thionite reduction.[54]

The rates for reduction of porcine plasma amine oxidase by substrate amines are much faster than the subsequent reoxidation rate; in contrast, both rates are slower but practically identical for the bovine plasma enzyme, whereas the plant amine oxidases display extremely fast rates of reaction with O_2.[46] If the reoxidation mechanism involves sequential single-electron-transfer steps, then the rate of such steps will be very sensitive to copper–quinone interactions. The distance and overlap between the Cu(II) $d_{x^2-y^2}$ orbital and the donor orbital on the reduced cofactor will be critical, for example. There may also be significanct Franck–Condon barriers to electron transfer, as the Cu(I) site appears to have a different structure from that of the Cu(II) site, both with respect to coordination number and geometry.[55] These factors could easily account for the rather large variations in reoxidation rates displayed by amine oxidases even though the active-site structure is remarkably conserved. In other words, the notable reactivity differences among amine oxidases may primarily reflect variations in the copper–quinone interactions among these enzymes. Possible differences in the Franck–Condon factors could be assessed by correlating Cu(I) site structures (deduced from EXAFS) with reactivity.

Summary

The data described in this paper establish the following:

(1) The Cu(II) site structure in *Arthrobacter* P1 methylamine oxidase is at least very similar to the Cu(II) site structures in eukaryotic amine oxidases.

(2) The organic cofactor in *Arthrobacter* P1 methylamine oxidase is identical with that found in other amine oxidases and is probably PQQ or a closely related compound. Moreover, the microenvironment of the quinone in methylamine oxidase is essentially the same as that in the plasma amine oxidases.

(3) Electron transfer between the reduced quinone and Cu(II) occurs in the presence of ligands that stabilize Cu(I), generating a semiquinone form of the *Arthrobacter* methylamine oxidase that is identical with the semiquinone forms previously generated in eukaryotic amine oxidases. Some of the unpaired spin density in the semiquinone must reside on the nitrogen derived from the substrate.

In addition, other new spectroscopic evidence for copper–quinone interactions has been presented.

Acknowledgment. We thank Dean Wilcox and Charles Dickinson for access to the EPR spectrometers at Dartmouth College and the University of Massachusetts. We also thank Judith Klinman and Peter Knowles for several helpful discussions. Research at Amherst College was supported by the NIH (Grant GM 27659 to D.M.D.); research at the Veterans Administration Medical Center and UCSF was supported by Program Project Grant HL-16251-16, the Veterans Administration, and a UCSF Academic Senate Grant (W.S.M.).

Registry No. Cu, 7440-50-8; NH_3, 7664-4-7; CN^-, 57-12-5; methylamine oxidase, 80891-30-1; phenylhydrazine, 100-63-0.

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Crystal and Molecular Structure of the Hexasaccharide Complex (*p*-Nitrophenyl α-maltohexaoside)$_2$·Ba(I$_3$)$_2$·27H$_2$O

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Abstract: *p*-Nitrophenyl α-maltohexaoside (**I**) [4-nitrophenyl penta[*O*-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside], an amylose fragment with a blocked reducing end, crystallizes as the complex (**I**)$_2$·Ba(I$_3$)$_2$·27H$_2$O in space group $P2_12_12_1$ with a = 33.732 (13) Å, b = 29.212 (10) Å, c = 14.442 (4) Å, and Z = 4. On the basis of 10055 counter X-ray Cu Kα diffraction data, the structure was determined by Patterson, direct, and difference Fourier methods and least-squares refined to R = 0.097 and R_w = 0.092 for 7590 independent reflections with $F_o > 3\sigma(F_o)$. The triiodide ions are almost linear and are arranged in the c-direction as an infinite zigzag chain with interunit angles from 121.3° to 166.1°. The structures of the two molecules of **I** in the asymmetric unit resemble cleaved cyclodextrins distorted in the form of lock washers with left-handed screw sense; all 12 glucoses are in the $^4C_1$ chair form. Two lock washers in opposite directions wrap around two I_3^- ions to form a left-handed antiparallel double helix. It is stabilized by van der Waals interactions with the polyiodide chain, as observed with amylose and cyclodextrins, and by both intramolecular interresidue and intermolecular O(2)---O(3′) hydrogen bonds, several of which are mediated and augmented by water bridges. The glucoses in the center of the molecules are more regularly arranged than those at the ends. They were used to mathematically construct an amylose antiparallel double helix with 2 × 8 glucoses per turn with a pitch height of 18.64 Å. In the crystal structure, adjacent double-helical complexes related by 2_1 screw symmetry along c are arranged such that an "infinite", wavy double helix is formed. It is stabilized by stacking interactions between the *p*-nitrophenyl groups, by hydrogen-bonded water molecules serving as intermolecular bridges, by interactions between I_3^- units, and by coordination of Ba^{2+} to four different molecules of **I**. The ligands are arranged symmetrically around Ba^{2+} in the form of a capped square antiprism with two O(5) atoms occupying the caps and glucoses chelated pairwise with their O(2), O(3) and O(5), O(6) oxygens, respectively. All except one of the 27 water molecules in the asymmetric unit are in direct hydrogen-bonding contact with the double helix. There is a characteristic, systematic hydration scheme such that glucose atoms O(2), O(3) and/or O(5), O(6) chelate water molecules to form five-membered cyclic structures, similar to the chelation of the Ba^{2+}. This motif in glucose hydration is so systematic that it will probably occur in other heavily hydrated crystalline amylose fragments and, above all, in solution.

The crystallization of oligosaccharides is particularly difficult, probably because the molecules are very flexible and adopt a variety of conformations that are stabilized by hydrogen bonds of intra- and intermolecular type. Only a few single crystal structure analyses are reported of longer oligomers, with the tetramer stachyose the largest characterized molecule so far.[1,2]

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We also have information from crystallographic studies about the structures of longer oligosaccharides that are part of glycoproteins[3] or are bound to an enzyme active site as in phosphorylase *a*.[4] In these cases, however, the atomic resolution is usually limited so that only the configuration of the carbohydrate can be given with some confidence, and details of molecular structure and of hydrogen-bonding schemes are obscured. On the other hand, there are the cyclic oligosaccharides of the cyclodextrin type that contain six, seven, or eight α-D-glucose units linked with α(1→4)-type glycosidic bonds. The cyclodextrins are more rigid due to the confinement of their annular structures, and they readily form suitable single crystals either alone (as hydrates) or as inclusion complexes.[5,6]

We have initiated a program where linear oligosaccharides of the amylose type are prepared,[7] crystallized, and subjected to X-ray analysis. This is the report on the first member of the series, a maltohexaoside whose reducing end is fixed in the α-position with a *p*-nitrophenyl group to avoid mutarotation. *p*-Nitrophenyl α-maltohexaoside (**I**) could not be crystallized as such but only as a complex with $Ba(I_3)_2$ and 27 water molecules. The triiodide forms a slightly zigzagged polyiodide chain around which the maltohexaoside molecules are wrapped in the form of an antiparallel, left-handed double helix. In a preliminary report, we have described this hexasaccharide molecule in relation to helices formed by amylose and to the conformation of the cyclodextrins.[8] In the present paper, the main focus is on experimental procedures, the molecular structure, and the hydration of the complex $(\mathbf{I})_2{\cdot}Ba(I_3)_2{\cdot}27H_2O$.

