(2)	0105(8)	-0003(1)	0005(3)	-0002(4)
C(4')	3647(2)	5277(4)	4999(6)	0013(1)	0026(3)	0078(7)	-0004(1)	0002(2)	-0007(4)
C(5')	3132(3)	4514(4)	5024(7)	0013(1)	0031(3)	0125(9)	-0004(2)	-0001(3)	-0014(5)
C(6')	2504(3)	4934(5)	4880(10)	0013(1)	0045(3)	0186(13)	0001(2)	0002(3)	-0027(6)
O(1')	3780(2)	2720(3)	5122(7)	0017(1)	0034(3)	0190(11)	-0005(1)	0019(3)	-0003(5)
O(2')	4862(2)	3525(3)	3941(5)	0012(1)	0041(2)	0105(6)	0004(1)	0004(2)	-0017(3)
O(3')	4767(2)	5333(3)	5006(6)	0009(1)	0026(2)	0160(7)	-0003(1)	0005(2)	-0017(4)
O(5')	3206(2)	3853(3)	3804(6)	0013(1)	0035(2)	0155(7)	-0000(1)	-0006(2)	-0032(4)
O(6')	2109(2)	4120(4)	5027(9)	0011(1)	0058(3)	0355(15)	-0006(1)	0004(3)	-0023(7)
βO(1')	3705(12)	2557(21)	2891(32)	1.3(2)					
WATER									
O(W1)	0604(2)	9689(4)	7737(6)	0014(1)	0107(4)	0136(8)	-0002(2)	-0002(2)	0059(5)
O(W2)	1104(3)	1906(7)	6561(10)	0016(2)	0048(8)	0190(15)	-0004(4)	-0002(5)	-0014(11)
O(W3)	1756(2)	9970(3)	6296(7)	0013(1)	0111(3)	0157(9)	0003(1)	0000(3)	0056(4)
O(W4)	4094(3)	1166(4)	9257(9)	0020(1)	0049(4)	0201(12)	0008(2)	0006(4)	0021(7)
O(W5)	3011(2)	2236(5)	9716(7)	0037(1)	0186(5)	0272(9)	0052(2)	0004(2)	-0004(6)
O(W6)	2325(2)	2438(4)	6900(7)	0023(1)	0083(3)	0260(10)	-0005(1)	-0004(3)	0001(5)
O(W7)	4733(3)	1952(4)	6727(6)	0029(2)	0062(3)	0139(8)	0002(2)	0013(3)	-0000(5)

[a] The values for calcium and bromide ions have been multiplied by 10^5; all other values have been multiplied by 10^4. Temperature factors are in the form $T = \exp(-\beta_{11}h^2 - \beta_{22}k^2 - \beta_{33}l^2 - 2\beta_{12}hk - 2\beta_{13}hl - 2\beta_{23}kl)$. The final value of the isotropic extinction parameter is $g = 0.038$ (4). The isotropic thermal parameter for βO(1') is shown. The refined occupancy parameters for atoms O(1') and βO(1') are 0.87 (1) and 0.11 (1), respectively; these parameters were refined independently and were not constrained to a total value of 1.0.

deviation. A final three-dimensional difference Fourier map showed several peaks and troughs with magnitudes ranging from 0.5 to 0.8 e/Å^3 in the immediate vicinities of the calcium and bromide ions, plus a peak of 0.9 e/Å^3 near atom O(6). There were no other peaks or troughs in excess of 0.5 e/Å^3 in this map.

A final difference Fourier map was also calculated with the hydrogen atoms and atom βO(1') omitted from the calculated structure factors. The electron densities at the hydrogen-atom positions had an average value of 0.78 e/Å^3 and ranged from 0.2 to 0.9 e/Å^3, with the 0.2 e/Å^3 value corresponding to atom H(O-6). Since the electron density at the position of atom H(O-6) was unusually low and there was an additional peak near atom O(6), the coordinates determined for H(O-6) might be incorrect. The residual electron density at the position of βO(1') was 1.4 e/Å^3.

During the refinement, both real and imaginary components of the anomalous dispersion correction factors were applied to the atomic scattering factors for bromide, calcium, and oxygen. The correction factors used were from the International Tables for X-Ray Crystallography.[27] After I refined the correct enantiomer (D-lactose), the coordinates were inverted and the incorrect enantiomer (L-lactose) was refined. During the refinement of the L-lactose enantiomer, it was assumed that all lactose sites were occupied by the α anomer. The L-lactose model refined to only $R = 0.057$ and goodness-of-fit = 3.41. By the use of the R-factor ratio test,[32] a comparison of the refinements of the correct and incorrect enantiomers indicates the D-lactose absolute configuration to be correct with a probable error of less than 0.5%.

