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GEL CHROMATOGRAPHIC INVESTIGATION OF THE COMPETITIVE BINDING OF MAGNESIUM AND COPPER(II) IONS WITH POLYPHOSPHATE IONS

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SUMMARY

An atomic-absorption flow detector combined with a gel chromatographic column (Sephadex G-25) was applied in the investigation of the competitive binding of magnesium and copper(II) ions with diphosphate and triphosphate ions. Some preliminary results are given and the practical utility of the theoretical equations presented in evaluating the competitive binding of magnesium and copper(II) ions is discussed.

INTRODUCTION

As described in previous papers[1,2] an atomic-absorption flow detector (AAD) combined with a gel chromatographic column is useful for obtaining quantitative information on the binding of magnesium ions with various polyphosphate ions such as diphosphate (pyrophosphate, P_2) and triphosphate (tripolyphosphate, P_3) ions. This method is based on the automatic monitoring of magnesium complexes of diphosphate and triphosphate ions, Mg–P_2 and Mg–P_3, which are formed according to the following equations during the elution of P_2 and P_3 through a column pre-equilibrated and eluted with a magnesium chloride solution of concentration known to be $[Mg]_0$:

$$Mg + P_2 \rightleftarrows Mg\text{–}P_2 \quad (1)$$

$$Mg + P_3 \rightleftarrows Mg\text{–}P_3 \quad (2)$$

The amounts of Mg–P_2 and Mg–P_3 complexes can be expressed as a function of $[Mg]_0$ and their stability constants, which are given by

$$K_{Mg\text{–}P_2} = \frac{[Mg\text{–}P_2]}{[Mg]_0\,[P_2]} \quad (3)$$

$$K_{Mg-P_3} = \frac{[Mg-P_3]}{[Mg]_0\,[P_3]} \tag{4}$$

where the square brackets represent molar concentrations[2].

Gel chromatographic techniques based on Hummel and Dreyer's method[3] have been increasingly applied to the characterization of metal–ligand binding in inorganic[1,2,4], bioinorganic[5–15] and environmental chemistry[16]. In such equilibrium experiments, the concentrations of free metal ions in the eluents are usually required to be very low[17], such as $[Mg]_0 = 10^{-5}\ M$ for a magnesium complex with a stability constant of 10^5. Therefore, it becomes important to have a theoretical basis for predicting the effect of trace amounts of foreign metal ions in the eluent on the formation of particular metal complexes. This work was aimed at obtaining such information by investigating the competitive binding of magnesium and copper(II) ions with diphosphate and triphosphate ions. In addition to the equations for magnesium complexes (eqns. 1–4), the complexation reactions and stability constants for copper(II) complexes can be given as follows:

$$Cu + P_2 \rightleftarrows Cu-P_2 \tag{5}$$

$$Cu + P_3 \rightleftarrows Cu-P_3 \tag{6}$$

$$K_{Cu-P_2} = \frac{[Cu-P_2]}{[Cu]_0\,[P_2]} \tag{7}$$

$$K_{Cu-P_3} = \frac{[Cu-P_3]}{[Cu]_0\,[P_3]} \tag{8}$$

where $[Cu]_0$ represents the molar concentration of copper(II) ions in the eluent.

Theoretical considerations will be presented on the assumption that magnesium and copper(II) ions compete with each other for the same binding sites on polyphosphate ions to form 1:1 metal complexes. Some preliminary experimental results will also be presented in order to examine the practical utility of the theoretical equations.

EXPERIMENTAL

Unless otherwise stated, all experiments were carried out as described previously[2] with a Sephadex G-25 column (90×1.5 cm I.D.). Each eluent contained 0.08 *M* ammonia–0.02 *M* ammonium chloride (pH 10) as background electrolytes, in addition to a desired amount of magnesium chloride and/or copper(II) chloride.

RESULTS AND DISCUSSION

An advantageous characteristic of the gel chromatographic equilibrium technique over the conventional static (batchwise) methods is that $[Mg]_0$ can be kept at a desired and pre-determined value. In other words, the equilibrium solution being examined is apparently "buffered" with respect to magnesium ions and the concentrations of ligand and metal complex are then dynamically adjusted to be in equi-

librium with $[Mg]_0$ through the process of their chromatographic migration[17]. To understand the basic principles, let us review briefly the results given previously[1,2].

