Calcium Binding to Galactose. Crystal Structure of a Hydrated α-Galactose–Calcium Bromide Complex

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Abstract: X-Ray diffraction data were used to determine the crystal structure of a hydrated calcium bromide complex of galactose, which is the principal carbohydrate moiety attached to hydroxylysine residues in bone collagen. Crystals of α-galactose–$CaBr_2 \cdot 3H_2O$ are orthorhombic, space group $P2_12_12_1$, with a = 19.388 (1), b = 8.746 (1), and c = 8.672 (1) Å. Intensity data for 1417 independent reflections were collected with an automated diffractometer. A trial structure was obtained by the heavy-atom method, and was refined by least squares to R = 0.048. The absolute configuration was confirmed by anomalous dispersion effects. An outstanding feature of the crystal packing is the interaction of galactose molecules with calcium ions. The calcium ions are coordinated to five hydroxyl groups from galactose molecules, and to three water molecules. Similar interactions may be involved in binding calcium minerals to bone collagen.

Many simple carbohydrates chelate calcium ions in aqueous solution[1] and in the solid state,[2-4] and calcium–carbohydrate interactions have been implicated in a variety of biological processes. We are currently investigating the crystal structures of calcium–carbohydrate complexes[2-4] to elucidate the structural factors involved in these interactions. In this paper we describe the crystal structure of a hydrated calcium bromide complex of α-galactose (Figure 1).

We are especially interested in the calcium binding properties of galactose, because this simple sugar is the major carbohydrate component of bone collagen.[5,6] Bone consists of calcium minerals deposited in organic matrices of which collagen is the major component,[7] and various physical and chemical studies have suggested that mineral deposition in bone may be partially controlled by the collagen matrix.[8-12] Electron microscopic studies indicate that, in mature bone, crystallites of mineral are in contact with collagen and are oriented with respect to the collagen fibrils,[7,13] so it is likely that the physical and mechanical properties of mature bone are influenced by mineral–collagen interactions. However, little is known about the types of interactions involved in mineral–collagen contacts, or about structural features of bond collagen that may be of importance in calcification processes. Although one might expect bone collagen to be endowed with certain special characteristics that enhance mineral–collagen interactions, various investigations have indicated that the primary and secondary structure of collagen from bones and from nonmineralizing tissues are nearly identical.[7] On the other hand, recent work has shown that the carbohydrate composition of bone collagen is strikingly different from that of soft-tissue collagen. In bone collagen, the glycosylated hydroxylysine residues (3–4 per molecule) contain a preponderance of galactose monosaccharides,[5,6] but in collagen from soft tissues[5,14] these residues contain principally glucosylgalactose disaccharides. We determined the crystal structure of this galactose–calcium bromide complex to examine the possibility that galactose moieties might provide effective sites for interactions with calcium salts.

Experimental Section

Clear, rectangular plates of the complex were obtained by the procedure of Hann and Hudson.[15] Weissenberg and oscillation photographs showed that the crystals are orthorhombic; the space group is $P2_12_12_1$, as indicated by the systematic absence of reflections $h00$ with h odd, $0k0$ with k odd, and $00l$ with l odd. The crystals are deliquescent; therefore, the crystal used for data collection was coated with a thin layer of epoxy glue. A crystal fragment with approximate dimensions of 0.10, 0.20, and 0.50 mm was mounted on a Picker FACS-1 diffractometer with its b axis slightly inclined to the ϕ axis of the diffractometer. Approximate cell parameters that were used in collecting intensity data were calculated by a least-squares analysis of the angular settings for eight medium-angle reflections (Cu Kα, λ 1.5418 Å).

Intensity data were collected with the diffractometer, by use of nickel-filtered copper radiation, a scintillation counter, and a θ–2θ scanning technique. Measurements were made for the 1417 reflections with $2\theta \leq 128°$. Three strong, medium-angle reflections were chosen as standards and monitored periodically; the intensities of these reflections did not vary significantly during the collection of intensity data. Immediately after data collection, accurate values for the cell parameters were determined by a least-squares analysis of 2θ values for 11 high-angle reflections (Cu $K\alpha_1$); these cell parameters were not significantly different from those obtained prior to the measurement of intensities. Crystal data are listed in Table I. The intensities were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus a correctional term $(0.04S)^2$, S being the scan count.