Results

Table II lists the final heavy-atom parameters and their estimated standard deviations. Table III gives the hydrogen-atom parameters and their estimated standard deviations. The estimated errors in positional coordinates are about 0.001 Å for bromide and calcium ions and 0.004–0.009 Å for carbon and oxygen atoms. A table of observed and calculated structure factors has been deposited.[33]

The crystal packing is shown in Figure 1. The lactose molecules, water molecules, and bromide ions form a cohesive hydrogen-bonded network, which appears to use all of the hydrogen atoms that are covalently bonded to oxygen atoms; however, several of the hydrogen bonds are unusually long. Hydrogen-bond lengths are given in Table IV. The calcium ion is surrounded by a shell composed of oxygen atoms from hydroxyl groups and water molecules. The bromide ions are hydrogen bonded to water molecules and to hydroxyl groups. There are no close contacts between calcium cations and bromide anions. The closest bromide–calcium contact is 4.94 Å, a distance 2 Å longer than the sum of the bromide and calcium ionic radii. Several of the water molecules and hydroxyl groups form bridges between calcium and bromide ions.

A prominent feature of this crystal structure is the in-

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(33) Observed and calculated structure factors, conformational torsion angles, and bond angles involving heavy atoms of lactose will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036, by referring to code number JACS-73-908. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche.

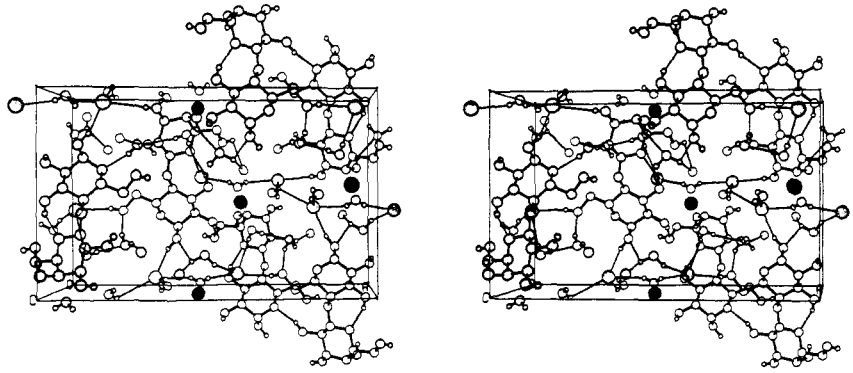

Figure 1. Stereodrawing showing the crystal packing as viewed down the c axis. The bromide ions are shown as dotted circles, and the calcium ions are depicted by solid black circles. Atom βO(1′) is not shown, and the only hydrogen atoms that are included are those which are bonded to oxygen atoms. Heavy lines represent covalent bonds and the smaller lines represent hydrogen bonds. (This drawing, as well as those shown in Figures 2–4, were prepared by using the program ORTEP[34]).

Table III. Hydrogen-Atom Parameters[a]

Atom	x	y	z	B, Å²
		Lactose		
H(C-1)	343	711	514	3.50
H(C-2)	356	726	817	1.75
H(C-3)	378	877	634	2.05
H(C-4)	422	939	643	1.72
H(C-5)	416	826	457	2.58
H(C-6)	527	800	585	2.63
H′(C-6)	513	882	483	2.63
H(O-2)	252	691	795	2.80
H(O-3)	305	913	835	2.20
H(O-4)	458	812	826	2.20
H(O-6)	552	770	390	2.80
H(C-1′)	378	289	305	2.62
H(C-2′)	428	433	292	2.28
H(C-3′)	427	440	601	1.73
H(C-4′)	363	563	413	1.75
H(C-5′)	315	419	592	2.24
H(C-6′)	245	528	397	2.70
H′(C-6′)	243	538	562	2.70
H(O-1′)	350	220	487	3.80
H(O-2′)	491	326	340	2.10
H(O-3′)	472	592	536	2.20
H(O-6′)	175	423	537	4.10
		Water		
H(W-1)	033	944	816	3.60
H′(W-1)	087	940	833	3.60
H(W-2)	101	238	654	3.20
H′(W-2)	143	210	670	3.20
H(W-3)	197	973	587	4.30
H′(W-3)	193	1018	677	4.30
H(W-4)	373	152	939	3.90
H′(W-4)	404	088	085	3.90
H(W-6)	217	291	624	5.10
H′(W-6)	254	252	726	5.10
H(W-7)	500	143	612	4.80
H′(W-7)	433	163	685	4.80

