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SODIUM BINDING TO D-GLUCURONATE RESIDUES: CRYSTAL STRUCTURE OF SODIUM D-GLUCURONATE MONOHYDRATE

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ABSTRACT

Three-dimensional X-ray diffraction data were used to determine the crystal structure of sodium β-D-glucuronate monohydrate, a model system for investigating the factors involved in the binding of sodium ions to D-glucuronate residues of glycosaminoglycans. Crystals of the salt are monoclinic, space group $P2_1$, with a = 9.206(3) Å, b = 7.007(2) Å, c = 7.378(3) Å, β = 96.84(3)°, and Z = 2. Intensity data for 858 reflections were measured with an automated diffractometer. A trial structure, obtained by direct methods, was refined by least squares to R = 0.035. An outstanding feature of the crystal packing is the interaction of D-glucuronate anions with sodium ions. The sodium ion is coordinated to three symmetry-related D-glucuronate anions and to one water molecule. The D-glucuronate anion binds sodium cations through the three following sites: one that involves a carboxyl oxygen atom combined with ring oxygen O-5; one that includes a single carboxyl oxygen atom, and one composed of the O-3–O-4 pair of hydroxyl groups.

INTRODUCTION

Sodium complexes of glycosaminoglycans appear to be involved in various biological processes, including sodium storage, electrolyte physiology, and maintainance of the organization and properties of extracellular matrices[1–3]. Although sodium–glycosaminoglycan complexes have been studied in aqueous solutions and in hydrated gels[1,4,5], little is known about the sites at which sodium is bound, or about the geometrical restraints that are imposed on the sodium–polysaccharide interactions. D-Glucuronic acid is a major component of many glycosaminoglycans. We report here the crystal structure of sodium β-D-glucuronate and describe the sodium interactions with the carbohydrate moiety.

EXPERIMENTAL

Clear, rectangular plates of sodium D-glucuronate monohydrate were grown by evaporating an aqueous solution that contained sodium D-glucuronate and calcium hydroxide. Weissenberg and oscillation photographs showed the crystals to be monoclinic, and space group $P2_1$ was indicated by the systematic absence of reflections $0k0$ with k odd. A crystal fragment, having the approximate dimensions of 0.11 × 0.35 × 0.31 mm, was mounted on a Picker FACS-1 diffractometer with its b axis slightly inclined to the ϕ axis of the diffractometer. Cell parameters, for use in collecting intensity data, were calculated by a least-squares analysis of the observed angular settings for twelve medium-angle reflections (CuK$\bar{\alpha}$, $\lambda = 1.5418$ Å). Intensity data were collected with the diffractometer, by use of a scintillation counter, nickel-filtered copper radiation, and a $\theta - 2\theta$ scanning technique. The scanning speed was 1°/min and the background was counted for 20 sec at each terminus of the scans. Measurements were made for the 858 symmetry-independent reflections having $2\theta < 128°$. Three strong, medium-angle reflections, which were monitored periodically, exhibited no significant variations in intensity during the period of data collection. Immediately after data collection, accurate values for the cell parameters were determined by a least-squares analysis of 2θ values for twelve high-angle reflections (CuKα_1, $\lambda = 1.54051$ Å); these cell parameters were not significantly different from those obtained prior to the measurement of intensities. Crystal data are listed in Table I.

Those reflections having scan counts below background level were given negative intensities and were retained in all subsequent calculations. All intensities were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus a correctional term $(0.03S)^2$, S being the scan count. The intensities and their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied by using the computer program ORABS[6].

