Multinuclear NMR and molecular modelling investigations on the structure and equilibria of complexes that form in aqueous solutions of Ca$^{2+}$ and gluconate

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**A R T I C L E   I N F O**

Article history:
Received 23 March 2010
Received in revised form 4 May 2010
Accepted 10 May 2010
Available online 16 May 2010

**Keywords:**
\(\phi\)-Gluconate
Calcium complex stability
Protonation
\(^1\text{H} \text{NMR}, \text{\(^{13}\text{C} \text{NMR}}, \text{\(^{43}\text{Ca} \text{NMR}\)}\)

**A B S T R A C T**

Complexation of \(\phi\)-gluconate (Gluc\(^-\)) with Ca$^{2+}$ has been investigated via \(^1\text{H}, \text{\(^{13}\text{C} \text{NMR}, \text{\(^{43}\text{Ca} \text{NMR}}\)}\) spectroscopy in aqueous solutions in the presence of high concentration background electrolytes (\(1 \leq I \leq 4 \text{ M (NaCl)}\) ionic strength). From the ionic strength dependence of its formation constant, the stability constant at 6 \(\leq \text{pH} \leq 11\) and at I \(\rightarrow 0\) M has been derived (log \(K_{1,1}^{\text{Ca}} = 1.8 \pm 0.1\)). The protonation constant of Gluc\(^-\) at I \(\rightarrow 1 \text{ M (NaCl)}\) ionic strength was also determined and was found to be log \(K_{a}^{\phi} = 3.24 \pm 0.01\) (\(^{13}\text{C} \text{NMR}\)) and log \(K_{a} = 3.23 \pm 0.01\) (\(^1\text{H} \text{NMR}\)). It was found that \(^1\text{H}\) and \(^{13}\text{C} \text{NMR}\) chemical shifts upon complexation (both with H$^+$ and with Ca$^{2+}$) do not vary in an unchanging way with the distance from the Ca$^{2+}$/H$^+$ binding site. From 2D \(^1\text{H} - \text{\(^{43}\text{Ca} \text{NMR}}\) spectra, simultaneous binding of Ca$^{2+}$ to the alcoholic OH on C2 and C3 was deduced. Molecular modelling results modulated this picture by revealing structures in which the Gluc\(^-\) behaves as a multidentate ligand. The five-membered chelated initial structure was found to be thermodynamically more stable than that derived from a six-membered chelated initial structure.

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1. Introduction

Polyhydroxy carboxylates are known to sequester calcium ions in solution.\(^1\) Under hyperalkaline conditions high stability Ca$^{2+}$ complexes may be formed (e.g., with isosaccharinate), where the metal ion is claimed to be bound via the alcoholate group(s) of the carbohydrate.\(^2,3\) However, at intermediate pH such deprotonation does not take place, and the contribution of the alcoholic OH to the coordination (if any) is expected to be relatively small. Therefore, the stability of the calcium complexes formed is very low, and the formation constant of the first stepwise complex, \(K_{1,1}\), defined as

\[
K_{1,1} = \frac{[\text{CaL}^\text{Ca}^{2+}]}{[\text{Ca}^{2+}][L]} \tag{1}
\]

(where L$^-$ denotes the polyhydroxy-carboxylate anion), is in the order of \(10^{-80}\) dm$^3$ mol$^{-1}$.\(^4\) As a result of this low stability, the experimental determination of the corresponding formation constants is inherently difficult, and accurate stability data are scarce.\(^5\) Not at all surprisingly, even less is known about the structure of these solution complexes.\(^1\)

