ESR Study of Manganese(II) Complexes in Aqueous Solutions

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Electron spin resonance spectroscopy was used to study manganese(II) complexes with several organic ligands in aqueous solutions. The concentration formation constants of manganese complexes with deprotonated (L) and monoprotonated (HL) phosphonoacetic acid (PAA) were determined at different ionic strengths. These values were extrapolated to the zero ionic strength in order to determine the thermodynamic values of the complexation constants. The values obtained are: log K_{HL}^{PAA} *= 3.5 ± 0.2, and log* K_{L}^{PAA} *= 6.3 ± 0.1. The formation constants of manganese complexes with phosphonoformic acid (PFA), 3-phosphonopropionic acid (3-PPA), and citric acid were measured at ionic strength of 0.05 and were found to be: log* K_{HL}^{PFA} *= 2.57 ± 0.05, log* K_{L}^{PFA} *= 5.34 ± 0.05, log* $K_{HL}^{3\text{-}PPA}$ *= 1.6 ± 0.2, log* $K_{L}^{3\text{-}PPA}$ *= 3.15 ± 0.04, and log* $K_{L}^{citric\ acid}$ *= 4.28 ± 0.04. In aqueous solutions manganese(II) ion does not appear to form complexes with the crown ethers 12C4, 15C5, or 18C6, while the cryptand C211-Mn(II) complex has a log* K_f *of 1.6 ± 0.1.*

Introduction

Electron spin resonance has been found to be a powerful technique for the investigation of biomacromolecular complexes with manganese ions. Studies in aqueous solutions have been conducted on complexes of manganese with nucleobases, nucleotides, nucleosides, and deoxyribonucleic acid [1], adenosine 3′,5′-mono-phosphate dependent protein kinase [2], nicotinamide adenine dinucleotide phosphate [3], tris-washed chlorophasts [4], adenosine triphosphate [5], isocitrate dehydrogenase [6], and a variety of other biomacromolecules. However, with the exception of Townsend's pioneering work in 1954 [7], there seems to have been little interest in the use of ESR for the investigation of manganese ion complexes with smaller ligands.

Phosphonoacetic acid (PAA, Fig. 1), has been found to be an effective inhibitor of certain forms of Herpes virus [8–10]. The proposed mode of action involves a competition with inorganic pyrophosphate in the DNA replication cycle [11]. This competition probably involves a complexation reaction with a metal ion; magnesium(II) and manganese(II) seem to be the most probable. The binding of magnesium and other metal ions to PAA and its analogs, phosphonoformic acid (PFA) and 3-phosphonopropionic acid (3-PPA), were the subject of a previous study of our group [12]. It was found that Mg^{2+} ion does indeed complex significantly with PAA and PFA.

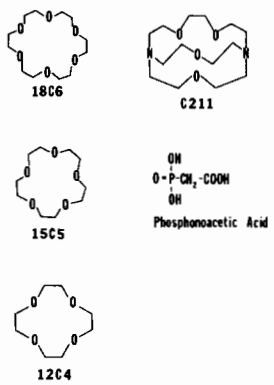

Fig. 1. Structural formulae of ligands used in this investigation.

In this paper we wish to report an exploratory study of the application of the electron spin resonance technique to studies of manganese(II) complexes with the biologically significant PAA, PFA and 3-PPA as well as with several crown ethers, 12C4, 15C5 and 18C6 and with a diazapolyoxa cryptand C211 (Fig. 1).

Experimental

Reagents

Phosphonoacetic acid and 3-phosphonopropionic acid (Richmond Organics) were both recrystallized as described earlier [12]. The observed melting points correspond to the literature values of 142–143 °C [13] and 178–180 °C [14] respectively. Phosphono-

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formic acid was obtained through the courtesy of Professor J. A. Boezi (Biochemistry Department of MSU) and was used as received. Crown ethers, twelve-crown-four (12C4) (Aldrich) and fifteen-crown-five (15C5) (Aldrich) were fractionally distilled under reduced pressure; eighteen-crown-six (18C6) (Aldrich) was recrystallized from acetonitrile. The cryptand C211 (Merck) was used without further purification.

