Dissociation Constants of Ethane-1-hydroxy-1,1-diphosphonate [EHDP] and Dichloromethylene-diphosphonate [Cl_2MDP] for H^+, Ca^{2+}, Mg^{2+} and Zn^{2+}

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Diphosphonates, Dissociation Constants

The dissociation constants of ethane-1-hydroxy-1,1-disphosphonate [EHDP] and dichloromethylene-diphosphonate [Cl_2MDP] for H^+, Ca^{2+}, Mg^{2+} and Zn^{2+} have been determined from pH-titration curves. EHDP forms more stable chelates with Ca^{2+}, Mg^{2+} and Zn^{2+} than Cl_2MDP. Cl_2MDP is a stronger acid than EHDP.

Introduction

The diphosphonates EHDP and Cl_2MDP have been used for studying bone metabolism and for treatment of bone diseases [1–4]. Both substances are adsorbed to apatite, EHDP being more strongly bound than Cl_2MDP [5, 6]. EHDP preferentially inhibits the growth of apatite crystals and mineralization, Cl_2MDP, however, especially inhibits resorption of bone [7]. As yet, there has been no explanation for this fundamental difference in the action of EHDP and Cl_2MDP.

For the interpretation of additional effects, it was suggested that EHDP chelates divalent cations and thus inhibits Ca^{2+}-dependent enzymes involved in the formation of collagen fibrils and Zn^{2+}-dependent pyrophosphatase [8].

For characterizing these effects of EHDP and Cl_2MDP quantitatively, we determined their dissociation constants for H^+, Ca^{2+}, Mg^{2+}, and Zn^{2+}.

Methods

pH titrations were performed at 25 °C with 10 ml of 1 mM solutions of EHDP and Cl_2MDP * in 0.1 M KCl by adding 10 μl portions of 0.1 N KOH and gassing with purified N_2. 10 mM $CaCl_2$, $MgCl_2$ or $ZnCl_2$ was added. The procedure was extensively described by Dietsch and Siegmund [9].

Abbreviations: EHDP, ethane-1-hydroxy-1,1-diphosphonate; Cl_2MDP, dichloromethylene-diphosphonate.

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According to Schwarzenbach [10], titration curves of weak acids are given by the equation

$$\sum_{j=0}^{n} (g-j) \cdot [H^+]^j \cdot K^H_{HjZ} = 0.$$

The symbols were used as defined by Schwarzenbach [10]. n, number of individual equilibrium constants; j, indicates which proton dissociates; m, number of dissociable protons; a, mol of KOH added per mol of ligand; g, real degree of protonation; $K^H_{H_2Z}$, K^H_{HZ} dissociation constants for the third and fourth H^+ ion, respectively

$$e.g.\ K^H_{HZ} = \frac{[H^+] \cdot [EHDP^{4-}]}{[H\,EHDP^{3-}]};$$

pK_a, negative logarithm of acid dissociation constant; pK_a', apparent pK_a in the presence of a divalent cation; K^M_{ZM}, K^M_{HZM} formation constants for the single protonated and nonprotonated complexes

$$e.g.\ K^H_{HZM} = \frac{[H\,EHDP^{3-}]\,[M^{2+}]}{[MH\,EHDP^{-}]};$$

pM, negative logarithm of divalent cation concentration.

By restriction to the dissociation constants $K^H_{H_2Z}$ and K^H_{HZ}, which correspond to the dissociation of the last two protons involved in complex formation one gets

$$g \cdot \frac{1}{K^H_{HZ}} + (g-2) \cdot [H^+]^2 . K^H_{H_2Z} = -(g-1) \cdot [H^+]. \quad (1)$$

g was determined according to Eqn (2)

$$g = m - a + \frac{[OH^-] - [H^+]}{[Z]} \quad (2)$$

and was substituted into Eqn (1) to obtain $K^H_{H_2Z}$ and K^H_{HZ}.

The association constants of the metal chelates were calculated from Eqn (3)

$$\log K = pK_a + pK_a' + pM . \qquad (3)$$

For detailed description of calculation and computer program see Dietsch and Siegmund [9] and Dietsch [11].