These statements hold even for the calcium complex(es) of the \(\phi\)-gluconate (Fig. 1, Gluc\(^-\) hereafter) ion. Gluc\(^-\) is perhaps the most common polyhydroxy carboxylate with biological relevance and wide-ranging pharmaceutical and industrial applications.\(^6\) From the literature the CaGluc\(^+(\phi)\) complex (or ion pair) is very weak.\(^4,7-10\) From H$^+$/Pt electrode potentiometry log \(K_{1,1} = 1.22\) was obtained (20 $^\circ$C, \(I = 0.20 \text{ M KCl}\)).\(^7\) While from ion-exchange measurements under similar conditions, log \(K_{1,1} = 1.22\) was found (25 $^\circ$C, \(I = 0.16 \text{ M KCl}\)).\(^8\) Ca$^{2+}$-ISE potentiometry yielded log \(K_{1,1} = 1.31\) (25 $^\circ$C, \(I = 0.70 \text{ M KNO}_3\))\(^6\) and 1.05 (25 $^\circ$C, \(I = 0.50 \text{ M NaCl}\)).\(^9\) At reasonably high [Gluc$^-\phi$]/[Ca$^{2+}\phi$] ratios (where the lower index \(T\) denotes total or analytical concentrations) noticeable formation of the Ca(Gluc)\(^+\) complex was also reported\(^9\) (hence the difference between the formation constants published in Refs.\(^4\) and\(^6\)). pH-potentiometric titrations\(^10\) resulted in log \(K_{1,1} = 1.21\) (25 $^\circ$C, \(I = 1 \text{ M NaClO}_4\)). The latter value is somewhat higher than expected, possibly due to complications caused by lactonisation of HGluc in acidic solutions.\(^11-13\) (see also below). From these data, and also on the basis of analogies with isosaccharinic acid, the formation constant at \(I \rightarrow 0\) M has been estimated to be log \(K_{1,1}^{\text{Ca}} = 1.72,14,15\)

The metal-binding site in CaGluc\(^-\) (i.e., the structure of the complex in solution) has not been clarified yet. It is plausible that the
(deprotonated) carboxylate moiety is involved in the complexation. However, on the basis of the formation constants, monodentate carboxylate coordination is highly unlikely, as simple monocarboxylates under similar conditions form much weaker complexes with Ca$^{2+}$ than does Gluc$^-$. Participation of alcoholic OH groups of C2 (five-membered chelate ring), C3 (six-membered chelate ring) or C6 (head-to-tail coordination or macro-chelate formation) in the complexation of the calcium are both possible. However, from the data published so far in the literature, none of them have been experimentally proven.

In the present work we embarked on the detailed multinuclear NMR (including $^1$H, $^{13}$C and $^{43}$Ca) characterisation of aqueous solutions containing Ca$^{2+}$ and Gluc$^-$ in the pH range of 6–11. To confirm the structural information obtained, the spectroscopic experiments were supplemented with quantum mechanical calculations. Our project aimed at (i) establishing whether multinuclear NMR spectroscopy was suitable for producing equilibrium constants of reasonable quality for the very weak complexes that form in these systems; (ii) determining the formation constants of the complex(es) formed in hypersaline solutions and to deduce the species formed during protonation and Ca$^{2+}$–complexation of the Gluc$^-$, that is, the stepwise Ca(Gluc)$^{+}$, or the binuclear Ca$_2$Gluc$^{3+}$ is detectable by the NMR techniques employed; (iv) quantitatively characterising the effects of complexation on the NMR chemical shifts of Ca$^{2+}$ and Gluc$^-$ and (iv) identifying the binding sites of the metal ion in the complex(es).

2. Experimental

2.1. Reagents and solutions

Stock solutions of each compound were prepared using anhydrous sodium gluconate (Sigma–Aldrich product, ≥99% purity), CaCl$_2$ (Reanal product, puriss grade) and NaCl (Spectrum 3D product, analytical reagent grade) dissolved in MilliQ Millipore deionised water. Prior to solution preparation the CaCl$_2$ was dried at 200 °C overnight to eliminate structural water and kept in a desiccator over P$_2$O$_5$. The H$^+$ concentration was set with a standardised HCl solution (E. Merck product, [HCl]$_T$ = 3.571 M). D$_2$O (E. Merck product, 99.95% purity) was finally added to each sample making up the concentration to 20% v/v.

The analytical concentration of gluconate, [Gluc$^-$]$_T$, in the test solutions was usually 0.200 M. In the calcium-containing systems, the concentration of CaCl$_2$ was systematically raised from 0.0200 to 0.4000 M, and the ionic strength was set with NaCl in the ionic strength range of 1.00–4.00 M. Such high ionic strength was necessary, because the two interacting electrolytes (CaCl$_2$ and NaGluc) needed to be present in sufficiently high concentrations for the NMR measurements. Experiments at I = 5.00 M (NaCl) failed due to the decreased solubility of Ca(Gluc)$_2$(s) at this high ionic strength. For the protonation constant determinations, the ratio of [HCl]$_T$:[Gluc$^-$]$_T$ was systematically raised from 0.00 to 1.00, and the ionic strength was set to 1.0 M with NaCl.

