

## First Sphere Coordination of Divalent Metal Cations by Cyclodextrin: Structure of the $\beta$ -Cyclodextrin–Calcium Chloride–Water (1/2/11.25) Compound

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### Abstract

The structure of the title compound provides the first illustration of direct coordination of a polyvalent cation, as well as an inorganic anion, by  $\beta$ -cyclodextrin ( $\beta$ -CD). Crystal data:  $P2_12_12_1$ ,  $Z = 4$ ,  $a = 15.875(1)$ ,  $b = 17.583(1)$ ,  $c = 24.270(2)$  Å,  $V = 6775(1)$  Å<sup>3</sup>,  $R = 0.059$  for 4744 unique observed reflections with  $I > 3\sigma(I)$ . There are two  $\text{CaCl}_2$  per  $\beta$ -CD, with no direct contacts between the ions. None of the ions are included within the cyclodextrin cavity. The cations are in direct interaction simultaneously with two or three  $\beta$ -CD molecules and an eightfold coordination occurs for both. No disorder was found except for the two water molecules situated inside the cavity. The crystalline form is a novel monomer-type structure. The  $\beta$ -CD molecules are stacked as monomer entities inclined at  $\sim 50^\circ$  along a twofold screw axis, such that they are arranged in a new herringbone-like scheme. The overall packing is stabilized by intermolecular interactions between cyclodextrin monomers and by direct coordination to the ions. Moreover, the cohesion is reinforced by hydrogen bonds involving water molecules arranged in infinite chain motifs traversing the whole structure.

### 1. Introduction

Chelation of calcium ions by carbohydrates is of importance both in the binding of calcium to carbohydrate cell surfaces (Siuzdak, Ichikawa, Caulfield, Munoz, Wong & Nicolaou, 1993) and in biological transport processes (Eggens, Feuderson, Toyokuni, Dean, Strood & Hakomori, 1989). Concerning the role of the polyvalent ions in the gelation behaviour of polysaccharides, it has been suggested that there are direct cross-links between the ions and the helical polysaccharide chains, giving rise to much stronger interactions which lead to gel formation (Dea, 1993). These examples point out the considerable interest of the cation–saccharide interactions in biological systems. However, these types of interactions are difficult to investigate with oligo- and polysaccharides due to crystallization problems arising for systems larger than trisaccharides. It is likely that the cyclodextrins will provide suitable models for potential binding of polyvalent cations by polysaccharides.

The cyclodextrins are cyclic oligosaccharides consisting of six, seven or eight  $\alpha,1-4$  linked glucopyranose units in the  ${}^4C_1$  chair conformation. They have an intramolecular cavity of relatively hydrophobic nature with the hydroxyl groups forming two hydrophilic faces (primary and secondary). They have attracted interest owing to their ability to form inclusion compounds with a wide variety of guest molecules (Szejtli, 1989). However, their interaction with metals has received less attention and structural characterization of the first sphere coordination of polyvalent metal cations by cyclodextrins remains largely an unexplored field.

The present study is a part of a more general research project concerning the direct interaction of polyvalent cations, of predominant importance in the bio-inorganic field, with cyclodextrins. Crystallographic investigations have demonstrated that *inclusion compounds* of  $\beta$ -cyclodextrin adopt a limited number of molecular packings with invariant motifs. The main focus of the present analysis tends to emphasize the ability of cyclodextrins to form new assemblies by direct interactions, *outside the cavity*, to the structural elements constituting their environment.

Inclusion compounds of  $\beta$ -cyclodextrin ( $\beta$ -CD) crystallize essentially as dimer layer-type structures in four classes of molecular packing (Le Bas & Rysanek, 1987; Mentzafos, Mavridis, Le Bas & Tsoucaris, 1991), whereas the hydrate of  $\beta$ -CD (Lindner & Saenger, 1982a) and inclusion complexes with small guest organic molecules crystallize (Saenger, 1984) in monomer-type structures, in a compact overall packing which was described as cage-type herringbone structures (monoclinic-HB structures). Crystal structures of ionic insertion  $\beta$ -CD compounds have been reported in three cases and solely with monovalent ions:  $\text{KI}_7$ , in a dimer layer-type structure (Betzel, Hingerty, Noltemeyer, Weber & Saenger, 1983),  $\text{HI}$  (Lindner & Saenger, 1982b) and  $\text{KOH}$  (Charpin, Nicolis, Villain, de Rango & Coleman, 1991) in monoclinic-HB structures. For both  $\text{K}^+$  systems, the monovalent cation, external to the cavity, lies in the intermolecular spaces.

We report here the first crystal structure of a  $\beta$ -CD complex with a first-sphere coordination of a polyvalent metal cation by cyclodextrin. The direct interaction of divalent cations as well as inorganic anions with  $\beta$ -cyclodextrin is illustrated.

## 2. Experimental

Crystallization of  $\beta$ -CD with polyvalent metal salts is considered to be rather difficult since the association constants are likely to be very low, as observed for inorganic monovalent ion complexes (Buvári & Barcza, 1979). This requires the use of aqueous solutions at high concentration of salts, but the solubility of  $\beta$ -CD increases considerably at such concentrations, as shown in recent investigations on a variety of chloride and nitrate salts (Coleman & Nicolis, 1993).

Suitable crystals for crystal analysis were obtained, after  $\sim 1$  year, by slow evaporation at room temperature of a highly concentrated  $\text{CaCl}_2/\beta$ -CD aqueous solution. A colourless single crystal of approximate dimensions  $0.8 \times 0.6 \times 0.4$  mm was sealed, in the presence of the mother liquor, in a thin glass capillary, tested by photographic methods and then mounted on an Enraf-Nonius CAD-4 diffractometer. Crystallographic data are summarized in Table 1.

The structure was solved using the program *SHELXS86* (Sheldrick, 1985). Due to the large number of parameters to be refined, only ions and O atoms were given anisotropic temperature factors; H atoms bonded to C atoms were introduced in ideal positions, but not refined. Extended maxima of electron density appear close to two water molecules situated within the cavity, especially near the partially occupied site for which the contribution remains unclear. All calculations were carried out on a Digital MicroVAX computer using the *SDP-Plus* package (B. A. Frenz & Associates, Inc., 1983). Molecular graphics were performed by the *SYBYL* program package (Tripos Associates, Inc., 1988) implemented on an Evans & Sutherland PS300 interactive graphics station. Final atomic coordinates and thermal parameters of the non-H atoms are given in Table 2.\*

## 3. Discussion

The crystal structure exhibits a novel cage-type packing of  $\beta$ -CD monomers in a new herringbone-like pattern. The structure reveals that two cations are trapped in the intermolecular spaces external to the cyclodextrin cavity, with two  $\text{CaCl}_2$  and 11.25 water molecules per  $\beta$ -CD molecule. There is no direct contact between the ions, all being simultaneously coordinated to O atoms of the  $\beta$ -CD molecule and water molecules (except one  $\text{Cl}^-$  anion, only connected to water molecules). No disorder was found on either the primary hydroxyl group or the ion and water molecule sites (except one water site which is not fully occupied).

