Complex Formation Equilibria of Monoisopropyl and P,P-Diisopropyl Esters of Clodronic Acid with Calcium and Magnesium Ions in Aqueous (CH₃)₄NCl Solutions

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The complex formation equilibria of the monoisopropyl ester and P,P-diisopropyl ester of clodronic acid with Ca²⁺ and Mg²⁺ ions have been studied by a series of EMF titrations in aqueous solutions at 25°C and I = 1.0 (Me₂NCl). Computer analysis of the potentiometric data showed that the complexation scheme can be expressed exclusively in terms of the mononuclear ML species. We found a linear correlation (r = 0.99) between β₁₀₁ (ML) and the sum of the logarithm of the acid constants (ΣpKₓ) of the studied esters, clodronic and etironic acids. Also there is a linear correlation (r = 1.00) between the logarithm of the overall stability constants of Mg²⁺ and Ca²⁺ complexes, and between log β₁₀₁ (ML) and log β₁₁₁ (HL) of these ligands.

Isopropyl esters of (dichloromethylene)-bisphosphonic acid (clodronic acid) belong to a group of gem-bisphosphonates, which contain a P–C–P bridge. They are chemically related to and mimic the physiologic behaviour of P–O–P compounds, pyrophosphates. Most importantly, both pyrophosphate and bisphosphonate ligands have very high affinity for calcium(II) in homogeneous solution and at the surfaces of minerals and bone. Unlike pyrophosphates, bisphosphonates are resistant to chemical and enzymatic hydrolysis and retain their activity in conditions in which pyrophosphates would be destroyed. For this reason bisphosphonates are widely used as therapeutic agents for diseases in the skeletal system and in soft tissues.¹⁻⁵

Clodronic acid, together with conventional cancer therapy, has been used successfully for treatment of hypercalcemia related to osteolytic metastases and malignancies. Clodronic acid has also yielded good results in the treatment of Paget’s disease and primary hyperparathyroidism.⁶,⁷ However, the therapeutic index and the absorption of clodronic acid are poor. The clinical properties of methylenebisphosphonates can be modified by varying the substituents at the bridge carbon and at the phosphate moieties.⁸ For this reason the isopropyl ester derivatives of clodronic acid, acting as prodrugs of clodronate or new bisphosphonate drugs, were synthesised. Also there are no data available on the complex formation equilibria of the studied esters with calcium and magnesium ions. The present paper describes study of the complexation of monoisopropyl and P,P-diisopropyl esters of clodronic acid with Ca(II) and Mg(II) ions in aqueous solutions under constant conditions, I = 1.0 (CH₃)₄NCl and 25°C by potentiometric measurements.

Experimental

Syntheses. The studied esters were prepared by Leiras Oy according to procedures described elsewhere.⁹,¹⁰

Reagents. Stock solutions of tetramethylammonium chloride, tetramethylammonium hydroxide and HCl were prepared and analysed as described previously.¹⁰ Calcium and magnesium stock solutions were prepared by dissolving the corresponding chlorides CaCl₂·2H₂O and MgCl₂·6H₂O (Merck p.a.). The metal ion content of the solutions was determined by titration with standard EDTA (Merck P.a.).¹¹

Potentiometric measurements. The potentiometric titrations were performed in a temperature-controlled room (25°C) at 25.0 ± 0.1°C. A locally constructed titration system consisting of a multichannel high-impedance amplifier, a Hewlett-Packard 3478A multimeter and Metrohm 665 Dosimat piston burette was used for the measurements. The equipment was controlled with an Amstrad PC 1640 HD 20 computer using the program TIT3.¹² The indicator electrode was an Orion research

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91-01 glass electrode and the reference electrode an Orion
90-02 double junction Ag/AgCl electrode. The outer
mante of the Ag/AgCl electrode was filled with 1.0 M
tetramethylammonium chloride solution. The measure-
ment of the free concentration of \( \text{H}^+ \) and the calibration
of the electrode system were carried out as described in
detail earlier.\textsuperscript{10}