[a] The positional parameters, which were determined from difference Fourier maps, have been multiplied by 10^3; the errors in these parameters are probably 0.1–0.3 Å.

teraction between lactose molecules and calcium ions. Figure 2 shows the environment of the calcium ion, which binds two lactose molecules and four water molecules. One lactose molecule is coordinated to the calcium ion through O(3) and O(4) of its galactose moiety and the second is coordinated through O(2′) and O(3′) of its glucose moiety. Thus, the calcium ion is surrounded by a shell composed of eight oxygen atoms: four from water molecules and four from lactose hydroxyl groups. There are no hydrogen bonds formed be-

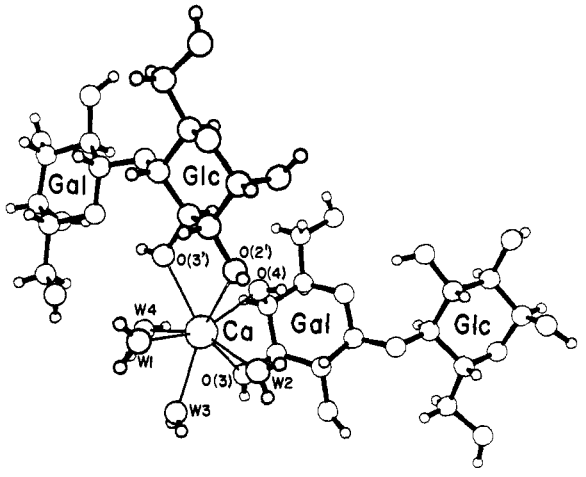

Figure 2. Environment of the calcium ion. Only the α anomer of lactose is shown.

tween oxygen atoms within this calcium cordination shell. The stereochemistry of the calcium ion coordination shell is shown in more detail in Figure 3. The eight oxygen atoms form a distorted square-antiprism with calcium–oxygen distances ranging from 2.379 to 2.538 Å. Within the calcium shell, there are several short (<2.85 Å) oxygen–oxygen contacts.

Figure 4 shows the lactose conformation. A table of conformational torsion angles is included in the microfilm edition of the journal, where the values are compared with those reported for the lactose molecule in the crystal structure of lactose monohydrate.[31,33] The conformation is in general agreement with the crystal structure of lactose monohydrate,[31,35] and that of the related disaccharide, cellobiose.[36–38] As in these structures, the disaccharide conformation is stabilized by an O(3′)–O(5) intramolecular hydrogen bond. The resulting torsion angles about the C(1)–O(1) and O(1)–C(4′) bridge bonds are in agreement with those of cellobiose and deviate only 10–20° from those of

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Table IV. Hydrogen Bond Distances

Donor atom	Hydrogen atom	Acceptor atom	Donor–acceptor distance, Å	Donor atom	Hydrogen atom	Acceptor atom	Donor–acceptor distance, Å
O(1′)	H(O-1′)	Br(2)[b]	3.15	O(W-2)	H′(W-2)	O(W-6)[a]	2.79
βO(1′)		Br(2)[b]	3.33	O(W-3)	H(W-3)	Br(2)[d]	3.26
O(2′)	H(O-2′)	O(6)[g]	2.69	O(W-3)	H′(W-3)	Br(2)[e]	3.36
O(3′)	H(O-3′)	O(5)[a]	2.76	O(W-4)	H(W-4)	O(W-5)[a]	2.82
O(6′)	H(O-6′)	Br(1)[b]	3.20	O(W-4)	H′(W-4)	O(3)[i]	3.32
O(2)	H(O-2)	O(5′)[c]	2.93	O(W-5)		O(2)[c]	2.95
O(3)	H(O-3)	Br(2)[d]	3.47	O(W-5)		O(W-6)[a]	2.91
O(4)	H(O-4)	O(W-7)[e]	2.84	O(W-6)	H(W-6)	O(6′)[a]	2.87
*O(6)[k]	H(O-6)	O(W-4)[e]	3.06	O(W-6)	H′(W-6)	O(W-5)[a]	2.91
		βO(1′)[f]	2.82	O(W-7)	H(W-7)	Br(1)[i]	3.22
O(W-1)	H(W-1)	Br(1)[h]	3.26	*O(W-7)	H′(W-7)	O(1′)[a]	2.74
O(W-1)	H′(W-1)	Br(2)[d]	3.45			O(W-4)[a]	2.84
O(W-2)	H(W-2)	Br(1)[b]	3.33				

Symmetry codes: [a] x, y, z. [b] $-x+1, y-1/2, -z+3/2$. [c] $-x+1/2, -y+1, z+1/2$. [d] $x-1/2, -y+3/2, -z+2$. [e] $-x+1, y+1/2, -z+3/2$. [f] $-x+1, y+1/2, -z+1/2$. [g] $-x+1, y-1/2, -z+1/2$. [h] $x-1, y, z$. [i] $x, y-1, z$. [j] $-x+3/2, -y+1, z-1/2$. [k] An asterisk indicates bifurcated hydrogen bonds.