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Table I. Crystal Data

Stoichiometry	$C_6H_{12}O_6$–$CaBr_2 \cdot 3H_2O$
Z	4
Space group	$P2_12_12_1$
a	19.388 (1) Å
b	8.746 (1) Å
c	8.672 (1) Å
ρ (calcd)	1.961 g cm^{-3}
ρ (obsd)	1.96 g cm^{-3}
μ	109.4 cm^{-1}

The intensities and their variances were corrected for Lorentz and polarization factors, absorption corrections were applied by using the computer program ORABS,[16] and the data were scaled by means of a Wilson[17] plot.

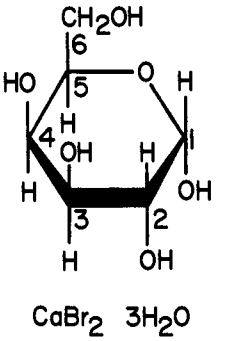

Figure 1. Structural formula of the hydrated galactose–calcium bromide complex.

Table II. Final Heavy-Atom Parameters and Their Standard Deviations[a]

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br(1)	1367 (1)	2210 (1)	1631 (1)	24 (1)	104 (1)	112 (1)	−6 (1)	2 (1)	−9 (1)
Br(2)	4588 (1)	2665 (2)	4467 (1)	34 (1)	279 (3)	82 (1)	49 (1)	5 (1)	17 (2)
Ca	3768 (1)	3163 (2)	9634 (2)	16 (1)	82 (2)	76 (2)	0 (1)	0 (1)	−4 (2)
C(1)	1126 (4)	3830 (9)	7156 (9)	15 (2)	86 (10)	71 (10)	3 (4)	2 (4)	4 (8)
O(1)	1069 (3)	4157 (6)	5551 (6)	18 (1)	98 (7)	79 (7)	−5 (2)	−10 (3)	16 (6)
C(2)	1729 (4)	4721 (8)	7773 (9)	17 (2)	71 (10)	71 (9)	2 (4)	2 (4)	4 (8)
O(2)	1682 (3)	6293 (6)	7285 (7)	20 (1)	64 (7)	95 (8)	4 (3)	0 (3)	0 (6)
C(3)	2411 (4)	4088 (10)	7236 (10)	14 (2)	112 (12)	93 (11)	−4 (4)	−6 (4)	13 (10)
O(3)	2968 (3)	4741 (7)	8115 (7)	18 (2)	92 (8)	152 (10)	−13 (3)	−21 (3)	31 (8)
C(4)	2458 (3)	2389 (8)	7399 (8)	14 (2)	77 (10)	79 (9)	−2 (4)	0 (3)	−4 (9)
O(4)	2597 (2)	1994 (6)	8982 (6)	16 (1)	90 (8)	88 (7)	−4 (3)	−3 (2)	22 (6)
C(5)	1820 (4)	1597 (9)	6749 (9)	15 (2)	98 (10)	66 (9)	5 (4)	0 (4)	−3 (8)
O(5)	1206 (2)	2255 (6)	7431 (6)	12 (1)	91 (7)	90 (7)	−2 (2)	1 (2)	17 (6)
C(6)	1783 (4)	−80 (9)	7047 (10)	19 (2)	71 (10)	109 (12)	−1 (4)	−9 (4)	−10 (9)
O(6)	1247 (3)	−785 (6)	6149 (7)	18 (2)	88 (8)	138 (9)	−2 (3)	−8 (3)	−25 (7)
W1	4909 (3)	2988 (7)	661 (7)	20 (2)	160 (10)	118 (9)	1 (3)	−7 (3)	36 (9)
W2	4073 (3)	1299 (7)	7802 (8)	27 (2)	110 (9)	126 (9)	1 (3)	12 (4)	−23 (8)
W3	4488 (3)	4568 (8)	7802 (7)	26 (2)	131 (10)	110 (9)	−4 (3)	8 (3)	10 (8)

[a] The values have been multiplied by 10^4. Temperature factors are in the form $T = \exp(-\beta_{11}h^2 - \beta_{22}k^2 - \beta_{33}l^2 - 2\beta_{12}hk - 2\beta_{13}hl - 2\beta_{23}kl)$. The final value of the isotropic extinction parameter is $g = 0.019$ (3).

