Computation of Metal Binding in Bi-Metal–Bi-Chelate Systems*

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ABSTRACT: In solutions containing two different metal ions and two metal-binding agents, calculation of the equilibrium concentrations of the various ion species for a given pH is complicated by competition among the ions. For a solution containing K^+, Mg^{2+}, adenosine triphosphate (ATP), and EDTA or PP_i, such as is often encountered in muscle biochemistry, the free magnesium concentration in the region above pH 6 is expressed as the solution of a cubic equation in $[Mg^{2+}]$. (This equation can be more generally applied to any system containing a monovalent cation, a divalent cation, and two metal-binding tetravalent acids for which only the final acid dissociation need be considered for one and the last two acid dissociations for the other.) The concentrations of the other ion species are readily found in terms of this free magnesium concentration. From known values of the equilibrium constants and specified total concentrations of the reactants, the level of Mg^{2+} and of other components in the system is calculated for a given pH with the aid of a digital computer. In the present work, the desired root of the cubic equation in each case was found by the computer using the Newton–Raphson method of successive approximations. Computations were made for the pH range from 6.00 to 9.35 and for a range of total concentrations of the reactants. Illustrative examples of the results show features such as the competition for magnesium between ATP and chelator, which can result in drastic shifts in bound magnesium with relatively small changes in the free magnesium concentration. Although a change in $[K^+]$ has little direct effect on EDTA binding of magnesium, the competition between K^+ and Mg^{2+} for ATP is such that a change in $[K^+]$ alters the amount of magnesium bound by ATP which, in turn, brings about a shift in the amount of Mg bound by EDTA.

Myosin ATPase[1] is well known for its sensitivity to metal ions. Many studies have been made of its pH dependence and the effect of modifiers such as EDTA. Since enzyme, substrate, and modifiers of the chelator type can all interact with metal ions (both monovalent and multivalent), it is important to know the concentrations of the various possible species present in solution in order to try to identify the active forms of the reactants.

Recently Offer (1964) has investigated myosin A ATPase activity at pH 8 in a reaction medium containing various concentrations of ATP, Mg, and EDTA. Taking into account the interaction of ATP with Mg^{2+} and K^+ and the interaction of EDTA with Mg^{2+}, he obtains an equation (applicable at pH 8) for the free magnesium concentration, $[Mg^{2+}]$, in terms of the total concentrations of ATP, EDTA, Mg, and K^+

$$[Mg^{2+}] = \frac{[Mg]_0}{10^{6.42}[EDTA]_0 + \dfrac{38{,}000[ATP]_0}{1 + 11[K^+]} + 1}$$

By using this equation to calculate the concentration of free magnesium in each reaction mixture, he finds a close correlation between the concentration of free magnesium and the myosin ATPase activity in that reaction mixture.

Since myosin ATPase under certain conditions shows a strong pH dependence, it is of interest to consider the effect of the hydrogen ion concentration on the various equilibrium concentrations of the reactants. The formation of the K–EDTA complexes can also be included [see Botts *et al.* (1965)]. In developing an equation to include such additional effects, the relationships in Chart I were taken into account. These assumptions lead to a cubic equation in free magnesium concentration, $[N^{2+}]$, with coefficients expressed in terms of knowable equilibrium constants and knowable concentrations of reactants

$$G[N^{2+}]^3 + \{G([Y_0] + [A_0] - [N_0]) + J + GF\}[N^{2+}]^2 + \{GF([Y_0] - [N_0]) + J([A_0] - [N_0]) + JF\}[N^{2+}] - JF[N_0] = 0$$

where

$$G \equiv K_1 + K_2K_5^{-1}[H^+]$$

$$F \equiv \{1 + K_7[K^+] + K_7K_8[K^+]^2 + K_9^{-1}[H^+]\}K_{10}^{-1}$$

$$J \equiv 1 + K_5^{-1}[H^+] + K_4[K^+] + K_3K_5^{-1}[H^+][K^+] + K_6^{-1}K_5^{-1}[H^+]^2$$

The cubic equation was solved with the aid of a digital

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[1] Abbreviation used in this work: ATP, adenosine 5′-triphosphate.

