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CALCIUM-CARBOHYDRATE BRIDGES COMPOSED OF UNCHARGED SUGARS

STRUCTURE OF A HYDRATED CALCIUM BROMIDE COMPLEX OF α-FUCOSE

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SUMMARY

X-ray diffraction data were used to determine the crystal structure of a hydrated $CaBr_2$ complex of α-fucose, a common terminal sugar of oligosaccharide chains on glycoproteins. Crystals of $C_6H_{12}O_5 \cdot CaBr_2 \cdot 3H_2O$ are orthorhombic, space group $P2_12_12_1$, with $a = 14.360(2)$, $b = 12.896(3)$, and $c = 8.043(1)$ Å. Intensity data for 1442 independent reflections were measured with an automated diffractometer. A trial structure, obtained by the heavy-atom method, was refined by least-squares to $R = 0.052$. Ca^{2+} is chelated by a pair of hydroxyl groups from each of two symmetry-related fucose molecules and is coordinated to three water molecules. Thus the structure consists of hydrated fucose-calcium-fucose bridges. The bridge geometry, which is dictated by the coordination requirements of Ca^{2+}, is like that of other calcium-carbohydrate complexes. Our results indicate that calcium-fucose interactions can provide an effective, stereospecific mechanism for cross-linking carbohydrate chains. Similar calcium-carbohydrate bridges may be involved in a variety of Ca^{2+}-dependent agglutination and adhesion processes.

INTRODUCTION

Ca^{2+} and carbohydrates appear to participate in a variety of biological adhesion and agglutination processes, particularly those occurring at cell surfaces. For example, Ca^{2+} and membrane-bound carbohydrate residues have been strongly implicated in cell-cell adhesion [1–7], cellular adhesion to extracellular aggregating factors [8], and binding of glycoproteins to cell surfaces [9–11]. Although calcium-carbohydrate complexes are thought to mediate many of these processes, little is known about the types of interactions that are likely to be involved. The interactions are generally presumed to be of the simple ionic type, with Ca^{2+} binding non-specifically to such anionic residues as the carboxyl groups of glucuronate [3] or sialic acid [6] moieties. However, many solution studies have shown that, in aqueous media, Ca^{2+} also binds to uncharged carbohydrates [12–15]; these observations

suggest that Ca^{2+} may interact with the carbohydrates of biological systems at uncharged sites. Recent crystallographic studies demonstrated that, in hydrated solid-state environments, neutral sugars chelate Ca^{2+} through sets of hydroxyl groups. These results indicated that Ca^{2+}, in conjunction with water molecules, can bind simultaneously to several uncharged sugar residues and thereby form hydrated carbohydrate-calcium-carbohydrate bridges [16–19].

In this paper we describe the crystal structure of a hydrated $CaBr_2$ complex of α-fucose, a common terminal residue of the oligosaccharide chains on glycoproteins and glycolipids [20]. We show that Ca^{2+} can form hydrated bridges between fucose moieties, and we suggest that similar interactions may provide an effective, stereospecific mechanism for cross-linking carbohydrate chains in biological systems.

EXPERIMENTAL

Evaporation of an approximately equimolar mixture of $CaBr_2$ and α-D-fucose yielded clear, rectangular plates of α-D-fucose · $CaBr_2$*. Weissenberg and oscillation photographs showed the crystal to be 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. Since the crystals are deliquescent, they were enclosed in thin-walled glass capillary tubes for X-ray analysis. A crystal fragment with approximate dimensions of 0.10, 0.20, and 0.50 mm was mounted on a Picker FACS-1 diffractometer with its b 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 eight medium-angle reflections (CuK$\bar{\alpha}$, $\lambda =$ 1.5418 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 1442 symmetry-independent reflections with $2\theta < 128°$. Immediately after data collection, accurate values for the cell parameters were determined by a least-squares analysis of 2θ values for 12 high-angle reflections (CuKα_1); these cell parameters were not significantly different from those obtained before intensities were measured. Table I lists crystal data. Reflections with scan counts below background level ("negative" intensities) were assigned intensities of 0.0 and were retained in all subsequent calculations. 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. Intensities and their variances were corrected for Lorentz and polarization factors, and crystal absorption corrections were calculated by using the computer program ORABS [21]; absorption by the glass capillary tube was ignored. The data were scaled by means of a Wilson [22] plot.

