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Complexation Properties of Phosphonocarboxylic Acids in Aqueous Solutions

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The concentration formation constants of phosphonoacetic acid (PAA) complexes with the Ca^{2+} and Mg^{2+} ions were determined in aqueous solution at 25°C by potentiometric and coulometric titrations at different ionic strengths and were extrapolated to $I=0$ in order to obtain thermodynamic values of the formation constants. Complexes were formed by the completely deprotonated $K_f(ML)$ and monoprotonated $K_f(MHL)$ forms of the PAA anion. The respective values for the complexes are: log $K_f(CaL) = 4.68 \pm 0.03$, log $K_f(CaHL) = 2.61 \pm 0.08$; log $K_f(MgL) = 5.58 \pm 0.09$, log $K_f(MgHL) = 3.0 \pm 0.3$. The enthalpy and entropy of complexation for the deprotonated Ca^{2+} and Mg^{2+} PAA species, determined from the temperature dependence of the log $K_f(ML)$, are: $\Delta H°(Ca) = 0.6 \pm 0.2$ kcal-mol^{-1}, $\Delta S°(Ca) = 21.4 \pm 0.6$ cal-mol^{-1}-K^{-1}, $\Delta H°(Mg) = 3.0 \pm 0.7$ kcal-mol^{-1}, and $\Delta S°(Mg) = 35 \pm 2$ cal-mol^{-1}-K^{-1}. It is seen therefore, that the complexes are entropy stabilized but enthalpy destabilized. Formation constants were also determined for Ca^{2+} and Mg^{2+} complexes with PAA analogs, phosphonoformic and 3-phosphonopropionic acids and the complexation of PAA was also studied at a single ionic strength, with Na^+, Ag^+, Tl^+, Sr^{2+}, Ba^{2+}, Cd^{2+}, Cu^{2+}, and Pb^{2+} ions.

KEY WORDS: phosphonocarboxylic acids; phosphonoacetic acid; calcium and magnesium complexes; enthalpy and entropy complexation; potentiometry; cyclic voltammetry; herpes virus.

1. INTODUCTION

Phosphonoacetic acid (PAA), synthesized in 1924 by Nylen,[(1)] initially generated little attention. It had a limited use as an extracting

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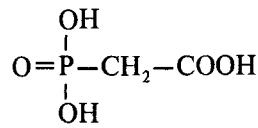
agent for some lanthanide series elements.[2] However, during a random testing of compounds with a cell culture screen, it was found that PAA is an effective inhibitor of herpes simplex viruses.[3,4] The discovery stimulated interest in this compound as a potential therapeutic agent. It has since been found to be active as as inhibitor of many forms of the herpes virus.[5] The mode of action was postulated to involve a competion with inorganic pyrophosphate in the DNA polymerase cycle.[6] Implicit in this hypothesis is the assumption of a complexation reaction involving PAA and a metal ion prior to its reaction with the enzyme DNA polymerase. Its general usage as a therapeutic agent for humans is precluded by severe toxic reactions to the drug in rabbits[7] and monkeys.[5] The symptoms, as well as the discovery of the deposition of PAA in bones, tend to implicate a side reaction with the Ca^{2+} ion.



It was of interest to us to study the complexing ability of PAA and of some of its analogs *vis-a-vis* biologically important metal ions, and to see whether there exists a correlation between such ability and the physiological properties of these ligands. As a preliminary step it was necessary to obtain precise information on the acidic properties of these compounds. In our previous publication[8] we reported the three acidity constants of PAA as well as those of phosphonoformic, and 3-phosphonopropionic acids. In the case of PAA, measurements of ^{13}C and ^{31}P chemical shifts also gave us the deprotonation sequence of the triprotic acid.

Simultaneously with our publication, Stunzi and Perrin reported[9] formation constants for PAA complexes with Mg^{2+}, Ca^{2+}, Cu^{2+} and Zn^{2+} ions in aqueous solutions which were 0.15*M* in KNO_3 at 37°C.