The measurements were carried out in an argon atmos-
phere using a constant ionic strength medium of 1.0 M
tetramethylammonium chloride by a series of potenti-
metric titrations. During the titration of acidic three com-
ponent metal–\( \text{H}^+ \)–ligand solutions, the ratio between the
total concentrations of ligand (\( \text{C}_L \)) and metal (\( \text{C}_M \)) was
held constant, and the total hydrogen ion concentration
(\( \text{C}_{\text{H}} \)) was varied by the addition of tetramethylammonium
hydroxide solution [\( \text{C}_n = 1.0, (\text{CH}_3)_4\text{NCl} \)] from a burette.
A total of five different titrations were carried out for each
metal–\( \text{H}^+ \)–ligand system within the limits: 0.0174 \( \leq \text{C}_M \leq 0.0059 \) \( \text{M} \),
0.0078 \( \leq \text{C}_L \leq 0.0128 \) \( \text{M} \) with
0.46 \( \leq \text{C}_L / \text{C}_L \), \( 0.2 \leq 2.2 \), and 2.9–3.6 \( \leq -\log [\text{H}^+] \leq 6.6–7.6 \)
(depending on the metal–\( \text{H}^+ \)–ligand system).

**Data treatment.** The equilibrium reactions in metal–\( \text{H}^+ \)–
ligand system can be described by two general reactions
(3) and (4):

\[
q\text{H}^+ + r\text{L}^- \leftrightarrow \text{H}_q\text{L}_r^-; \quad \beta_{\text{q,r}}
\]

\[
p\text{M}^{2+} + q\text{H}^+ + r\text{L}^- \leftrightarrow \text{M}_p\text{H}_q\text{L}_r^-; \quad \beta_{\text{p,q,r}}
\]

where eqn. (3) denotes the acid–base properties of the
ligand, eqn. (4) denotes the formation of the metal–ligand
complexes and \( n \) is 2 (\( P.P \)-disopropyl ester) or 3
(monoisopropyl ester). For reaction (3) the values of the
overall protonation constants (\( \beta_{\text{q,r}} \)) were determined ear-
lier\textsuperscript{10} and were considered as known in calculations of
overall stability constants (\( \beta_{\text{p,q,r}} \)) for reaction (4). The mass
balance and equilibrium conditions used in our calcula-
tions are as given by eqns. (5)–(7):

\[
\text{C}_M = [\text{M}^{2+}] + \Sigma \Sigma q \beta_{\text{p,q},r} [\text{M}^{2+}] [\text{H}^+] [\text{L}^-]^r
\]

\[
\text{C}_H = [\text{H}^+] - k_w/\text{[H}^+\]}

\[
+ \Sigma \Sigma q \beta_{\text{q,r}} [\text{M}^{2+}] [\text{H}^+] [\text{L}^-]^r
\]

\[
\text{C}_L = [\text{L}^-] + \Sigma \Sigma q \beta_{\text{q,r}} [\text{M}^{2+}] [\text{H}^+] [\text{L}^-]^r
\]