We arrived at a suitable trial structure by the heavy-atom method, using one of the Br^- as the heavy atom. The trial structure was refined by using a modified version of the full-matrix least-squares programs ORFLS [23, 24]. 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

* The D-isomer of α-fucose was used for this crystallographic analysis. The crystal structure of the corresponding $CaBr_2$ complex of α-L-fucose, the common isomer in glycoproteins and glycolipids, would be the mirror image of that described here.

TABLE I

CRYSTAL DATA

Stoichiometry	$C_6H_{12}O_5 \cdot CaBr_2 \cdot 3H_2O$
Z	4
Space group	$P2_12_12_1$
a	14.360 (2) Å
b	12.896 (3) Å
c	8.043 (1) Å
ρ (calculated)	1.864 g · cm^{-3}
ρ (observed)	1.86 g · cm^{-3}
μ (CuK$\bar{\alpha}$)	106.9 cm^{-1}

$1/\sigma^2(Fo^2)$. Scattering factors for the non-hydrogen atoms were from the International Tables for X-ray Crystallography [25], and hydrogen atom scattering factors were from Stewart et al. [26]. Coordinates for those hydrogen atoms bonded to carbon atoms, except those of the methyl group, were calculated by assuming tetrahedral coordination around the carbon atoms and C-H bond distances of 0.95 Å. The hydrogen atoms of the methyl and hydroxyl groups and of the water molecules were located in difference Fourier maps that were calculated during the latter stages of refinement. 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 and anisotropic temperature parameters and Zachariasen's [27] isotropic extinction parameter g (as formulated by Coppens and Hamilton [28]) were included in the refinement. As the refinement proceeded, the coordinates of the hydrogen atoms attached to oxygen atoms were improved by the use of difference Fourier maps.

The final R index ($\Sigma||Fo|-|Fc||/\Sigma|Fo|$) is 0.052, and the goodness-of-fit ($[\Sigma w(Fo^2-Fc^2)^2/(m-s)]^{1/2}$, where m is the number of reflections used and s is the number of parameters refined) is 1.43. During the last cycle of refinement, no parameter shifted more than one-fifth of its estimated standard deviation. A final difference Fourier map showed several peaks and troughs in the vicinities of the Br^- that ranged in magnitude from 0.5 to 0.7 e/Å^3; no other peaks or troughs exceeded 0.5 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 the non-hydrogen atoms. The correction factors of Cromer and Liberman were used [29]. After the correct enantiomer (D-fucose) was refined, coordinates were then inverted and the incorrect enantiomer (L-fucose) was refined. The L-fucose model refined to only $R = 0.061$ and goodness-of-fit $= 1.72$. A comparison of the two enantiomers, by use of the R-factor ratio test [30], indicated that the absolute configuration of D-fucose is correct with a probable error of less than 0.5 %.

RESULTS

Table II lists the final non-hydrogen atom parameters and their estimated standard deviations; the estimated errors in positional coordinates are about 0.001 Å for Br^- and Ca^{2+} and 0.006–0.011 Å for carbon and oxygen atoms. Tables of hydrogen-atom parameters, calculated and observed structure factors, bond angles, con-

TABLE II

HEAVY-ATOM PARAMETERS AND THEIR STANDARD DEVIATIONS

All values have been multiplied by 10^4. Temperature factors are coefficients in the expression $T = \exp(-\beta_{11}h^2 - \beta_{22}k^2 - \beta_{33}l^2 - 2\beta_{12}hk - 2\beta_{13}hl - 2\beta_{23}kl)$. The final value of the isotropic extinction parameter is $g = 0.006$ (2).