2.2. NMR experiments

The NMR spectra of the solutions were recorded on a Bruker Avance DRX 500 NMR spectrometer equipped with a 5-mm inverse broadband probe-head furnished with z-oriented magnetic field gradient capability. In the NMR measurements the magnetic field was stabilised by locking it to the $^2$D signal of the solvent. The sample temperature was set to 25 ± 1 °C during all data acquisitions. For the individual samples 32–64 scans were taken to record $^1$H NMR spectra and 256–512 scans (with the proton decoupler turned on) to obtain the $^{13}$C spectra. Up to 64 k scans were taken to record $^{43}$Ca NMR spectra. The $^1$H–$^{43}$Ca correlations were detected by 2D HMQC (heteronuclear multiple quantum coherence) experiment (via heteronuclear zero and double quantum coherence). For creation of the anti-phase magnetisation, the 1/(2J$_{CH}$) delay was optimised, and a final 250 ms was used, which corresponds to an $^1$H–$^{43}$Ca coupling constant of 2 Hz. Spectra were acquired with 128 increments in the indirect dimension and with 256 scans using a recycle delay of 2 s. No decoupling was applied during acquisition.

The pH of the solutions ($p$H$_{observed}$) was determined with a pH-sensitive glass electrode (Metrohm 6.0234.100) and an Orion 710A pH meter, calibrated according to the procedure described in the literature. In these solutions the 20% v/v D$_2$O was replaced with distilled water. To calculate the pH in the target solutions a 4:1 H$_2$O–D$_2$O mixture ($p$H$_{mixture}$), the approximation used in the literature ($p$D = $p$H + 0.4015), was used assuming a linear variation of the pH with deuteron replacement (i.e., $p$H$_{mixture}$ = $p$H$_{observed}$ + 0.08).

The equilibrium constants and the limiting chemical shifts of the species formed during protonation and Ca$^{2+}$–complexation of Gluc$^-$ were calculated with aid of the PSEQUAD suite. Data fitting was performed by fixing the limiting chemical shifts of the Gluc$^-$ calculating hereby the stability/acidity constants and the limiting chemical shifts of the CaGluc$^+$ or HGluc, respectively.

2.3. Molecular modelling

Two structural varieties of the CaGluc$^+$ complex cations (assuming five- or six-membered rings containing the Ca$^{2+}$ ion) were studied computationally by Hartree–Fock ab initio calculations applying the 6–31 G* basis set included in the HyperChem program package. Full geometry optimisations were performed on the isolated ions. Calculations were considered to be over when the gradient norm reached 0.1.

For modelling the aqueous solution of the complexes, the calculations were performed with the PM3 semiempirical quantum chemical method, also included in the HyperChem package. The geometries of the two structural variations of the complex ions were optimised applying explicit water molecules. It means that the geometries of a solvent cage consisting of 216 water molecules and the complex cations were fully optimised until the gradient norm became smaller than 0.1. Through applying explicit water molecules the model system approaches reality to a great extent. However, the size of the system increased considerably, and, now, semiempirical calculations could only be used. The initial geometries of the naked cations were taken from the ab initio (HF 6–31 G* basis set) calculations previously performed on the isolated cations. After optimisation applying explicit water molecules the water molecules were removed, and a single point energy calculation was performed on the ligands, and the values (reflecting the stabilities of the complex cations) were compared and related to experimental findings.
Figure 2. $^{13}$C NMR spectra of solutions with $[\text{NaGluc}]_T = 0.200 \text{ M}$ with peak assignments at the two limiting, 0.0 (a) and 1.4 (b) $[\text{Ca}]_T:[\text{Gluc}^-]_T$ ratios.