A Sephadex G-25 column is pre-equilibrated with an eluent (pH 10) containing magnesium ions, with $[Mg]_0 = 1 \cdot 10^{-5}\ M$. A sample solution containing P_2 (100 nmole), P_3 (100 nmole) and magnesium ions (300 nmole) is applied to the pre-equilibrated column and then eluted with the solution used to equilibrate the column. The absorbance at 285.2 nm (A_{Mg}) corresponding to the total magnesium concentration in the effluent is monitored with an AAD and plotted against the retention time. The resulting elution profile is shown schematically in Fig. 1. A_{Mg} increases above the baseline level to form three positive peaks at the elution positions of Mg–P_3, Mg–P_2 and free magnesium ions. The height of the horizontal baseline corresponds to $[Mg]_0$, which is also the concentration level of free magnesium ions in the zones of Mg–P_2 and Mg–P_3. On the basis of the standardized area below the baseline level, the peak areas for Mg–P_2 and Mg–P_3 can be easily translated into the respective amounts of Mg–P_2 and Mg–P_3 (Q_{Mg-P_2} and Q_{Mg-P_3})[17]. Q_{Mg-P_2} and Q_{Mg-P_3} can be correlated with their stability constants by the following equations:

$$Q_{Mg-P_2} = \frac{[Mg]_0 K_{Mg-P_2} Q_{P_2}}{[Mg]_0 K_{Mg-P_2} + 1} = \bar{n}_{Mg-P_2} Q_{P_2} \tag{9}$$

$$Q_{Mg-P_3} = \frac{[Mg]_0 K_{Mg-P_3} Q_{P_3}}{[Mg]_0 K_{Mg-P_3} + 1} = \bar{n}_{Mg-P_3} Q_{P_3} \tag{10}$$

where Q_{P_2} and Q_{P_3} represent the total amounts of P_2 and P_3 applied, respectively, and $\bar{n}$ is the average number of magnesium ions bound to a ligand. It should be noted that each $\bar{n}$ value is dependent on $[Mg]_0$ but is not affected by the presence of other ligands[17].

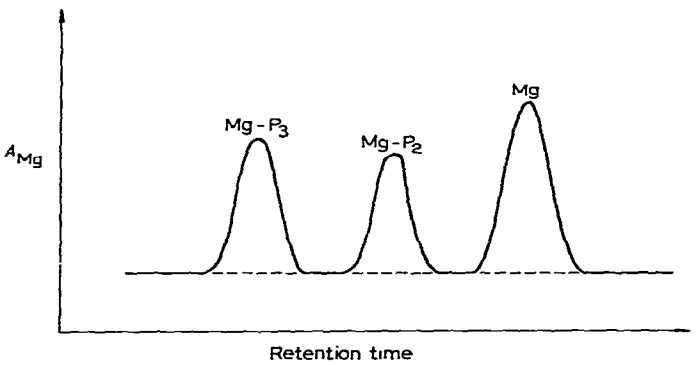

Fig. 1. Schematic representation of an elution profile for a mixed solution of diphosphate, triphosphate and magnesium ions.

Eqns. 9 and 10 mean that, if $[Mg]_0$ is given, Q_{Mg-P_2} and Q_{Mg-P_3} can be calibrated against Q_{P_2} and Q_{P_3}, respectively, to give a linear relationship. This relationship is very useful analytically in the indirect determination of polyphosphate ions[1]. Further-

more, $\bar{n}$ values can be easily determined from the slopes, thus permitting the subsequent calculation of the stability constants. For example, the conditional stability constants for $Mg–P_2$ and $Mg–P_3$ at pH 10 have been evaluated to be roughly log $K_{Mg-P_2} = 5.6$ and log $K_{Mg-P_3} = 5.9$ (ref. 2).

Copper(II) complexes

If an eluent contains copper(II) ions instead of magnesium ions, eqns. 9 and 10 for magnesium complexes are replaced with the following equations for the copper(II) complexes $Cu–P_2$ and $Cu–P_3$:

$$Q_{Cu-P_2} = \frac{[Cu]_0 K_{Cu-P_2} Q_{P_2}}{[Cu]_0 K_{Cu-P_2} + 1} = \bar{n}_{Cu-P_2} Q_{P_2} \tag{11}$$

$$Q_{Cu-P_3} = \frac{[Cu]_0 K_{Cu-P_3} Q_{P_3}}{[Cu]_0 K_{Cu-P_3} + 1} = \bar{n}_{Cu-P_3} Q_{P_3} \tag{12}$$