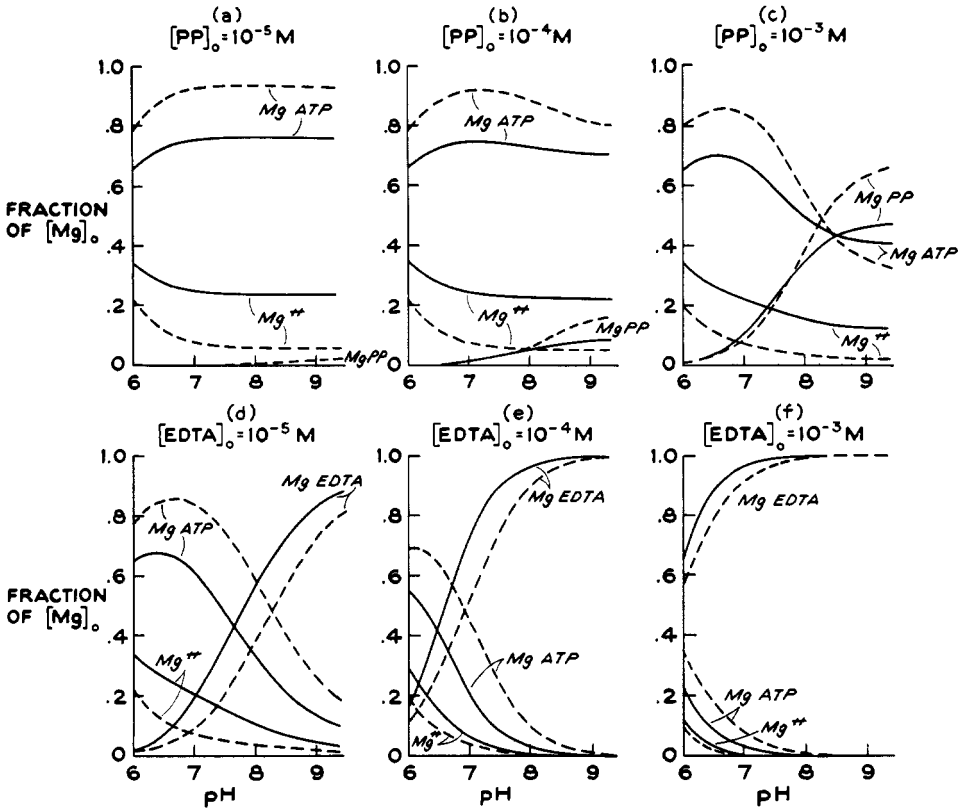

FIGURE 1: Distribution of magnesium as a function of pH for various total concentrations of PP_i or EDTA as indicated: $[ATP]_0$ = 0.653 mM and $[Mg]_0$ = 10^{-5} M; solid lines, $[K^+]$ = 0.60 M; dashed lines, $[K^+]$ = 0.06 M.

CHART I

$[A_0]$ = total concentration of ATP, $[ATP]_0$
$[Y_0]$ = total concentration of chelator, $[EDTA]_0$ or $[PP_i]_0$
$[N_0]$ = total concentration of magnesium, $[Mg]_0$
$[K^+]$ = potassium ion concentration
$[H^+]$ = hydrogen ion concentration
$[A_0] = [A^{4-}] + [NA^{2-}] + [KA^{3-}] + [K_2A^{2-}] + [HA^{3-}]$
$[Y_0] = [Y^{4-}] + [HY^{3-}] + [H_2Y^{2-}] + [KY^{3-}] + [HKY^{2-}] + [NY^{2-}] + [HNY^{1-}]$
$[N_0] = [N^{2+}] + [NY^{2-}] + [HNY^{1-}] + [NA^{2-}]$
$K_1 = [NY^{2-}]/([N^{2+}][Y^{4-}])$
$K_2 = [HNY^{1-}]/([N^{2+}][HY^{3-}])$
$K_3 = [HKY^{2-}]/([K^+][HY^{3-}])$
$K_4 = [KY^{3-}]/([K^+][Y^{4-}])$
$K_5 = [H^+][Y^{4-}]/[HY^{3-}]$
$K_6 = [HY^{3-}][H^+]/[H_2Y^{2-}]$
$K_7 = [KA^{3-}]/([K^+][A^{4-}])$
$K_8 = [K_2A^{2-}]/([K^+][KA^{3-}])$
$K_9 = [A^{4-}][H^+]/[HA^{3-}]$
$K_{10} = [NA^{2-}]/([N^{2+}][A^{4-}])$