Figure 3. $^1$H NMR spectrum of a solution with $[\text{NaGluc}]_T = 0.200 \text{ M}$ with peak assignments.
3. Results and discussion

3.1. Effect of protonation and Ca\(^{2+}\) complexation on the \(^{13}\)C and \(^1\)H NMR spectra of Gluc

In the \(^{13}\)C NMR spectrum of a NaGluc solution at pH 6–11, six well-defined peaks for the six carbon atoms of the Gluc\(^-\) could be observed (Fig. 2). In the order of the increasing field, these peaks were assigned to C1, C2, C4, C5, C3 and C6, respectively (Figs. 1 and 2). This assignment is in agreement with those previously published\(^{12,13}\) and was confirmed on the basis of the 2D \(^1\)H–\(^{13}\)C NMR spectrum of a NaGluc solution (spectrum not shown). Sharp NMR peaks can be observed during protonation or complexation of the Gluc\(^-\). The \(^{13}\)C NMR spectra of the two limiting [Ca\(^{2+}\)]:[Gluc\(^-\)] ratios (Fig. 2) indicate that the chemical exchange rate is much faster than the NMR time scale.

In the \(^1\)H NMR spectrum of NaGluc (Fig. 3) the signals of the H2 and H3 protons are well resolved; however, those of H4–H6 are not (this is mainly due to the double quartet of H6 around 3.60 and 3.75 ppm, respectively).

When the pH of a NaGluc solution (I = 1 M NaCl, [Gluc\(^-\)]\(_I\) = 0.200 M) was varied from ~6.5 to ~1.8 by adding HCl, the \(^{13}\)C NMR chemical shifts of the ligand gradually moved upward (Table 1), while the \(^1\)H NMR resonances shifted downfield (see inset in Fig. 4).

Besides the six primary carbon peaks for the carbon atoms of the Gluc\(^-\), the development of additional peaks was seen in the \(^{13}\)C NMR spectrum upon the decrease of pH, due to the lactonisation of HGluc.\(^{12}\) Lactonisation was mainly observed under pH ~3.8, and the intensities of the relevant peaks increased with time and decrease in pH; however, the chemical shifts of these small extra peaks remained pH independent. On the other hand, the gradual shift of the six sharp intense peaks of the (not lactonised) Gluc\(^-\) with the pH (Fig. 4) made it possible to extract the dissociation constant of HGluc from these data. The protonation constant,

\[
K_a = \frac{[\text{HGluc}]}{[\text{H}^+][\text{Gluc}^-]} \quad (2)
\]

calculated using the chemical shift variation of every carbon atom was found to be log \(K_a = 3.24 \pm 0.01\). The difference between our and the literature\(^{12}\) data (log \(K_a = 3.30 \pm 0.02\) at I = 0.1 M NaClO\(_4\)) is most likely due to the difference in ionic strengths and the possible influences of the calibration protocol for the glass electrode. On the basis of the pH-dependent \(^1\)H NMR chemical shift variations of the hydrogen atoms on C2 and C3 (H2A, H2B and H3 in Fig. 4), respectively, the acidity constant was found to be log \(K_a = 3.23 \pm 0.01\), a result that is practically identical to that gained from \(^{13}\)C NMR measurements.

3.2. Determination of the formation constants of CaGluc\(^+\)

The variation of the \(^1\)H NMR chemical shifts of Gluc\(^-\) upon Ca\(^{2+}\) addition was in the order of 0.03–0.06 ppm even for the (non-exchangeable) protons closest to the carboxylate end. Because of this and the complex and overlapping spectra of the non-exchanging protons on C4, C5 and C6, the \(^1\)H NMR data proved to be unsuitable for determining formation constants for the complexes between Ca\(^{2+}\) and Gluc\(^-\).

In the \(^{13}\)C NMR spectra of Gluc\(^-\), significant variations were seen as a result of Ca\(^{2+}\) complexation (Table 1 and Fig. 5). To obtain formation constants and complex compositions, two kinds of fitting protocols were used: in one, only the variations C1 and C3 chemical shifts upon Ca\(^{2+}\) complexation were used, and in the second, chemical shift changes for all six carbon atoms were used together (Table 1) The limiting chemical shifts for the Gluc\(^-\) ion were held constant during optimisations. The fitting parameters (that is, the agreement between the observed and calculated chemical shifts) were found to be excellent for the first protocol and reasonable for the second one, and the formation constants obtained for both protocols are practically identical. (Note that in some cases no convergence could be achieved by using the second protocol; see Table 1.)