Competitive binding of magnesium and copper(II) ions

A major aim of this paper is to present theoretical considerations on the competitive binding of magnesium and copper(II) ions with polyphosphate ions. If an eluent contains both magnesium and copper(II) ions, Q_{P_2} and Q_{P_3} are expressed by the following equations:

$$Q_{P_2} = Q'_{Mg-P_2} + Q'_{Cu-P_2} + q_{P_2} \tag{13}$$

$$Q_{P_3} = Q'_{Mg-P_3} + Q'_{Cu-P_3} + q_{P_3} \tag{14}$$

where Q' represents the amount of each metal complex in the presence of magnesium and copper(II) ions in the eluent and q is the amount of each free ligand. It should be noted that eqns. 13 and 14 are independent of each other, although the free metal concentrations, $[Mg]_0$ and $[Cu]_0$, are common to two equilibrium systems.

Eqns. 13 and 14 can be rearranged as follows:

$$Q'_{Mg-P_2} = \frac{[Mg]_0 K_{Mg-P_2} Q_{P_2}}{1 + [Mg]_0 K_{Mg-P_2} + [Cu]_0 K_{Cu-P_2}} = \bar{n}'_{Mg-P_2} Q_{P_2} \tag{15}$$

$$Q'_{Mg-P_3} = \frac{[Mg]_0 K_{Mg-P_3} Q_{P_3}}{1 + [Mg]_0 K_{Mg-P_3} + [Cu]_0 K_{Cu-P_3}} = \bar{n}'_{Mg-P_3} Q_{P_3} \tag{16}$$

$$Q'_{Cu-P_2} = \frac{[Cu]_0 K_{Cu-P_2} Q_{P_2}}{1 + [Mg]_0 K_{Mg-P_2} + [Cu]_0 K_{Cu-P_2}} = \bar{n}'_{Cu-P_2} Q_{P_2} \tag{17}$$

$$Q'_{Cu-P_3} = \frac{[Cu]_0 K_{Cu-P_3} Q_{P_3}}{1 + [Mg]_0 K_{Mg-P_3} + [Cu]_0 K_{Cu-P_3}} = \bar{n}'_{Cu-P_3} Q_{P_3} \quad (18)$$

where $\bar{n}'$ is the average number of magnesium or copper(II) ions bound to a ligand in the presence of magnesium and copper(II) ions.

By monitoring the atomic absorption due to magnesium (A_{Mg}) and copper (A_{Cu}) in the effluent, two elution profiles can be obtained as shown schematically in Fig. 2. This illustration is based on the assumption that $[Cu]_0 K_{Cu-P_2} > [Mg]_0 K_{Mg-P_2}$ and $[Cu]_0 K_{Cu-P_3} < [Mg]_0 K_{Mg-P_3}$. It is expected that Cu–P_2 and Cu–P_3 complexes are eluted at the same retention times as those of the corresponding magnesium complexes. Under such conditions, the predominant formation of Cu–P_2 and Mg–P_3 complexes is suggested by the following equations:

$$\frac{Q'_{Cu-P_2}}{Q'_{Mg-P_2}} = \frac{[Cu]_0 K_{Cu-P_2}}{[Mg]_0 K_{Mg-P_2}} > 1 \quad (19)$$

$$\frac{Q'_{Cu-P_3}}{Q'_{Mg-P_3}} = \frac{[Cu]_0 K_{Cu-P_3}}{[Mg]_0 K_{Mg-P_3}} < 1 \quad (20)$$

A peak for free copper(II) ions, as well as for free magnesium ions, is also expected to appear, although the practical detection of the former peak was impossible because of its marked broadening, probably due to adsorption.