computer using the Newton–Raphson method of successive approximations. For each set of numerical coefficients, the desired positive root of the equation was taken to be an $[N^{2+}]$ value which, when substituted into the cubic equation, gave a residual of less than 10^{-20}.

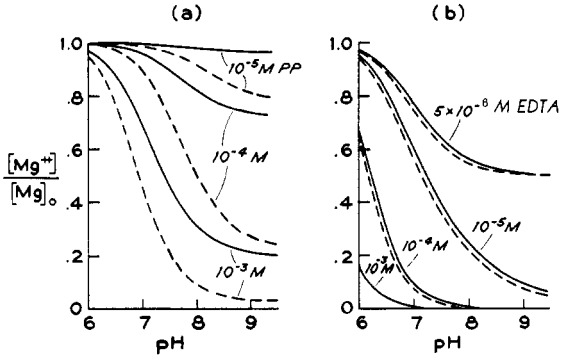

FIGURE 2: Free magnesium levels in the absence of ATP, with various total concentrations of PP_i or EDTA as indicated: $[Mg]_0$ = 10^{-5} M, $[ATP]_0$ = 0; solid lines, $[K^+]$ = 0.60 M; dashed lines, $[K^+]$ = 0.06 M. Total concentrations of (a) PP_i and (b) EDTA are indicated on the graphs.

(Actually, the successive approximations were limited to 25 iterations; in all of the test cases this was sufficient to reduce the residual to the specified level.) Calculations were made for all possible combinations of the following values and for two different chelators (EDTA or PP_i)

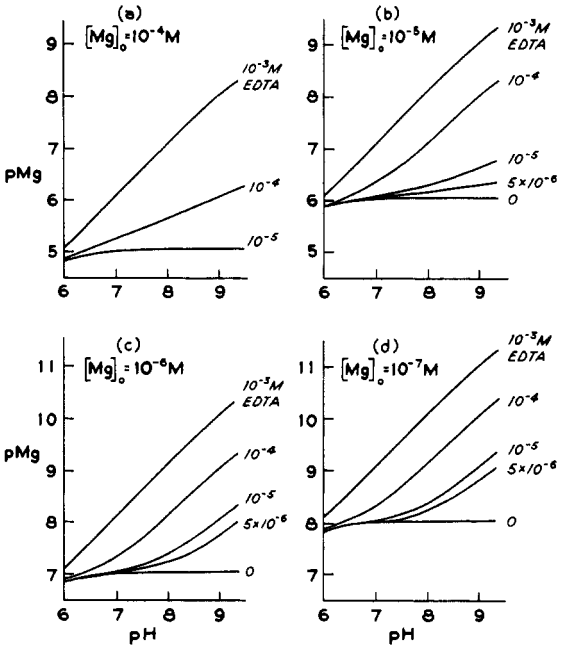

FIGURE 3: Variation of pMg = $-\log [Mg^{2+}]$ with pH for different concentrations of $[Mg]_0$ and $[EDTA]_0$; $[ATP]_0$ = 2.00 mM; $[K^+]$ = 0.6 M. In (a), the curve (not shown) for $[EDTA]_0 = 0$ levels off at pMg = 5.03 and is almost coincident with that for 10^{-5} M EDTA.