It is assumed that the formation of the CaGluc\(^+\) complex, only, was sufficient to reasonably describe all the experimental data points obtained with chemically meaningful log \(K_{1,1}\) values and acceptable standard deviations. However, small but systematic differences between the observed and calculated values were seen (Fig. 5) at the highest [Gluc\(^-\)]\(_I\):[Ca\(^{2+}\)]\(_I\) ratios, that is, where the formation of the Ca(Gluc)\(_2\)\(^0\) complex was the most favoured. (The three sets of data at 1.0 M ionic strength but different pHs (~6, ~8 and ~11) were also fitted together (Table 1) to increase the confidence level for the results. Similar behaviour was observed, which supports the formation of the Ca(Gluc)\(_2\)\(^0\) complex.) The extent of this deviation, however, was too small to make the extraction of log \(K_{1,2}\) possible from our \(^{13}\)C NMR data; nevertheless, the existence of these effects indicate the possible formation of

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<th>(\log K_{1,2})</th>
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\(^a\) Calculated by simultaneously fitting the chemical shift variations observed for all the carbon atoms of Gluc\(^-\) upon Ca\(^{2+}\) addition.

\(^b\) Calculated by simultaneously fitting the chemical shift variations observed for C1 and C3 of Gluc\(^-\) upon Ca\(^{2+}\) addition.

\(^c\) Failure in optimisation.

\(^d\) Fit for all three sets of data (pH ~6, ~8 and ~11) together.

\(^e\) The protonation constant of Gluc\(^-\), \(\log K_a\) as defined in Eq. 2.

\(^f\) The differences between the limiting chemical shifts of the carbon atoms of HGluc and Gluc\(^-\) (\(\Delta \delta = \delta_{\text{HGluc}} - \delta_{\text{Gluc}^-}\)).

\(^g\) From the data relating to C1, only.
Ca(\text{Gluc})_2^{0}$ complex already suggested in some reports\textsuperscript{9} and ignored elsewhere.\textsuperscript{4}

As expected, the log $K_{1,1}$ values obtained at various pH values at $6 < \text{pH} < 11$ were practically identical (because the gluconate ion was fully deprotonated and the formation of the first stepwise calcium–hydroxo complex, Ca(OH)$^+$, was negligible\textsuperscript{22} even at pH $\sim 11$).

The log $K_{1,1}$ versus $I$ curve passes through a smooth minimum (Fig. 6). By including data from other literature sources obtained at ionic strengths $< 1$ M, an extended Debye–Hückel treatment\textsuperscript{23} was used with

$$\log K_{1,1} = \log K_{1,1}^0 - A \frac{\sqrt{I}}{1 + \sqrt{I}} + BI$$

(3)

where the Debye–Hückel constant $A = 2.046$, and the empirical parameter $B$ was found to be 0.089. From this calculation, $\log K_{1,1}^0 = 1.8 \pm 0.1$ was obtained, with reasonable agreement with other values from the literature.\textsuperscript{2,14,15}
On the basis of the data in Table 1, it is striking that the $^{13}$C NMR limiting chemical shifts ($\delta_{\text{Ci}}$) observed for Gluc$^-$/C0 and calculated for CaGluc$^+$ show systematic downfield variation with increasing ionic strength. The extent and direction of this shift are similar to those reported by Zhang et al.\textsuperscript{12} obtained for solutions of pH > 12 and with increasing pH. Zhang et al. argued that such variations were due to deprotonation of the aliphatic alcohol of Gluc$^-$. However, on the basis of the present data, it seems that such variations in the $^{13}$C NMR chemical shifts can be caused by interactions with Na$^+$ ions (i.e., breaking of the inner-molecular H-bonding of Gluc$^-$), as suggested in Ref.\textsuperscript{24}. The differences between the limiting chemical shifts of Gluc$^-$ and HGluc, that is, 

$$\Delta \delta = \delta_{\text{HGluc}} - \delta_{\text{Gluc}}$$

\text{(4)}

Figure 6. The formation constant of the CaGluc$^+$ complex as a function of the ionic strength (NaCl).\textcircled{O}: present work; $\Delta$: values from Refs. 4 and 10, which were not included in the optimisation; $\bullet$: values from Refs. 7–9. Solid line: calculated on the basis of the extended Debye–Hückel treatment (Eq. 3), with log $K_{11}^0 = 1.8$.