We arrived at a suitable trial structure by the heavy-atom method as follows: coordinates for one bromide ion were determined from a sharpened Patterson map, coordinates for the second bromide ion and for the calcium ion were determined from a sum-function superposition of sharpened Patterson maps translated to the first bromide ion position, and the remaining nonhydrogen atoms were located in a Fourier map that was calculated by using phase angles derived from the three ions. The trial structure was refined by using a modified version of the full-matrix least-squares program ORFLS.[18,19] The quantity minimized was $\Sigma w(F_o^2 - F_c^2/k^2)^2$, where k is a scale factor and weight w is equal to $1/\sigma^2(F_o^2)$. Scattering factors for the nonhydrogen atoms were from the ref 20, and hydrogen atom scattering factors were from Stewart, Davidson, and Simpson.[21] Coordinates for those hydrogen atoms bonded to carbon atoms were calculated by assuming tetrahedral coordination around the carbon atoms and C–H bond distances of 0.9 Å. All those hydrogen atoms bonded to oxygen atoms were located in difference Fourier maps that were calculated during the latter stages of refinement. The hydrogen atoms were assigned the isotropic temperature factors of the heavy atoms to which they are bonded, and were included in the calculation of structure factors but not in the least-squares refinement. The heavy-atom positional parameters, the anisotropic temperature factors, and Zachariasen's[22] isotropic extinction parameter g (as formulated by Coppens and Hamilton[23]) were included in the refinement. As the refinement proceeded, the coordinates of the hydrogen atoms that are attached to oxygen atoms were improved by use of difference Fourier maps.

The final R index ($\Sigma ||F_o| - |F_c|| / \Sigma |F_o|$) is 0.048, and the goodness-of-fit $\{[\Sigma w(F_o^2 - F_c^2/k^2)^2/(m - s)]^{1/2}$, where m is the number of reflections used and s is the number of parameters refined$\}$ is 2.07. During the last cycle of refinement, no parameter shifted more than one-fifth of its estimated standard deviation. A final difference Fourier map was calculated with the hydrogen atoms omitted from the calculated structure factors. The electron densities at the hydrogen atom positions had an average value of 0.7 e/Å^3 and ranged from 0.4 to 0.9 e/Å^3. The remainder of the map showed several peaks and troughs of magnitudes ranging from 0.5 to 1.0 e/Å^3 in the vicinities of the bromide ions; there were no other peaks or troughs in excess of 0.5 e/Å^3.

During the refinement, both real and imaginary components of the anomalous dispersion correction factors were applied to the atomic scattering factors for the nonhydrogen atoms. The correction factors used were from Cromer and Liberman.[24] After we refined the correct enantiomer (D-galactose), the coordinates were inverted and the incorrect enantiomer (L-galactose) was refined. The L-galactose model refined to only $R = 0.059$ and goodness-of-fit = 2.60. By use of the R-factor ratio test,[25] a comparison of the refinements of the correct and incorrect enantiomers

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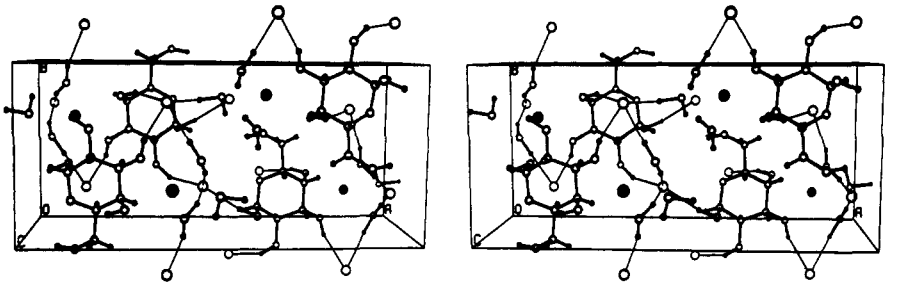

Figure 2. Stereo drawing showing the crystal packing as viewed down the c axis. Bromide ions are depicted as circles and calcium ions as solid black balls. Covalent and hydrogen bonds are represented, respectively, by heavy and light lines. (This drawing and those in Figures 3–5 were prepared by using the program ORTEP. C. K. Johnson, "ORTEP, A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations, Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965).

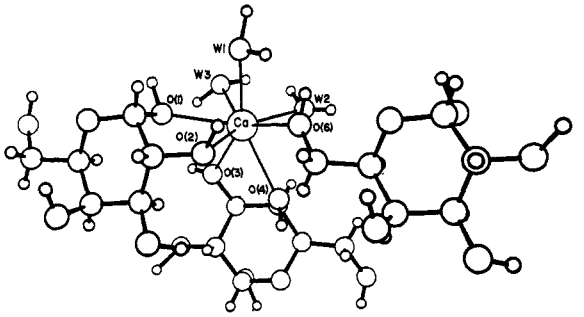

Figure 3. Environment of the calcium ion.

indicates the D-galactose absolute configuration to be correct with a probable error of less than 0.5%.