Experimental Section

Commercially available *p*-nitrophenyl α-maltohexaoside (**I**; Boehringer-Mannheim) is very soluble in water. This is a common feature of the linear oligosaccharides and contrasts with the cyclically closed cyclodextrins, which are less soluble, with the least soluble β-cyclodextrin at 1.87 g/100 mL of solution.[6] Many attempts to crystallize **I** as such or as a complex with alcohols or with fatty acids and with dimethyl sulfoxide failed, and we resorted to polyiodide complexes. From our experience with α-cyclodextrin–polyiodide complexes, we knew that the type of the formed complex and the space group of the crystallized material can depend on the counter cation.[9] For this reason, iodides of H^+, of NH_4^+, of alkali and alkaline earth metal ions, and of Tl^+, Zn^{2+}, and Cd^{2+} were dissolved in water, and the proper amount of iodine was added to form the triiodide complexes. These solutions were usually 0.1 M and were mixed with an aqueous solution of 0.1 M **I** so that the polyiodide was in molar excess of ~10%. When the solutions were allowed to evaporate, most of the polyiodides formed microcrystals with **I** that were unsuitable for detailed X-ray single crystal structure analysis. With Ba^{2+} as the counterion, however, a brownish precipitate formed immediately after the solutions were mixed. This precipitate consisted of well-shaped needlelike crystals that could be grown larger in the form of brown laths when solutions with optimum concentration conditions were sealed in melting point capillaries and left over several days.

Crystals used for X-ray diffraction experiments were sealed in quartz capillaries. Systematic extinction of odd-index axial reflections indicated the orthorhombic space group $P2_12_12_1$. The unit cell constants a = 33.732 (13), b = 29.212 (10), and c = 14.442 (4) Å were obtained from least-squares refinement of accurately determined angular positions of 20 reflections measured on a STOE four-circle diffractometer (Ni-filtered Cu Kα radiation). The final structure analysis shows that the crystal asymmetric unit contains two molecules of **I**, two triiodide anions, one barium ion, and 27 water molecules, $(C_{42}H_{65}O_{33}N)_2{\cdot}Ba(I_3)_2{\cdot}27H_2O$, yielding a molecular weight of 3424 and a calculated density of D_x = 1.598 g/cm^3, with F_{000} = 6544 and μ = 135 cm^{-1}. A total of 10055 X-ray intensity data were collected with three standard reflections monitored every hour. The data were corrected for the usual geometrical factors, for crystal decay (which did not exceed 10%), and for absorption by using the ψ-scan method.[10]

The heavy atom positions were derived by Patterson and direct methods,[11] and all the other non-hydrogen atoms were located from a series of difference Fourier maps.[12] In full-matrix least-squares refinement cycles, the heavy atoms were treated anisotropically and the C, N, and O atoms isotropically. Hydrogen atom positions were not located, but the H-atoms of C–H bonds were included in the refinement as fixed idealized isotropic contributions [d(CH) = 0.96 Å]. The refinement converged at R = 0.097 and R_w = 0.092 [$w^{-1} = \sigma^2(F_o)$] for 7590 structure factors with $F_o > 3\sigma(F_o)$. All atomic positions in the crystal structure are fully occupied except for the water molecules W100/101 and W270/271 with 1:1 disorder and the 2:1 disordered water molecules W200/201 and W240/241. In initial least-squares cycles, the two positions of each of the disordered water oxygens were refined with coupled occupancy. In final refinement cycles, these atomic positions were treated with fixed occupancy and thermal parameters were refined isotropically.

Results and Discussion

The atomic coordinates and temperature factors are available as supplementary material. Some geometrical data for the two molecules of **I**, termed $\mathbf{I}_1$ and $\mathbf{I}_2$, are given in Table I and Figure 1. The glucoses are numbered from the *p*-nitrophenyl-substituted reducing end, A–F in molecule $\mathbf{I}_1$ and G–L in molecule $\mathbf{I}_2$. In the text, the atom numbering is followed by a letter identifying the glucose; e.g., O(2)K means atom O(2) in glucose K. Primed numbers, e.g., O(3′), mark atoms in an adjacent glucose.

The intra- and intermolecular O···O distances between glucose units that we associate with O—H···O hydrogen bonds are indicated in Figure 1a. They are given in the supplementary material, together with hydrogen-bond distances to water molecules. The geometry of the polyiodide chain is described in Figure 1b, and the coordination of the Ba^{2+} cation is given in Figure 1c and in Table II.

An Antiparallel Double Helix as the Main Structural Motif. The asymmetric unit of this crystal structure contains a complex with the formula $(\mathbf{I})_2{\cdot}Ba(I_3)_2{\cdot}27H_2O$. The two molecules of **I** are arranged in the form of a left-handed, antiparallel double helix wound around a polyiodide chain consisting of two I_3^- units. The 2_1 screw axis parallel to c produces a "coherent" polyiodide and left-handed amylose double helix; see Figure 2.

(a) Relation to Cyclodextrins. The structures of the two individual maltohexaoside molecules **I** are reminiscent of the cyclodextrins. In a "Gedankenexperiment", an α-cyclodextrin can be cleaved at one of the glycosyl C(1)–O(4′)–C(4′) bonds, ond one of the ends is moved up, the other down to produce a lock-washer-type structure with a left-handed twist, as in Figure 3. In a certain sense this retains the central cavity of the cyclodextrin. It is of hydrophobic character because it is covered by ether-like O(4) and O(5) atoms and by hydrogen atoms attached to C(1), C(3), and C(5). This is one of the reasons why the cyclodextrins and the maltohexaoside double helix have the ability to form inclusion complexes, with the polyiodides being well-known guest molecules.[9] The outer surfaces of the cyclodextrins and of the left-handed lock-washer molecules of **I** are predominantly hydrophilic. Because the hydroxyl groups of O(2) and O(3) are on one side and that of O(6) is on the other, intramolecular, interresidue hydrogen bonds between the O(2) and O(3) hydroxyls of adjacent glucoses can form, which contribute to the conformational stability of the cyclic and linear oligosaccharides. As we shall see later, the intermolecular hydrogen bonds observed in the double helix formed by two lock washers in opposite directions are analogous to those found in some of the cyclodextrin crystal structures.