where \( \text{C}_M \), \( \text{C}_H \) and \( \text{C}_L \) are total concentrations and
[\( \text{M}^{2+} \)], [\( \text{H}^+ \)] and [\( \text{L}^- \)] are free concentrations of metal,
\( \text{H}^+ \) and ligand, respectively. Generally, in titrations the
total concentrations \( \text{C}_M \), \( \text{C}_H \) and \( \text{C}_L \) are known and the
free concentration of \( \text{H}^+ \) can be measured. In each
experimental point [\( \text{M}^{2+} \)] and [\( \text{L}^- \)] can be calculated us-
ing eqns. (5) and (7) with the assumption of certain val-
ues of \( \beta_{\text{q,r}} \) and \( \beta_{\text{p,q,r}} \). The search for a model (\( \text{p,q,r} \)) and
for corresponding overall stability constants (\( \beta_{\text{p,q,r}} \)) that give
the 'best' fit to experimental data was carried out using
modified version SCOGS21 of the nonlinear least-
squares computer program SCOGS.\textsuperscript{14} The error squares
sum \( U = \Sigma (V_{\text{calc}} - V_{\text{obs}})^2 \) was minimized (\( V_{\text{calc}} \) is the cal-
culated and \( V_{\text{obs}} \) the added titrant volume at the point \( i \)).
The quantities of \( V_{\text{calc}} \) were calculated from input data
using current estimates of the constants, and the mea-
sured \( -\log [\text{H}^+] \).\textsuperscript{14} The statistical part of SCOGS2 cal-
culates the \( P \)-value as well as an agreement index \( R \) for each
set of constants. At the 95\% confidence level the
\( P \)-value of the model should exceed 0.05. The \( P \)-value
is a measure for the normal distribution of the
(\( V_{\text{calc}} - V_{\text{obs}} \)- residuals). The agreement index is cal-
culated from eqn. (8):

\[
R = (\Sigma (V_{\text{calc}} - V_{\text{obs}})^2/\Sigma (V_{\text{obs}})^2)^{1/2}
\]

(8)

The best set of constants gives the smallest \( R \) value.
\( \bar{n} \), the average number of ligands bound to metal ion, is
calculated from eqn. (9):

\[
\bar{n} = (\Sigma (C_{\text{L}} - (C_{\text{H}} - [\text{H}^+] - k_w/[\text{H}^+]))/C_{\text{H}})/C_{\text{M}}
\]

(9)

where \( \bar{n}_{\text{H}} \), the average number of \( \text{H}^+ \) bound to each
ligand, is calculated using the known values of protona-
tion constants.

**Results and discussion.**

The potentiometric data consisted of five titrations and
109–179 experimental points for each metal–ligand sys-
tem. The treatment of the potentiometric data was ini-
itated by making Bjerrum plots of \( \bar{n} \) vs. \( \log \text{[L]} \) (Fig. 1).
The experimental \( \bar{n} \)-curves seem to be only a function of
\( \log \text{[L]} \), and all the curves coincide for the whole
\( -\log [\text{H}^+] \). \( C_L \) and \( C_M \) region over which the measure-
ments were carried out, and no significant deviation from
the calculated \( \bar{n} \)-curve was observed. This indicates that
the values of the stability constants of Mg\textsuperscript{2+} and Ca\textsuperscript{2+}
complexes do not depend upon the total concentration of
the ligand or the free concentration of the metal and that
the system can be described solely by the stepwise for-
mation of ML\textsubscript{r} complexes. Further, the plots show that
complexes with a ligand-to-metal ratio higher than one are
hardly formed, because of the low \( \bar{n} \)-values. Computer
analyses revealed that the ML complexes explain the ex-
perimental data fairly well. However, in the case of \( P.P \)-
disopropyl ester the precipitation in metal containing
solutions limits the operational \( -\log [\text{H}^+] \) range used in
calculations. The precipitation took place at about
\( -\log [\text{H}^+] \geq 7.0 \) and the precipitate is probably the ML
complex.