In this paper we wish to report thermodynamic values for the formation constants of the PAA-Mg^{2+} and PAA-Ca^{2+} complexes for completely deprotonated and monoprotonated PAA at 25°C, the enthalpy and the entropy of the complexation reactions as well as the complexation studies with some analogs of PAA and with several additional metal ions.

2. EXPERIMENTAL

2.1. Reagents

Phosphonoacetic acid and 3-phosphonopropionic acid (3-PPA) (Richmond Organics) were both recrystallized as described earlier.[8] The observed melting points agree well with the literature values of 142-143°C[1] and 178-180°C[15] respectively. Phosphonoformic acid was obtained through the courtesy of Professor J. A. Boezi (Biochemistry Department, Michigan State University) and was used as received.

The following chemicals were of reagent grade and were used without further purification: barium chloride (Mallinckrodt), cadmium nitrate tetrahydrate (J. T. Baker), calcium chloride (MCB), copper(II) chloride (MCB), iron(II) chloride (MCB), lead(II) nitrate (Fischer), magnesium chloride (Alfa), potassium chloride (Mallinckrodt), potassium hydroxide (MCB), silver chloride, sodium iodide (Fischer), and thallium(I) nitrate (Alfa).

Tetrabutylammonium bromide (Bu_4NBr, Eastman) was purified by previously described techniques,[8] while tetraethylammonium perchlorate (Et_4NClO_4, Eastman) was recrystallized twice from water and vacuum dried at 50°C for 24 h. Water was purified as described earlier.[8]

2.2. pH Electrode

The method used to determine the formation constants of Mg^{2+}, Sr^{2+}, and Ba^{2+} complexes with PAA has been described elsewhere.[8] The only deviation was that prior to the coulometric titration of the ligand, the respective metal ions were added to the ligand, at approximately the same concentration as that of the ligand.

2.3. Selective Ion Electrodes

The potentiometric measurements of Ca^{2+} ion concentration were carried out with an Orion calcium selective electrode (model 93-20) and reference electrode (model 90-01). The pH was adjusted utilizing the previously described coulometric equipment,[8] and was measured with an Orion combination pH electrode (model 91-05).

Potentiometric measurements of the sodium ion solutions were done with a Corning sodium ion selective electrode (NAS 11-18) and a standard calomel reference electrode. The technique involves the same procedures as that used for the calcium potentiometric experiments.

2.4. Cyclic Voltammetry

Cyclic voltammograms were obtained with a PAR model 174A polarographic analyzer. The experiments were conducted in a glass cell in which the reference electrode was separated from the working compartment by a glass frit. Prior to measurements nitrogen was passed through for 10 min. The working electrode was a hanging mercury drop, the counter electrode, a Pt wire, and a standard calomel electrode was used as reference. In all cases the ligand was present at a concentration of from 10-50 times that of the metal ion in order to satisfy the restrictions of the Lingane equation (see below) and its modifications.

All cells were thermostatted to a constant temperature of about 25°C. The measurements were carried out in a grounded Faraday cage to reduce electrical noise. An air-driven magnetic stirrer was used for the same reason.

2.5. Titration Procedures

The procedures for the titrations of the calcium ion were conducted as follows: The electrodes (calcium, reference, and pH) were placed in 20 ml of a constant ionic strength solution. Calcium chloride solution was added gradually, the pH adjusted coulometrically and the emf measured. A plot of the emf *vs.* Ca^{2+} ion concentration gave the calibration curve. The same solution was then back-titrated with a PAA solution (or of one of its analogs).

A nonlinear least-squares program KINFIT 4[10] was used to fit the calibration curve, and a general equilibrium-solving program MINIQUAD 76A[11] was used for the formation constants.