Tetraethylammonium perchlorate (TEAP, Eastman) was recrystallized twice from water and vacuum dried at or below 50 °C for 24 h. Manganese-(II) chloride hexahydrate (Baker) was dried at 220 °C for 48 h, then vacuum dried at 120 °C for 24 h. Tetramethylammonium hydroxide (Eastman) was obtained as a 10% solution in water. A tris buffer was made with gold label tris-(hydroxymethyl)amino-methane (Alfa) and reagent grade perchloric acid. Reagent grade citric acid (MCB) was dried at 110 °C.

Measurements

The spectra were obtained with a Varian E-4 EPR Spectrometer operating at 9.11 GHz. The modulation frequency is 100 kHz with a peak-to-peak amplitude of 12.5 gauss. A quartz aqueous flat cell (Wilmad WG-812) was used for all measurements.

Figure 2 shows a calibration curve with the resonance intensity plotted as a function of the manganese ion concentration. It is seen that the intensity of the resonance varies linearly with the concentration of the free Mn^{2+} ion. The tris buffer and supporting electrolyte were found to have no effect on the resonance signal. In all complexation studies, the total metal ion concentration was held constant at 1 m*M*. In the case where the formation constant with a completely deprotonated phosphono-carboxylic acid or with cryptand C211 was measured, the solution was buffered to a pH of 8. Complexes with monoprotonated phosphonocarboxylic acids were studied at a pH of 4.

All solutions were deoxygenated with a stream of nitrogen for 15 min prior to the addition of the manganese ion. The thermodynamic formation constants were derived using a non-linear least-squares curve fitting program KINFIT4 [15].

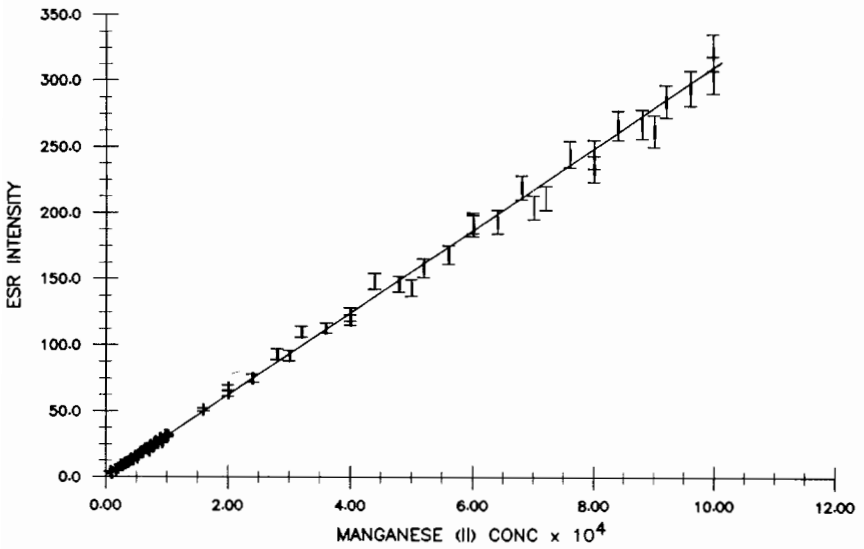

Fig. 2. A plot of Mn^{2+} concentration *vs.* the intensity of the ESR signal.

TABLE I. The Formation Constants of a Manganese(II)–Citrate Complex.

log K	Supporting Electrolyte	Method	Reference
3.6	KCl	potentiometry (pH) visible spectroscopy (competition)	16
3.69	NaCl	radio isotope ion-exchange	18
3.83	NaCl	radio isotope ion-exchange	17
3.74 ± 0.03	TMAC	MnESR	this work
4.28 ± 0.04	TEAP	MnESR	this work
4.15 ± 0.02[a]	TMAC	potentiometry (pH)	19

[a]Corrected for the $Mn^{2+}Cl^-$ ion pair formation.