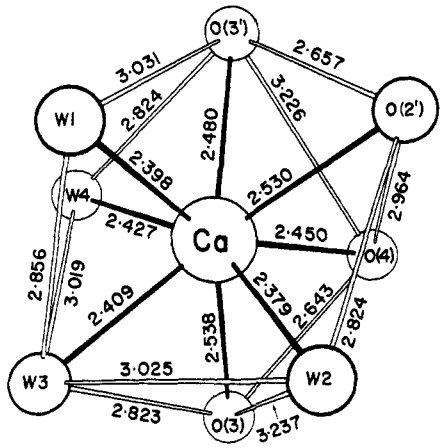

Figure 3. Stereochemistry of the calcium ion coordination shell. All oxygen–oxygen contacts shorter than 3.25 Å are shown, along with the calcium–oxygen distances. W-1, W-2, W-3, and W-4 are oxygen atoms of water molecules.

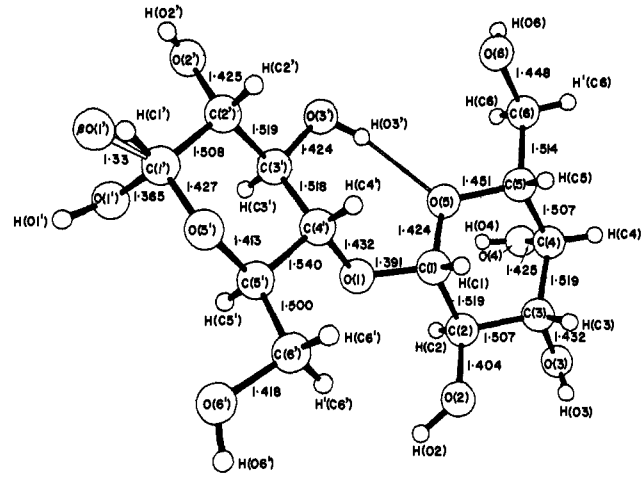

Figure 4. Bond lengths involving nonhydrogen atoms of the lactose molecule. The estimated standard deviations in bond lengths are 0.007–0.01 Å, except for the C(1′)–βO(1′) bond length, which has a standard deviation of about 0.03 Å.

lactose monohydrate. The conformations of the galactose and glucose moieties in this complex vary slightly from those found in the monohydrate structure; the greatest differences occur at the sites where calcium ions bind to the molecule. The torsion angles about the C–O bonds are markedly different in the two structures, probably because of differences in the hydrogen bonding schemes.

Bond lengths involving heavy atoms are shown in Figure 4. These values are not significantly different from those in the crystal structure of lactose monohydrate,[31,35] which were discussed in some detail by Fries, *et al.*[31] A table of bond angles involving the heavy atoms of lactose and the corresponding values for lactose monohydrate[31] is included in the microfilm edition of the journal. Of several significant differences between the bond angles in this structure and those in lactose monohydrate, the biggest occur at the calcium binding sites.

Discussion

It has been demonstrated that lactose interacts with calcium ions in aqueous systems,[1,22,23] and crystalline lactose–calcium salt complexes have been isolated from water[22,23] and from methanol.[39] However, no previous structural studies of these complexes have been reported, and little is known about the specific factors that govern lactose–calcium interactions in solution or in the solid state. The results described here indicate that lactose can chelate calcium ions by using the O(2′)–O(3′) pair of hydroxyl groups of the glucose moiety and the O(3)–O(4) pair of hydroxyl groups of the galactose moiety. In view of the extensive hydration in this crystal structure (7 mol of water per mole of lactose) and the presence of water molecules in the calcium ion coordination shell, it is reasonable to assume that these lactose–calcium interactions are typical of those that occur in aqueous systems. Thus the calcium binding in water can be attributed to a simple substitution of lactose hydroxyl groups for water molecules in the calcium hydration shell.