(b) Geometrical Details of *p*-Nitrophenyl α-Maltohexaoside Molecules $\mathbf{I}_1$ and $\mathbf{I}_2$. The crystal structure determination is not

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Table I. Some Geometrical Data Describing the Conformations of the Two Molecules I_1 (Glucoses A–F) and I_2 (Glucose G–L)

	glucose puckering parameters[a]			torsion angles[c]			glycosidic bond angle				
	QT, Å	θ_2, deg[b]	Φ_2, deg[b]	χ, deg	ϕ, deg	ψ, deg	C(4)–O(1′)–C(1′), deg	C(1)···C(4), Å	O(4)···O(4′), Å	O(4)–O(4′)–O(4″), deg	O(3)···O(2′), Å
molecule I_1											
p-nitrophenyl									4.50[d]		
glucose A	0.498	6.5	172	−67 (3)	73 (3)[d]		116 (2)[d]	2.90 (3)	4.50	137[d]	2.70
glucose B	0.568	8.2	−73	−57 (3)	103 (2)	109 (2)	118 (2)	2.91 (4)	4.64	146	2.91
glucose C	0.581	10.3	−76	−64 (3)	94 (2)	101 (2)	114 (2)	2.93 (4)	4.54	142	2.98
glucose D	0.530	5.8	108	−65 (3)	93 (3)	109 (3)	120 (2)	2.72 (4)	4.59	139	3.05
glucose E	0.476	7.1	−137	−71 (2)	96 (3)	112 (3)	119 (2)	2.92 (4)	4.50	139	2.68
glucose F	0.527	4.4	92	−69 (2)	112 (2)	115 (2)	113 (2)	2.93 (4)			
mean values[e]	0.530			−66	100	109	117	2.89	4.55	142	2.86
molecule I_2											
p-nitrophenyl									4.43[d]		
glucose G	0.551	4.3	47	−60 (3)	82 (3)[d]		116 (2)[d]	2.93 (3)	4.38	131[d]	2.83
glucose H	0.582	5.7	70	−60 (2)	107 (2)	111 (2)	116 (2)	2.91 (4)	4.42	144	>3.50
glucose I	0.651	12.7	−129	−63 (3)	80 (3)	86 (3)	112 (2)	2.99 (4)	4.43	132	2.87
glucose J	0.618	4.4	94	−60 (3)	105 (3)	120 (2)	112 (2)	2.87 (4)	4.58	136	2.84
glucose K	0.597	3.5	79	−71 (2)	94 (3)	109 (3)	126 (2)	2.79 (4)	4.46	140	>3.50
glucose L	0.572	14.3	166	52 (4)	45 (2)	73 (3)	119 (2)	2.79 (4)			
mean values[e]	0.595				86	100	117	2.90	4.45	138	2.85
sucrose[13]	0.556	5.2	183.7								

[a] Cremer and Pople parameters QT (total puckering amplitude). [b] θ_2 and Φ_2 are spherical polar angles. [c] Torsion angles are defined as follows: χ, O(5)–C(5)–C(6)–O(6); ϕ, C(4)–O(4)–C(1′)–O(5′); ψ, C(3)–C(4)–O(4)–C(1′). [d] Data involving *p*-nitrophenyl groups. [e] Averaged values only given for small variations of the parameters (*p*-nitrophenyl-involving data ignored).

Table II. Distances and Angles of Barium Coordination by Water (W) and Glucose Oxygen Atoms[a]

Distances from Ba (x, y, z)

atom		distance, Å
water	W1	2.72 (2)
	W2	2.72 (2)
glucose A (x, y, z − 1)	O2	2.74 (2)
	O3	2.91 (2)
glucose F (−0.5 + x, 0.5 − y, −z)	O5	3.13 (1)
	O6	2.88 (2)
glucose G (−0.5 + x, 0.5 − y, 1 − z)	O2	2.84 (2)
	O3	2.92 (1)
glucose K (x, y, z)	O5	3.15 (1)
	O6	2.87 (2)

Selected Angles around the Central Atom

atoms	angle, deg
W1–Ba–W2	70.3 (6)
O2 A–Ba–O3 A	58.3 (5)
O2 G–Ba–O3 G	58.1 (4)
O5 F–Ba–O6 F	54.5 (4)
O5 K–Ba–O6 K	55.0 (5)
O5 K–Ba–O5 F	157.6 (4)

[a] Esd's and symmetry operations are given in parentheses.

very accurate concerning the atomic positions of the C, N, and O atoms because barium and iodine dominate the X-ray diffraction, and the resolution of the data is limited to 1.2 Å. The estimated standard deviations obtained from the least-squares correlation matrix are in the range of 0.02–0.04 Å for the bond lengths between C, O, and N atoms and 2–5° for the bond angles. All the bond distances and bond angles are within the 3σ ranges of those usually observed for glucose units in the cyclodextrins and in other related molecules.[6] Of interest are the bond angles at the glycosidic oxygen atoms, shown in Table I. They are in the range of 112–126° with a mean of 117°. This is smaller than the average of 119% found in α-cyclodextrin and larger than the average of 112.6° in γ-cyclodextrin and compares more closely to the average found in β-cyclodextrin, 117.7°.[6] Other data, such as mean virtual bond lengths O(4)···O(4′) of 4.50 (9) Å, mean O(2)···O(3′) hydrogen-bond distances of 2.86 (2) Å, and mean O(4)···O(4′)···O(4″) angles of 139.8°, were discussed in the previous paper.[8]

(c) Conformation of the Two *p*-Nitrophenyl α-Maltohexaoside Molecules I_1 and I_2. In Table I are given some conformational parameters that characterize the overall shape of the two molecules I_1 and I_2. The 12 glucose residues are all in the $^4C_1$ conformation with some degree of flexibility, which is obvious from the Cremer and Pople parameters.[13] The variations in the total puckering amplitude QT and the angular variables θ_2 and Φ_2 indicate that there are some distortions from ideal $^4C_1$ geometry, which is characterized by the parameters for sucrose given for comparison in Table I.

In a glucose, the rotation of the O(6) hydroxyl group around the C(5)–C(6) bond is limited to preferred conformations with torsion angle χ, O(5)–C(5)–C(6)–O(6), in the −gauche range, sometimes in the +gauche range but never trans (Table I). In the two molecules of **I**, all the torsion angles are −gauche with a rather narrow distribution of −57° to −71°, except for the terminal glucose unit L where the torsion angle is +gauche, 52°. This less preferred conformation is obviously stabilized by a number of hydrogen bonds; see Figure 1a.