\* Lists of structure factors, anisotropic displacement parameters and H-atom coordinates have been deposited with the IUCr (Reference: PA0290). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 1. *Experimental details*

Crystal data	
Chemical formula	$\text{C}_{42}\text{H}_{70}\text{O}_{35} \cdot (\text{CaCl}_2)_2 \cdot 11.25\text{H}_2\text{O}$
Chemical formula weight	1559.4
Cell setting	Orthorhombic
Space group	$P2_12_12_1$
$a$ (Å)	15.875 (1)
$b$ (Å)	17.583 (1)
$c$ (Å)	24.270 (2)
$V$ (Å <sup>3</sup> )	6775 (1)
$Z$	4
$D_x$ (Mg m <sup>-3</sup> )	1.53
Radiation type	Mo $K\alpha$
Wavelength (Å)	0.71073
No. of reflections for cell parameters	25
$\theta$ range (°)	6–15
$\mu$ (mm <sup>-1</sup> )	0.424
Temperature (K)	291
Crystal form	Plate
Crystal size (mm)	$0.8 \times 0.6 \times 0.4$
Crystal colour	Colourless
Data collection	
Diffractometer	Enraf-Nonius CAD-4
Data collection method	$\omega$ - $2\theta$ scan
Absorption correction	Refined from $\Delta F$ (DIFABS; Walker & Stuart, 1983)
No. of measured reflections	8094
No. of independent reflections	8094
No. of observed reflections	4744
Criterion for observed reflections	$I > 3\sigma(I)$
$\theta_{\text{max}}$ (°)	28
$(\sin \theta/\lambda)_{\text{max}}$ (Å <sup>-1</sup> )	0.66
Range of $h, k, l$	$-20 \rightarrow h \rightarrow 0$ $0 \rightarrow k \rightarrow 23$ $0 \rightarrow l \rightarrow 32$
No. of standard reflections	3
Frequency of standard reflections	Every 60 min
Intensity decay (%)	4.6
Refinement	
Refinement on	$F$
Minimization	Sum of $w\Delta F^2$
$R$	0.059
$wR$	0.076
$S$	1.24
No. of reflections used in refinement	4744
No. of parameters used	636
H-atom treatment	Theoretical: not refined
Weighting scheme	$w = \{([F^2 + 2F\sigma(F)]^{1/2} - F)^2 + (0.04F)^2 + 3\}^{-1}$
$(\Delta/\sigma)_{\text{max}}$	0.32
$\Delta\rho_{\text{max}}$ (e Å <sup>-3</sup> )	0.82
$\Delta\rho_{\text{min}}$ (e Å <sup>-3</sup> )	-0.60
Extinction method	None
Source of atomic scattering factors	<i>International Tables for X-ray Crystallography</i> (1974, Vol. IV)
Computer programs	
Data collection	<i>SDP-Plus</i> (B. A. Frenz & Associates, Inc., 1983)
Cell refinement	<i>SDP-Plus</i>
Data reduction	<i>SDP-Plus</i>
Structure solution	<i>SHELXS86</i> (Sheldrick, 1985)
Structure refinement	<i>SDP-Plus</i>
Molecular graphics	<i>ORTEP</i> (Johnson, 1965) and <i>SYBYL</i> (Tripos Associates, Inc., 1988)

### 3.1. Macrocycle conformation and local environment of the $\beta$ -CD molecule

The cyclodextrin macrocycle conformation is distorted probably due to the coordination of the O atoms to

Table 2. Fractional atomic coordinates and equivalent isotropic displacement parameters ( $\text{\AA}^2$ )
$$B_{\text{eq}} = (4/3)\sum_i \sum_j \beta_{ij} a_i \cdot a_j$$