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br (1)	1416 (1)	886 (1)	640 (1)	62 (1)	55 (1)	143 (2)	8 (1)	−28 (1)	− 6 (1)
Br (2)	8314 (1)	223 (1)	982 (1)	60 (1)	92 (1)	114 (2)	−11 (1)	−16 (1)	− 3 (1)
Ca	4884 (1)	865 (1)	764 (2)	47 (1)	39 (1)	115 (3)	5 (1)	1 (1)	1 (2)
C (1)	4694 (7)	3415 (7)	5373 (11)	52 (6)	43 (7)	102 (14)	1 (5)	− 9 (8)	−10 (8)
C (2)	4925 (6)	3263 (7)	3523 (10)	38 (5)	27 (6)	101 (13)	− 1 (4)	− 2 (6)	−10 (7)
C (3)	4075 (6)	3040 (6)	2479 (10)	46 (5)	29 (6)	102 (14)	12 (4)	5 (7)	− 7 (8)
C (4)	3456 (6)	2253 (7)	3250 (12)	38 (5)	40 (6)	158 (15)	3 (4)	− 5 (8)	3 (9)
C (5)	3228 (6)	2596 (7)	4984 (12)	36 (4)	36 (6)	164 (15)	2 (5)	24 (8)	− 2 (8)
C (6)	2567 (7)	1900 (8)	5938 (15)	64 (6)	57 (8)	228 (23)	3 (6)	60 (12)	15 (13)
O (1)	4344 (4)	4432 (4)	5529 (7)	66 (4)	38 (4)	91 (9)	− 1 (3)	24 (5)	−16 (5)
O (2)	5418 (4)	4175 (5)	2988 (7)	50 (3)	44 (4)	113 (10)	−14 (4)	32 (5)	− 2 (6)
O (3)	4391 (4)	2671 (4)	873 (7)	59 (4)	40 (4)	88 (9)	2 (3)	7 (6)	2 (6)
O (4)	3944 (4)	1272 (4)	3165 (8)	55 (4)	26 (4)	164 (12)	3 (3)	25 (6)	4 (6)
O (5)	4072 (4)	2657 (5)	5928 (8)	51 (4)	40 (4)	127 (11)	− 1 (3)	24 (6)	14 (6)
O (W1)	3559 (4)	502 (6)	− 791 (9)	60 (4)	103 (6)	162 (12)	6 (4)	−14 (7)	−35 (8)
O (W2)	6204 (5)	1006 (6)	2482 (10)	71 (5)	64 (6)	222 (15)	7 (4)	−41 (8)	−12 (8)
O (W3)	5789 (10)	1616 (6)	−1311 (16)	292 (14)	39 (6)	570 (36)	34 (8)	325 (20)	50 (12)