Results

Table II lists the final heavy-atom parameters and their estimated standard deviations. Table III gives the hydrogen atom parameters, as determined directly from difference Fourier maps. The estimated errors in positional coordinates are about 0.001 Å for bromide and calcium ions and 0.004–0.009 Å for carbon and oxygen atoms. A table of observed and calculated structure factors has been deposited.[26]

Table III. Hydrogen Atom Parameters[a]

Atom	x	y	z	B, Å^2
H(C1)	73	414	767	1.70
H(O1)	57	370	540	2.59
H(C2)	173	470	881	1.56
H(O2)	130	700	800	2.39
H(C3)	247	432	622	2.36
H(O3)	305	570	765	2.01
H(C4)	280	201	678	2.40
H(O4)	220	180	935	1.97
H(C5)	180	176	572	1.91
H(C6)	170	−26	804	3.07
H′(C6)	219	−53	678	3.07
H(O6)	80	−60	620	2.91
H(W1)	480	200	100	3.60
H′(W1)	550	280	50	3.60
H(W2)	405	30	785	3.72
H′(W2)	440	200	700	3.72
H(W3)	460	380	700	3.43
H′(W3)	420	560	750	3.43

[a] The positional parameters, which were determined from difference Fourier maps, have been multiplied by 10^3; the errors in these positions are probably 0.1–0.3 Å.

(26) See paragraph at end of paper regarding supplementary material.

The crystal packing is shown in Figure 2. Hydrogen bond lengths and angles are given in Table IV. The calcium ion is surrounded by a shell composed of oxygen atoms from hydroxyl groups and water molecules. The bromide ions are hydrogen bonded to water molecules and to hydroxyl groups. The closest bromide–calcium contact is 4.5 Å, a distance 1.5 Å longer than the sum of the bromide and calcium ionic radii. Several of the water molecules and hydroxyl groups form bridges between the calcium and bromide ions.

Table IV. Hydrogen Bond Distances (Å) and Angles (deg)

Donor	Hydrogen	Acceptor[a]	Donor–Acceptor	Hydrogen–Acceptor	Angle
O(1)	H(O1)	Br(2) c	3.283	2.2	170
O(2)	H(O2)	Br(2) b	3.238	2.2	160
O(3)	H(O3)	Br(1) b	3.229	2.3	160
O(4)	H(O4)	Br(1) d	3.317	2.6	150
O(6)	H(O6)	W(1) e	2.986	2.5	110
W(1)	H′(W1)	Br(1) f	3.460	2.5	140
W(2)	H(W2)	Br(1) g	3.343	2.6	150
W(2)	H′(W2)	Br(2) a	3.285	2.3	150
W(3)	H(W3)	Br(2) a	3.342	2.4	160
W(3)	H′(W3)	Br(1) b	3.424	2.3	170

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

Figure 3 shows the environment of the calcium ion, which binds to three symmetry-related galactose molecules and to three water molecules. One galactose molecule is coordinated to the calcium ion through the O(1) and O(2) hydroxyl groups, another through the O(3) and O(4) hydroxyl groups, and a third molecule binds through its O(6) hydroxyl group. Thus, the calcium ion is surrounded by a shell composed of eight oxygen groups. There are no hydrogen bonds formed between oxygen atoms within this calcium coordination shell. The stereochemistry of the calcium ion coordination shell is shown in more detail in Figure 4. The eight oxygen atoms form a distorted square-antiprism with calcium–oxygen distances ranging from 2.35 to 2.55 Å. Within the calcium shell, there are several short (<2.85 Å) oxygen–oxygen contacts.

Figure 5 shows the galactose conformation and its heavy-atom thermal ellipsoids. Conformational torsion angles are listed in Table V, bond lengths are shown in Figure 5, and bond angles are given in Table VI.

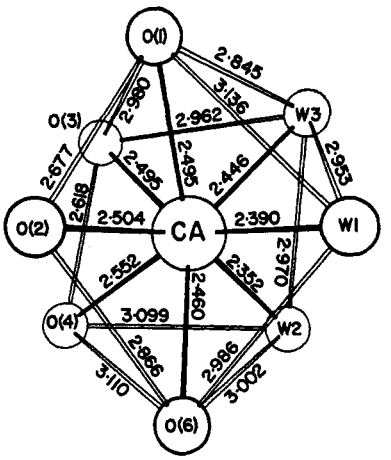

Figure 4. Stereochemistry of the calcium ion coordination shell. All oxygen–oxygen contacts shorter than 3.25 Å are shown, as are the calcium–oxygen distances. W1, W2, and W3 are oxygen atoms of water molecules.