Results

Tab. I contains the pK_a-values of EHDP and Cl_2MDP, Tab. II the logarithms of the complex binding constants determined from the pH titration

Tab. I. pK_a-values of EHDP and Cl_2MDP at 25 °C in 0.1 M KCl. The pK_{a_1}-values of EHDP and Cl_2MDP are not detectable by this method, because they are strong acids. Mean ± S.E., $n=10$.

	pK_{a_1}	pK_{a_2}	pK_{a_3}	pK_{a_4}
EHDP	<2	2.5±0.2	6.89±0.01	10.60±0.02
Cl_2MDP	<2	2.3±0.2	5.82±0.01	8.84±0.01

Tab. II. Logarithms of the stability constants of the chelates of EHDP and Cl_2MDP with Ca^{2+}, Mg^{2+} and Zn^{2+} at 25° in 0.1 M KCl. Mean ± S.E. $n=10$.

Me^{2+}	EHDP		Cl_2MDP	
	$\log K^M_{HZM}$	$\log K^M_{ZM}$	$\log K^M_{HZM}$	$\log K^M_{ZM}$
Ca	3.0	6.0	2.86±0.01	4.71±0.01
Mg	3.03±0.02	6.17±0.02	2.92±0.01	4.75±0.01
Zn	5.00±0.04	8.19±0.04	4.61±0.01	6.70±0.02

curves [not shown]. The titration curve of EHDP with Ca^{2+} was performed only in the presence of 5 mM Ca^{2+}, because at higher Ca^{2+} concentrations and at higher pH values $Ca[OH]_2$ was formed. Because there was not a large excess of Ca^{2+}, the Ca^{2+}-ion concentration cannot be considered as being constant during the titration. Thus the values of the stability constants of CaEHDP [Tab. II] are probably not as precise as the other values and therefore S. E. was not given. The stability constants increase in the order Ca < Mg < Zn in agreement with the Irving-Williams series.

EHDP forms stronger complexes with Ca^{2+}, Mg^{2+} and Zn^{2+} than Cl_2MDP or pyrophosphate. Probably the OH group from the hydroxyethyl group is involved in the chelate formation, resulting in a stronger chelate effect.

Cl_2MDP is a stronger acid than EHDP [Table I] or phosphoric acid. This property can be attributed to the inductive effect of the two chlorine atoms on the methylene group. A well known analogous example is trichloroacetic acid which is a stronger acid than acetic acid.

Discussion

The physico-chemical constants may give an explanation for the different pharmacological behaviour of EHDP and Cl_2MDP. Since EHDP has a higher affinity for Ca^{2+} ions, EHDP could also be more strongly bound to Ca^{2+} ions at the growing points of the apatite crystal [active growth sites]. A similar mechanism has been proposed by Meyer and Nancollas [12]. In the growth of apatite crystals, EHDP may compete with phosphate for the Ca^{2+} ions at the growing points of apatite crystals. [Phosphate has a lower affinity for Ca^{2+} then EHDP.] Thus EHDP inhibits mineralization more strongly than Cl_2MDP. At higher concentrations the Cl_2MDP can also compete successfully with the phosphate to inhibit mineralization.

In the resorption of bone the osteoclasts are involved. Rowe and Hausmann [13] suggested that the inhibition of bone resorption by diphosphonates is due to abnormal osteoclasts, however, there was no sufficient quantitative correlation.

Therefore, we will propose another mechanism for the inhibition of bone resorption by Cl_2MDP based on the fact, that Cl_2MDP is a stronger acid than EHDP.

There is evidence, that the resorption of apatite and bone involves H^+ ions, which are formed by carbonic anhydrase of the osteoclasts [14–17]. The weaker acid EHDP, adsorbed to apatite, is more easily protonated than the stronger acid Cl_2MDP by the local H^+ ion activity at the apatite crystal near the surface of the osteoclasts. The protonated acids [EHDP, phosphoric acid] may be dissolved from the apatite crystal, whereas the stronger acid Cl_2MDP remains deprotonated and adsorbed to the apatite and, unlike EHDP- inhibits bone resorption.

As there is an equilibrium in the bone between adsorbed and dissolved EHDP or Cl_2MDP, the dissolved diphosphonate can bind some Mg^{2+}, Ca^{2+} and Zn^{2+} in the extracellular fluid [ECF]. However, the concentration of dissolved diphosphonates in the ECF is low compared to the concentration of Ca^{2+} and Mg^{2+}, therefore, a significant reduction of the Ca^{2+} and Mg^{2+} ion activity *in vivo* may be excluded. Since most of the Zn^{2+} is bound to proteins, the ion activity of Zn^{2+} is unknown, and it remains open, whether the Zn^{2+} activity is reduced *in vivo*.

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