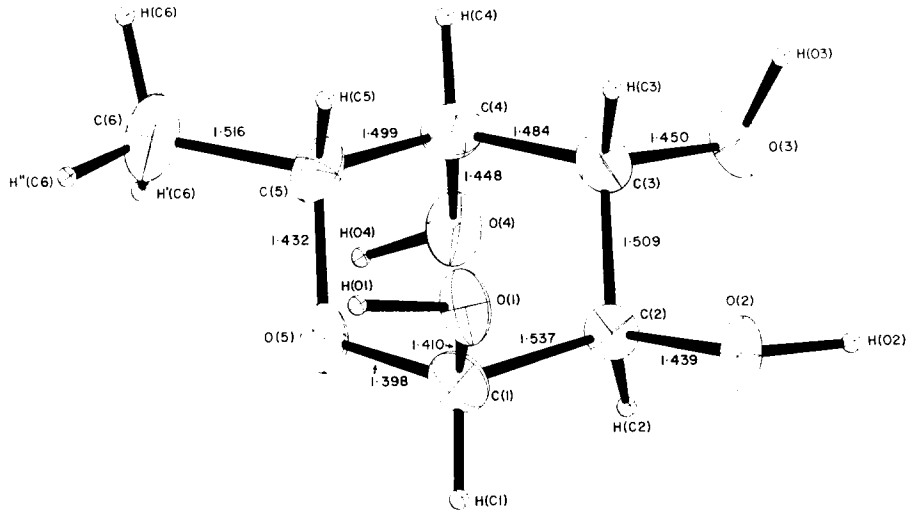

Fig. 1. Conformation and bond lengths of the fucose molecule. Estimated standard deviations in bond lengths are 0.09–0.013 Å. Non-hydrogen atoms are represented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 30 % probability; hydrogen atoms are represented by spheres of 0.07 Å radii. (This drawing and those in Figs 2 and 3 were prepared by using the computer program ORTEP [36].)

formational torsion angles, and hydrogen-bond distances and angles have been deposited*. Fig. 1 depicts the fucose conformation, atomic thermal ellipsoids, and those bond lengths that involve only non-hydrogen atoms.

No direct contacts exist between Ca^{2+} and Br^-; the closest bromide-calcium distance is 4.8 Å, which is 1.8 Å greater than the sum of the ionic radii for these two ions [31]. All hydroxyl- and water-oxygen atoms are coordinated to Ca^{2+}; none of these oxygen atoms serves as a hydrogen-bond acceptor in the crystal structure. All hydrogen atoms from the hydroxyl groups and from the water molecules from hydrogen bonds to Br^-.

Fig. 2 shows the environment of Ca^{2+}, which is coordinated to two symmetry-related fucose molecules and to the three water molecules. Ca^{2+} is chelated by one fucose molecule through its O(1)–O(2) pair of hydroxyl groups, and by the second fucose molecule through its O(3)–O(4) pair of hydroxyl groups. Ca^{2+} is thus surrounded by a coordination polyhedron composed of seven oxygen atoms: four from hydroxyl groups and three from water molecules. The stereochemistry of the coordination polyhedron is shown in greater detail in Fig. 3. The seven oxygen atoms assume a pentagonal-bipyramidal configuration, with calcium-oxygen distances that range from 2.32 to 2.44 Å.

* These tables are deposited with, and can be obtained upon request from: Elsevier Publishing Company, BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/024/76959/000 (1975) 000.

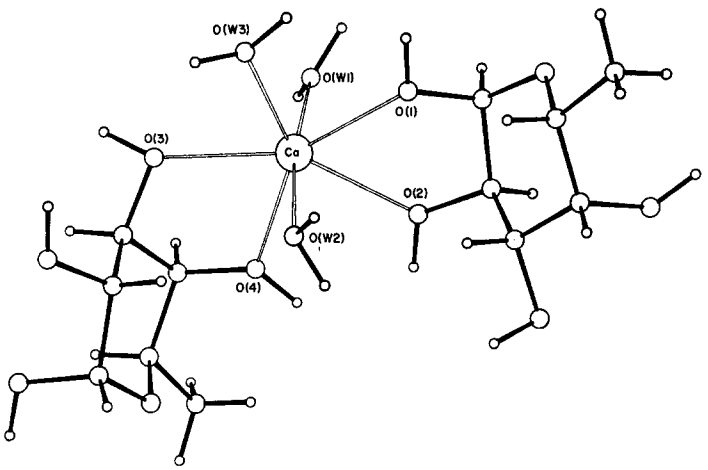

Fig. 2. Environment of Ca^{2+} in the fucose · $CaBr_2$ · $3H_2O$ complex.

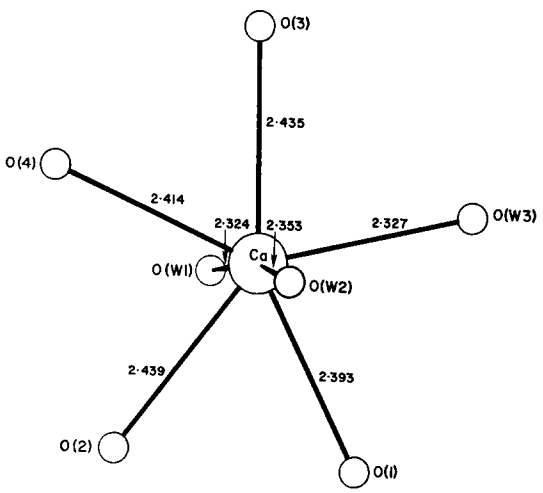

Fig. 3. Stereochemistry of Ca^{2+} coordination shell in the fucose · $CaBr_2$ · $3H_2O$ complex. Calcium-oxygen distances are given. O (W1), O (W2), and O (W3) are oxygen atoms of water molecules.

