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CALCIUM INTERACTIONS WITH D-GLUCANS: CRYSTAL STRUCTURE OF α,α-TREHALOSE–CALCIUM BROMIDE MONOHYDRATE

WILLIAM J. COOK AND CHARLES E. BUGG
Institute of Dental Research and Department of Biochemistry, University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294 (U. S. A.)
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ABSTRACT

Three-dimensional X-ray diffraction data were used to determine the crystal structure of α,α-trehalose–calcium bromide monohydrate, a model system for investigation of factors involved in the binding of calcium ions to D-glucans of dental plaques. Crystals of $C_{12}H_{22}O_{11}\cdot CaBr_2\cdot H_2O$ are orthorhombic, space group $C222_1$, with $a = 11.058(1)$ $b = 11.573(1)$, $c = 15.101(1)$ Å, and $Z = 4$. Intensity data for 925 independent reflections were measured with an automated diffractometer. A trial structure, obtained by the heavy-atom method, was refined by least-squares to $R = 0.03$. An outstanding feature of the crystal packing is the interaction of trehalose molecules with calcium ions. Each calcium is coordinated to hydroxyl groups from four symmetry-related D-glucose moieties, thereby cross-linking the trehalose molecules. Similar interactions between calcium ions and the D-glucose residues of extracellular D-glucans may be of importance in the agglutination processes involved in dental-plaque formation.

INTRODUCTION

There is considerable evidence that extracellular D-glucans play a prominent role in the etiology of dental caries[1–6]. Several strains of cariogenic streptococci produce copious quantities of D-glucans as products of sucrose metabolism[1–7]. These D-glucans, which are highly branched polymers of D-glucose having α-(1 → 6) and α-(1 → 3) linkages[8–11], are major components of dental plaques, and it has been suggested that they contribute to the agglutination processes involved in plaque formation[2,3,4,12]. In support of this premise, it has been shown that high-molecular weight D-glucans agglutinate cariogenic streptococci[2,12], and that they adhere to apatite[2] and enamel[13] surfaces. In the presence of saliva[3], D-glucans form insoluble precipitates that contain significant amounts of calcium; as these precipitates can be solubilized by ethylenedinitrilotetraacetic acid, a calcium-chelating agent, it appears that they are stabilized by interactions that involve calcium ions.

Little is known about interactions that occur within dental plaques, or about the specific contributions of D-glucans to plaque formation. However, a number of studies have demonstrated that calcium ions bind to carbohydrates in aqueous solutions[14], as well as in hydrated solid-state systems[15-18]. In our laboratory, crystallographic investigations have indicated that calcium–carbohydrate interactions provide an effective mechanism for cross-linking carbohydrate molecules[15-18]. Thus, it is reasonable to assume that calcium–glucan interactions occur within dental plaque, and that these interactions contribute to those agglutination processes involved in plaque formation. To investigate the structural factors that are likely to govern calcium–D-glucan interactions, we are currently examining a series of crystal structures in which calcium salts are complexed with oligosaccharides composed of D-glucose residues. In this paper we describe the crystal structure of a hydrated calcium bromide complex of α,α-trehalose (α-D-glucopyranosyl α-D-glucopyranoside).

EXPERIMENTAL

Clear, prismatic crystals of the complex were grown by evaporating an aqueous solution that contained an approximately equimolar mixture of α,α-trehalose and calcium bromide. Weissenberg and oscillation photographs showed the crystals to be orthorhombic, space group $C222_1$, as indicated by the systematic absence of reflections hkl with $(h+k)$ odd and $00l$ with l odd. A crystal fragment with approximate dimensions of 0.2 × 0.2 × 0.2 mm was mounted on a Picker FACS-1 diffractometer with its c 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 observed angular settings for twelve medium-angle reflections (CuKα, $\lambda = 1.5418$ Å). Intensity data were collected with the diffractometer, by use of a scintillation counter, nickel-filtered copper radiation, and a $\theta-2\theta$ scanning technique. The scanning speed was 1.0°/min, and the background was counted for 40 sec at each terminus of the scans. Measurements were made for each of the 925 independent reflections with $2\theta \leq 128°$. Three strong, medium-angle reflections were monitored periodically and showed no significant variations in intensity during the period of data collection. Immediately after data collection, accurate values for the cell parameters were determined by a least-squares analysis of 2θ values for twelve high-angle reflections (CuKα_1, $\lambda = 1.54051$ Å); these cell parameters were not significantly different from those obtained prior to the measurement of intensities. Crystal data are listed in Table I.