Figure 7. $^{43}$Ca NMR spectra of an aqueous solution with [CaCl$_2$] = 0.200 M in the presence of [NaGluc] = 0.200 M. Inset: the spectrum of the same solution without added NaGluc.
for the carbon atoms C1–C6 in Table 1, are all of the same sign (i.e., the displacement of the $^{13}$C NMR chemical shifts are directed towards the lower frequency with decreasing pH). However, the $\Delta \delta$ values do not change monotonously with the distance from C1 of the carboxylate group undergoing protonation. This is most likely to be due to the differences in conformational changes caused by protonation on C1, which are the most pronounced at C4.

The situation is more complex with regard to the $\Delta \delta$ values of Gluc$^-$ and CaGluc$^+$. The displacements are towards the higher frequencies for C1 and C2 and towards the lower frequencies for C3–C6. $\Delta \delta$ is the largest for C2 at each ionic strength. The $\Delta \delta$ on C3 upon Ca$^{2+}$ binding is larger than that upon protonation. From these subtle variations, which result from several coexisting and (sometimes) opposite effects, the structure of the CaGluc$^+$ complex, that is, the binding site of Ca$^{2+}$ cannot be unambiguously deduced.

### 3.3. The structure of the CaGluc$^+$ complex

The identification of the binding sites was experimentally approached via 2D $^1$H–$^{43}$Ca NMR measurements. For this, first the $^{43}$Ca NMR spectra of solutions containing CaCl$_2$ (0.200 M) and various amounts of NaGluc (0.100–0.400 M) were recorded. Because of the low abundance of the $^{43}$Ca isotope (0.135 at %), acquisition of each spectra took several days (64000 scans); therefore, these experiments were restricted to a few selected samples. Upon addition of Gluc$^-$, the peak of the hydrated Ca$^{2+}$ ion gradually moved towards the lower frequencies and significantly broadened (Fig. 7), that is, the full width at the half-height (FWHH) increased from 1.2 to 13.1 Hz. On the basis of the $^{43}$Ca chemical shift variation, and taking into consideration the formation constants shown in Table 1, the limiting chemical shift of Ca$^{2+}$ in the CaGluc$^+$ complex can be approximated as $\sim -4.4$ ppm (relative to the hydrated Ca$^{2+}$ ion).

The 2D $^1$H–$^{43}$Ca NMR spectrum of a solution containing [CaCl$_2$]$_T$ = [NaGluc]$_T$ = 0.200 M is shown in Figure 8. The $^1$H–$^{43}$Ca correlations were detected by 2D HMQC heteronuclear multiple quantum coherence (HMQC)$^{25}$ experiment (via heteronuclear zero and double quantum coherence). For creation of the anti-phase magnetisation, the 1/(2-$J_{\text{C,Ha}}$) delay was optimised and a final 250 ms was used, which corresponds to an $^1$H–$^{43}$Ca coupling constant of 2 Hz. Spectra were acquired with 128 increments in the indirect dimension and with 256 scans and using a recycle delay of 2 s. No decoupling was applied during acquisition. From this spectrum it seems plausible that the Ca$^{2+}$ ion interacts with the OH on C2 and C3 simultaneously. Since the interaction with the carboxylate oxygen (on C1) is plausible, two possible scenarios may be suggested. In one, bonding isomerism takes place according to Scheme 1. According to this model two isomers of the calcium gluconate complex are formed in aqueous solution as a five- and a six-membered chelated structure, and they are in equilibrium. On the basis of qualitatively examining the 2D $^1$H–$^{43}$Ca NMR spectrum, the predominant structure is the five-membered chelate structure of the two bonding isomers.
In the second scenario Gluc\(^{-}\) acts as a tridentate ligand binding via the carboxylate and the aliphatic alcohols on both C2 and C3.

For clarifying the more likely scenario, all structure types were modelled as isolated ions (HF–6–31 G\(^{*}\) ab initio calculations) as well as in aqueous solutions by applying explicit water molecules (PM3 semiempirical calculations). The ab initio calculations resulted in structures displayed in Figures 9 and 10 together with all Ca–O distances applying the numbering scheme of Scheme 1.