Interactions of calcium ions with hydroxyl groups also take place in the crystal structures of other sugar–calcium salt complexes. For example, in the crystal structures of the hydrated calcium bromide complexes of galactose and *myo*-inositol, the calcium ions are

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coordinated to the sugars through hydroxyl groups.[13] Likewise, in the cyclohexaamylose–potassium acetate[40] and sucrose–sodium bromide[41] complexes, the alkali metals interact with the carbohydrates by binding to hydroxyl groups. Calcium–hydroxyl interactions also occur in the crystal structures of calcium salts of sugar acids; in the crystal structures of the calcium salts of 5-keto-D-gluconic acid,[42] arabonic acid,[43] α-D-gluco-isosaccharinic acid,[44] lactobionic acid,[45] and garcinia acid,[46] the calcium ions are coordinated to hydroxyl groups, along with the negatively charged carboxyl groups. The geometry of the calcium ion coordination shell in the lactose complex (Figure 3) is closely related to that found in the calcium bromide complexes of galactose and *myo*-inositol, and in the calcium salts of sugar acids. In these crystal structures the calcium ions are also surrounded by square-antiprism coordination shells composed of eight oxygen atoms, and both calcium–hydroxyl and calcium–water distances are in agreement with those depicted in Figure 3.

Comparison of the stereochemistry of the galactose and glucose moieties in this complex with that in the crystal structure of lactose monohydrate[31] suggests that the calcium interactions are responsible for small conformational changes at the calcium binding sites. Calcium binding to the glucose moiety is accompanied by a decrease of 7° in the O(2′)–C(2′)–C(3′)–O(3′) torsion angle, and a decrease of 5° in the C(3′)–C(2′)–O(2′) bond angle, with a resultant 0.2-Å decrease in the spacing between O(2′) and O(3′). Calcium binding to the galactose moiety of lactose is accompanied by decreases of 9° in the O(3)–C(3)–C(4)–O(4) torsion angle, 5° in the C(4)–C(3)–O(3) bond angle, and 0.2 Å in the O(3)–O(4) spacing. Analogous conformational changes were observed in the calcium bromide complexes of galactose and *myo*-inositol, where calcium binding to adjacent hydroxyl groups is responsible for decreases of about 0.2 Å in the spacings between the hydroxyl oxygen atoms.[13]

The finding that this crystal structure contains a mixture of the α and β anomers of lactose is not completely unexpected, since crystals of lactose monohydrate[31] and of *N*-acetyl-D-glucosamine[47] also contain mixtures of α and β anomers. The crystal packing in this complex is such that both the α- and β-oxygen atoms can be accommodated in the lattice without suffering any unusual contacts. As shown in Table IV, it appears that both O(1′) and βO(1′) serve as donors in the formation of hydrogen bonds to a single bromide ion, while O(1′) accepts a hydrogen bond from water oxygen atom O(W-7) and βO(1′) accepts a hydrogen bond from atom O(6). The hydrogen bonds to atoms O(1′) and βO(1′) appear to be bifurcated, with O(W-7) and O(6) forming additional hydrogen bonds to atom O(W-4). The results of the least-squares refinement indicate that approximately 10–14% of the lactose sites are occupied by the β anomer. Thus the α/β ratio is only slightly lower than that for lactose monohydrate where the β anomer accounted for 6–9% of the lactose.[31]

Numerous studies have demonstrated that lactose stimulates intestinal absorption of calcium ions. There is little agreement about the mechanism that might account for this effect, but Charley and Saltman have presented evidence that calcium–lactose complexes may be involved.[1] Although the lactose effect has been most extensively studied, it has been shown that a number of other simple carbohydrates and polyols are also capable of stimulating calcium absorption.[15,16,19] In fact, it appears that most sugars and polyols which are poorly absorbed and are thus able to reach the lower intestinal tract will increase calcium absorption.[19–21] As emphasized by Wasserman and Kallfelz,[48] any tenable explanation of the ability of lactose to promote calcium absorption must also take into account the similar stimulating effects exerted by the related carbohydrates and polyols. This crystal-structure analysis demonstrates that lactose binds calcium ions by a simple chelation process that involves pairs of adjacent hydroxyl groups. There is no apparent evidence that the interactions involved in this complex are specific for lactose. Similar hydroxyl–calcium interactions account for the solid state binding of calcium ions by α-galactose,[13] *myo*-inositol,[13] and β-D-mannofuranose,[49] and are probably also responsible for the binding of calcium to a variety of sugars and polyols in aqueous systems.[11,50,51] Thus lactose is not unique in its ability to bind calcium ions, and it is likely that the other carbohydrates and polyols which increase calcium absorption would be capable of forming calcium complexes that are analogous to the lactose–calcium complex described here. It is not clear what role these complexes play in calcium metabolism, but, as suggested by Charley and Saltman,[1] they might enhance calcium absorption either by serving as carriers that transport calcium across intestinal membranes or by inhibiting the precipitation of insoluble calcium salts within the intestine.

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