3. RESULTS AND DISCUSSION

3.1. Formation Constants of Metal Ion-PAA Complexes

The calibration curves for the potentiometric methods were computer fitted to the equation

$$E = E^{\circ\prime} + 2.303(RT/nF)\log([X] + Z) \tag{1}$$

where E is the observed potential, $E^{\circ\prime}$ is the sum of the standard, junction, and asymmetry potentials, and Z is the concentration of the impurity. In the case of the pH measurments $E^{\circ\prime}$ and Z were the fitted parameters, while in the calcium and sodium ion selective electrode

Table I. Formation Constants of Metal Ion Complexes with PAA in Aqueous Solutions at 25°C

Ion	log K_f (ML)	log K_f (MHL)
Na^{+} [a,d]	1.43±0.02	0.79±0.05
Ag^{+} [b,d]	3.11±0.05	2.70±0.1
Tl^{+} [b,e]	2.51±0.03	
Cd^{2+} [c,e]	3.90±0.1	1.90±0.1 [h]
Cu^{2+} [b,e]	8.00±0.1	4.20±0.1
Pb^{2+} [b,e]	7.00±0.1	4.10±0.1
Mg^{2+} [b,f]	4.50±0.02	2.56±0.05
Mg^{2+} [g]	3.90	1.84
Ca^{2+} [b,d]	3.67±0.02	2.10±0.06
Ca^{2+} [g]	3.18	1.62
Sr^{2+} [b,f]	3.67±0.02	2.56±0.06
Ba^{2+} [b,f]	3.67±0.02	2.50±0.06

[a] I=0.078. [b] I=0.05. [c] I=0.4. [d] Ion selective electrode. [e] Cyclic voltammetry. [f] Coulometric titrations. [g] Ref. 9; 37°C and I=0.15. [h] ML_2.

measurements, the slope (2.303 RT/nF) was also a fitted parameter. In all experiments, the calculated slope was within the specified literature range.[12] When the calibration procedure was completed, the same solution was used for the metal-ligand titration in order to minimize changes in the junction potential.

The cyclic voltammetry data were fitted to the Lingane[13] equation

$$(E_{1/2})_c - (E_{1/2})_f = (RT/nF)\ (\ln K_f - p \ln C) \qquad (2)$$

where $(E_{1/2})_c$ and $(E_{1/2})_f$ are the half-wave potentials of the complexed and the free metal ion, n is the number of electrons transferred, K_f is the formation constant, C, the analytic concentration of ligand, and p is the coordination number of the metal ion. With a weak complex, a simplified Buck[14] equation was used

$$(E_{1/2})_c - (E_{1/2})_f = (RT/nF)[\ln K_f - p \ln C - \ln(1 + 1/CK_f)] \qquad (3)$$

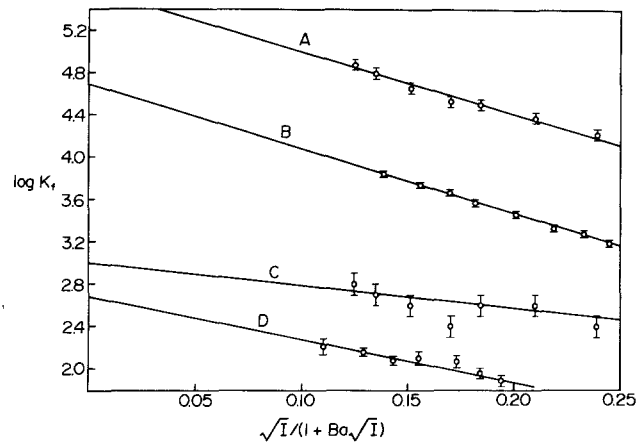


Fig. 1. A plot of $I^{1/2}/(1 + BaI^{1/2})$ *vs.* log K_f for Mg and Ca complexes with PAA to determine the thermodynamic log K_f. A, MgPAA; B, CaPAA; C, MgHPAA; D, CaHPAA.

The cathodic and anodic peak-to-peak separation was independent of the sweep rate, it was 60±2 mV for a one-electron process, and 30±2 mV for a two-electron process. These results are consistent with the requirements for the reversible behavior for the amalgam-forming redox couple.