DISCUSSION

As in other crystalline calcium-halide complexes of uncharged carbohydrates [16–19], fucose chelates Ca^{2+} through sets of hydroxyl groups. Interactions of this type have also been observed in aqueous solution [12–15], where a wide variety of simple, uncharged sugars bind Ca^{2+} by substituting hydroxyl groups for the water molecules in the Ca^{2+} hydration shell. Crystallization experiments indicate that calcium-hydroxyl interactions are of general importance in the transition from aqueous solution to condensed, hydrated phases. Almost all our attempts to crystallize uncharged carbohydrates from aqueous calcium-halide solutions have produced

hydrated carbohydrate-calcium halide complexes. Even carbohydrates that possess anionic moieties bind Ca^{2+} through sets of hydroxyl groups, or through sets of hydroxyl groups acting in concert with the anionic substituents [32–35]. Anionic substituents may enhance the Ca^{2+}-binding properties of carbohydrates, but they are clearly not required for the formation of calcium-carbohydrate complexes in aqueous solution or in the solid-state.

Calcium-carbohydrate interactions in aqueous solution appear to be governed by the availability of sets of oxygen atoms that are in the proper geometrical arrangement to substitute for water molecules in the Ca^{2+} hydration shell [13]. The importance of this geometry in the solid phase is readily seen in the crystal structure of the fucose · $CaBr_2$ complex, as well as in all other crystal structures of calcium-carbohydrate complexes that have been examined. In each of these crystal structures, the carbohydrate moieties are positioned around Ca^{2+} in geometrical arrangements that permit the chelating oxygen atoms to form calcium contacts ranging in length from 2.3 to 2.6 Å. Since all of the chelating oxygen atoms must form suitable calcium-oxygen contacts simultaneously, the interactions are necessarily subject to appreciable geometrical constraints. In addition to these constraints, it appears that particular coordination geometries may be preferred by Ca^{2+}. Only two general types of calcium-coordination polyhedra have been found in the many crystal structures (approximately twenty) of calcium-carbohydrate salts and complexes that have been examined. In most cases, Ca^{2+} is coordinated to eight oxygen atoms in distorted square-antiprism arrangements. In other calcium-carbohydrate crystal structures, including the fucose · $CaBr_2$ complex, Ca^{2+} is coordinated to seven oxygen atoms, which form pentagonal-bipyramidal polyhedra around Ca^{2+}. In view of the geometrical constraints observed for calcium-carbohydrate interactions in aqueous solution and in crystal structures, it is likely that these interactions display considerable stereospecificity in a wide range of other environments, including those of biological systems.

A particularly prominent feature of the crystal packing scheme in the fucose · $CaBr_2$ complex is the role that Ca^{2+} plays in cross-linking fucose molecules. As shown in Fig. 3, Ca^{2+} is bound to two symmetry-related fucose molecules and to three water molecules so that hydrated fucose-calcium-fucose bridges are formed. Similar carbohydrate-calcium-carbohydrate bridges have been found in all of the crystal structures of calcium-carbohydrate salts and complexes that have been examined. Considering the role of water molecules in these bridges, it is reasonable to assume that related bridging interactions occur in other condensed, hydrated phases containing carbohydrates and Ca^{2+}. If so, these interactions could provide an effective, stereospecific mechanism for cross-linking carbohydrate chains in biological systems, and might thereby contribute to a variety of calcium-dependent agglutination and adhesion processes.

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