The relative orientation between the glucose units is given by the two glycosidic torsion angles ϕ, C(4)–O(4)–C(1′)–O(5′), and ψ, C(3)–C(4)–O(4)–C(1′), where the primed atoms belong to the adjacent glucose unit. We have not chosen the torsion angles where hydrogen atoms are used as reference points, because hydrogen atoms were not located in this crystal structure. The torsion

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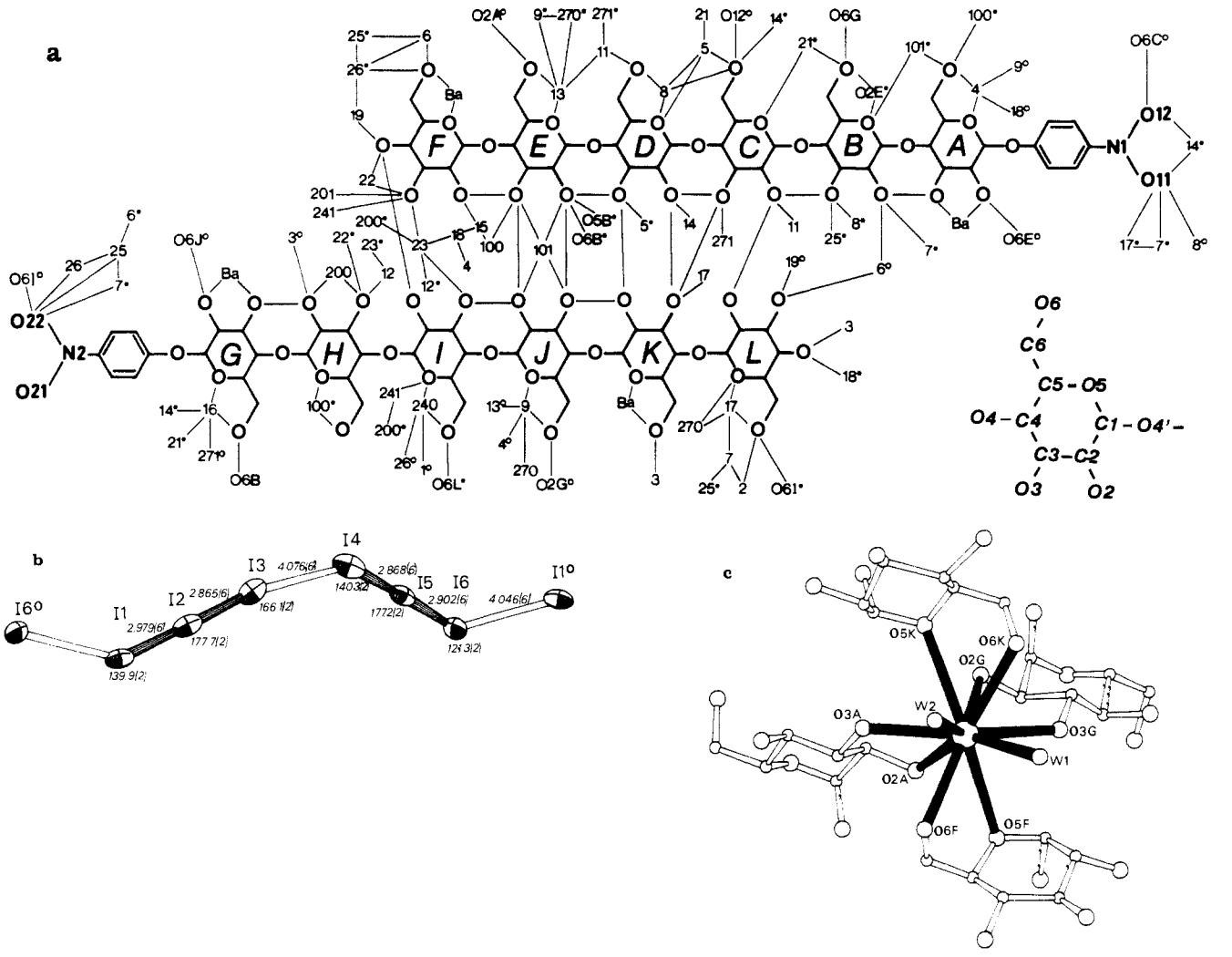

Figure 1. (a) Atomic numbering for monosaccharide units and glucose numbering scheme in molecules I_1 and I_2. Thin lines indicate O---O distances <3.5 Å, which we associate with possible O–H---O hydrogen bonds; the barium coordination is also given. Water oxygen atoms are only given by their numbers; glucose hydroxyl groups are differentiated by O, i.e., O6H is O(6) of glucose H. Contacts within the infinite helix are indicated by ° and those between symmetry-related infinite helices by *. (b) Geometrical details of the polyiodide chain. The iodine atoms I1 to I6 are located within the antiparallel double helix; I6° and I1° belong to adjacent asymmetric units related by the 2_1 screw axis along *c*. (c) Barium ion coordination. Bond distances are given in Table III. The four glucoses belong to four different molecules; see symmetry information in Table III.

angles ϕ and ψ are comparable for all the glycosidic links in molecule I_1, where ϕ is in the range 93–112° and ψ in the range 101–115°. As a consequence, the O(2) and O(3) hydroxyl groups of adjacent glucoses are so close together that hydrogen bonds can form; see Figure 1a and Table I. In molecule I_2 the variations in glycosidic torsion angles are much wider, from 45° to 107° for ϕ and from 73° to 120° for ψ. Consequently, interresidue hydrogen bonds between O(2) and O(3) hydroxyl groups of adjacent glucoses H, I and K, L cannot form (Table I). As a compensation, these hydroxyl groups are engaged in a number of intermolecular hydrogen-bond interactions, as in Figure 1a. Moreover, the O(2) hydroxyls of glucoses I and L are in van der Waals contact to the polyiodide chain (see section d), suggesting that the disruption of the interresidue O(2)---O(3′) hydrogen bonds is due to imperfect spatial fitting of the polyiodide chain into the cavity of an "ideal" double helix with all O(2)---O(3′) hydrogen bonds formed.

The *p*-nitrophenyl groups attached to the glucoses A and G are in approximately vertical orientations to the pyranose six-membered rings. Torsion angles ϕ are 73 (3)° for molecule I_1 and 82 (3)° for I_2, and torsion angles C(1)–O(1)–C(1′)–C(2′) are in the cis range, −2 (4)° for molecule I_1 and −8 (4)° for molecule I_2. Similar orientations were also observed for other sugar residues, e.g., in α-laminaribiose octaacetate, where the acetyl groups are nearly cis-planar to the exocyclic sugar C–O bonds with which they are esterified.[14]

(d) Polyiodide Chain. The polyiodide chain in this complex consists of I_3^- units, as seen in Figure 1b. The two symmetrically independent I_3^- units are linear, 177.7 (2)° and 177.2 (2)°, with I–I distances in the range 2.865 (6)–2.979 (6) Å, as usually observed,[15,16] The distances between the triiodide units, 4.046 (6) and 4.076 (6) Å, are shorter than the expected van der Waals distance of 4.3 Å.[17] The more obtuse angles formed by the triiodide units, 166.1 (2)° and 140.3 (2)°, occur between the triiodide units within the helical molecular complex, whereas the more acute angles, 121.3 (2)° and 139.9 (2)°, are between adjacent complexes. The zigzag arrangement of the polyiodide chain impedes strong charge-transfer interactions and permits van der Waals contacts to two O(2) hydroxyl and two water oxygen atoms, with distances as follows: I1---O(2)L, 3.69 Å; I1---W6, 3.61 Å; I5---O(2)I, 3.57 Å; I6---W3, 3.76 Å (esd's at 0.02–0.04 Å). The zigzag contrasts with the linear or near-linear polyiodide chains observed in the blue-black starch–iodine[18] and α-cyclo-