The best set of overall stability constants obtained with
the program SCOGS2 are given in Table 1. Because the
program gave a \( P \)-value range 0.051–0.52 and an \( R \)-index
range 0.0014–0.0062 for stability constants calculated
from individual titrations, statistically reliable constants
were obtained over the \( -\log [\text{H}^+] \). \( C_L \) and \( C_M \) region
used in the calculations. The monoisopropyl ester forms
stronger complexes with Ca\textsuperscript{2+} and Mg\textsuperscript{2+} than the \( P.P \)-
diisopropyl ester. Probably only one negatively charged phosphonate group from \( PP \) diisopropyl ester and both phosphonate groups from monoisopropyl ester are involved in the complex formation, resulting in a stronger chelate effect for the monoisopropyl ester. Also both ligands bind the \( Mg^{2+} \) ion more strongly than the \( Ca^{2+} \) ion, which is thus in agreement with the Irving–Williams order of stabilities.\(^{15}\) Figure 2 shows \( log \beta_{101} \) of the \( Mg^{2+} \) and \( Ca^{2+} \) complexes as a function of the sum of the logarithm of the acid constants (\( \Sigma pK_a \)) of the monoisopropyl ester, the \( PP \) diisopropyl ester of clodronic acid,\(^{10}\) clodronic acid and etitronic acid.\(^{16}\) The linearity observed in both cases emphasizes the dominance of ionic interactions in these systems.\(^{17}\) Deviation from unit slope in both cases is attributed to a change in the nature of bonding between the donor group and the metal ions as well as to

\[
\begin{array}{|c|c|c|c|c|c|c|}
\hline
M^{2+} & -log [H^+] & C_L/mM & C_M/mM & log \beta_{101} & \Sigma pK_a & log \beta_{1011} \\
\hline
PP-diisopropyl ester of clodronic acid & & & & & & \\
Ca & 2.9–6.6 & 7.8–11.5 & 16.7–6.2 & 1.91±0.02 & 6.43\(^a\) & 6.13\(^a\) \\
Mg & 3.0–6.6 & 7.8–11.5 & 17.4–6.4 & 2.02±0.02 & & \\
\hline
Monoisopropyl ester of clodronic acid & & & & & & \\
Ca & 3.6–7.4 & 8.6–12.8 & 16.0–5.9 & 3.29±0.02 & 8.43\(^a\) & 7.24\(^a\) \\
Mg & 3.7–7.6 & 8.7–12.8 & 17.5–6.5 & 3.65±0.02 & & \\
\hline
Clodronic acid & & & & & & \\
Ca & & & & 5.4\(^b\) & 17.71\(^b\) & 9.55\(^b\) \\
Mg & & & & 5.89\(^b\) & & \\
\hline
Etitronic acid & & & & & & \\
Ca & & & & 7.17\(^b\) & 22.13\(^b\) & 11.14\(^b\) \\
Mg & & & & 7.24\(^b\) & & \\
\hline
\end{array}
\]

\(^a\) Values are taken from Ref. 10. \(^b\) Values are taken from Ref. 16.
Fig. 2. Log $\beta_{ML}$ as a function of $\Sigma pK_a$ for Mg$^{2+}$ (A) and Ca$^{2+}$ (B) of (1) the $P,P$-disopropyl ester of clodronic acid, (2) the monoisopropyl ester of clodronic acid, (3) clodronic acid and (4) etironic acid.

solvation effects.$^{18}$ A plot of log $\beta_{101}(ML)$ against log $\beta_{011}(HL)$ (Fig. 3), and a plot of log $\beta_{101}$ of Mg$^{2+}$ complexes against those of Ca$^{2+}$ complexes (Fig. 3) gave straight lines of unit slope. These signify that in the first case the equilibrium constants of proton displacement reactions$^{19}$

$$M^{2+} + HL^{1-n} \rightleftharpoons ML^{2-n} + H^+$$  

(10)

and in the second case the equilibrium constants of central ion exchange reactions:

$$Mg^{2+} + CaL^{2-n} \rightleftharpoons MgL^{2-n} + Ca^{2+}$$  

(11)

are equal for these four ligands. The above-mentioned linear relationship equations are these:

$$\log \beta_{101} (MgL) = 0.3052 \Sigma pK_a + 0.5265 \quad r = 0.98$$  

(12)

$$\log \beta_{101} (CaL) = 0.3059 \Sigma pK_a + 0.2596 \quad r = 0.99$$  

(13)

$$\log \beta_{101} (MgL) = 1.0232 \log \beta_{011} (HL) - 4.0123 \quad r = 1.00$$  