Those reflections with scan counts below background level ("negative" intensities) were assigned intensities of 0.0 and were retained in all subsequent calculations. All 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[19]. Data were then scaled by means of a Wilson[20] plot.

The observed density (Table I) is consistent with a unit cell containing four trehalose–$CaBr_2 \cdot H_2O$ formula units, which indicates that the asymmetric unit

TABLE I

CRYSTAL DATA

Stoichiometry	$C_{12}H_{22}O_{11} \cdot CaBr_2 \cdot H_2O$
Z	4
Space group	$C222_1$
a	11.058(1) Å
b	11.573(1) Å
c	15.101(1) Å
ρ (calculated)	1.924 g cm^{-3}
ρ (observed)[a]	1.91 g cm^{-3}
μ	86.8 cm^{-1}

[a] The density was measured by flotation in a tetrabromoethane–carbon tetrachloride mixture.

contains half of the formula unit, with the calcium ion, the water molecule and atom O-1 of trehalose all lying on twofold axes. Assuming this to be the case, we arrived at a suitable trial structure by the heavy-atom method as follows: coordinates for the bromide ion were determined from a sharpened, three-dimensional Patterson map; coordinates for the calcium ion were determined from a sum-function superposition of sharpened Patterson maps translated to the bromide-ion position; and the remaining nonhydrogen atoms were located in a Fourier map that was calculated by using phase angles derived from the two ions. The trial structure was refined by using a modified version of the full-matrix least-squares program ORFLS[21,22]. The quantity minimized was $\Sigma w(Fo^2 - Fc^2/k^2)^2$, where k is a scale factor and weight w is equal to $1/\sigma^2(Fo^2)$. Scattering factors for the nonhydrogen atoms (Ca^{2+}, Br^{-1}, C, O) were from the *International Tables for X-Ray Crystallography*[23], and hydrogen atom scattering factors were from Stewart, Davidson, and Simpson[24]. 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 0.95 Å. All hydrogen atoms bonded to oxygen atoms 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 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[25] isotropic extinction parameter g [as formulated by Coppens and Hamilton[26]] were included in the refinement. As the refinement proceeded, coordinates of hydrogen atoms attached to oxygen atoms were improved by the use of difference Fourier maps.

The final R index $(\Sigma||Fo|-|Fc||/\Sigma|Fo|)$ including all reflections is 0.030. The goodness-of-fit $\{[\Sigma w(Fo^2 - Fc^2/k^2)^2/(m-s)]^{\frac{1}{2}}$, where m is the number of reflections used and s is the number of parameters refined$\}$ is 1.42. During the last cycle of refinement no parameter shifted more than one-fifth of its estimated standard deviation. In a final difference Fourier map, which was calculated with the hydrogen atoms omitted from the calculated structure-factors, the electron densities at the hydrogen-atom positions had an average value of 0.9 $e/\text{Å}^3$ and ranged from 0.6 to 1.1 $e/\text{Å}^3$. This map showed no other peaks or troughs in excess of 0.4 $e/\text{Å}^3$.