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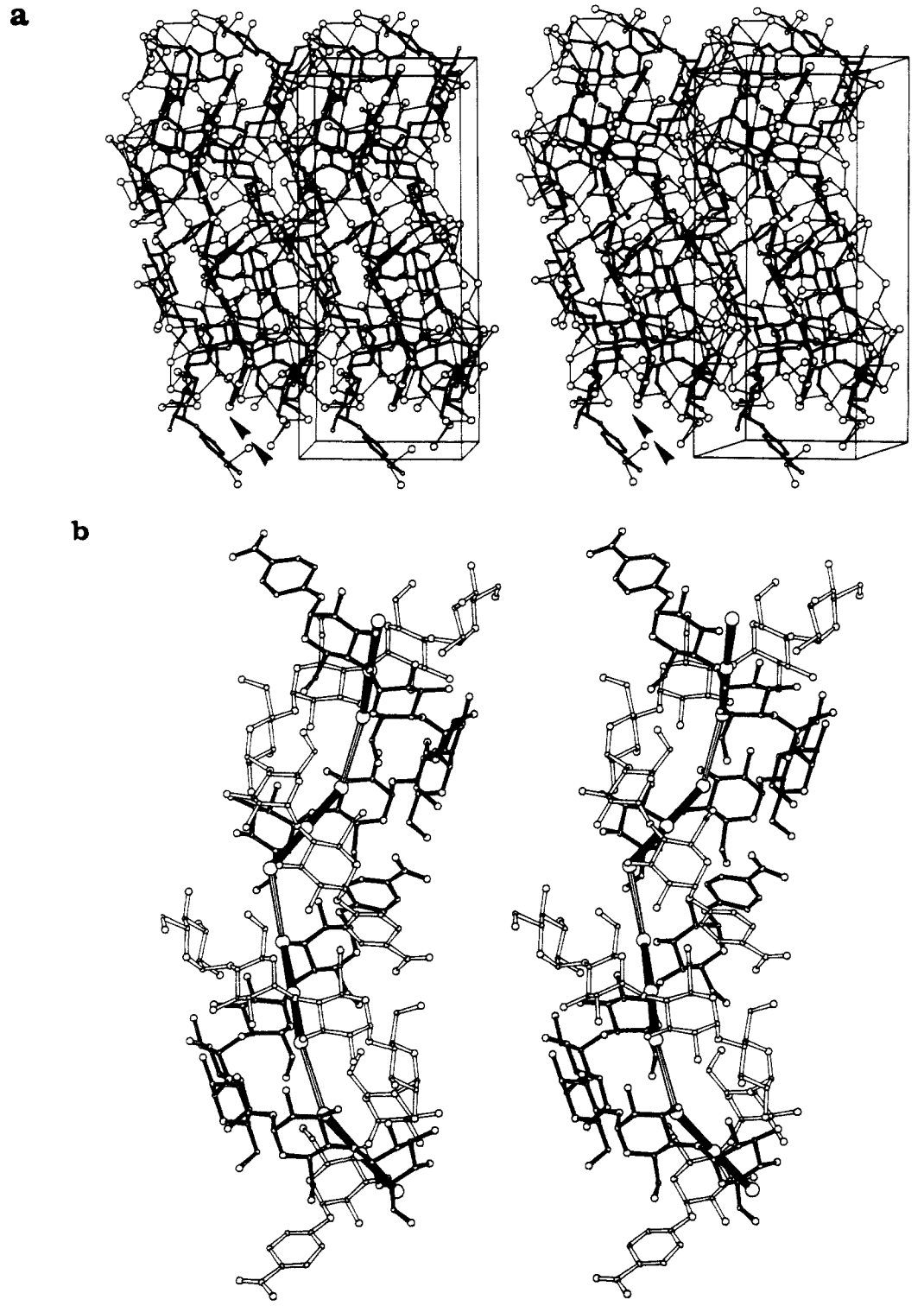

Figure 2. (a) Stereoview of the unit cell content. Atoms drawn with increasing radii indicate C, N < O, OH glucose < O_{water}, I < Ba^{2+}. Covalent I–I bonds are drawn with very thick lines, short I---I distances with three lines, other covalent bonds with thick lines, and hydrogen-bonding and Ba---O coordination distances with thin lines. The view is approximately along *b*, showing the coordination of four molecules of I by the barium ion (center, drawn shaded). At the upper and lower ends of this structure, there is a gap to insert the *p*-nitrophenyl group of the adjacent molecules, indicated here by two arrows only for the lower ends for clarity. (b) Stereoview down *c*, showing two asymmetric units of the "coherent" double-helical structure. Ba^{2+} and water molecules are omitted for clarity. Molecule I_1 is shown with solid bonds and I_2 with open bonds.

dextrin complexes,[9,19] which are not in contact with oxygen atoms, and explains why the color of the crystals of $(I)_2 \cdot Ba(I_3)_2 \cdot 27H_2O$ is only brown.

(e) Hydrogen-Bonding Interactions Stabilizing the Double Helix. There are a number of intramolecular O(2)---O(3′) hydrogen bonds discussed in (c) that add to the conformational stability of the two molecules I_1 and I_2. In connection with the antiparallel double helix, the intermolecular hydrogen bonds are of interest. They are formed between glucose hydroxyl groups in the central, more regular part of the double helix. These interactions involve the O(2) and O(3) hydroxyls of glucoses D, K and E, J, so that the O(2) hydroxyl of one glucose is linked with the O(3) hydroxyl of the other. Since in molecule I_1 the O(2) and O(3) hydroxyls in adjacent glucoses form intrastrand hydrogen bonds (which are broken between glucoses H, I and K, L of molecule I_2 due to

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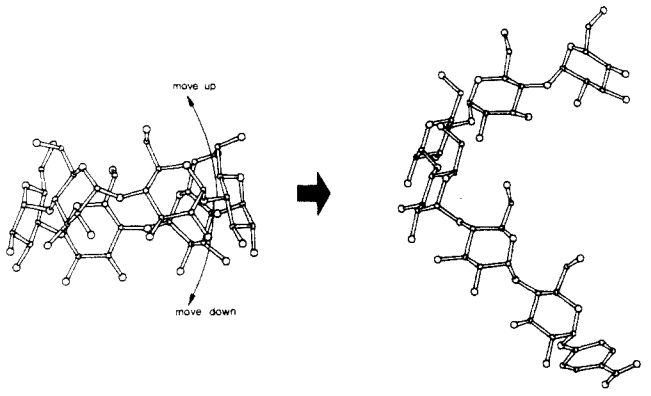

Figure 3. Structural relation between the cyclically closed α-cyclodextrin (left) and the left-handed helical form of **I** (right).

rotation about the glycosidic link), we anticipate that, in a more regular arrangement, inter- and intramolecular hydrogen bonds between O(2) and O(3) hydroxyls would form quadrilateral structures. These are possible in the idealized double helix constructed on the basis of this crystal structure, vide infra, and a comparable arrangement was observed in (α-cyclodextrin)$_2$·LiI_3·I_2·$8H_2O$.[9]

Since the O(2), O(3) sides of the two molecules are associated through hydrogen bonding, the O(6) hydroxyls on the other side should also associate, as shown in the regular structure, vide infra. In the distorted double helix of this crystal structure, only one such O(6)···O(6′) hydrogen bond of 2.81 (4) Å is formed between glucoses B and G belonging to the same asymmetric unit (Figure 1a). These interactions are more regularly formed in (α-cyclodextrin)$_2$·$Cd_{0.5}I_5$·$27H_2O$.[9]

Factors Contributing to the Formation and Stabilization of the "Infinite" Double Helix. The individual short helical duplexes formed by molecules of **I** constitute one asymmetric unit and are about one turn long. The duplexes are considerably tilted and produce a wavy "infinite" double helix that we associate with interactions due to complex formation with polyiodide and barium cations, *p*-nitrophenyl stacking forces, polyiodide binding, and hydrogen bonding.