Normalized structure-factors (E values) for use in direct methods were derived from the reduced intensity-data by means of a Wilson (1942) plot[7]. The multisolution, tangent-formula program MULTAN[8] was then used to generate and refine 16 different sets of normalized structure-factor phases. For those phase sets having the "best" associated figures of merit, E-maps were computed. None of the

TABLE I

CRYSTAL DATA FOR SODIUM β-D-GLUCURONATE MONOHYDRATE

Stoichiometry	$C_6H_9O_7 \cdot Na \cdot H_2O$
Z	2
Space group	$P2_1$
a	9.206(3) Å
b	7.007(2) Å
c	7.378(3) Å
β	96.84(3)°
ρ (calculated)	1.66 gcm^{-3}
ρ (observed)[a]	1.67 gcm^{-3}
μ (CuK)$\bar{\alpha}$	17.6 cm^{-1}

[a] The density was measured by flotation in a benzene–1,1,2,2-tetrabromoethane mixture.

maps, however, revealed a plausible molecular structure. (After the phase problem had been solved, these maps were re-examined. One map did, in fact, contain a fragment of the glucuronate molecule, correctly oriented with respect to the crystallographic axes, but incorrectly positioned with respect to the crystallographic symmetry elements.)

A compilation of experimental *E*-statistics at this point showed departures of $\langle E^2 \rangle$ from the expected (theoretical) value of unity for several reflection classes, notably for $h = 0$. We accordingly chose to renormalize the *E*-values so that $\langle E^2 \rangle$ approximates 1 for separate classes of reflection indices, parity groups, and $\sin\theta/\lambda$ intervals[9]. Again, using the program MULTAN[8], another 16 phase models were generated. An *E*-map computed from the most consistent of these phase sets readily revealed the positions of all nonhydrogen atoms.

The trial structure was refined by using a modified version of the full-matrix, least-squares program ORFLS[10,11]. The quantity minimized was $\sum w(Fo^2 - Fc^2/k^2)^2$, where k is a scale factor and the weight, w, is equal to $1/\sigma^2$ (Fo^2). Scattering factors for the nonhydrogen atoms (Na^+, C, O) were obtained from the *International Tables for X-ray Crystallography*[12], and hydrogen atom scattering-factors were those of Stewart *et al.*[13]. Positions of hydrogen atoms were identified from difference Fourier maps that were calculated during the later stages of refinement; all hydrogen atoms except one bonded to the water oxygen atom were located.

Included in the final cycles of refinement were all nonhydrogen-atom positional and anisotropic-temperature parameters, as well as Zachariasen's[14] isotropic-extinction parameter[15] g. All hydrogen-atom positional parameters and isotropic temperature-factors were also included, except for the hydrogen atom bonded to C-1 and the water hydrogen atom H-W. Although the positions of these two hydrogen atoms are chemically realistic, their temperature factors consistently refine to physically unrealistic values (negative for H-1 and high positive for H-W). These two atoms were given fixed parameters and were included in structure-factor calculations but not in refinement. During the last cycle of refinement, no parameter shifted more than one-fourth of its standard deviation. The final R index $(||\sum Fo| - |Fc||/\sum|Fo|)$ based on all reflections is 0.035. The goodness-of-fit, $\{\sum w(Fo^2 - Fc^2/k^2)^2/(m - s)\}^{1/2}$, where m is the number of reflections used and s is the number of parameters refined, is 2.57. In a final, difference Fourier map there were two peaks of 0.4–0.6 eÅ^{-3} in magnitude; these had no obvious chemical interpretations. No other peaks of this map exceeded 0.3 eÅ^{-3} in magnitude.

RESULTS

Heavy-atom and hydrogen-atom parameters, together with their estimated standard deviations, are listed in Tables II and III, respectively. Estimated errors in positional parameters are about 0.002 Å for the sodium ion, 0.004 Å for carbon and oxygen atoms, and 0.05 Å for hydrogen atoms. A table of observed and calculated structure factors is deposited with, and can be obtained from Elsevier Scientific

TABLE II

HEAVY-ATOM PARAMETERS AND THEIR ESTIMATED STANDARD DEVIATIONS[a]