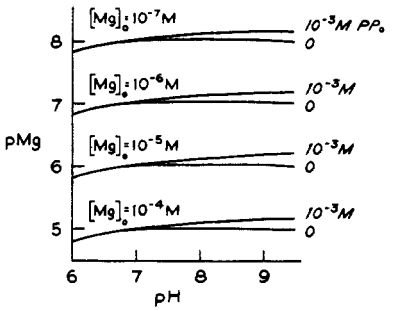

FIGURE 4: Variation of pMg = $-\log [Mg^{2+}]$ with pH for different concentrations of $[Mg]_0$ and $[PP_i]_0$; $[ATP]_0$ = 2.00 mM; $[K^+]$ = 0.6 M.

$[A_0]$ = 0, 0.653, 1.00, 2.00 mM
$[N_0]$ = 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M
$[Y_0]$ = 0, 5×10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M
pH = 6.00, 6.35, 6.70, 7.00, 7.35, 7.70, 8.00, 8.35, 8.70, 9.00 9.35
$[K^+]$ = 0.60, 0.06 M

Values used for the equilibrium constants are listed in Table I and were assumed to be the same for both K^+ concentrations. For each combination of concentrations, the computer expressed $[N^{2+}]$, $-\log[N^{2+}]$, and the concentrations of various forms of ATP and Y:

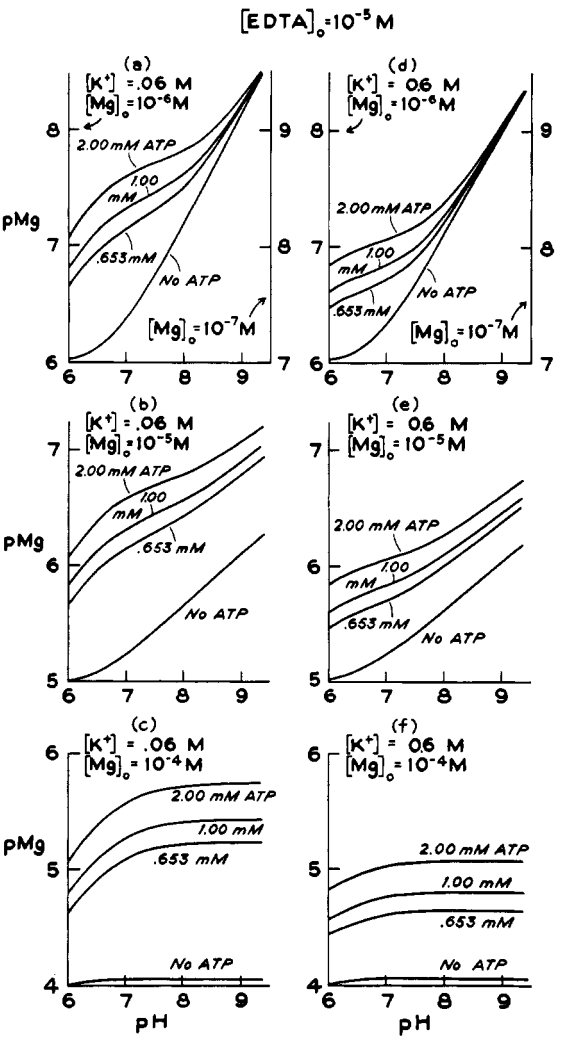

FIGURE 5: Variation of pMg = $-\log [Mg^{2+}]$ with pH for different concentrations of $[Mg]_0$, $[ATP]_0$, and $[K^+]$; $[EDTA]_0 = 10^{-5}$ M. Note that the curves in (a) and (d) apply also for $[Mg]_0 = 10^{-7}$ M when the right-hand ordinate scale is used. (In (a) with $[ATP]_0 = 0$, the curve for $[Mg]_0 = 10^{-7}$ M actually lies slightly above that shown for $[Mg]_0 = 10^{-6}$ M; at pH 9.35 the pMg value for $[Mg]_0 = 10^{-7}$ would be 9.52 instead of 9.47.)

$[NA^{2-}]$, $[KA^{3-}]$, $[K_2A^{2-}]$, $[A^{4-}]$, $[HNY^{1-}]$, $[NY^{2-}]$, $[KHY^{2-}]$, $[KY^{3-}]$, $[Y^{4-}]$, and $[HY^{3-}]$.