Table V. Conformational Torsion Angles Involving Heavy Atoms[a]

Angle	Deg
C(1)–C(2)–C(3)–C(4)	−46.8
C(2)–C(3)–C(4)–C(5)	48.0
C(3)–C(4)–C(5)–O(5)	−52.8
C(4)–C(5)–O(5)–C(1)	60.0
C(5)–O(5)–C(1)–C(2)	−59.1
O(5)–C(1)–C(2)–C(3)	51.0
O(1)–C(1)–C(2)–O(2)	49.1
O(2)–C(2)–C(3)–O(3)	71.3
O(3)–C(3)–C(4)–O(4)	43.5
O(4)–C(4)–C(5)–C(6)	−47.8
C(4)–C(5)–C(6)–O(6)	−168.6
O(5)–C(5)–C(6)–O(6)	70.1

[a] A positive torsion angle is defined for a view along the middle bond as a clockwise twist of the bond furthest from the viewer with respect to that which is nearest. The estimated standard deviations are about 0.7°.

Table VI. Bond Angles Involving Heavy Atoms[a]

Angle	Deg	Angle	Deg
C(1)–C(2)–C(3)	112.4	C(3)–C(2)–O(2)	108.4
C(2)–C(3)–C(4)	112.9	C(2)–C(3)–O(3)	110.4
C(3)–C(4)–C(5)	111.6	C(4)–C(3)–O(3)	107.4
C(4)–C(5)–O(5)	109.5	C(3)–C(4)–O(4)	109.8
C(5)–O(5)–C(1)	114.3	C(5)–C(4)–O(4)	113.3
O(5)–C(1)–C(2)	111.2	C(4)–C(5)–C(6)	115.0
O(5)–C(1)–O(1)	111.7	O(5)–C(5)–C(6)	106.3
C(2)–C(1)–O(1)	107.7	C(5)–C(6)–O(6)	111.3
C(1)–C(2)–O(2)	109.9		

[a] The estimated standard deviations are about 0.4°.

The crystal structure of free galactose has not been determined. However, except for structural differences at the sites where calcium ions bind to the sugar, the structure of the galactose molecule is in agreement with that found for the galactose moiety in the crystal structure of lactose monohydrate.[27] The magnitudes of the C(3)–C(4)–C(5)–O(5), O(3)–C(3)–C(4)–O(4), O(4)–C(4)–C(5)–C(6), and C(4)–C(5)–C(6)–O(6) torsion angles are from 6 to 12° smaller than in the crystal structure of lactose monohydrate, and C(4)–C(3)–O(3) and C(5)–C(4)–O(4) bond angles are 4–5° smaller than in lactose monohydrate. On the other hand, in the crystal structure of the lactose–calcium bromide complex,[13] where calcium is also chelated to the O(3)–O(4) pair of hydroxyl groups, the conformation in the region of the C(3)–C(4) bond is in agreement with that found in the galactose complex. These conformational changes resulting from calcium chelation are similar to those noted in the crystal structures of other carbohydrate–calcium complexes,[12] in which calcium chelation by pairs of adjacent hydroxyl groups also results in a 0.2 Å decrease in the spacing between the hydroxyl oxygen atoms.

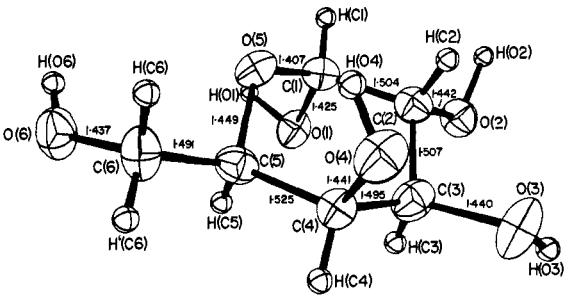

Figure 5. Conformation of the galactose molecules. Nonhydrogen atoms are represented by thermal ellipsoids that are defined by the principal axes of thermal vibration and scaled to include 50% probability. Hydrogen atoms are represented by spheres of 0.1 Å radius. Bond lengths that involve nonhydrogen atoms are given; estimated standard deviations are 0.008–0.010 Å.