Atom	X	Y	Z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Na	2827(2)	2500(0)	1259(2)	349(8)	242(8)	430(8)	11(7)	122(6)	−29(7)
C-1	2630(4)	9495(6)	5295(5)	265(16)	221(18)	245(15)	26(15)	109(13)	−55(14)
C-2	1501(4)	8109(6)	4415(5)	219(16)	237(18)	236(17)	−3(15)	54(14)	0(14)
C-3	2270(4)	6358(5)	3764(5)	293(18)	185(18)	216(16)	−12(15)	79(14)	21(15)
C-4	3424(4)	6933(5)	2542(5)	253(16)	180(17)	232(16)	6(13)	88(13)	−19(14)
C-5	4408(3)	8528(6)	3428(5)	229(15)	178(17)	231(17)	13(15)	80(13)	−1(14)
C-6	5373(4)	9270(6)	2026(4)	274(18)	219(17)	213(15)	−23(15)	60(13)	−11(16)
O-1	1945(3)	11122(5)	5856(4)	287(13)	291(15)	433(15)	26(12)	63(11)	−158(13)
O-2	545(3)	7582(5)	5730(4)	306(13)	380(15)	358(13)	−67(14)	194(11)	−76(14)
O-3	1294(3)	5095(5)	2704(4)	368(15)	263(15)	333(15)	−106(13)	153(11)	−62(13)
O-4	4221(3)	5283(4)	2201(4)	359(14)	202(14)	340(15)	14(11)	176(12)	−51(12)
O-5	3523(2)	10073(4)	3924(3)	287(13)	181(12)	284(12)	2(11)	98(9)	−12(11)
O-6	4879(3)	10627(5)	1055(4)	481(16)	303(15)	431(16)	128(13)	237(13)	158(13)
O-6′	6537(3)	8392(4)	1860(3)	258(12)	312(14)	304(13)	39(11)	106(10)	51(11)
O-W	411(4)	11357(8)	664(5)	644(22)	799(30)	597(21)	−142(23)	−53(17)	−126(22)

[a] All values have been multiplied by 10^4. Temperature factors are in the form $T = \exp[-2\pi^2 (U_{11}h^2a^{*2} + .. + 2U_{12}hka^*b^* + \ldots)]$. The final value of the isotropic extinction parameters is $g = 0.020(6)$. The y coordinate of the sodium ion was not refined.

TABLE III

HYDROGEN-ATOM PARAMETERS AND THEIR ESTIMATED STANDARD DEVIATIONS

Atom	X	Y	Z	U(Å)^2
H-1	325	899	637	7
H-2	103(4)	862(5)	341(6)	8(8)
H-3	272(4)	574(6)	476(5)	11(8)
H-4	309(5)	750(7)	131(6)	29(10)
H-5	501(4)	807(6)	451(5)	22(10)
HO-1	267(6)	1179(10)	657(7)	56(16)
HO-2	−13(8)	696(11)	520(10)	71(20)
HO-3	107(7)	455(14)	322(12)	92(31)
HO-4	462(5)	548(9)	133(7)	43(14)
H-W	4	1083	−26	127

[a] All parameters have been multiplied by 10^3. Isotropic temperature factors are in the form $T = \exp[-8\pi^2U \sin^2\theta/\lambda^2]$.

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Fig. 1 shows the crystal-packing and hydrogen-bonding schemes. All hydrogen atoms that are covalently bonded to oxygen atoms participate in hydrogen bonding,

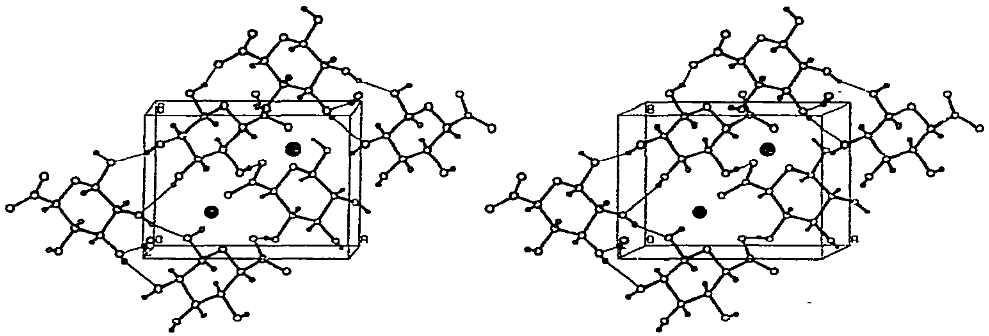

Fig. 1. Stereo unit-cell drawing of the crystal structure of sodium β-D-glucuronate monohydrate. Covalent and hydrogen bonds are denoted by heavy and thin lines, respectively.