Results and Discussion

Formation Constants of Manganese Ion with Citric Acid

The general validity of the technique was confirmed by a study of the known manganese citrate complex. Table I shows a comparison of the literature values of the formation constant of this complex with that obtained in this study. Both tetramethylammonium chloride and tetraethylammonium perchlorate were used as the supporting electrolytes. Chlorides have also been used as supporting electrolytes in previously reported studies [16–18]. As seen from Table I our values obtained with TMAC agree reasonably well with the literature values. However, a later study [19] showed that when a chloride was used as the 'inert' supporting electrolyte, $Mn^{2+}Cl^-$ ion pairs are formed. The chloride ion, therefore, competes with the ligand for the Mn^{2+} ion. Since we used tetraethylammonium perchlorate as the supporting electrolyte for all subsequent work, the formation of the ion pairs was minimized and should not affect the values of the formation constants. The formation constant we obtained, after correction for the difference in the ionic strength by using the Debye-Huckel equation and an ion size parameter of 6 Å [20], agrees with that in reference 17 within 0.09 logK units. It is seen therefore, that the ESR spectroscopy is quite applicable to the determination of formation constants for the complexes of the Mn^{2+} ion.

Formation Constant of the Manganese Ion-PAA Complexes

The concentration formation constants were determined at different ionic strengths so that a thermodynamic formation constant could be calculated upon extrapolation to zero ionic strength. The purpose of determining a thermodynamic formation constant was twofold; the thermodynamic formation constant has a greater fundamental significance than a formation constant measured at a 'high and constant ion strength' and the equilibrium conditions at other ionic strengths can be easily calculated. In addition, the variation of $\log K_L$ values as a function of the ionic strength give some indication on the precision of the experimental technique.

The data were fitted using the Debye-Huckel equation for evaluating activity coefficients,

$$\log K^c_{ML} = \log K^t_{ML} - \frac{12A\sqrt{I}}{1 + Ba\sqrt{I}} \quad (1)$$

and

$$\log K^c_{MHL} = \log K^t_{MHL} - \frac{8A\sqrt{I}}{1 + Ba\sqrt{I}} \quad (2)$$

where A = 0.510 and B = 0.328×10^8 in aqueous solutions at 25 °C. All other symbols have their usual meaning [12]. The theoretical slopes of the $\log K_c$ *vs.* $\sqrt{I}/(1 + Ba\sqrt{I})$ for the mono- and deprotonated manganese complexes with PAA are −4.07 and −6.11. A least squares analysis of the data, using *a* and K^t as parameters, yielded the graphs shown in Fig. 3 and the extrapolated log K values are given in Table II. The ion size parameter of 6 Å obtained for the Mn^{2+} ion compares well with Kielland's value of 6 Å [20] and Stokes value of 4.7 Å [21].

Some Analogs of PAA

The formation constants of PFA and 3-PPA complexes with Mn^{2+} ion were determined at I = 0.05.

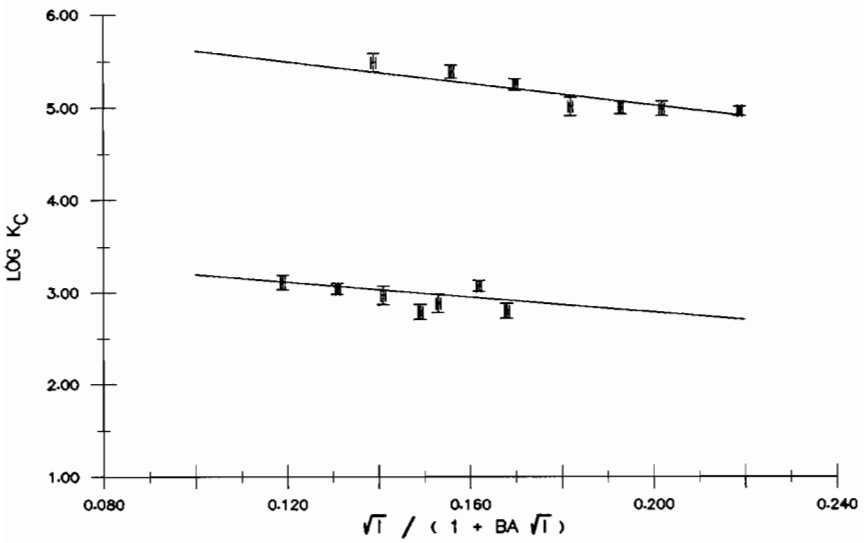

Fig. 3. Variation of log K_c of the PAA·Mn^{2+} complexes, as a function of the ionic strength. Upper plot-deprotonated ligand; lower plot-monoprotonated ligand.