During the refinement, both real and imaginary components of the anomalous-dispersion correction factors were applied to the atomic scattering factors for bromide, calcium, oxygen, and carbon. The correction factors used were those from Cromer and Liberman[27]. After refining the correct enantiomer ("D"-trehalose), the coordinates were inverted and the incorrect enantiomer ("L"-trehalose) was refined to only $R = 0.046$ and goodness-of-fit = 2.34. By use of the R-factor ratio test[28], a comparison of the refinements of the correct and incorrect enantiomers indicates that the "D"-trehalose absolute configuration is 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. The estimated errors in positional coordinates are about 0.001 Å for the bromide and calcium ions and 0.003–0.005 Å for carbon and oxygen atoms. A table of observed and calculated structure-factors will be furnished by the authors upon request.

The crystal-packing and hydrogen-bonding schemes are shown in Fig. 1. Atom O-1 of trehalose is located on the crystallographic twofold axis at $x = 0.5$, $z = 0.25$, and the two D-glucose moieties are related to each other by this twofold axis. Both the calcium ion and the water molecule are situated on the twofold axis at

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}
Br	6999(5)	12848(5)	11230(4)	482(5)	511(5)	376(3)	−54(4)	−45(3)	193(3)
Ca	20889(13)	50000	0	398(11)	295(10)	113(6)	0	0	2(6)
C-1	4025(4)	2906(4)	2192(3)	32(4)	23(3)	14(2)	−3(3)	−2(2)	−1(2)
C-2	3187(4)	3653(4)	1635(3)	34(4)	28(3)	11(2)	−4(3)	−1(2)	0(2)
C-3	2605(5)	4604(4)	2182(3)	41(4)	32(4)	13(2)	0(3)	−2(3)	5(2)
C-4	2017(4)	4129(4)	3022(3)	36(4)	33(4)	11(2)	2(3)	2(3)	−3(2)
C-5	2886(4)	3329(4)	3511(3)	29(4)	30(3)	10(2)	−6(3)	−1(2)	2(2)
C-6	2302(5)	2701(4)	4272(3)	44(5)	48(4)	17(2)	−4(4)	3(3)	8(3)
O-1	5000	3575(4)	2500	19(3)	26(3)	15(2)	0	−2(2)	0
O-2	3749(3)	4116(3)	864(2)	35(3)	43(3)	11(2)	−4(2)	4(2)	3(2)
O-3	1746(3)	5140(3)	1603(2)	53(3)	38(3)	12(2)	19(3)	−3(2)	0(2)
O-4	1653(3)	5073(3)	3582(2)	44(3)	41(3)	18(2)	6(3)	7(2)	−4(2)
O-5	3347(3)	2449(3)	2913(2)	35(3)	26(2)	14(2)	−3(2)	1(2)	3(2)
O-6	3167(3)	1991(3)	4725(2)	52(3)	30(2)	14(2)	−5(2)	−1(2)	4(2)
O-W	−12(5)	5000	0	29(4)	88(5)	22(2)	0	0	−4(3)

[a] Values from bromide and calcium ions have been multiplied by 10^5 and all others 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 $g = 0.007(3)$. O-W is the oxygen atom of the water molecule. Parameters listed without standard deviations were not refined.

TABLE III

HYDROGEN-ATOM PARAMETERS[a]

Atom	x	y	z	B (Å^2)
H-1	434	230	185	1.28
H-2	256	318	140	1.27
H-3	320	515	236	1.55
H-4	131	371	286	1.59
H-5	355	377	374	1.50
H-6	166	223	403	2.14
H-6′	195	325	466	2.14
H(O-2)	430	475	100	1.63
H(O-3)	145	560	170	1.86
H(O-4)	230	540	375	1.92
H(O-5)	380	260	480	2.20
H-W	−55	495	−40	2.37

[a] Positional parameters, which are multiplied by 10^3, were either calculated or determined from difference Fourier maps. Each hydrogen atom was assigned the approximate isotropic temperature factor of the nonhydrogen atom to which it is bonded.