TABLE IV

HYDROGEN-BOND DISTANCES AND ANGLES

Donor atom	*Hydrogen atom*	*Acceptor atom*	*Donor–acceptor distance (Å)*	*Hydrogen–acceptor distance (Å)*	*Donor–hydrogen–acceptor angle (degrees)*
O-1	HO-1	O-6'[a]	2.600	1.71	159
O-2	HO-2	O-1[b]	2.652	1.85	164
O-3	HO-3	O-2[b]	2.788	2.23	156
O-4	HO-4	O-6[c]	2.643	1.88	165
O-W	H-W	O-3[d]	2.914	2.12	165

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

with the possible exception of the one hydrogen atom of water whose position could not be determined. Hydrogen-bond distances and angles are listed in Table IV. Fig. 2 shows the environment of the sodium ion, which is coordinated to a water molecule and to three symmetry-related D-glucuronate ions. One of the D-glucuronate ions chelates the sodium ion through the O-3–O-4 pair of hydroxyl groups, the second through the ring oxygen atom (O-5) combined with the carboxyl oxygen atom O-6, and the third binds sodium through the carboxyl oxygen atom O-6'. This results in a coordination polyhedron around sodium composed of six oxygen atoms: one from a water molecule, two from hydroxyl groups, two from the carboxyl group, and one from the ring oxygen (O-5). Fig. 3 shows that these six oxygen atoms form a slightly distorted, octahedral arrangement around the sodium ion. The sodium–oxygen distances range from 2.32 to 2.62 Å. This coordination geometry is similar to that found in other sodium–carbohydrate complexes[16–19].

Fig. 4a shows the D-glucuronate conformation, sodium binding-sites, thermal ellipsoids, and bond lengths. Fig. 4b shows those bond angles that involve only nonhydrogen atoms. Table V lists conformational torsion-angles.

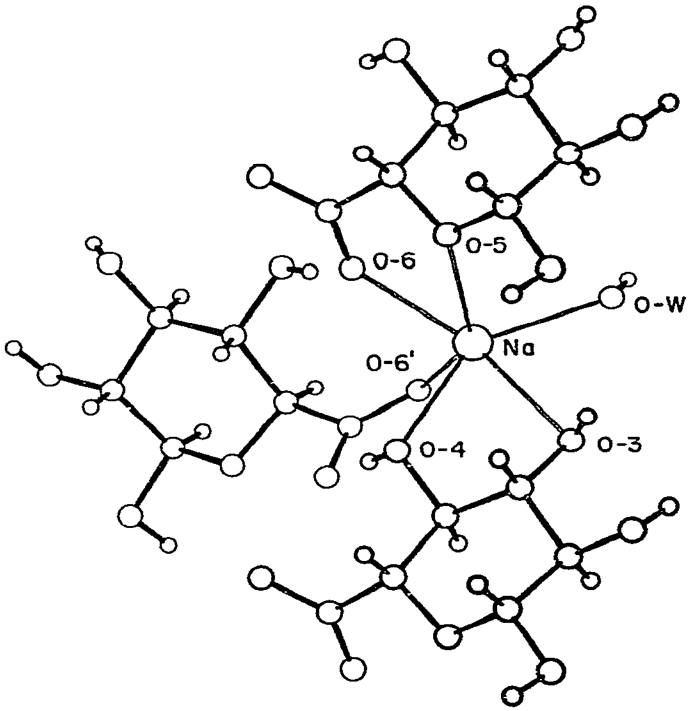

Fig. 2. Sodium environment for sodium β-D-glucuronate monohydrate. The sodium ion is coordinated to three β-D-glucuronate anions and to one water molecule. O-W designates the oxygen atom of the water molecule.