TABLE II. The Thermodynamic Formation Constants of Some Divalent Cation-PAA Complexes.

Divalent Cation	K^t_{ML}	K^t_{MHL}	A	Reference
Ca^{2+}	4.68 ± 0.03	2.61 ± 0.08	5.2	12
Mg^{2+}	5.58 ± 0.09	3.00 ± 0.3	3.1	12
Mn^{2+}	6.30 ± 0.1	3.50 ± 0.2	6	this work

TABLE III. The Formation Constants of Manganese(II) Complexes with PAA, PFA, and 3-PPA at I = 0.05.

	K^c_{ML}	K^c_{MHL}
PFA	5.34 ± 0.05	2.57 ± 0.05
PAA	5.25 ± 0.06	2.97 ± 0.06
3-PPA	3.15 ± 0.04	1.60 ± 0.2

During the titration the ionic strength did not vary by more than 10%. The results, along with the formation constant of the Mn(II)–PAA complex at I = 0.05, are shown in Table III. Phosphonoformic and phosphonoacetic acids exhibit approximately the same biological activity, and they do have an approximately equal complexing ability towards the Mn^{2+} ion. On the other hand, 3-PPA exhibits no inhibitory effects on Herpes virus and also has a deprotonated formation constant two orders of magnitude smaller than that of PAA or PFA [22]. It seems therefore, that at least the biological activity and the complexing ability of the phosphonocarboxylic acids exhibit similar trends.

Some Neutral Ligand Complexes with Manganese

To demonstrate the viability of manganese ESR for the study of non-protic ligands, Mn^{2+} complexes with several crown ether ligands and one cryptand were investigated. The cavity size of 12C4 fits best the crystallographic diameter of the manganese(II) ion [23]. However, the crystallographic diameters are not always the best criteria for choosing a 'fit' between cavity and ion size [24]. Therefore, crown ethers with larger cavity sizes were also tested because ions may form stronger complexes with a larger crown than is indicated by the best match between ion and cavity size [25, 26].

There was no evidence for the complexation of crown ethers 12C4, 15C5, and 18C6 with the manganese(II) ion. Within the experimental error, there was no decrease in the intensity of the manganese aquo resonance upon addition of the respective ligand as the ligand/Mn^{2+} mole ratio was varied from 0.5 to 10.0. It was also found that there was no significant line broadening of the signal within these mole ratio ranges.

The lack of complexation with the crown ethers is probably due to strong solvation of the Mn^{2+} ion by water and no ligand field stabilization energy in its complexes [27].

On the other hand, the addition of the cryptand C211 to an aqueous solution of Mn^{2+} salt resulted in a decrease of the Mn^{2+} ion concentration as a result of complex formation. Analysis of the result yielded constant, log $K_f = 1.6 \pm 0.1$.

An interesting reaction occurs during the manganese complexation study with C211. Usually manganese(II) ion undergoes slow oxidation in basic aqueous solutions [27]. The addition of C211 greatly accelerates this oxidation. This acceleration is dependent on the concentration of C211. Since the existence of a manganese(II) ion complex with C211 has been demonstrated, the oxidation rate enhancement is probably associated with some phenomena occurring due to the complexation. It seems that the cryptand may stabilize a higher oxidation state of manganese to such an extent that the thermodynamic driving force for the oxidation is greatly increased. This increase probably is the driving force responsible for the enhancement of the oxidation rate.

Conclusions

It is seen from the above results that electron spin resonance spectroscopy is indeed a very valuable spectroscopic technique for the determination of stabilities of manganese(II) complexes. The precision of measurements and the useful analytical concentration range (10^{-3} to 10^{-6} *M*) compares favorably with other electrochemical or spectroscopic techniques. With continuous improvements in ESR instrumentation, the precision and, therefore, the usefulness of this technique should increase further.

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