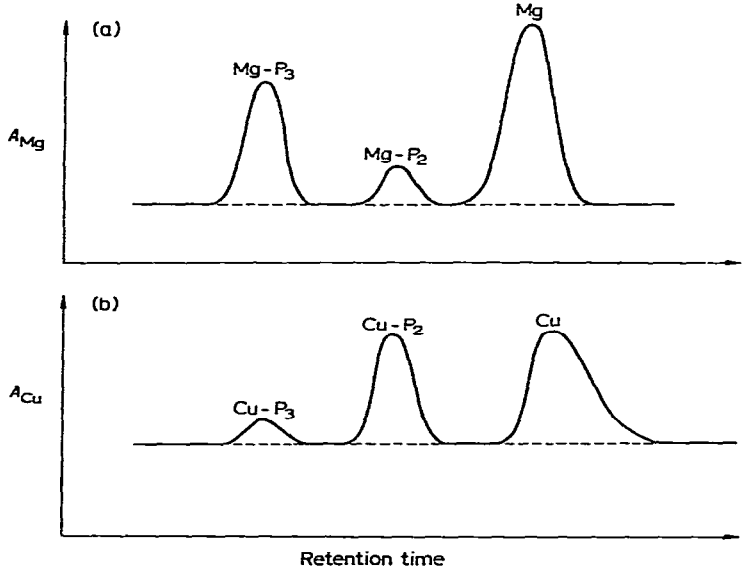

Fig. 2. Schematic representation of an elution profile for a mixed solution of diphosphate, triphosphate, magnesium and copper(II) ions. Absorbances due to magnesium (a) and copper (b) are selectively monitored.

Practical application

Some preliminary experimental results carried out at $[Mg]_0 = 1.0 \cdot 10^{-5}\ M$ and $[Cu]_0 = 5.0 \cdot 10^{-5}\ M$ are presented in order to examine the practical utility of eqns. 9–18 in evaluating the competitive reactions. The amount of each metal complex was calibrated against Q_{P_2} or Q_{P_3}, for magnesium complexes in Fig. 3 and for copper(II) complexes in Fig. 4. Good linearity was observed for each calibration graph.

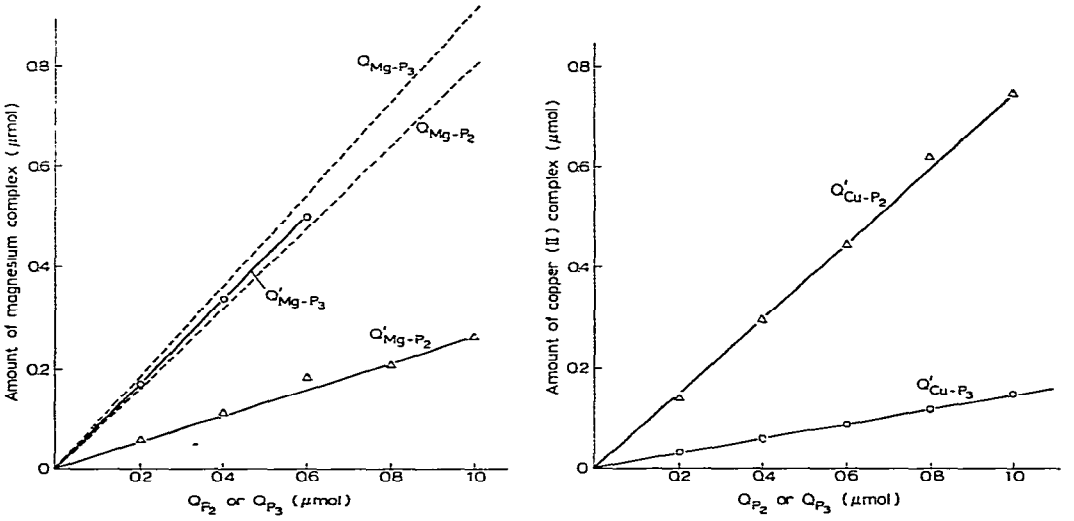

Fig. 3. Experimental plots for eqns. 9, 10, 15 and 16.

Fig. 4. Experimental plots for eqns. 17 and 18.

The broken lines in Fig. 3 represent data taken from previous papers[1,2] for magnesium complexes in the absence of copper(II) ions (eqns. 9 and 10). The solid lines in Fig. 3 show the calibration graphs for magnesium complexes in the presence of copper(II) ions. The formation of the $Mg–P_2$ complex is greatly depressed by the presence of copper(II) ions (eqn. 15), in contrast to the lesser inhibition by copper(II) ions of the formation of the $Mg–P_3$ complex (eqn. 16). As $[Mg]_0$, $[Cu]_0$ and K_{Mg-P_2} are known and $\bar{n}_{Mg-P_2}$ and $\bar{n}'_{Mg-P_2}$ are given by the slopes in Fig. 3, the conditional stability constant for the $Cu–P_2$ complex can be roughly evaluated from the equation

$$\frac{\bar{n}'_{Mg-P_2}}{\bar{n}_{Mg-P_2}} = \frac{1 + [Mg]_0 K_{Mg-P_2}}{1 + [Mg]_0 K_{Mg-P_2} + [Cu]_0 K_{Cu-P_2}} \qquad (21)$$

to be $K_{Cu-P_2} = 2.0 \cdot 10^5$. In the same way, K_{Cu-P_3} was calculated to be as low as $1.1 \cdot 10^4$.