	x	y	z	$B_{\text{eq}}$
O41	0.6080 (4)	0.0309 (3)	0.2812 (2)	1.7 (1)
C11	0.6856 (5)	0.2334 (5)	0.2114 (3)	1.4 (1)
C21	0.5921 (5)	0.2265 (4)	0.2268 (3)	1.4 (1)
C31	0.5788 (5)	0.1614 (5)	0.2660 (3)	1.6 (1)
C41	0.6110 (5)	0.0888 (4)	0.2390 (3)	1.4 (1)
C51	0.7031 (5)	0.0992 (5)	0.2228 (3)	1.7 (1)
C61	0.7402 (6)	0.0323 (5)	0.1925 (4)	2.2 (2)
O21	0.5636 (4)	0.2958 (3)	0.2525 (2)	1.9 (1)
O31	0.4907 (3)	0.1586 (3)	0.2775 (2)	1.9 (1)
O51	0.7129 (4)	0.1637 (3)	0.1868 (2)	1.7 (1)
O42	0.6922 (4)	-0.1292 (3)	0.3996 (2)	1.8 (1)
C12	0.5534 (5)	-0.0322 (5)	0.2740 (3)	1.6 (1)
C22	0.5081 (5)	-0.0435 (5)	0.3289 (3)	1.8 (1)
C32	0.5740 (5)	-0.0570 (5)	0.3740 (3)	1.8 (1)
C42	0.6276 (5)	-0.1258 (5)	0.3586 (3)	1.5 (1)
C52	0.6677 (5)	-0.1138 (5)	0.3008 (3)	1.6 (1)
C62	0.7134 (6)	-0.1834 (5)	0.2781 (4)	2.2 (2)
O22	0.4599 (4)	0.0228 (4)	0.3417 (3)	2.3 (1)
O32	0.5365 (4)	-0.0669 (4)	0.4265 (2)	2.5 (1)
O52	0.6024 (4)	-0.0980 (3)	0.2609 (2)	1.5 (1)
O43	0.9390 (4)	-0.1264 (3)	0.4951 (3)	1.7 (1)
C13	0.7196 (5)	-0.2016 (5)	0.4183 (3)	1.7 (1)
C23	0.7190 (5)	-0.1977 (5)	0.4814 (3)	1.7 (1)
C33	0.7922 (5)	-0.1481 (5)	0.5016 (3)	1.5 (1)
C43	0.8732 (5)	-0.1751 (5)	0.4767 (3)	1.6 (1)
C53	0.8649 (5)	-0.1699 (5)	0.4145 (3)	2.0 (1)
C63	0.9447 (7)	-0.1939 (6)	0.3876 (4)	3.0 (2)
O23	0.6405 (4)	-0.1731 (4)	0.5029 (2)	2.2 (1)
O33	0.7915 (4)	-0.1513 (4)	0.5609 (2)	2.2 (1)
O53	0.7988 (4)	-0.2208 (3)	0.3975 (2)	2.1 (1)
O44	1.0926 (4)	0.0547 (3)	0.5508 (2)	2.1 (1)
C14	1.0135 (5)	-0.1605 (5)	0.5159 (3)	1.9 (1)
C24	1.0215 (6)	-0.1423 (5)	0.5759 (4)	2.2 (2)
C34	1.0253 (6)	-0.0579 (5)	0.5833 (4)	2.0 (2)
C44	1.1007 (5)	-0.0261 (5)	0.5498 (3)	1.7 (1)
C54	1.0934 (6)	-0.0518 (5)	0.4895 (4)	2.0 (2)
C64	1.1702 (6)	-0.0304 (6)	0.4566 (4)	2.7 (2)
O24	0.9483 (4)	-0.1696 (4)	0.6047 (3)	2.7 (1)
O34	1.0345 (4)	-0.0352 (4)	0.6404 (2)	2.4 (1)
O54	1.0837 (4)	-0.1324 (3)	0.4870 (2)	2.3 (1)
O45	1.0756 (4)	0.2876 (3)	0.5028 (2)	2.1 (1)
C15	1.1458 (5)	0.0948 (5)	0.5861 (3)	1.5 (1)
C25	1.0933 (5)	0.1524 (5)	0.6179 (3)	1.8 (1)
C35	1.0544 (5)	0.2087 (5)	0.5785 (3)	1.9 (1)
C45	1.1221 (5)	0.2463 (5)	0.5434 (3)	1.7 (1)
C55	1.1759 (6)	0.1853 (5)	0.5155 (4)	2.2 (2)
C65	1.2511 (6)	0.2179 (6)	0.4853 (4)	2.9 (2)
O25	1.0274 (4)	0.1184 (3)	0.6502 (2)	1.9 (1)
O35	1.0099 (4)	0.2636 (3)	0.6110 (2)	2.3 (1)
O55	1.2085 (4)	0.1339 (3)	0.5569 (2)	2.1 (1)
O46	0.9242 (4)	0.3644 (3)	0.3625 (2)	1.9 (1)
C16	1.0970 (5)	0.3628 (5)	0.4922 (3)	1.9 (1)
C26	1.0167 (6)	0.4108 (5)	0.4990 (4)	2.4 (2)
C36	0.9514 (5)	0.3858 (5)	0.4563 (3)	1.8 (1)
C46	0.9894 (5)	0.3908 (5)	0.3989 (3)	1.8 (1)
C56	1.0680 (5)	0.3408 (5)	0.3962 (3)	2.0 (1)
C66	1.1150 (6)	0.3384 (6)	0.3430 (4)	2.7 (2)
O26	0.9839 (5)	0.3982 (4)	0.5536 (3)	3.0 (1)
O36	0.8778 (4)	0.4335 (4)	0.4617 (3)	2.8 (1)
O56	1.1263 (4)	0.3695 (4)	0.4367 (2)	2.3 (1)
O47	0.7302 (4)	0.2491 (3)	0.2594 (2)	1.6 (1)
C17	0.9269 (5)	0.3915 (5)	0.3084 (4)	2.0 (1)
C27	0.8416 (6)	0.4289 (5)	0.2963 (4)	2.3 (2)
C37	0.7722 (5)	0.3703 (5)	0.2924 (3)	1.8 (1)
C47	0.7954 (5)	0.3042 (5)	0.2558 (3)	1.6 (1)
C57	0.8774 (5)	0.2696 (5)	0.2776 (3)	1.8 (1)
C67	0.9100 (6)	0.2006 (5)	0.2468 (4)	2.1 (2)
O27	0.8257 (4)	0.4823 (4)	0.3390 (3)	3.4 (1)
O37	0.6941 (4)	0.4029 (4)	0.2743 (3)	2.4 (1)
O57	0.9411 (4)	0.3290 (4)	0.2724 (2)	2.1 (1)
O61	0.8274 (4)	0.0462 (4)	0.1812 (3)	2.5 (1)
O62	0.6536 (5)	-0.2417 (4)	0.2659 (3)	3.0 (1)

Table 2 (cont.)

	x	y	z	$B_{\text{eq}}$
O63	0.9409 (4)	-0.1966 (4)	0.3277 (3)	3.0 (1)
O64	1.1612 (5)	-0.0490 (5)	0.4001 (3)	3.9 (2)
O65	1.2914 (4)	0.1604 (4)	0.4531 (3)	3.7 (2)
O66	1.1829 (5)	0.2899 (5)	0.3451 (3)	4.4 (2)
O67	0.9135 (4)	0.2134 (4)	0.1889 (3)	2.7 (1)
Ca1	0.8395 (1)	0.1456 (1)	0.11123 (7)	1.89 (3)
Ca2	0.4346 (1)	0.2834 (1)	0.30973 (7)	1.57 (3)
Cl1	0.1207 (2)	0.1680 (2)	0.9139 (1)	3.48 (5)
Cl2	0.9012 (2)	0.9710 (2)	0.2862 (1)	4.91 (7)
Cl3	1.1969 (2)	0.8888 (2)	0.6801 (2)	5.71 (8)
Cl4	0.1563 (3)	0.5613 (2)	0.9780 (2)	5.95 (8)
OW1	0.6619 (4)	0.8531 (4)	0.1355 (3)	3.5 (1)
OW2	0.6889 (5)	0.7199 (4)	0.6007 (3)	2.9 (1)
OW3	0.6809 (5)	0.9762 (4)	0.5638 (3)	3.5 (2)
OW4	0.6583 (5)	0.6818 (4)	0.1544 (3)	3.2 (1)
OW5	0.5170 (4)	0.8879 (4)	0.6061 (3)	3.9 (2)
OW6	0.8937 (5)	0.5929 (5)	0.4677 (3)	4.6 (2)
OW7	0.8910 (5)	0.6544 (5)	0.3075 (2)	5.2 (2)
OW8	0.7113 (5)	0.4932 (5)	0.1782 (3)	4.6 (2)
OW9	0.8318 (6)	0.6358 (6)	0.1957 (4)	6.7 (2)
OW10	0.0434 (6)	0.0529 (6)	0.3575 (4)	6.5 (3)
OW11	0.4571 (9)	0.3550 (8)	0.5717 (6)	10.1 (4)
OW12	0.285 (3)	0.424 (2)	0.614 (2)	6.3 (9)

the ions, although the average bond distances and angles remain in the normal range for the seven independent glucopyranose residues. The geometrical data describing the conformation of the  $\beta$ -CD molecule are summarized in Table 3.