It was of interest to us to calculate the thermodynamic values of the formation constants, at least for the Ca^{2+} and Mg^{2+} ions to see how the formation of those complexes varied with the ionic strength of the medium, since this factor could have an important influence on the physiological properties of the ligands.

Using the Debye-Hückel equation to evaluate the ionic activity coefficients

$$-\log \gamma_i = \frac{AZ^2{}_i I^{1/2}}{1 + B\mathring{a}I^{1/2}} \tag{4}$$

the formation constants for the deprotonated and monoprotonated species are given by,

$$\log K_f^c(\mathrm{ML})\mathrm{H} = \log K_f^t(\mathrm{ML}) - \frac{12AI^{1/2}}{1 + B\mathring{a}I^{1/2}} \tag{5}$$

$$\log K_f^c(\mathrm{MHL}) = \log K_f^t(\mathrm{MHL}) - \frac{8AI^{1/2}}{1 + B\mathring{a}I^{1/2}} \tag{6}$$

where the superscripts t and c indicate the thermodynamic and concentration (at ionic strength I) formation constants and where the other symbols have their usual meaning. In aqueous solutions at 25°C, constants $A = 0.510$ and $B = 0.328 \times 10^8$. The concentration formation constants were determined at different ionic strengths and plotted against $I^{1/2}/(1 + Ba\mathring{A}I^{1/2})$. The results are shown in Fig. 1.

The theoretical slopes of the plots of log K_f *vs.* $I^{1/2}/(1 + Ba\mathring{A}I^{1/2})$ for the mono- and deprotonated complexes are -4.07 and -6.11, respectively. The ion size parameter a was then adjusted so that the experimental slopes for the plot were equal to the theoretical slopes, within the experimental error. The resulting ion size parameters are, 3.05 for Mg^{2+} and 5.18 Å for Ca^{2+} ions. These values appear to be reasonable given the estimated radii of the hydrated ions.[16,17]

3.2. PAA Complexes with Other Metal ions

Concentration formation constants of PAA complexes with Na^+, Ag^+, Tl^+, Cd^{2+}, Cu^{2+}, Pb^{2+}, Sr^{2+} and Ba^{2+} ions were determined by several electrochemical techniques at a constant ionic strength, the results are shown in Table I. It is seen that both mono- and the deprotonated forms of PAA are effective complexing agents for a number of metal ions. The alkali metals form the weakest complexes, followed by the alkaline earths, and the strongest complexing ability is shown by the transition metal ions. Within the alkaline earth series, the strength of the complex tends to decrease slightly with increasing ion size.

In cases where comparisons are possible, our results seem to agree well with those of Stunzi and Perrin[9] when the differences in temperature and in ionic strength of the solutions are taken into account. For example, if we use the Debye-Hückel equation and the temperature coefficient of K_f to recalculate the data given in Table I for the Mg-PAA and Ca-PAA complexes (completely deprotonated ligand) to $I = 0.15$ and 37°C the log K_f values are 3.8_6 and 3.0_2, as compared with Stunzi and Perrin values of 3.90 and 3.18, respectively.

3.3. The Entropy and Enthalpy of Complexation

The concentration formation constants for the Ca^{2+} and Mg^{2+} ions with the completely deprotonated PAA anion were measured at several different temperatures. The respective temperature ranges were 0-60 and 5-40°C and were dictated by the behavior of the respective electrodes which tended to become erratic outside these limits.

Table II. Thermodynamic Complexation Parameters for the Complexation of PAA and the Pyrophosphate Anion with Ca^{2+} and Mg^{2+} Ions in Aqueous Solutions at 25°C

Complex	log K_f	$\Delta G^{o\,a}$	$\Delta H^{o\,a}$	$\Delta S^{o\,b}$	Ref.
Mg^{2+}·PAA	5.58±0.09	-7.20±0.7	3.0±0.7	35.0±2	this work
Ca^{2+}·PAA	4.68±0.03	-6.38±0.07	0.6±0.2	21.4±0.7	this work
Mg^{2+}·$P_2O_7^{4-}$	5.45	-7.43	3	43	18
Ca^{2+}·$P_2O_7^{4-}$	5.4	-7.3	4.6	46	19

[a]kcal-mol^{-1}. [b]cal-mol^{-1}-K^{-1}.