The approach based on eqn. 21 indicates that the competitive binding of copper(II) ions can be evaluated indirectly without the measurement of the peak for the copper(II) complex. This indirect method may be useful for complexes of metals that cannot be detected by the atomic-absorption method.

An alternative approach for evaluating the competitive binding is based on the detection of not only magnesium complexes but also copper(II) complexes. The amounts of copper(II) complexes can be determined directly from the slopes in Fig. 4. The finding that $\bar{n}'_{Cu-P_2}$ is greater than $\bar{n}'_{Cu-P_3}$ is consistent with the prediction from the results in Fig. 3. The stability constant for the $Cu-P_2$ complex can be evaluated according to the equation

$$\frac{\bar{n}'_{Cu-P_2}}{\bar{n}'_{Mg-P_2}} = \frac{[Cu]_0 K_{Cu-P_2}}{[Mg]_0 K_{Mg-P_2}} \tag{22}$$

The K_{Cu-P_2} value thus evaluated was $2.3 \cdot 10^5$, which is in agreement with the value of $2.0 \cdot 10^5$ estimated by eqn. 21. On the other hand, a value of $2.7 \cdot 10^4$ for K_{Cu-P_3} is considerably greater than the $1.1 \cdot 10^4$ given by the indirect method. This discrepancy may be ascribed partly to the uncertainty of the latter value, which was evaluated indirectly from the small difference between $\bar{n}_{Mg-P_3}$ and $\bar{n}'_{Mg-P_3}$ shown in Fig. 3. Further investigations under selected conditions will be required in order to account for such a discrepancy. One of the possible factors that may participate in the complexation reactions, but that has not been considered in the above discussion, will be mentioned below.

As noted in the introductory section, all of the theoretical discussion is based on the assumption that magnesium and copper(II) ions compete with each other for the same binding sites to form 1:1 complexes. This assumption may be acceptable for magnesium complexes at such a low concentration as 10^{-5} *M* of free magnesium ions[18,19]. However, there is no reason to rule out the possibility of simultaneous binding of two copper(II) ions or of magnesium and copper(II) ions to the binding sites on triphosphate ions. Ellison and Martell[18] have suggested that more than 1 mole of copper(II) ions per mole of triphosphate ions may become bound in the presence of excess of copper(II) ions. If the simultaneous binding of magnesium and copper(II) ions takes place to form metal complexes of the type $Mg-P_3-Cu$, it is likely that the stability constant for $Cu-P_3$ may be underestimated by the indirect method (Fig. 3), but overestimated by the direct method (Fig. 4). This factor will be taken into consideration in subsequent studies.

It is well known that copper(II) ions react with ammonia to form amine complexes. As the eluents used in our experiments contain 0.08 *M* ammonia–0.02 *M* ammonium chloride as background electrolytes, $[Cu]_0$ is regarded as the total concentration of all copper(II) ions, including ammine complexes. Consequently, the K_{Cu-P_2} and K_{Cu-P_3} obtained denote the conditional stability constants as defined by Ringbom[20].

Although the preliminary results described above are insufficient for a detailed discussion to be based on them, it may be meaningful to note the rounded-off values $2 \cdot 10^5$ and $2 \cdot 10^4$ as the conditional stability constants for $Cu-P_2$ and $Cu-P_3$ complexes in order to demonstrate the unusual relationship between them, *i.e.*, $K_{Cu-P_2} > K_{Cu-P_3}$. This unusual order was also indicated by the ion-exchange method[21] and is of interest in relation to the fact that for most divalent metal ions the stability constant of the triphosphate complex is greater than that of diphosphate complex[22], *e.g.* $K_{Mg-P_3} > K_{Mg-P_2}$.

Eqns. 11 and 15 suggest that careful preparation of an eluent is needed for the determination of the stability constant of the $Mg–P_2$ complex in order to eliminate interfering cations such as calcium ions, whose stability constant for the diphosphate complex has been given as $\log K_{Ca-P_2} = 5.34$ (ref. 18). It can be readily calculated that about 5% of the $Mg–P_2$ complex will be diminished by the presence of calcium ions at a concentration as low as 10^{-6} *M* in the eluent if $[Mg]_0 = 10^{-5}$ *M*. Even the contaminating level in de-ionized water becomes serious in some biochemical experiments[14,15]. It should be noted that the contaminants in the eluent, but not in a sample solution, must be carefully eliminated.

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