The O(4) plane is inclined at 135 and 107°, respectively, to the *ab* and *ac* planes. The distances O(4) $n$ —O(4) $n + 1$  range from 4.23 to 4.54 Å. The tilt angles between the O(4) plane and the least-squares planes through the atoms C(1), C(4), O(4) and O(4') range from 5.8 to 36.9°, indicating distortions of the ring greater than those ordinarily observed in  $\beta$ -CD complexes for both monoclinic herringbone- and dimer layer-type structures.

The torsion angles concerning the orientation of the C(6)—O(6) bonds show two different types of conformation, *gauche-gauche* for two residues, G2 and G7, and *gauche-trans* for the others. This implies that the C(6)—O(6) bonds point away from the centre for G2 and G7 and to the centre for the other five. This is rather unusual as in the previously reported  $\beta$ -CD structures, the *gauche-gauche* conformation is preferred.

The O(2)—O(3) distances between adjacent glucose units reveal the probable existence of the usual intramolecular interglucose hydrogen bonds stabilizing the 'round' shape of the ring. They vary between 2.71 and 2.89 Å, except O32—O23 and O36—O27, 3.11 and 3.20 Å, respectively.

The local environment of the  $\beta$ -CD molecule, with ions and water molecules, is illustrated in Fig. 1 and shown schematically in Fig. 2. There is a very high occurrence of direct interaction with the ions and their symmetry and translation-related counterparts, one  $\beta$ -CD molecule interacting simultaneously with five calcium and six chloride ions. All residues participate directly in coordination to the ions, involving 14 out of the 21

Table 3. Geometrical data describing the conformation of the  $\beta$ -CD molecule

Residue	Tilt ( $^{\circ}$ )	O4 distance from O4 plane ( $\text{\AA}$ )	O2 distance from O2/O3 plane ( $\text{\AA}$ )	O3 distance from O2/O3 plane ( $\text{\AA}$ )	O6 distance from O6 plane ( $\text{\AA}$ )	O5—C5—C6—O6 torsion angle ( $^{\circ}$ )
G1	22.1 (6)	-0.116	0.421	0.002	0.482	60.3 (8)
G2	5.8 (19)	-0.446	-0.634	-0.744	-0.900	-51.8 (8)
G3	14.4 (5)	0.443	-0.339	0.158	0.541	57.2 (10)
G4	26.9 (4)	0.135	1.171	0.738	0.542	63.1 (10)
G5	11.4 (12)	-0.423	-0.349	-0.682	-0.412	68.8 (9)
G6	6.1 (15)	0.021	-0.746	-0.525	-0.393	62.0 (10)
G7	36.9 (4)	0.385	0.697	0.832	0.157	-67.8 (8)

hydroxyl groups, except G6 which is only connected to water molecules. Five residues are simultaneously linked to several ions, the residue G1 being connected to one anion and two cations. Moreover, the hydroxyl groups participate strongly in the hydration, some binding several water molecules simultaneously.

### 3.2. Arrangement of $\beta$ -CD molecules

The  $\beta$ -CD molecule, inclined at  $\sim 50^{\circ}$  with respect to a twofold screw axis parallel to  $a$ , generates, through a network of intermolecular hydrogen bonds, an infinite chain (Fig. 3) which forms a new herringbone-

Table 4. Intermolecular hydroxyl group short contacts ( $\text{\AA}$ )

O21—O25'	2.860 (8)	O36—O65'	2.980 (10)
O31—O35'	3.048 (8)	O31—O62''	3.070 (9)
O22—O26'	2.922 (9)	O63—O67'''	2.832 (9)
O36—O55'	2.971 (9)	O33—O51''	3.065 (7)

Symmetry codes: (i)  $x - \frac{1}{2}, \frac{1}{2} - y, 1 - z$ ; (ii)  $1 - x, y + \frac{1}{2}, \frac{1}{2} - z$ ; (iii)  $2 - x, y - \frac{1}{2}, \frac{1}{2} - z$ ; (iv)  $-x + \frac{1}{2}, -y, z + \frac{1}{2}$ .

like pattern (HB chain). The crystallographic symmetry induces a second HB chain with the opposite direction ('antiparallel'). Each HB chain is stacked along the  $b$  axis, forming sheets parallel to the  $ab$  plane (Fig. 4): there are, then, two 'antiparallel' sheets, HBA and HBB, alternately packed along the  $c$  axis (Fig. 5).

This arrangement is stabilized by eight intermolecular hydrogen bonds between hydroxyl groups: five ensure a strong cohesion inside the HB chains and three link antiparallel HB chains (Table 4), but, between  $\beta$ -CD molecules located in parallel HB chains, within the HBA and HBB sheets, there exist no short contacts with (O...O) distances less than 3.4  $\text{\AA}$ .

### 3.3. Calcium coordination

The two independent  $\text{Ca}^{2+}$  cations, Ca1 and Ca2, located between the HB chains play an essential role in the cohesion of the whole structure by cross-linking simultaneously  $\beta$ -CD monomers situated in adjacent antiparallel HB chains (Fig. 3b and Fig. 5). The cation Ca1 serves as a bridge between two antiparallel HB chains by linking two  $\beta$ -CD units, one through three atoms of the primary hydroxyl side, the other through two secondary hydroxyls, while the Ca2 cation bridges two  $\beta$ -CD molecules of the same HB chain to one of an antiparallel HB chain (Fig. 6a).

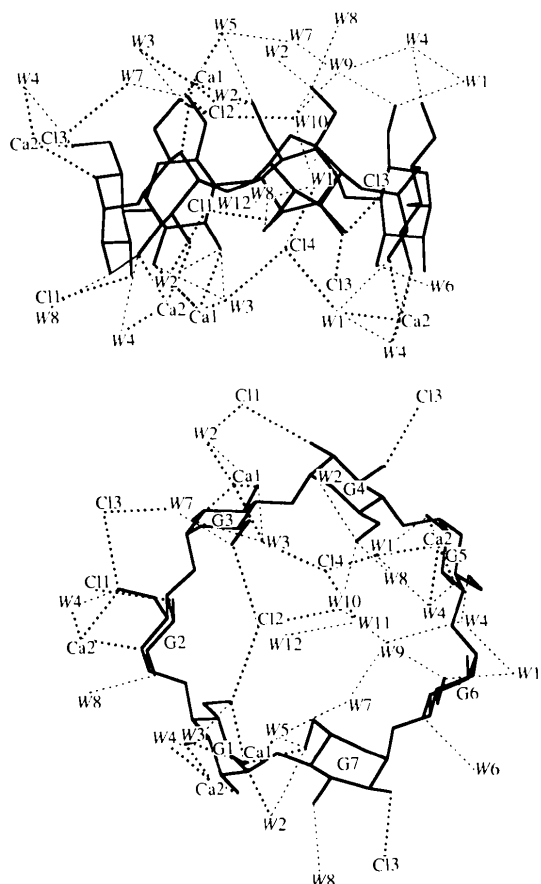


Fig. 1. The  $\beta$ -CD molecular unit plus significant interacting ions and water molecules.