Much to our surprise, no five-membered chelate ring could be found (Fig. 9). Instead, the Ca\(^{2+}\) entered into bonding interactions with O(C1), O(C2), O(C3) and O(C6) at the same time. This was the optimum geometry whether calculations were started from a five-membered chelate ring or from the structure where Gluc\(^{-}\) was assumed to act as tridentate ligand. Actually, the Ca\(^{2+}\) ion comfortably nestled itself among as many oxygens as possible.

When calculation was started from a six-membered ring, in the optimum structure there were bonding interactions between the Ca\(^{2+}\) ion and the O(C1), O(C3) and O(C6) at the same time (Fig. 10). Chemical intuition, as well as the comparison of the computed total energies \((E_{\text{tot}} = -900023.04 \text{ kcal/mol}, E_{\text{tot}0} = -90004.39 \text{ kcal/mol})\), revealed that the previous arrangement was more favourable thermodynamically than this one.

Modelling an aqueous solution modified, though not very significantly, the situation (Fig. 11). In the complex ion starting from the five-membered chelate, the Ca\(^{2+}\) was even better embedded in the hug of oxygens than in the isolated form [there were bonding interactions with O(C1), O(C2), O(C3), O(C5) and O(C6)], while in the complex ion starting from the six-membered chelate there were the same number of bonding interactions [with O(C1), O(C3) and O(C5)] as in the isolated form. The difference in stabilities (actually in the enthalpies of formation) is 130 kcal/mol in favour of the ion starting from the five-membered chelate. (Probably this high value is an overestimation of the real-life stability difference.)

To answer the originally posed question, we can say that Scheme 1 is basically correct with the modification that, although the actual structures are related to the five- or six-membered chelate, in both bonding isomers, the gluconate acts as multidentate ligand.

4. Conclusions

Complexation of \(\text{\textit{D}-gluconate (Gluc\(^{-}\) with Ca\(^{2+}\)}\) has been investigated via \(^1\text{H}, \(^{13}\text{C}\) and \(^{43}\text{Ca}\) NMR spectroscopy in aqueous solutions in the presence of a high concentration of background electrolytes \((1 \text{ M} \leq I \leq 4 \text{ M (NaCl) ionic strength})\). Assumption of a 1:1 complex, only, was sufficient to describe all the experimental observations; however, at high \([\text{Gluc}\(^{-}\)]/\text{[Ca}\(^{2+}\)\)] ratios, formation of small amounts of the Ca(Gluc\(^{2-}\))\(^5\) solution species was also inferred. From the ionic strength dependence of its formation constant, which was determined from the chemical shift variation upon \([\text{Ca}\(^{2+}\)]\), the stability constant at \(I \to 0 \text{ M}\) has been derived \((\log K_{\text{1,1}} = 1.8 \pm 0.1)\). The formation constant was found to be pH independent at \(6 \leq \text{pH} \leq 11\). The chemical exchange between the CaGlu\(^{2+}\) complex and its constituents was found to be fast on the NMR time scale, and the limiting chemical shifts for the CaGlu\(^{2+}\) complex have been calculated. It was found that the variation of the \(^{1}\text{H}\) and \(^{13}\text{C}\) NMR chemical shifts upon complexation (both with H\(^{+}\) and with Ca\(^{2+}\)) does not vary in an unchanging way with the distance from the Ca\(^{2+}/\text{H}^{+}\)-binding site. From these measurements the protonation constant of Gluc\(^{-}\) at \(I = 1 \text{ M (NaCl) ionic strength}\) was also determined and was found to be log \(K_{\text{p,2}} = 3.24 \pm 0.01 \text{ (\(^{13}\text{C}\) NMR)}\) and log \(K_{\text{p,1}} = 3.23 \pm 0.01 \text{ (\(^{1}\text{H}\) NMR)}\), which is in good agreement with independent literature data. From 2D \(^{1}\text{H}/^{43}\text{Ca}\) NMR spectra, the binding of Ca\(^{2+}\) to the alcoholic OH on C2 and C3 can be deduced,
from which has been suggested that a five- and a six-membered chelated ring system coexists in the CaGluc+ complex. Molecular modelling results (ab initio calculations on the isolated species and semiempirical results on moieties in aqueous solution) modulated this picture by revealing structures in which the Gluc behaves as multidentate ligand. Calculations, which coincide with the experimental findings, indicate that the complex ion derived from the five-membered chelate initial structure is thermodynamically more stable than that derived from the six-membered chelated initial structure.

References