$y = 0.5$, $z = 0$. All hydrogen atoms that are covalently bonded to oxygen atoms participate in hydrogen-bonding. Hydrogen-bond distances and angles are listed in Table IV. The calcium ion is surrounded by oxygen atoms, and the bromide ion is hydrogen bonded to hydroxyl groups. The bromide ions are not in the immediate coordination polyhedron around the calcium ion; the closest calcium–bromide contact is 4.59 Å, a distance 1.60 Å longer than the sum of the ionic radii for these two ions.

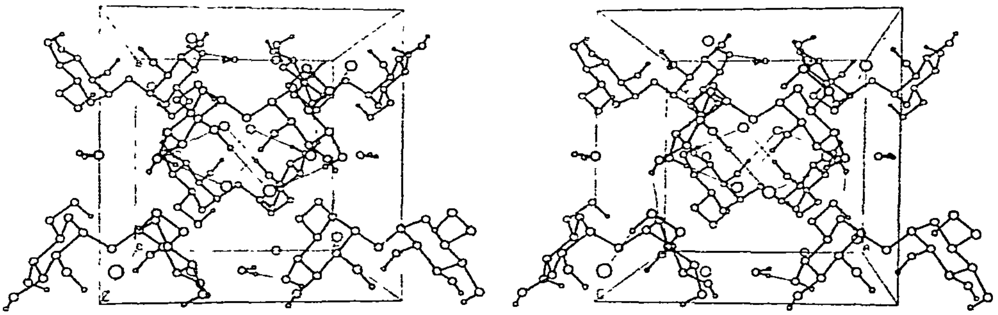

Fig. 1. Stereo drawing of the crystal structure as viewed down the c axis. Only hydrogen atoms bonded to oxygen atoms are included. Heavy lines represent covalent bonds and fine lines represent hydrogen bonds. (This drawing and those in Figs. 3–5 were prepared with the program ORTEP[29]).

Fig. 2 shows the environment of the calcium ion, which is coordinated to the water molecule and to four symmetry-related D-glucose moieties. Two of the D-glucose residues chelate the calcium ion through atoms O-2 and O-3, and the other two bind through their O-6 hydroxyl groups. The calcium ion is thus surrounded by a coordination polyhedron composed of seven oxygen atoms: one from the water