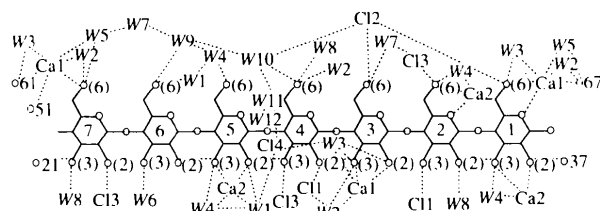


Fig. 2. Schematic representation of short contacts involving primary and secondary hydroxyl groups.

Crystallographic investigations of calcium salt complexes with disaccharides composed of pyranose residues, such as lactose or  $\alpha,\alpha$ -trehalose, have previously demonstrated chelation of calcium through the O(2) and O(3) pairs of the hydroxyl groups (Bugg & Cook, 1972; Cook & Bugg, 1973), but O(6), O(5) chelation does not occur. In this calcium salt complex with  $\beta$ -CD, we observe the systematic chelation of the cation by either O(2),O(3) or O(5),O(6) oxygens of the glucopyranose units, as previously observed for the barium ion in the hexasaccharide complex (*p*-nitrophenyl  $\alpha$ -maltohexoside)<sub>2</sub>.Ba(I<sub>3</sub>)<sub>2</sub>.27H<sub>2</sub>O (Ding, Steiner & Saenger, 1991). Moreover, we also have a case where the  $\beta$ -CD unit acts as a tridentate ligand, one cation being simultaneously coordinated to the O(5) and O(6) oxygens of a glucopyranose unit and

to the O(6) hydroxyl group of an adjacent one. This shows that the cyclodextrin is able to provide three O atoms to a cation even if it does not possess the 'axial-equatorial-axial' hydroxyl sequence, considered as the most favourable for an efficient complexation of a cation by sugar (Angyal, 1974). In fact, this is the probable reason for local macrocycle distortions as large as the tilt angle observed (36.9°) for residue G7.

The Ca1 cation has five and Ca2 six coordination sites occupied by O donor atoms of the  $\beta$ -CD unit, with water molecules on the remaining sites, three for Ca1 and two for Ca2, yielding an overall coordination number of eight for both (Fig. 7). Eightfold coordination of the calcium is common and has been found in calcium salts of mono- and disaccharides such as calcium D-galactarate, tetrahydrate (Sheldrick, Mackie & Akkrig, 1989) and the calcium bromide complexes of lactose, galactose and inositol (Bugg & Cook, 1972), the calcium

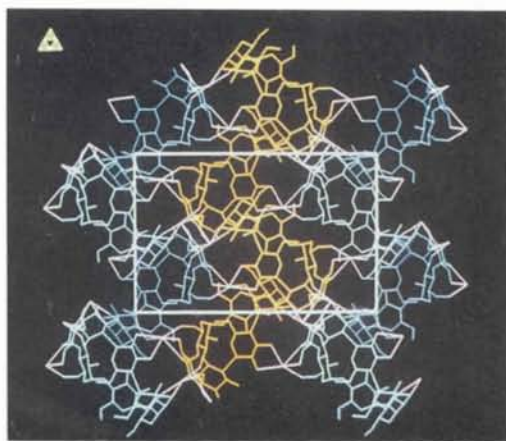
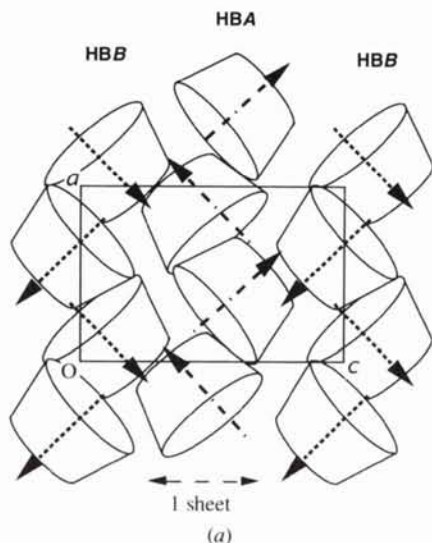


Fig. 3. (a) Schematic representation of the two 'antiparallel' HB chains forming, along the perpendicular *b* axis, two antiparallel sheets: HBA ( $y = 1/4$ ) and HBB ( $y = 3/4$ ). (b) Projection of the structure on the *ac* plane showing the two (blue and yellow) HB chains and the two cations ( $\text{Ca}^{2+}$  in purple, *c* horizontal axis).

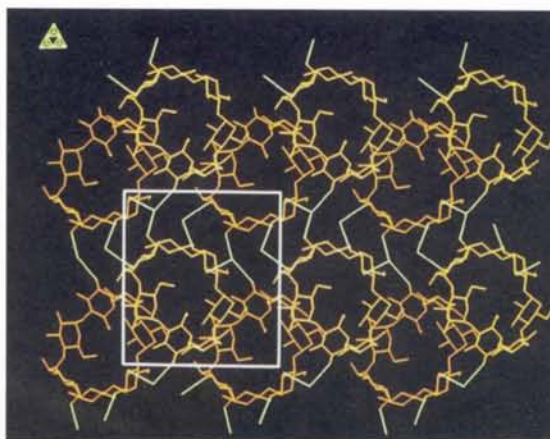


Fig. 4. Section parallel to the *ab* plane representing one sheet of parallel HB chains (HBA) and showing the role of two anions (green) in the stacking of the HB chains within a sheet (*b* horizontal axis).

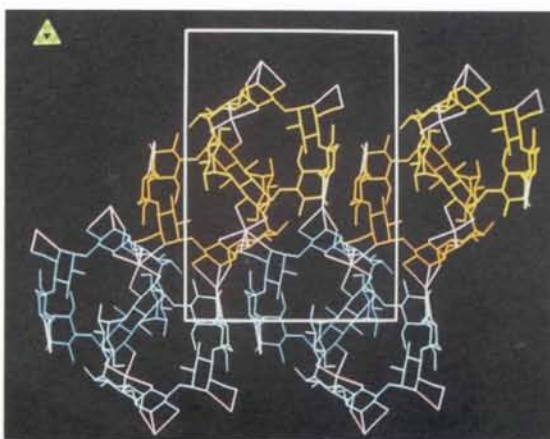


Fig. 5. Projection of the structure on the *bc* plane illustrating the role of the cations (purple) in the stacking of the 'antiparallel' HB chains (*b* horizontal axis).

coordination being achieved by four, five or six hydroxyl groups of different saccharide molecules and by water molecules.