Since comparisons at different ionic strengths are not necessarily valid, one may consider that the ionic strength for the lower potassium salt concentration is maintained at the level of the higher concentration by use of an appropriate amount of an indifferent (nonbinding) salt. (It is clear that the presence of such a salt would not alter the form of the cubic equation in $[N^{2+}]$ since no additional equilibria are involved.) The equilibrium constants substituted in the equation should then be those applicable at this fixed ionic strength. As indicated in Table I, when affinity constants at 25° and the desired ionic strength are not available in the litera-

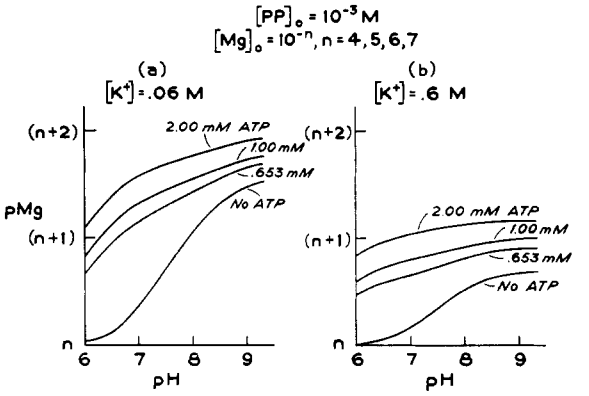

FIGURE 6: Variation of pMg = $-\log [Mg^{2+}]$ with pH for different concentrations of $[ATP]_0$, $[Mg]_0$, and $[K^+]$; $[PP_i]_0 = 10^{-3}$ M. Curves shown are for $[Mg]_0 = 10^{-7}$ M. As indicated by the ordinate scale labeling, the corresponding curves for the other values of $[Mg]_0$ are essentially superimposable on these curves (deviation is generally within 0.02 and not more than 0.04 of a pMg unit).

TABLE I: Values Assumed for Equilibrium Constants.

Constants	EDTA	PP_i	ATP
K_1	0.490×10^{9a}	0.501×10^{6b}	$K_7 = 9^c$
K_2	0.191×10^{3a}	0^b	$K_8 = 0.6^c$
K_3	0.49^c	4^c	$K_9 = 0.18 \times 10^{-6c}$
K_4	9.2^c	2.1×10^{2c}	$K_{10} = 3.8 \times 10^{4d}$
K_5	0.35×10^{-10c}	0.37×10^{-9c}	
K_6	0.51×10^{-6c}	0.43×10^{-6c}	

[a] Schwarzenbach and Ackermann (1947); 20°, 0.1 M KCl. [b] Vasil'ev (1957); 19°, 0.02 M KNO_3. [c] Botts *et al.* (1965); 25°. [d] Burton (1959).

ture, we have used, as approximations, values obtained at somewhat different ionic strengths and temperatures. However, the general results depend on features which would not be seriously affected by a refinement of these affinity constant values; the constant for the formation of the MgEDTA complex would remain several orders of magnitude greater than that for the formation of MgPP or MgATP, and the constant for forming KPP would remain several times greater than that for K-EDTA or KATP.

It is seen that the possibility of formation of a complex involving the binding of both a monovalent and a divalent metal ion is not considered among the equilibria listed above. The affinity constants for the formation of these complexes are not available at present. In general, however, one would anticipate that such complexes constitute a fairly minor fraction of the bound metal concentration. To form such a complex, one of the metal