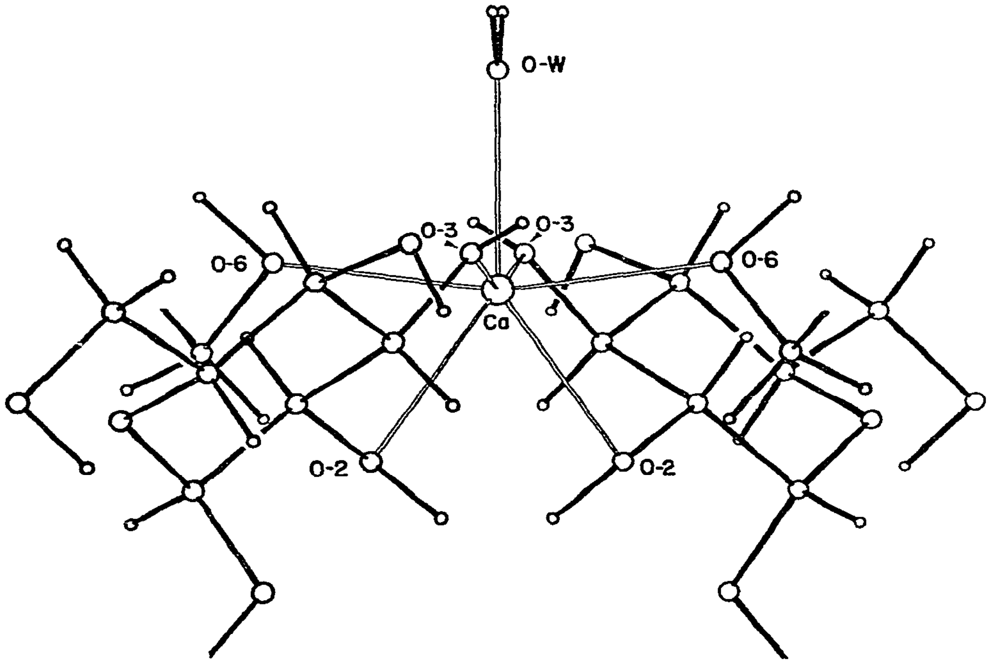

Fig. 2. Environment of the calcium ion, which is coordinated to four D-glucose moieties and to the water molecule. Only portions of the D-glucose residues are shown. The Ca–O-water line coincides with a twofold crystallographic axis. Part of the calcium environment is also shown in Fig. 5.

TABLE IV

HYDROGEN-BOND DISTANCES AND ANGLES

Donor atom	*Hydrogen atom*	*Acceptor atom*[a]	*Donor–acceptor distance* (Å)	*Approximate hydrogen–acceptor distance* (Å)	*Approximate donor–hydrogen–acceptor angle*
O-2	H(O-2)	Br *a*	3.333	2.4	170°
O-3	H(O-3)	O-5 *b*	2.773	2.2	140°
O-4	H(O-4)	Br *b*	3.277	2.4	170°
O-6	H(O-6)	Br *c*	2.164	2.4	130°
O-W	H-W	O-4 *d*	2.807	2.0	170°

[a] Symmetry codes: *a*: $x+1/2, y+1/2, z$; *b*: $-x+1/2, y+1/2, -z+1/2$; *c*: $-x+1/2, -y+1/2, z+1/2$; *d*: $-x, -y+1, z-1/2$.

molecule, and six from hydroxyl groups. These seven oxygen atoms assume a pentagonal bipyramidal arrangement. The calcium–oxygen distances range from 2.323 to 2.474 Å, in agreement with those found in the crystal structure of fucose–$CaBr_2$ trihydrate[30], a complex in which the calcium ion is also coordinated to seven oxygen atoms. On the average, these calcium–oxygen contacts are slightly shorter than those found in calcium–carbohydrate complexes that display eightfold calcium-coordination.

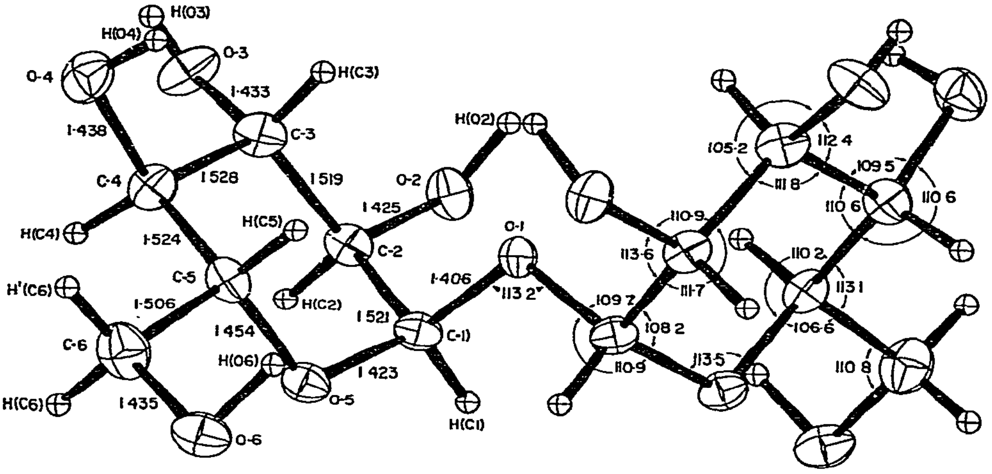

Fig. 3. Conformation of the trehalose molecule. Nonhydrogen atoms, represented by thermal ellipsoids that are defined by the principal axes of thermal vibration, are scaled to include 50% probability. Hydrogen atoms are represented by spheres of 0.07 Å radius. Bond lengths and angles involving nonhydrogen atoms are given; estimated standard deviations are about 0.006 Å and 0.3°, respectively. A twofold crystallographic axis passes through O-1 and lies nearly parallel to the plane of the paper.