The Ca—O distances are as expected for this type of coordination (Einspahr & Bugg, 1980); the shortest distances occur with OW, the largest with O(5) (Table 5a). The polyhedron formed by the oxygen ligands can be described as a trigonal dodecahedron for Ca2 and a slightly distorted square antiprism for Ca1. This distortion results from steric constraints due to the coordination by polydentate ligands.

### 3.4. The chloride anions

None of the anions are included within the cavity, but different types of interaction occur: two anions, Cl1 and Cl3, located between the intermolecular spaces are linked to two or three  $\beta$ -CD molecules, whereas the two other anions lying at the level of the hydroxyl groups are not directly involved in the stability of the structure. The geometry of the coordination is either tetrahedral or trigonal. The Cl—O distances (Table 5b) involving the hydroxyl groups are, all but one, smaller than those usually observed for Cl—O water in  $\text{CaCl}_2$  hydrates (Agron & Busing, 1991), as well as in chloride salts of saccharides.

The anions Cl1 and Cl3 participate strongly in the cohesion of the structure by cross-linking, along the *b* axis, adjacent parallel HB chains (Fig. 4), but neither is directly linking adjacent antiparallel HB chains. These anions are both simultaneously connected with residues G2 and G4 of  $\beta$ -CD units located in adjacent parallel HB chains (Fig. 6b). Moreover, Cl3 links two  $\beta$ -CD units of the same chain. All but one of these associations concern

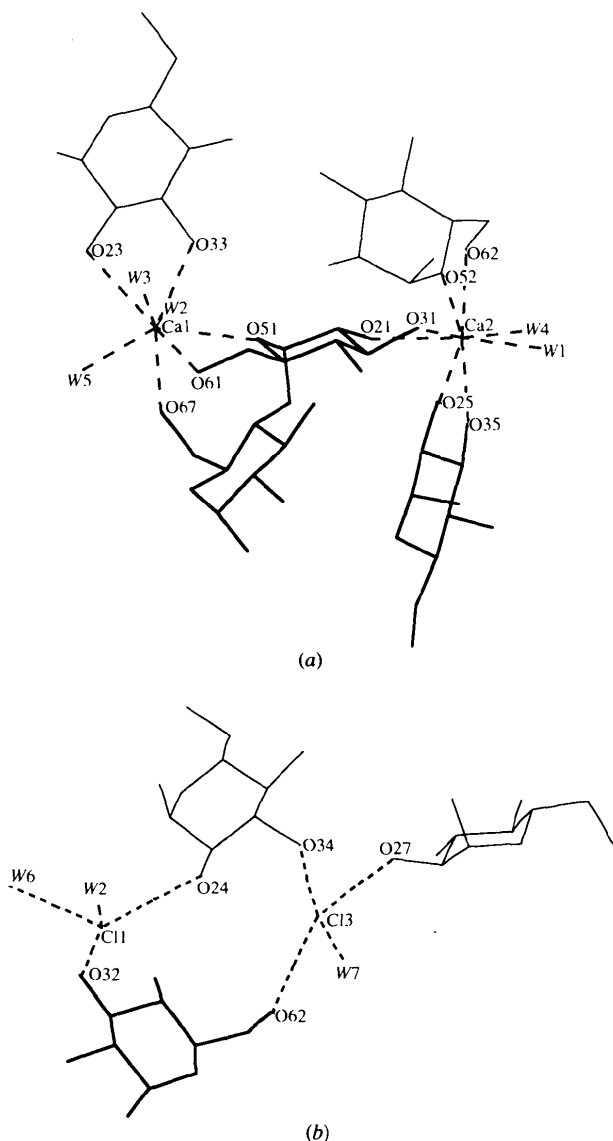


Fig. 6. Environment of the ions (the different line thickness has no physical significance, but simply serves to differentiate between the chains): (a) calcium ion coordination showing the contribution of the two cations in the stacking of antiparallel HB chains; (b) chloride ion Cl1 and Cl3 coordination showing the contribution of these anions in the stacking of parallel HB chains.

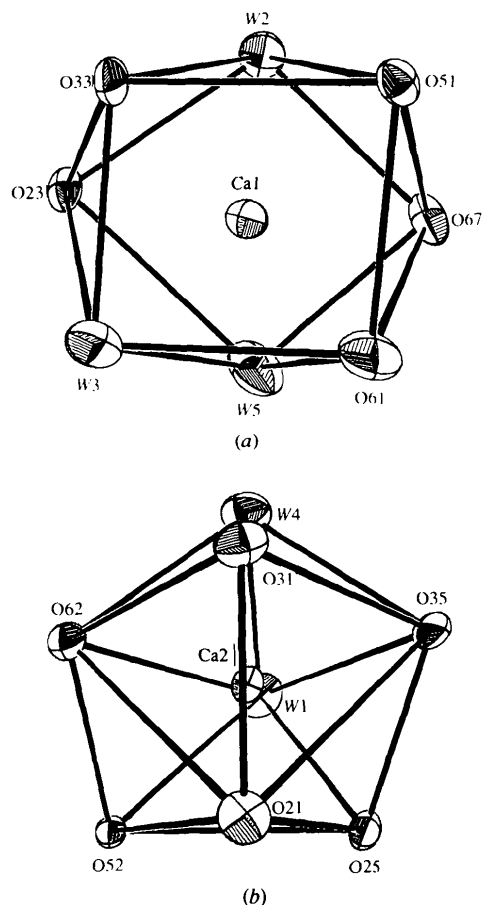


Fig. 7. ORTEP (Johnson, 1965) view of the coordination polyhedra of the two cations. Displacement ellipsoids are drawn at 50% probability for all ions and atoms.