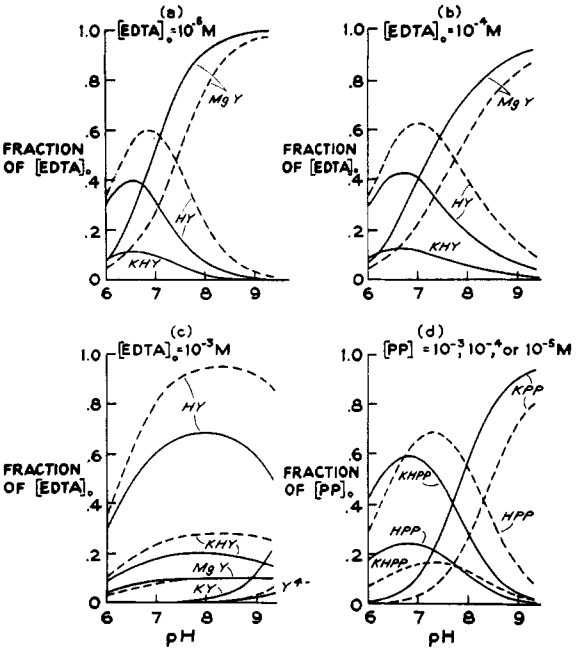

FIGURE 7: Distribution of EDTA or PP_i among the various possible ionic species for different concentrations of the chelator; $[ATP]_0$ = 2.00 mM, $[Mg]_0 = 10^{-4}$ M; solid lines: $[K^+]$ = 0.6 M; dashed lines: $[K^+]$ = 0.06 M. In the case of PP_i(d), the distribution is essentially the same for $[PP_i]_0 = 10^{-3}$, 10^{-4}, and 10^{-5} M, as indicated.

ions would have to bind to an anion species bearing not more than three negative charges, and possibly only one. The ratio, $[MgKY^{1-}]/[KY^{3-}]$, might be roughly comparable to that of $[MgHY^{1-}]/[HY^{3-}]$, or less than 1:50 for EDTA in the presence of the highest concentration of magnesium used here. The uncharged species, Mg-KHY, would be expected to form to an even smaller extent (unless precipitation took place).

Results and Discussion

Figure 1 shows the distribution of magnesium between its complexed forms and its free ionized state for different chelator concentrations. In the absence of chelator (EDTA or PP_i) and with $[ATP]_0$ in excess of $[Mg]_0$, a large fraction of the magnesium is in the form of MgATP. While addition of a chelator further reduces the level of free magnesium, Mg^{2+}, the bulk of the chelator-bound magnesium comes from competition with MgATP for its bound magnesium.

All of these metal-binding compounds (ATP, EDTA, and PP_i) have much greater affinities for Mg^{2+} than for K^+. With ATP and PP_i, however, the difference in affinities is less pronounced so that the presence of K^+ in much higher concentrations than Mg^{2+} can affect considerably the Mg-binding capacity of these compounds. Figures 1 and 2a show that a 10-fold reduction in $[K^+]$ from 0.6 to 0.06 M can alter extensively both

the level of free magnesium and the concentrations of the complexed forms of magnesium. Figure 1a, in which the PP_i concentration is so low that essentially no MgPP is formed, illustrates the effect of $[K^+]$ on the Mg-binding capacity of ATP alone. Since the final acid dissociation of ATP occurs largely below pH 7, the curves in this figure are nearly pH independent above pH 7.5. In Figure 2b, it is seen that with EDTA in the absence of ATP, a 10-fold change in $[K^+]$ has virtually no effect on the distribution of Mg between the bound and free states. Therefore, when both ATP and EDTA are present (Figure 1d–f), the change in the MgEDTA curves with $[K^+]$ must stem largely from the ATP-imposed changes in $[Mg^{2+}]$. The ability of ATP to dictate these changes (despite its lower affinity for Mg^{2+}) depends on the fact that, for comparable total concentrations of ATP and EDTA, $[ATP^{4-}] \gg [EDTA^{4-}]$ in the pH range under study. Thus, even when [MgEDTA] greatly exceeds [MgATP] as in Figure 1f, the ATP interaction with K^+ is still a dominant factor in allowing more binding of Mg to ATP (and, therefore, less to EDTA) in 0.06 M K^+ than in 0.6 M K^+.