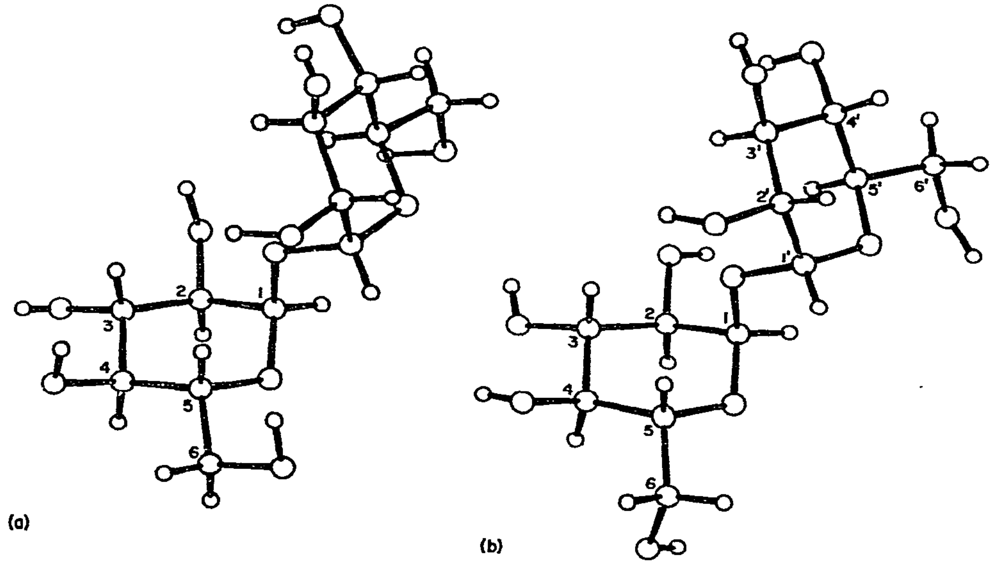

Fig. 4. Comparison of the trehalose conformations found in (*a*) the α,α-trehalose–calcium bromide monohydrate complex and (*b*) the crystal structure of α,α-trehalose dihydrate[32]. The hexose rings of the lower D-glucose moieties are shown in identical orientations in (*a*) and (*b*).

Fig. 3 shows the trehalose conformation, thermal ellipsoids, and those bond lengths and angles that involve only nonhydrogen atoms. Conformational torsion angles are listed in Table V. As depicted in Fig. 4, the conformation is in general agreement with that found in the crystal structure of α,α-trehalose dihydrate[32]. The torsion angles about the C-1–O-1 bridge bonds between the D-glucose residues differ by only 2–6° from the corresponding values in trehalose dihydrate. The conformation of the D-glucose moiety in this complex varies only slightly from that in the trehalose dihydrate structure; no intra-ring torsion angles differ by more than 8° in the two structures, and the conformation about the C-5–C-6 bond is in agreement with that found for one of the D-glucose moieties in the dihydrate structure. The bond lengths are in agreement with those of trehalose dihydrate. There are several significant differences (3–7°) in bond angles involving atoms O-2 and O-3, the hydroxyl groups that are chelated to the calcium ion, but the other bond angles are in agreement with those of the dihydrate. It appears that calcium chelation by the O-2–O-3 pair of hydroxyl groups results in significant structural changes at the binding site. Relative to trehalose dihydrate, there are decreases of 4–5° in the O-2–C-2–C-3–O-3 torsion angles and decreases of 4–7° in the C-2–C-3–O-2 bond angle, which result in decreases of 0.1 to 0.2 Å in the O-2–O-3 spacings. These structural changes at the chelation site are similar to those observed in other calcium–carbohydrate complexes[15].