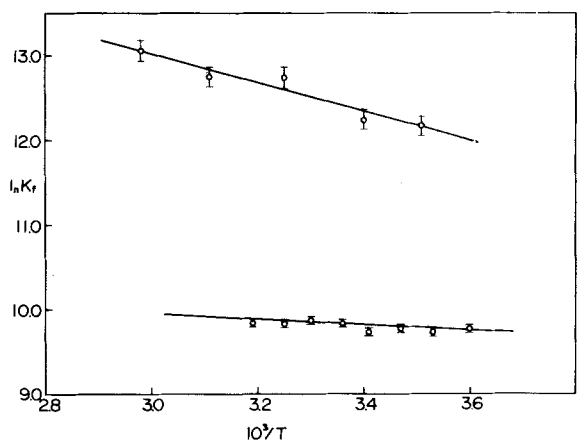


Fig. 2. A plot of 10^3(temperature)$^{-1}$ *vs.* lnK_f to determine ΔS^o and ΔH^o of complexation for deprotonated Mg-PAA, upper trace, and Ca-PAA, lower trace.

The plots of log K_f *vs.* $1/T$ are shown in Fig. 2. The values of ΔH^o and ΔS^o, obtained from these plots in the usual manner, are given in Table II. It is interesting to note that in both cases the complexes are entropy stabilized, but enthalpy destabilized.

The postulated mode of action against herpes virus involves competition with the pyrophosphate moiety (via a magnesium complex) for the active site on the enzyme.[6] Thermodynamic data for the magnesium and calcium complexes with the pyrophosphate[18,19] are shown

in Table II. It is seen that PAA can compete with the pyrophosphate for the Mg^{2+} ions. Phosphonoacetate is a more effective selective complexing agent, since the pyrophosphate will not effectively differentiate between Mg^{2+} and Ca^{2+} ions, while PAA can. The extent of complexation of PAA with Ca^{2+} would suggest that the biological side effect due to such a complexation are possible.

Table III. Complexation Constants of Some PAA Analogs at 25°C and an Ionic Strength of 0.05

	PFA	PAA	3-PPA
$\log K_f$ (MgL)	3.59±0.05	4.50±0.05	2.28±0.05
$\log K_f$ (MgHL)	1.70±0.30	2.60±0.1	1.70±0.1
$\log K_f$ (CaL)	3.55±0.05	3.67±0.02	2.38±0.02
$\log K_f$ (CaHL)	1.84±0.04	2.10±0.06	1.64±0.07

3.4. Some Analogs of PAA

The therapuetic effect of PAA led to the hypothesis that other phosphonocarboxylic acids might have a similar therapeutic effect. Recent studies suggest that phosphonoformic acid (PFA) is also an effective inhibitor of some herpes virus,[20] while 3-PPA is not. The formation constants of Mg^{2+} and Ca^{2+} complexes with PFA and 3-PPA were determined potentiometrically (as described in Section 2.3) at 25°C and a constant ionic strength of 0.05. The ionic strength was found to vary during the course of a single titration by approximately 5% in the worst case, but in most cases, the variation was 2% or less. The contribution to the ionic strength from the charged species, ligand and metal ion, was approximately 10%. This can be corrected by summing the contribution from the supporting electrolyte and from the mean contribution of other charged species over the course of an experiment. The ionic strength therefore is reported to only one significant figure.

The results obtained are compared with those for PAA in Table III. It is clear that PFA and PAA form complexes of approximately the same strength while 3-PPA has a much weaker complexing ability. In general these results are consistent with the hypothesis that the inhib-

itory mode of action involves a complexation reaction with the magnesium ion.

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