Discussion

Interaction between galactose molecules and calcium ions is an outstanding feature of this crystal structure (Figure 3). The crystal packing scheme is such that all five hydroxyl groups of the galactose molecule are involved in calcium binding; thus the hydroxyl groups and water molecules form a cohesive shell that encompasses the calcium ion. As discussed previously,[12–14] hydroxyl–calcium interactions are of major importance in the crystal structures of other carbohydrate–calcium halide complexes, and in the crystal structures of calcium salts of sugar acids. Interactions of this type also account for the binding of calcium ions to uncharged carbohydrates in aqueous systems.[28] In view of the role that water molecules play in the galactose–calcium bromide complex, it is likely that the interactions involved are typical of those that occur in aqueous environments, where galactose could bind calcium ions by simply substituting hydroxyl groups for water molecules in the calcium hydration shell. Complexes of this type might be expected to occur between calcium ions and the galactose moieties of bone collagen, and might provide an effective mechanism for collagen–mineral interactions.

Solution studies show that simple carbohydrates bind calcium ions strongly only if they can provide sites with three or more hydroxyl groups in a geometrical arrangement that is suitable for calcium coordination.[28] As predicted by the solution studies,[28] our results indicate that galactose can provide only a single hydroxyl group or pairs of hydroxyl groups for calcium binding. Consequently, single, isolated ga-

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lactose moieties of bone collagen are not likely to have a high affinity for calcium ions. However, if two or more galactose moieties were in close proximity to each other on the collagen matrix and were geometrically situated so that each could utilize hydroxyl groups for calcium coordination, then a strong binding site could result. Such a site is found in the crystal structure of this galactose–calcium complex where the crystal packing permits three galactose molecules to assume an orientation that leads to interactions of five hydroxyl groups with the calcium ions. Similar binding sites might be provided by adjacent galactose moieties within the collagen molecules, or by neighboring galactose residues from different molecules in the collagen fibrils. Since triple-stranded bone collagen molecules contain two identical α_1 chains aligned in register,[1] the galactose moieties that are present on α_1 chains would always occur as adjacent pairs in the collagen molecules. A strong binding site for calcium ions should result if these pairs of galactose moieties from the two α_1 chains assume steric configurations with several hydroxyl groups in the proper orientation for calcium coordination. Similarly, galactose moieties from neighboring tropocollagen molecules might combine to form effective calcium binding sites. If two or more galactose moieties with the proper spatial arrangement are required for strong calcium binding, then the availability of such sites would be dependent upon both the primary structure of the collagen molecules and the packing pattern of collagen molecules within the collagen fibrils, two properties that are probably subject to cellular control.

It is noteworthy that galactose monosaccharides account for the majority of the carbohydrate in bone collagen, as contrasted to the glucosylgalactose residues that predominate in soft-tissue collagen.[8–10] If the carbohydrate moieties play some significant role in collagen–calcium interactions, there is no obvious reason why these interactions should be more favorable with galactose monosaccharides than with glucosylgalactose disaccharides. Since lactose (a glucose–galactose disaccharide) binds calcium ions in aqueous solution[11] and in the solid state,[12,13] it is reasonable to expect that the disaccharides of collagen could also bind calcium ions. However, the packing patterns of carbohydrate moieties within collagen fibrils are likely to be considerably different for galactose monosaccharides than for the bulkier disaccharides. Assuming that two or more carbohydrate moieties must combine together in a given geometrical arrangement to generate a strong calcium-binding site, then such packing factors could be of major importance and might explain the preponderance of galactose monosaccharides in bone collagen.

It is possible that galactose–calcium complexes may provide effective nucleation sites for growth of calcium minerals within the collagenous matrix of bone. Solution studies which demonstrated the importance of calcium binding to uncharged oxygen atoms of proteins[29] prompted Urry[30] to propose a theory of calcification in which the major driving force is the affinity of calcium ions for neutral sites. According to this theory, calcium ions bind to neutral sites, which become positively charged as a result of the bound calcium, and the calcification process is then propagated by the migration of neutralizing phosphate and carbonate ions to these sites. Binding of calcium ions to the neutral galactose moieties of bone collagen might serve as a suitable mechanism for the initial step in the process described by Urry. Even when bound by several hydroxyl groups of galactose moieties, calcium ions would be able to accommodate additional ligands in their coordination polyhedra. In the crystal structure of this galactose–calcium bromide complex the additional ligands are provided by water molecules. During calcification, these sites might be occupied by oxygen atoms from phosphate or carbonate ions, thus leading to the formation of mineral nuclei that initiate calcification.

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Supplementary Material Available. Observed and calculated structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20 × reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-6442.

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