(14)

Fig. 3. (A) Log $\beta_{ML}$ vs. log $\beta_{ML}$. (B) Log $\beta_{MgL}$ vs. log $\beta_{HL}$ and (C) log $\beta_{CaL}$ vs. log $\beta_{HL}$ for (1) the $P,P$-disopropyl ester of clodronic acid, (2) the monoisopropyl ester of clodronic acid, (3) clodronic acid and (4) etironic acid.

$$\log \beta_{101} (CaL) = 1.0252 \log \beta_{011} (HL) - 4.2868 \quad r = 1.00$$  

(15)

$$\log \beta_{101} (MgL) = 0.9979 \log \beta_{011} (CaL) - 0.2667 \quad r = 1.00$$  

(16)

In order to visualize the composition of the solutions studied in this work two distribution diagrams in the $H^+ - Mg^{2+}$-monoisopropyl ester/$P,P$-disopropyl ester
solutions are given in Fig. 4. The program SOLGASWATER\textsuperscript{20} was used to calculate these diagrams. Also this program was used to calculate the simplified model of $H^+ - Ca^{2+} - Mg^{2+} - \text{monoisopropyl ester} / P, P'-\text{diisopropyl ester}$ systems in human blood serum. The protonation constants from Ref. 10 and the stability constants obtained in the present study have been applied together with average concentrations of the studied esters (0.010 mM, $Mg^{2+}$ (0.85 mM) and $Ca^{2+}$ (2.40 mM) ions in blood serum.\textsuperscript{16} The distribution diagrams are shown in Fig. 5. The physiological pH of human serum is about 7. As can be seen, at this pH 76$\%$ of the monoisopropyl ester is bound to the complexes $CaL^-$ (42$\%$) and $MgL^-$ (34$\%$). In addition there exists free ligand in the forms of $HLL^-$ (15$\%$) and $L^3-$ (9$\%$). In the case of $P, P'$-diisopropyl ester the situation is totally different: 80$\%$ of the ligand is free in the forms of $L^2-$ (70$\%$) and $HL^-$ (10$\%$). The relative amounts of the complexes $CaL$ and $MgL$ are 14 and 6$\%$, respectively. The behaviour of the present ligands differ from that of clodronic acid. The distribution diagrams show clearly that for these two esters the calcium containing species are not predominant in human serum. In the case of clodronic acid about 80$\%$ of the ligand is bound to $Ca^{2+}$ ion.\textsuperscript{16}

![Distribution diagrams](image)

Fig. 4. Distribution diagrams in the (A) $H^+ - Mg^{2+} - Ca^{2+} - \text{monoisopropyl ester}$ and (B) $H^+ - Mg^{2+} - P, P'-\text{diisopropyl ester}$ of clodronic acid solutions $(C_{Ca} = 10.0 \text{ mM}, C_{Mg} = 10.0 \text{ mM})$. $F_i$ is defined as the ratio between the bound ligand content and the total ligand content.

![Simplified model calculation](image)

Fig. 5. Simplified model calculation on (A) $H^+ - Mg^{2+} - Ca^{2+} - \text{monoisopropyl ester}$ and (B) $H^+ - Mg^{2+} - Ca^{2+} - P, P'-\text{diisopropyl ester}$ of clodronic acid systems in human serum $(C_{Ca} = 0.01 \text{ mM}, C_{Mg} = 0.85 \text{ mM}, C_{Ca} = 2.4 \text{ mM})$. $F_i$ is defined as the ratio between the bound ligand content and the total ligand content. The physiological free hydrogen ion concentration is marked with a dashed line.

References

14. (a) Sayce, I. G. Talanta 15 (1968) 1397; (b) Ibid. 18 (1971) 653; (c) Sayce, I. G. and Sharma, V. S. Ibid. 19 (1972) 831.

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