Table 5. Coordination distances (Å)

(a) Cations			
Ca1...O51 <sup>1</sup>	2.739 (6)	Ca2...O52 <sup>2a</sup>	2.762 (6)
Ca1...O61 <sup>1</sup>	2.444 (7)	Ca2...O62 <sup>2a</sup>	2.349 (7)
Ca1...O67 <sup>1</sup>	2.521 (7)	Ca2...O21 <sup>1</sup>	2.484 (6)
Ca1...O23 <sup>1b</sup>	2.692 (6)	Ca2...O31 <sup>1</sup>	2.495 (6)
Ca1...O33 <sup>1b</sup>	2.413 (6)	Ca2...O25 <sup>1</sup>	2.469 (6)
Ca1...W2 <sup>1m</sup>	2.420 (7)	Ca2...O35 <sup>1</sup>	2.412 (6)
Ca1...W3 <sup>1m</sup>	2.453 (7)	Ca2...W1 <sup>1m</sup>	2.368 (7)
Ca1...W5 <sup>1m</sup>	2.357 (7)	Ca2...W4 <sup>1m</sup>	2.475 (7)
(b) Anions			
C11...O32 <sup>1m</sup>	3.080 (7)	C13...O62 <sup>1m</sup>	2.981 (7)
C11...O24 <sup>1m</sup>	3.091 (7)	C13...O34 <sup>1</sup>	3.060 (7)
C11...W2 <sup>1a</sup>	3.176 (8)	C13...O27 <sup>1m</sup>	3.087 (7)
C11...W6 <sup>1a</sup>	3.173 (8)	C13...W7 <sup>1m</sup>	3.187 (9)
C12...O61 <sup>1a</sup>	3.101 (7)	C14...W1 <sup>1m</sup>	3.141 (8)
C12...O63 <sup>1a</sup>	3.179 (8)	C14...W3 <sup>1a</sup>	3.155 (8)
C12...W10 <sup>1m</sup>	3.19 (1)	C14...W11 <sup>1a</sup>	3.25 (1)

Symmetry codes: (i)  $x, y, z$ ; (ii)  $-x + \frac{1}{2}, -y, z - \frac{1}{2}$ ; (iii)  $-x + \frac{1}{2}, -y + 1, z - \frac{1}{2}$ ; (iv)  $1 - x, y + \frac{1}{2}, \frac{1}{2} - z$ ; (v)  $x - \frac{1}{2}, \frac{1}{2} - y, 1 - z$ ; (vi)  $1 - x, y - \frac{1}{2}, \frac{1}{2} - z$ ; (vii)  $-x + \frac{1}{2}, -y, z + \frac{1}{2}$ ; (viii)  $1 - x, y + \frac{1}{2}, \frac{1}{2} - z$ ; (ix)  $1 - x, y - \frac{1}{2}, \frac{1}{2} - z$ ; (x)  $x, y + 1, z$ ; (xi)  $x + 1, y + 1, z$ ; (xii)  $\frac{1}{2} + x, \frac{1}{2} - y, 1 - z$ ; (xiii)  $x + \frac{1}{2}, -y + \frac{1}{2}, 1 - z$ ; (xiv)  $x - \frac{1}{2}, \frac{1}{2} - y, 1 - z$ ; (xv)  $-x + \frac{1}{2}, -y + 1, z + \frac{1}{2}$ .

secondary hydroxyl groups. The coordination of each anion includes water molecules, two for C11 and one for C13, yielding an overall coordination number of 4 in a distorted tetrahedron configuration, for both.

The anion C12 lies at the level of the primary hydroxyl groups (Fig. 1), coordinated to two O(6) atoms of the same  $\beta$ -CD and one O water, OW10, at 0.4 Å above the plane of these three atoms. The C14 anion lies at the level of the secondary hydroxyl groups (Fig. 1), exclusively coordinated to three water molecules, one inside and the other two outside the cavity. It is at 0.4 Å above the plane of these three water molecules which is itself inclined at 117° on the O(4) least-squares plane.

### 3.5. The water molecules

In the asymmetric crystal unit, 11 out of the 12 water sites are present with full occupancy. Nine are located outside of the cavity and one, OW10, situated close to the primary hydroxyl face. Two water molecules (OW11 and OW12) are within the cavity [at 0.4 and -0.3 Å above the O(4) least-squares plane], affected by higher thermal motion than the others and with an occupancy factor of 0.25 for OW12. They do not form hydrogen bonds with the macrocycle.

Most of the water molecules are engaged in coordination to the ions, six either with a cation or an anion, three others being shared between one cation and one anion. All external water molecules may be involved in direct hydrogen bonding contact with hydroxyl groups (Figs. 1 and 2). Five of them are in short contact with secondary hydroxyls and all but OW6, with primary hydroxyl groups. However, in the absence of the experimental determination of the H-atom positions, the many short contacts involving O atoms (hydroxyl groups and water molecules) are simply given here for (O...O) distances less than 3.1 Å (Table 6).

Table 6. Short contacts (Å) involving water molecules and primary or secondary hydroxyl groups

W1...W4 <sup>1</sup>	3.05 (1)	W4...O65 <sup>1m</sup>	2.755 (9)
W1...O25 <sup>1</sup>	3.067 (9)	W5...W6 <sup>1m</sup>	2.67 (1)
W1...O66 <sup>1m</sup>	2.74 (1)	W5...W7 <sup>1m</sup>	2.99 (1)
W2...O33 <sup>1a</sup>	2.952 (9)	W5...O67 <sup>1a</sup>	2.90 (1)
W2...O64 <sup>1a</sup>	3.04 (1)	W6...O36 <sup>1</sup>	2.82 (1)
W2...O67 <sup>1a</sup>	2.933 (9)	W7...W9 <sup>1</sup>	2.89 (1)
W3...O23 <sup>1a</sup>	3.079 (9)	W7...O63 <sup>1a</sup>	2.78 (1)
W3...O33 <sup>1a</sup>	2.848 (9)	W8...O22 <sup>1m</sup>	2.808 (9)
W3...O56 <sup>1m</sup>	2.849 (9)	W8...O37 <sup>1</sup>	2.84 (1)
W3...O61 <sup>1m</sup>	2.88 (1)	W8...O64 <sup>1m</sup>	2.87 (1)
W4...W9 <sup>1</sup>	3.04 (1)	W9...W10 <sup>1m</sup>	2.78 (1)
W4...O31 <sup>1m</sup>	2.914 (9)	W9...O66 <sup>1m</sup>	2.89 (1)
W4...O35 <sup>1a</sup>	3.028 (9)	W10...W11 <sup>1a</sup>	2.73 (2)
W4...O62 <sup>1a</sup>	3.022 (9)	W10...O64 <sup>1a</sup>	2.79 (1)

Symmetry codes: (i)  $x, y, z$ ; (ii)  $-x + \frac{1}{2}, -y + 1, z - \frac{1}{2}$ ; (iii)  $2 - x, y + \frac{1}{2}, \frac{1}{2} - z$ ; (iv)  $x, y + 1, z$ ; (v)  $x - \frac{1}{2}, -y + \frac{1}{2}, 1 - z$ ; (vi)  $-x + \frac{1}{2}, -y + 1, z + \frac{1}{2}$ ; (vii)  $x - \frac{1}{2}, \frac{1}{2} - y, 1 - z$ ; (viii)  $1 - x, y + \frac{1}{2}, \frac{1}{2} - z$ ; (ix)  $x - 1, y, z$ .