(a) Ba^{2+} Coordination. The Ba^{2+} cation plays a central role in this crystal structure as it links individual double helices related by the 2_1 screw operation along *c*; see Figure 2. It is also connected with glucoses of an adjacent infinite double helix and serves as a "lateral" link, thereby stabilizing the whole crystal structure (Figure 1c).

At the interface between the individual double helices, glucose A of one molecule of $\mathbf{I}_1$ [atoms O(2), O(3)] and glucose F of a symmetry-related molecule of $\mathbf{I}_1$ [atoms O(5), O(6)] are coordinated to the same Ba^{2+}. Likewise, this Ba^{2+} connects symmetry-related glucoses G [atoms O(2), O(3)] and K [atoms O(5), O(6)] of two different molecules of $\mathbf{I}_2$. The glucoses coordinate as chelating ligands to form five-membered rings with the metal ion. As indicated in Figure 1c and in Table II, the coordination displays pseudo-2-fold rotational symmetry, with two of the glucose units (F, K) coordinated with their O(5) and O(6) atoms and the other two (A, G) with their O(2) and O(3) atoms. In addition, the coordination of Ba^{2+} includes two water molecules, yielding an overall coordination number of 10,

The shape of the polyhedron formed by the oxygen ligands can be described as a square antiprism with capped squares. The ideal polyhedron is distorted because of the sterical restriction of the chelating ligands, limited flexibility of the α-maltohexaoside chains, and different binding affinities of the oxygen ligands. These are reflected in a systematic trend in the Ba^{2+}···O coordination distances (Table II): O_{water} < O(2) < O(6) < O(3) < O(5). The two O(5) oxygens represent the caps of the square antiprism. A view down the O(5)···Ba^{2+} distances onto the corresponding square planes of the coordination polyhedron shows O_{water}, O(3), O(2), and O(6) in clockwise order. All Ba^{2+}-coordinating oxygens are in hydrogen-bonding distances (see supplementary material), so

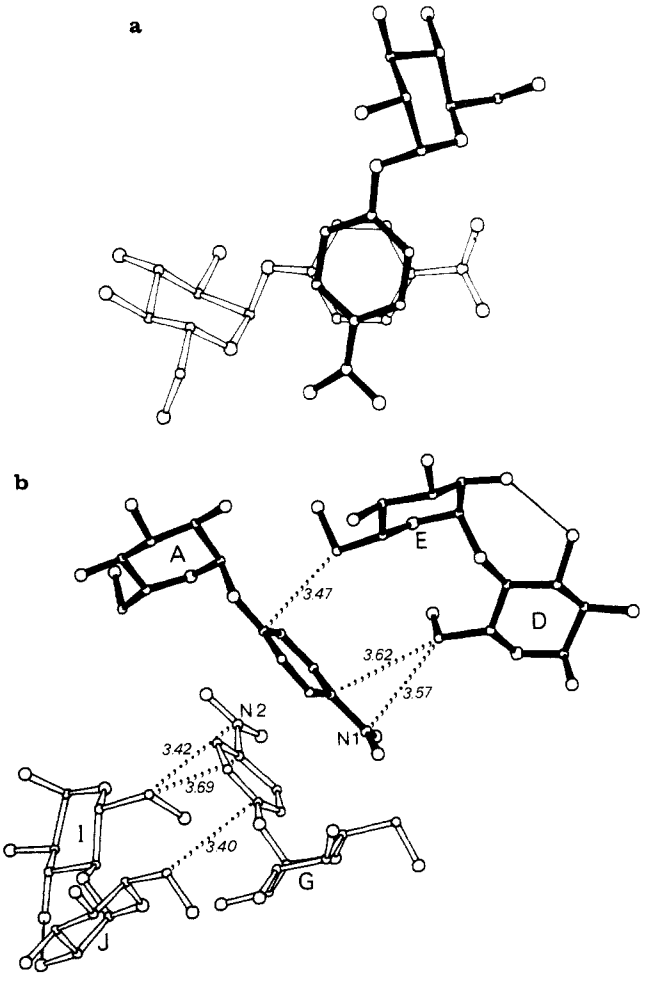

Figure 4. (a) Stacking interactions of *p*-nitrophenyl moieties of adjacent helical duplexes. The benzene rings are parallel (dihedral angle 29°) at 3.6-Å distance and rotated relative to each other by ~90°. (b) Further stabilization of this geometry by the interlock interactions *p*-nitrophenyl···C(6)—O(6) (glucose) that involve two molecules of $\mathbf{I}_1$ (solid bonds) and two molecules of $\mathbf{I}_2$ (open bonds). Glucoses D, E, and G belong to the asymmetric unit (*x*, *y*, *z*); glucoses I, J, and A are related by the symmetry operation (0.5 + *x*, 0.5 − *y*, 1 − *z*).

that this polyhedron fits without problems into the extended hydrogen-bonding network. This and the favorable coordination geometry appear to be the reasons why only barium among all investigated polyiodide counterions yielded suitable single crystals with **I**.

(b) Stacking of the *p*-Nitrophenyl Groups. Due to the antiparallel alignment of molecules of **I**, the double-helical complex has one *p*-nitrophenyl group at each end. The 2-fold screw axis parallel to *c* produces the "coherent, infinite" double helix and arranges the terminal *p*-nitrophenyl groups such that they are parallel, at 3.6 Å distance, and oriented at right angles, as in Figure 4. In fact, not only do they stack but one *p*-nitrophenyl group is interlocked between the *p*-nitrophenyl group and the C(6)–O(6) group of two central glucoses (D, E in $\mathbf{I}_1$ and I, J in $\mathbf{I}_2$) of the *adjacent* double-helical complex related by 2_1 screw symmetry along *c*; see Figure 4b.