TABLE V

CONFORMATIONAL TORSION ANGLES INVOLVING ONLY NONHYDROGEN ATOMS OF THE TREHALOSE MOLECULE[a]

O-5–C-1–C-2–C-3	56.5°
C-1–C-2–C-3–C-4	−50.6°
C-2–C-3–C-4–C-5	47.9°
C-3–C-4–C-5–O-5	−52.2°
C-4–C-5–O-5–C-1	61.9°
C-5–O-5–C-1–C-2	−63.1°
O-1–C-1–C-2–O-2	62.0°
O-2–C-2–C-3–O-3	57.8°
O-3–C-3–C-4–O-4	−70.4°
O-4–C-4–C-5–C-6	66.6°
C-4–C-5–C-6–O-6	−177.9°
O-5–C-5–C-6–O-6	61.1°
O-5–C-1–O-1–C-1	−11.2°
C-2–C-1–O-1–C-1	108.6°

[a] Estimated standard deviations are about 0.5°. The signs of the angles correspond to the notation of Klyne and Prelog[31].

DISCUSSION

Calcium binding to the D-glucose moieties of trehalose is a prominent feature of this crystal structure. As depicted in Fig. 2, the calcium ion is tightly imbedded in a coordination polyhedron that contains six hydroxyl groups from the sugar moieties.

The D-glucose residues bind to calcium through their O-2, O-3, and O-6 hydroxyl groups. It is noteworthy that the O-2–O-3 pair of hydroxyl groups, which is involved in calcium chelation, is also utilized by the D-glucose moiety of lactose for calcium-binding in the crystal structures of lactose–calcium bromide heptahydrate and lactose–calcium chloride heptahydrate. Therefore, it appears that this pair of hydroxyl groups provides a particularly effective site for the binding of calcium ions to D-glucose residues.

Calcium interactions with hydroxyl groups are of major importance in other crystal structures of calcium–carbohydrate complexes[15–18], and are believed to be responsible for calcium binding to a variety of uncharged carbohydrates in aqueous solution[1,33,34]. Solution studies have indicated that hexoses bind calcium ions strongly only at sites having an axial–equatorial–axial arrangement of three hydroxyl groups[33,34], apparently because this arrangement allows simultaneous chelation of all three hydroxyl groups to a single calcium ion. Since D-glucose does not possess this arrangement, saccharides composed of D-glucose might be expected to interact only weakly with calcium ions. However, several crystallographic studies in this laboratory have demonstrated that carbohydrates often chelate calcium ions through pairs of hydroxyl groups[15,30], so it appears that, at least in hydrated solid-state environments, two hydroxyl groups can serve as an effective calcium-chelation site. The crystallographic studies show that calcium interactions with simple pairs of hydroxyl groups are sufficiently favorable to compete successfully with cation–anion coulombic attraction; in all calcium halide–carbohydrate complexes that have been examined, the calcium ions are coordinated to hydroxyl groups and water molecules with no direct contacts between calcium cations and halide anions. Calcium interactions with pairs of hydroxyl groups are also strong enough to produce noticeable changes in the conformation of the sugar moieties at the calcium-binding sites. In all cases, calcium-chelation by a pair of hydroxyl groups has been found to produce both torsion-angle and bond-angle changes that cause a decrease of 0.1–0.2 Å in the intramolecular spacing between the hydroxyl oxygen atoms. Whereas calcium ions may not bind as strongly to simple sugar moieties as to multidentate chelating agents, it is clear that calcium–carbohydrate interactions such as those in the trehalose–calcium bromide complex are sufficiently effective to be of major importance in condensed systems.