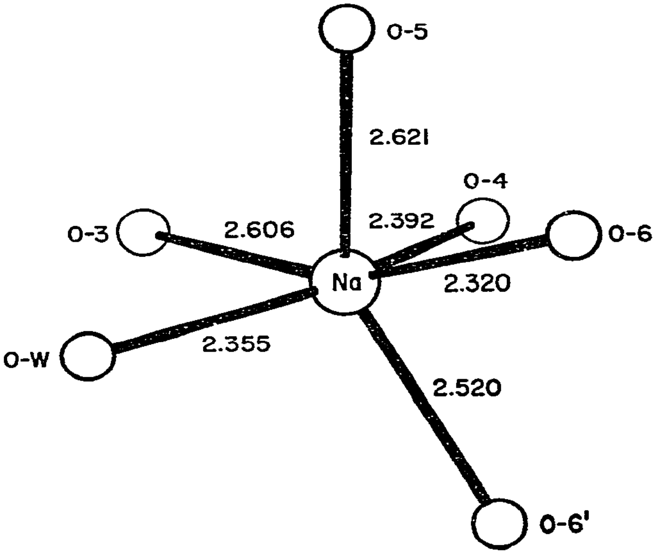

Fig. 3. Geometry of the sodium ion coordination-shell. Estimated standard deviations of Na–O distances are about 0.004–0.005 Å. O-W designates the oxygen atom of the water molecule.

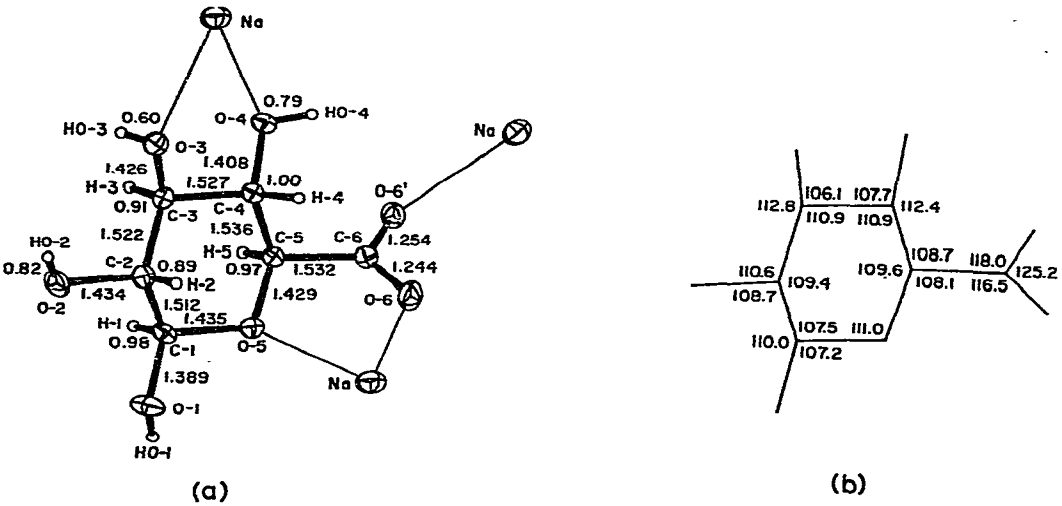

Fig. 4. (a) Conformation of the β-D-glucuronate anion. Estimated standard deviations in bond lengths are about 0.006 Å for bond lengths that involve nonhydrogen atoms and 0.05 Å for those that involve hydrogen atoms. Nonhydrogen atoms are depicted by thermal ellipsoids that are scaled to include 35%. The sodium-binding sites are shown. (b) Bond angles for the nonhydrogen atoms. Estimated standard-deviations are about 0.3°.

TABLE V

CONFORMATIONAL TORSION-ANGLES THAT INVOLVE ONLY NONHYDROGEN ATOMS OF THE β-D-GLUCURONATE ANION[a]