The stacks formed by the *p*-nitrophenyl groups appear to serve three structural functions. First, they contribute to the overall stability of the infinite double helix by hydrophobic and dispersion interactions between the aromatic residues. Second, the stacks have a van der Waals thickness of 7.3 Å that is comparable to that of a glucose unit measured from C(6) to O(3), 7.8 Å (carbon–oxygen distance 5.0 Å, plus 2 × 1.4 Å van der Waals radii).[17] Consequently, *p*-nitrophenyl stacks can compete in packing arrangements with glucose units, which gives rise to the smooth substitution of a glucose unit by a *p*-nitrophenyl stack in the infinite double helix. Third, a shown in Figure 2a, there is a gap at each

Table III. Geometrical Data of the Idealized Amylose Antiparallel Double Helix Constructed on the Basis of the Central Part of the Double-Helical Complex (Glucoses C and D)[a]

pitch height, Å	18.64
glucose residues per turn	8
van der Waals diameter, Å	
for central cavity	5.0
for outer-helix boundaries	15.9
hydrogen-bonding distances, Å	
intrastrand O(2)···O(3′)	2.49, 3.11 alternating
interstrand O(2)···O(3′)	3.24, 3.40 alternating
interstrand O(6)···O(6′)	2.93

atom	x, Å	y, Å	z, Å	atom	x, Å	y, Å	z, Å
C1 C	−2.913	5.247	0.636	C1 D	1.959	5.871	2.585
C2 C	−1.606	5.960	−0.039	C2 D	3.145	5.682	2.202
C3 C	−0.404	5.419	0.335	C3 D	3.834	4.369	2.619
C4 C	−0.133	5.325	1.880	C4 D	3.710	4.253	4.222
C5 C	−1.516	4.461	2.363	C5 D	2.339	4.451	4.774
O5 C	−2.582	5.212	1.946	O5 D	1.839	5.624	4.210
O2 C	−1.957	5.933	−1.528	O2 D	3.345	5.686	0.835
O3 C	0.501	6.283	−0.261	O3 D	5.206	4.223	2.413
C5 C	−1.442	4.330	3.992	C5 D	1.982	4.428	6.297
O6 C	−1.247	5.720	4.616	O6 D	2.704	5.625	6.829
O4′ C	1.049	4.896	2.239	O4′ D	4.039	3.052	4.770

[a] The given Cartesian coordinates of two glucose residues form one helical strand (eight glucose molecules) by using the symmetry operations: $y, -x, 4.66 + z$; $-x, -y, 9.32 + z$; $-y, x, 13.98 + z$. The average rise per residue is 2.33 Å. The antiparallel double helix is completed by using the symmetry operation $y, x, 18.64 - z$ for the octamer above.

end of the duplex between the *p*-nitrophenyl group of molecule $\mathbf{I}_1$ and glucose I of molecule $\mathbf{I}_2$ and between the *p*-nitrophenyl group of molecule $\mathbf{I}_2$ and glucose D of molecule $\mathbf{I}_1$; see Figure 4b. This gap accommodates the nitrophenyl groups of the contiguous duplex, which are tightly interlocked.

(c) Continuity of the Polyiodide Chain. The polyiodide chain in complexes with cations or small molecules favors zigzag structures with angles of about 90° between I_2 and/or I_3^- units.[15,16] This is similar to polyiodide in crystalline β-cyclodextrin–polyiodide[20] but contrasts with the linear polyiodide chains in crystalline complexes with α-cyclodextrin[9,19] and in blue starch–iodine. Since polyiodide and iodine have a strong tendency to form complexes with cyclic oligosaccharides and helical polysaccharides, we infer that the association of **I** with polyiodide was a necessary condition for successful crystallization. It appears that the polyiodide zigzag, which is midway between a linear arrangement and the more common 90° angles, is a compromise to satisfy the conditions of optimum interaction (~90°) between I_3^- units and the packing requirements of the double helices formed by **I** (~180°). In any case there will be a cohesive force between the triiodide units as the nonbonded I···I distances are all considerably shorter than the sum of van der Waals radii, 4.3 Å.

Geometry of an Idealized Antiparallel Double Helix Formed by Amylose. If at least two adjacent units of an oligomer molecule are oriented such that they form a helical structure, mathematical methods can be used to derive the parameters of this helix. On the basis of the central part (glucoses D, E) of the more regular molecule $\mathbf{I}_1$ in this crystal structure, we used the algorithm described in refs 21 and 22 to construct an idealized antiparallel double helix with the atomic parameters and geometrical features described in Table III herein and in Figure 4 of ref 8. This double helix contains 2 × 8 glucoses in one turn with pitch height 18.64 Å. It is stabilized by a hydrogen-bonding pattern that is also observed for α-cyclodextrin[9] and of course in the present crystal structure, i.e., intermolecular O(6)---O(6′), as between glucoses B and G, and four-membered cycles formed by intra- and intermolecular O(2)---O(3′) hydrogen bonding, as between glucoses D, K and E, J. The central cavity of the double helix has a van der Waals diameter of approximately 5 Å and considerably exceeds the 4.3-Å diameter expected for a polyiodide chain. We assume this to be the reason why the duplex formed in this crystal structure is somewhat collapsed and does not exhibit a more ideal double-helical structure.

Table IV. Ring Puckering Parameters Follwoing Cremer and Pople[13] for the Five-Membered Rings Formed by Coordination of Water Molecules, Hydroxyl Group, or Barium Ion to Glucose Oxygens

coordinating atom	five-membered ring via[a]	glucose residue	QT, Å	Φ_2, deg	ring conformation[b]
Ba	O2, O3	A	0.42	95.9	$^2T_3$
Ba	O2, O3	G	0.43	105.0	E_3
W101	O2, O3	E	0.48	85.2	$^2T_3$
W200	O2, O3	H	0.74	137.5	^4E
W101	O2, O3	J	0.83	146.7	^4E
W22	O4, O3	F	0.63	40.2	E_1
Ba	O5, O6	F	0.56	119.4	$^4T_3$
Ba	O5, O6	K	0.57	110.8	E_3
W4	O5, O6	A	0.58	116.2	E_3
O2 E	O5, O6	B	0.44	113.2	E_3
W8	O5, O6	D	0.85	146.1	^4E
W13	O5, O6	E	0.67	122.4	$^4T_3$
W16	O5, O6	G	0.65	132.1	$^4T_3$
W100	O5, O6	H	0.52	111.1	E_3
W240	O5, O6	I	0.73	133.5	$^4T_3$
W9	O5, O6	J	0.50	122.0	$^4T_3$
W17	O5, O6	L	0.47	−75.4	^3E
furanoid ring of sucrose[13]			0.353	265.1	$^3T_2$

[a] The ring order starts from the coordinating atom to the first listed glucose oxygen. [b] Twist (*T*) and envelope (*E*) conformations according to Altona and Sundaralingam.[26]

The overall geometry of the individual strand in the double helix is similar to that adopted by heptaamylose in the complex with the enzyme phosphorylase *a*.[4]

Heavy and Systematic Hydration of the Hexaamylose Double Helix. Monosaccharides crystallize predominantly in anhydrous form or as low hydrates, because the nearly spherical molecules pack easily in the crystal lattice. This is different from the disaccharides and higher saccharides, which frequently if not always cocrystallize with one or several water molecules. In the polymeric amylose of the A-type, the parallel double helices are closely packed, probably without water molecules, whereas in the B-type, the packing is more loose and few water molecules fill the space between the helices.[23,24]

In contrast, the antiparallel double helix formed by **I** is heavily hydrated (Figures 1a and 2a). All potential hydrogen-bond donors and acceptors of the two molecules in the asymmetric unit, including most of the O(5) ring oxygen atoms, are involved in hydrogen bonding to water molecules. The direct glucose---glucose hydrogen bonds are of the intramolecular type or are intermolecular but intrahelical. The contacts between the individual double helices, be they within the infinite helices or between them, are mediated by water of hydration molecules and by the Ba^{2+} cation.