As depicted in Figs. 1 and 2, calcium–D-glucose interactions lead to the formation of trehalose–calcium–trehalose bridges. The calcium ion binds to four symmetry-related D-glucose moieties, thereby linking four trehalose molecules together. Calcium–carbohydrate bridges are also found in the other crystal structures of calcium–carbohydrate complexes. In all complexes that have been examined, the calcium ions are coordinated to two or more carbohydrate molecules, with the resultant formation of carbohydrate–calcium–carbohydrate linkages that are dominant features of the crystal structures. In view of the prominent role played by calcium–carbohydrate bridges in a variety of hydrated solid-state environments, it appears likely that related interactions also occur in biological systems. Linkages of this type could provide an effective mechanism for the agglutination of polysaccharides by calcium ions, in which case they might exert an important influence on the formation of insoluble dental plaques.

Calcium binding to D-glucose residues of plaque D-glucans might be expected to resemble interactions in the trehalose–calcium bromide complex. Our results demonstrate that 1-substituted D-glucose moieties can chelate calcium ions through the O-2–O-3 pair of hydroxyl groups. In D-glucans, this pair of hydroxyl groups would be available on those D-glucose residues that are linked through their 1- and 6-positions. Since plaque D-glucans contain significant numbers of α-(1 → 6) linkages[8–10], it is likely that an appreciable fraction of the D-glucose residues would be able to offer their O-2–O-3 hydroxyl groups for calcium-chelation.

The geometry of the D-glucose–calcium–D-glucose bridges that involve the O-2–O-3 pairs of hydroxyl groups in the trehalose complex is depicted in Fig. 5. As suggested in this figure, similar interactions might also occur between the α-(1 → 6) linked D-glucose residues of D-glucans and serve to cross-link the D-glucan molecules in dental plaque. When chelated by two pairs of hydroxyl groups (Fig. 5) calcium ions would also be expected to bind several additional ligands, as the calcium-coordination shell would be only partially filled by the four hydroxyl groups. In dental plaque, these additional coordination sites might be occupied by water molecules, other carbohydrate moieties, or anions such as phosphate and carbonate ions. For example, the calcium ions might also chelate to carbohydrates from cell surfaces, and thus incorporate bacteria into the D-glucan–calcium precipitates. Alternatively, by interacting with phosphate ions on the surface of enamel, the calcium ions might effectively bind the D-glucan complexes to tooth surfaces. Finally, by binding carbonate and phosphate ions within dental plaques, the calcium–D-glucan complexes might provide suitable nucleation sites for the calcification processes that produce dental calculus.

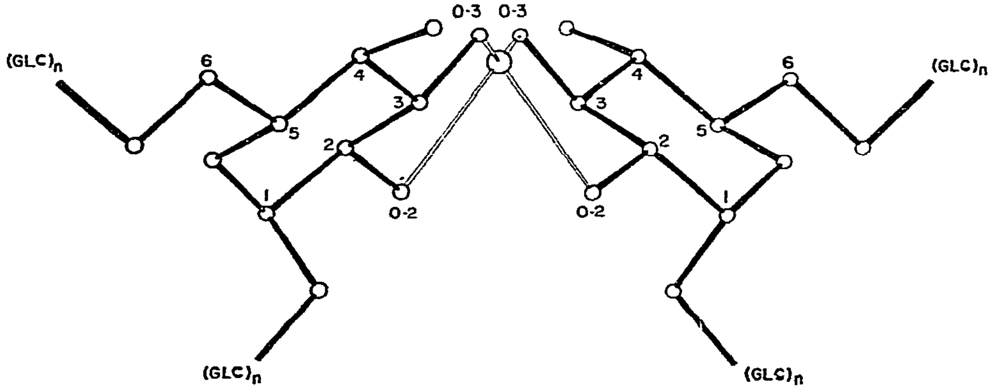

Fig. 5. Model for the calcium–D-glucose interactions that might be involved in cross-links between D-glucan molecules. The D-glucose–calcium–D-glucose bridge is taken from the trehalose–calcium bromide monohydrate complex. The calcium ion is represented by a dotted circle; hydrogen atoms are not shown.

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