All but three of the water molecules are highly hydrogen bonded to each other, as shown by the many short contacts between them. They no doubt form rings and infinite chain-like motifs, some passing through the cavity (Fig. 1).

### 3.6. Factors contributing to the stabilization of the structure

The two cations, two of the anions and nine of the water molecules are located between the two antiparallel HB chains of  $\beta$ -CD molecules. The overall packing, illustrated in Fig. 8, is stabilized by intermolecular interactions between cyclodextrin monomers and direct coordination to the ions, giving rise to lateral direct cross-links, in two perpendicular directions, between the HB chains and the ions. The cohesion inside the HB chains is ensured by strong intermolecular hydrogen bonding between the hydroxyl groups of the  $\beta$ -CD and by direct interactions involving one cation and one anion. There are no intermolecular interactions between  $\beta$ -CD units in adjacent HB chains forming sheets parallel to the *ab* plane. However, two anions serve as intermolecular bridges between parallel HB chains and then play an essential role in the stability of the structure. The cohe-

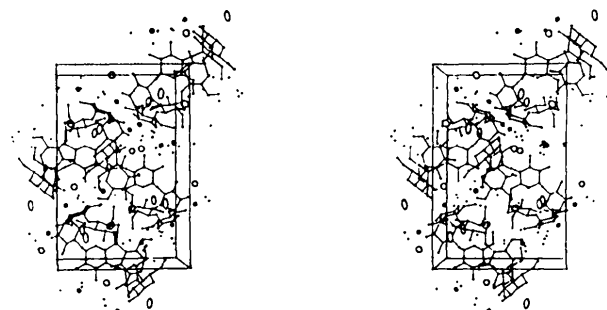


Fig. 8. Stereoscopic ORTEP (Johnson, 1965) view of the unit cell. Displacement ellipsoids are drawn at 50% probability for all ions.

sion of the alternate stacking of the 'antiparallel' sheets along the *c* axis is ensured by intermolecular interactions between hydroxyl groups of the  $\beta$ -CD units and by direct coordination of Ca cations cross-linking antiparallel HB chains. Moreover, the cohesion is reinforced by hydrogen bonding involving water molecules, although none appear to be essential to the stability of the structure.

### 3.7. Comparison with the monoclinic-HB structure of the potassium hydroxide/ $\beta$ -CD complex

In the monoclinic-HB structures, the  $\beta$ -CD monomers inclined at  $45^\circ$  along the twofold screw axis form herringbone chains which are also arranged in sheets stacked along a crystallographic axis. In the potassium hydroxide complex structure, the monovalent cation ( $K^+$ ) is inserted in the external lattice by replacing one of the water molecules of the related structures, without any significant change in the structure. However, no disorder either in the hydroxyl groups or the water positions appeared, contrary to that often encountered in structures of this class (Charpin, Nicolis, Villain, de Rango & Coleman, 1991). The occurrence of direct interactions of the ions with the  $\beta$ -CD molecule is much less frequent than in the calcium salt complex structure, partially due to the different stoichiometry (only one cation per  $\beta$ -CD) and also arising from the location of the counter-ion ( $OH^-$ ), proposed to be inside the cavity.

In the calcium salt complex structure, the intra-HB chain organization is much expanded along the twofold screw axis (Fig. 9), the repeat unit being larger by a factor of 1.5. This may result from a more sterically demanding local environment around the  $\beta$ -CD monomer. The arrangement of the parallel HB chains inside a sheet appears different, since the  $\beta$ -CD unit does not present the same orientation to the generating

translation axis. Thus, the alternate stacking of the antiparallel sheets leads to a tighter overall HB chain packing, the molecular area per chain in the plane perpendicular to the HB axis being 30% smaller. This may be related to the lateral cross-links between the HB chains, involving the ions.

The overall packing in the potassium hydroxide complex is partly stabilized by intermolecular interactions between  $\beta$ -CD monomers, ensuring the cohesion inside the HB chains and in the stacking of the sheets. However, no direct contacts exist between  $\beta$ -CD molecules located in HB chains of different sheets. The monovalent cation participates to the cohesion inside the HB chain and inside a sheet by cross-linking four  $\beta$ -CD molecules: this contribution is similar to that of one chloride anion in the calcium salt complex structure. However, the cohesion of the packing of the parallel sheets is ensured by hydrogen bonding involving two doublets of water molecules.

Thus, contrary to that observed in the calcium salt complex structure, the role of the external water molecules is essential to ensure the stability of this monoclinic-HB structure.

## 4. Concluding remarks

This new monomer-type structure is the first example illustrating the insertion of divalent cationic salts in the external lattice of a  $\beta$ -CD crystal structure. A new type of HB chain stacked differently from the previously known monoclinic-HB chain-like structures is observed. The influence of the presence of divalent cationic salts inside the structure is characterized by the absence of disorder, particularly in the population and position of water molecule sites, and by the occurrence of frequent direct interactions with the  $\beta$ -CD molecule involving a divalent cation as well as a chloride anion, with no direct contacts between the ions.

The crystal structure of this compound illustrates how the interactions between  $\beta$ -cyclodextrin and divalent cations differ from those of monovalent cations and how inorganic anions may interact directly with cyclodextrins. Further work is required to determine the influence of size or charge of cations.

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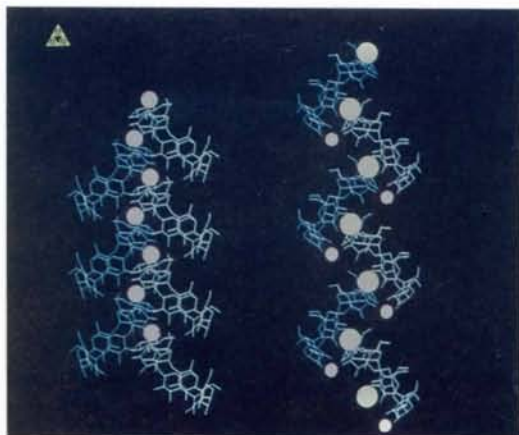


Fig. 9. Comparison between the HB chains, shown perpendicular to the HB axis, illustrating the role of the ions in the intra-HB chain organization: (left) potassium hydroxide complex ( $K^+$ , spheres); (right) calcium chloride complex ( $Cl_3$ , large spheres and  $Ca_2$ , small spheres).



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