If the number of hydrogen-bonded water molecules around O(2), O(3), and O(6) hydroxyl groups is compared, it is obvious that the glucose O(2), O(3) hydroxyls which are involved in intra- and interstrand hydrogen bonding are least hydrated (glucoses D, E of molecule $\mathbf{I}_1$ and glucoses J, K of molecule $\mathbf{I}_2$). The other glucose O(2), O(3) hydroxyl groups are more heavily hydrated, which we associate with more severe deviations from helical symmetry and with end effects. In contrast, the O(6) hydroxyls that are at the periphery of the helix owing to the –gauche orientation of the O(5)–C(5)–C(6)–O(6) torsion angles are all well hydrated and frequently engaged in hydrogen bonding to more than one water molecule.

A characteristic feature of the hydration pattern is the *systematic chelation of water molecules* by glucose O(2), O(3) and

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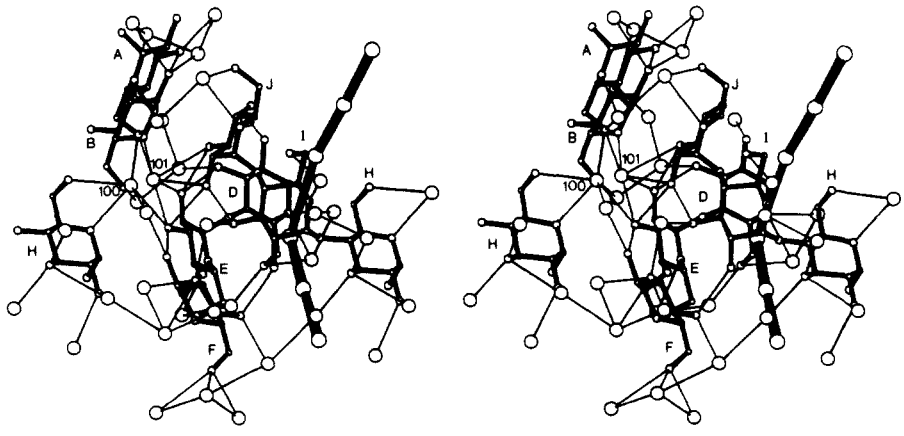


Figure 5. Stereoview illustrating the hydrogen-bonding contacts to the disordered water sites W100 and W101. These sites connect one double-helical duplex, indicated by the polyiodide chain segment, with an adjacent one, represented (to the left) by glucoses A and B of molecule I_1 and H of molecule I_2.

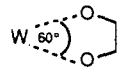
glucose O(5), O(6) atoms to form five-membered ring structures. This pattern is reminiscent of the chelation of the Ba^{2+} ion and shows comparable Cremer and Pople puckering parameters of the five-membered rings; see Table IV and Figure 1c. The puckering amplitudes QT in these chelate rings are in the range 0.42–0.85 Å and the preferred conformations are of the E_3, ^4E envelope and the related $^2T_3$, $^4T_3$ twist forms. The chelate formed by W17 and O(5), O(6) of glucose L is exceptional: it has the conformation ^3E, as the torsion angle O(5)–C(5)–C(6)–O(6), 52°, is in the unusual +gauche range (see Table I).

The water chelation by O(5), O(6) atoms dominates and is observed in glucoses A, D, E, G, H, I, J, and L in Figure 1a. In one case, the water molecule in this bridge is substituted by the O(2)E hydroxyl of a neighboring infinite helix that links the O(5), O(6) oxygens of glucose B. Glucoses E, H, and J show chelation of water molecules through the O(2), O(3) hydroxyl groups. This arrangement is comparable with hydrogen-bonding contacts in the channel-type structure of α-cyclodextrin–1-propanol, where the O(3) hydroxyl acts like the bridging water molecule to O(2), O(3) hydroxyls of a symmetry-related cyclodextrin ring.[25]

Similar to this pattern, the O(3), O(4) hydroxyls of the terminal glucose unit (F) in molecule I_1 are connected by a water molecule. Counting this case and including the four Ba^{2+}-coordinated glucose units, there are a total of 17 five-membered ring configurations in the present crystal structure. In a variant of this chelation scheme, a water molecule connects the O(5) of one glucose and the O(6) of the *adjacent* glucose; see Figure 1a, glucoses A, B and B, C.

The high affinity of the water molecule for O(2), O(3) or O(5), O(6) chelation is well illustrated by the disordered water molecule in sites W100 and W101. These sites are statistically half-occupied and at 1.45 (3) Å distance so that they cannot be filled simultaneously. As shown in Figures 1a and 5, sites W101 is chelated by the O(2), O(3) hydroxyls of glucoses E and J belonging to molecules I_1 and I_2 in the duplex, and it bridges O(6) of glucose A and O(5) of glucose B, both in molecule I_1. Site W100 is chelated by O(5), O(6) ofglucose H and is part of a four-membered ring formed by site 100, water 15, O(3)E, and O(2)F. It appears that there is a stereochemical conflict in this region of the crystal structure—the space filled by sites W100 and W101 is too small for two water molecules and too wide for just one molecule. The statistical distribution over two sites is an obvious compromise to satisfy the conditions of optimum chelation.

All water molecules except W1 are in direct hydrogen-bonding contact to oxygen atoms of molecules I_1 and I_2, excluding the glycosidic O(4) atoms, which are sterically hidden. They are also hydrogen-bonded to each other and form several chainlike motifs and three-membered rings; see Figure 1a. The many contacts between water molecules correlate with high coordination numbers, which can only be given here for O···O distances. Within the 3.5-Å hydrogen-bond cutoff criterium, they vary considerably from 3- to 7-fold coordination. Most of the higher coordination numbers are associated with the chelation that gives rise to 60° angles at the water molecule



and consequently permits several other ligands to approach. Also, the higher coordination numbers are due to disordered water sites, which contribute to the coordination of well-ordered water molecules or are themselves highly coordinated. In any case, the crystal structure of this hexasaccharide double helix illustrates that oligosaccharides differ largely from the mono- or disaccharides in their hydration behavior, which should be taken into account in model-building studies where glucoses are surrounded by water.

Acknowledgment. We gratefully acknowledge financial support by the Deutsche Forschungsgemeinschaft (Sa 196/12-2) and by the Fonds der Chemischen Industrie.

Registry No. $2I{\cdot}Ba(I_3)_2{\cdot}27H_2O$, 125250-23-9.

Supplementary Material Available: Listings of fractional atomic coordinates, temperature factors (isotropic for C, N, and O; anisotropic for Ba and I), and intra- and intermolecular O···O distances <3.5 Å that are indicative of hydrogen bonding (11 pages); a table of observed and calculated structure amplitudes (32 pages). Ordering information is given on any current masthead page.

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