Bond	*Angle (degrees)*	*Bond*	*Angle (degrees)*
O-5-C-1-C-2-C-3	62.1	C-3-C-4-C-5-O-5	−53.0
O-5-C-1-C-2-O-2	−177.1	C-3-C-4-C-5-C-6	−170.9
O-1-C-1-C-2-C-3	178.5	O-4-C-4-C-5-O-5	−173.6
O-1-C-1-C-2-O-2	−60.6	O-4-C-4-C-5-C-6	68.6
C-1-C-2-C-3-C-4	−53.5	C-4-C-5-O-5-C-1	64.1
C-1-C-2-C-3-O-3	−172.2	C-6-C-5-O-5-C-1	−177.7
O-2-C-2-C-3-C-4	−173.2		
O-2-C-2-C-3-O-3	68.1	C-5-O-5-C-1-C-2	−68.9
		C-5-O-5-C-1-O-1	172.9
C-2-C-3-C-4-C-5	48.8		
C-2-C-3-C-4-O-4	172.1	C-4-C-5-C-6-O-6	90.8
O-3-C-3-C-4-C-5	171.5	C-4-C-5-C-6-O-6′	−84.4
O-3-C-3-C-4-O-4	−65.2	O-5-C-5-C-6-O-6	−28.0
		O-5-C-5-C-6-O-6′	156.8

[a] Estimated standard deviations are about 0.4°. The signs of the angles correspond to the notation of Klyne and Prelog[25].

DISCUSSION

As shown in Fig. 4, the D-glucuronate anion binds sodium cations at three sites; two are chelation sites that involve a pair of oxygen atoms acting in concert, and the third involves a single oxygen atom from the carboxyl group. These three binding regions might all be involved in interactions between sodium ions and the D-glucuronate residues of glycosaminoglycans. However, we consider that the chelation site composed of the atoms O-6 and O-5 is likely to be of particular importance. The O-1 and O-4 atoms of D-glucuronate residues in polysaccharides are frequently involved in glycosidic linkages; although such acetal oxygen atoms might be as suitable as hydroxyl groups for binding metals, crystallographic studies of metal–oligosaccharide complexes have provided no examples wherein glycosidic oxygen atoms bind metal ions. Therefore, the O-3–O-4 site may be of only minor importance in sodium interactions with D-glucuronate residues of glycosaminoglycans. In general, cation-binding sites that involve a single ligand are not as strong as chelation sites that provide two or more ligands in a proper geometrical arrangement for interacting with the metal ion. Thus it is reasonable to assume that the O-5–O-6 site is much more favorable than a site that uses a single carboxyl oxygen atom. This assumption is supported by extensive evidence that metals bind much more strongly to those carboxylic acids having oxygen atoms at the alpha position than to simple unsubstituted carboxylic acids[20]. The probable importance of the O-5–O-6 chelation site is further supported by the finding that calcium, which has about the same ionic radius as sodium, binds to this site in the crystal structure of α-D-glucuronate ·CaBr · trihydrate[21].

The major conformational flexibility within the D-glucuronate anion is the orientation of the carboxyl group about the C-5–C-6 bond. In the crystal structure of the sodium salt, the carboxyl group is tilted about 30° out of the plane that is defined by atoms O-5, C-5, and C-6. This conformation is essentially the same as those in the crystal structures of the potassium and rubidium salts of D-glucuronic acid[22], and that in the crystal structure of an aldotriouronic acid[23]. However, a different conformation, wherein the carboxyl group is nearly coplanar with atoms O-5, C-5, and C-6, was found in the crystal structure of the calcium bromide salt of D-glucuronic acid[21].

As depicted in Fig. 2, the sodium ion, which is coordinated to three symmetry-related D-glucuronate anions, serves as a bridge between the sugar moieties. This general feature is also found in the crystal structures of calcium–carbohydrate complexes[20]. In all of the calcium–carbohydrate complexes that have been examined thus far, the calcium ions are coordinated to two or more carbohydrate moieties, usually together with several water molecules, resulting in hydrated carbohydrate–calcium–carbohydrate bridges. The properties of these calcium–carbohydrate bridges have been discussed in detail, in conjunction with speculations about the possible roles they might play in various biological adhesion and agglutination processes[20,24]. Although interactions of sodium with carbohydrates may be somewhat weaker than calcium interactions, many of the qualitative postulates about the cross-linking potential of such interactions should be applicable to sodium–carbohydrate complexes. Linkages such as that shown in Fig. 2 may provide a mechanism for stabilizing the networks of glucosaminoglycans in extracellular matrices.

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