When PP_i is substituted for EDTA the situation is more complicated. The affinity constant for the formation of KPP^{3-} is some 20 times greater than that for the formation of $KATP^{3-}$, and the affinity constant for $MgPP^{2-}$ formation is about 13 times greater than for $MgATP^{2-}$ (see Table I). In the lower pH region where $[ATP^{4-}] \gg [PP^{4-}]$ the ATP effect dominates, whereas in the higher pH region the PP_i effect becomes more prominent. This results in an actual crossing of the two curves for MgATP in Figure 1c (and also of those for MgPP); thus, for lower pH values ATP binds Mg better in 0.06 M K^+ than in 0.6 M K^+, but for higher pH values the reverse is true. It is interesting that over the pH range from 7.0 to 9.3, for which MgPP in 0.6 M K^+ undergoes a large, almost 5-fold increase in concentration, the corresponding Mg^{2+} concentration changes by less than a factor of 2 (Figure 1c).

In several of the cases illustrated here (*e.g.*, Figures 1e, f, and 2b) the fraction of the magnesium in the free form becomes relatively small in the higher pH range. Even small concentrations of magnesium, however, may have considerable effect on sensitive enzymatic reactions or phenomena such as superprecipitation and relaxation in muscle preparations. In order to show the differences in $[Mg^{2+}]$ for relatively low concentrations, it is convenient to plot $pMg = -\log[Mg^{2+}]$. Figure 3 illustrates the effect of $[EDTA]_0$ on pMg for given total concentrations of ATP, K^+, and Mg. When $[Mg]_0$ is relatively low (Figure 3c, d), even a small concentration of EDTA (10^{-5}, 5×10^{-6} M) can bring about a 10-fold or more change in $[Mg^{2+}]$ between pH 8.0 and 9.0. By contrast, Figure 4 shows that for the same K^+ and ATP concentrations as in Figure 3, even 10^{-3} M PP_i causes only about a twofold change in $[Mg^{2+}]$ over this same pH range. So, for low magnesium concentrations (10^{-7}, 10^{-6}, and even 10^{-5} M), 5×10^{-6} M EDTA is much more effective than 10^{-3} M PP_i in reducing the Mg^{2+} concentration.

Figures 3 and 4 have a fixed total ATP concentration. The effect of varying $[ATP]_0$ is shown in Figure 5 with $[EDTA]_0 = 10^{-5}$ M and in Figure 6 with $[PP_i] = 10^{-3}$ M. As indicated by the variable ordinate scale in Figures 6b and 5a, d, the corresponding curves for the different Mg concentrations can be superimposed. (In Figure 6a this, too, is approximately true; however, the curves for 10^{-7} M Mg lie slightly above their counterparts for 10^{-4} M Mg when the ordinate scale numbers are decreased by three.) Again the effect of potassium binding is illustrated by the fact that, in the presence of ATP and/or PP_i, all of the curves for 0.06 M K^+ (Figure 5, a–c and Figure 6a) lie well above their counterparts for 0.6 M K^+ (Figure 5, d–f and Figure 6b), indicating a severalfold lowering of the $[Mg^{2+}]$ in going from 0.6 M to 0.06 M K^+.

With PP_i and EDTA the final acid dissociation occurs, or begins to occur, largely in the pH range covered in these studies. Figure 7 shows the fractions of EDTA and PP_i in their various ionic forms for given total concentrations of the reactants. As $[EDTA]_0$ is progressively increased from 10^{-5} to 10^{-3} M, the fractional distribution of EDTA among its different forms can shift considerably (Figure 7a–c). By contrast, under the same conditions the fractional distribution of PP_i is essentially unchanged (Figure 7d). This is mainly due to the relatively small amount of PP_i appearing as MgPP, even when $[Mg]_0$ exceeds $[PP]_0$ by 10-fold. Although Figure 7d superficially resembles Figure 7a or 7b, it is seen that the sigmoid curves in the one case represent a potassium complex and in the other a magnesium complex. In 0.6 M K^+ above pH 7, over 80% of the PP_i is bound to potassium (KHPP or KPP) and less than 4% to magnesium when $[ATP]_0 = 2.00$ mM; in the absence of ATP, however, the level of MgPP is considerably higher.

From the examples given here, it is seen that the competition between two metal-binding substances for magnesium, and the competition between two metals for the same chelator, can result in concentrations of the various components of the system which are not readily predictable simply from a knowledge of individual equilibrium constants. In a separate paper we will discuss the implications of this work as applied to the myosin ATPase system.

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