# A COMMENT ON THE ROLE OF MAGNESIUM IN THE CONTROL OF MUSCLE GLYCOGEN SYNTHETASE KINASE

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Received 3 October 1969

Huijing and Larner [1, 2] found that cyclic AMP (cAMP) increased the activity of transferase I kinase of rabbit muscle. The cyclic nucleotide appeared to lower (from 2 to 0.5 mM) the $K_m$ of the kinase towards its trinucleotide substrate. The authors also suggested that an additional influence of cAMP was to lower (from 50 to 1 mM) the $K_a$ of the enzyme towards magnesium, which they postulated to be an activator, with compulsory binding order such that magnesium had to bind first. The values of $K_a$ they deduced must be dubious, since in their calculation they used concentrations of total added magnesium as opposed to that of the free magnesium ion. When the concentration of ATP substantially exceeds that of added magnesium the concentration of $Mg^{2+}$ falls to a very low level. Moreover the method of kinetic analysis used by Huijing and Larner was based on a model [3] in which substrate functions both as substrate and also as activator. This is not really applicable in the present case because the concentration of magnesium when acting as an activator $[Mg^{2+}]$ is quite different from its concentration as a substrate, i.e. [MgATP] and in fact varies inversely with the latter. Since the results of the kinetic analysis have been published several times [1,2,4,5] it seemed of interest to see whether similar conclusions, presumably with different numerical values, would result from re-analysis of the above data using the calculated values of MgATP and $Mg^{2+}$ likely to be present when the stated total quantities of metal and nucleotide were in solution.

Concentrations of complexes and of free ions (tables 1 and 2) were calculated by computer using a programme that recalculated the association constants from the results to check their accuracy. The binding capacity of glycerophosphate, present in the assay medium at 4.8 mM, was taken into account as well as that of ATP, although it made little difference to the kinetic results. Appreciable glycerophosphate-magnesium complex was formed only when $[Mg]_T$ was greater than $[ATP]_T$. The stability constant for magnesium glycerophosphate is given as 63.1 $M^{-1}$ [6], that of MgATP was tried at $1 \times 10^4$, $5 \times 10^4$ and $8 \times 10^4$ $M^{-1}$ [7, 8].

Huijing and Larner found that when $[Mg]_T$ was held constant and [ATP] increased, activity rose through a peak at a [Mg] approx. equal to [ATP] then declined. The decline they attributed to chelation of Mg by excess ATP. When [ATP] was held constant and $[Mg]_T$ increased, there was only a slight increase over a range of low $[Mg]_T$, activity then rose sharply and finally tapered off though continuing to rise when $[Mg]_T$ exceeded that of [ATP]. Their points are shown in figs. 1 and 2.

The following models were first tested to see if they fitted the published experimental data (in a manner similar to that described for pyruvate carboxylase by Blair [8]): (1) requirement for $Mg^{2+}$ and MgATP with random binding order, (2) compulsory binding order with $Mg^{2+}$ binding first, (3) compulsory binding order with MgATP binding first – this very obviously showed no tendency to fit the data. Both (1) and (2) gave partial satisfaction. However, the problem arose in finding values of $K_a$ for $Mg^{2+}$ which were satisfactory for the different situations represented in figs. 1 and 2. Particularly with random binding order activity decreased very sharply when [ATP] exceeded $[Mg]_T$ with quite low values for $K_a$ (e.g. 0.05 mM) whereas low values of $K_a$ favoured rise in activity in the plots varying [Mg] with constant [ATP] for smaller $[Mg^{2+}]$ than was previously observed. Activity diminished less rapidly in the presence of excess ATP for a given value

Table 1
Concentration of $Mg^{2+}$, MgATP and Mg-glycerophosphate complexes and $ATP^{4-}$, and ionic stength, for varying concentrations of total ATP.

| $ATP_T$ | $MgATP^{2-}$ | $ATP^{4-}$ | $Mg^{2+}$ | MgGlyc.-P | Ionic strength | |
|---|---|---|---|---|---|---|
| | | | | | actual | nominal |
| 1 | 0.992 | 0.0083 | 2.38 | 0.628 | 0.026 | 0.0364 |
| 2 | 1.98 | 0.0250 | 1.59 | 0.433 | 0.0289 | 0.0464 |
| 3 | 2.93 | 0.0700 | 0.828 | 0.241 | 0.0320 | 0.0564 |
| 4 | 3.69 | 0.309 | 0.238 | 0.0712 | 0.0366 | 0.0664 |
| 5 | 3.91 | 1.09 | 0.0714 | 0.0216 | 0.0451 | 0.0764 |
| 6 | 3.95 | 2.05 | 0.0385 | 0.0116 | 0.0548 | 0.0864 |
| 7 | 3.97 | 3.03 | 0.0262 | 0.0079 | 0.0664 | 0.0964 |
| 8 | 3.97 | 4.03 | 0.0197 | 0.0060 | 0.0746 | 0.1064 |
| 9 | 3.98 | 5.02 | 0.0158 | 0.0048 | 0.0845 | 0.1164 |
| 10 | 3.98 | 6.02 | 0.0132 | 0.0040 | 0.0945 | 0.1264 |
| 11 | 3.99 | 7.01 | 0.0114 | 0.0034 | 0.105 | 0.1364 |
| 12 | 3.99 | 8.01 | 0.0099 | 0.0030 | 0.114 | 0.1464 |
| 13 | 3.99 | 9.01 | 0.0089 | 0.0027 | 0.124 | 0.1564 |
| 14 | 3.99 | 10.0 | 0.0080 | 0.0024 | 0.134 | 0.1664 |
| 15 | 3.99 | 11.0 | 0.0072 | 0.0022 | 0.164 | 0.1764 |
| 16 | 3.99 | 12.0 | 0.0066 | 0.0020 | 0.154 | 0.1864 |

All concentrations are mM; ionic strength as M. $[Mg]_T$ = 4 mM, $[Glycerophosphate]_T$ = 4.8 mM, pH 7.8. Caffeine (3 mM) and mercaptoethanol (30 mM) were also present. Stability constant for MgATP was taken as $5 \times 10^4$ $M^{-1}$.

Table 2
Concentration of $Mg^{2+}$, $MgATP^{2-}$ and Mg-glycerophosphate complexes and $ATP^{4-}$, and ionic strength, for various concentrations of total magnesium.

| $[Mg]_T$ | $MgATP^{2-}$ | $ATP^{4-}$ | $Mg^{2+}$ | MgGlyc.-P | Ionic strength | |
|---|---|---|---|---|---|---|
| | | | | | actual | nominal |
| 1 | 0.9963 | 7.00 | 0.00285 | 0.0009 | 0.0710 | 0.075 |
| 2 | 1.99 | 6.01 | 0.00663 | 0.0020 | 0.0700 | 0.078 |
| 3 | 2.98 | 5.02 | 0.0119 | 0.0036 | 0.0690 | 0.081 |
| 4 | 3.97 | 4.03 | 0.0198 | 0.0060 | 0.0681 | 0.084 |
| 5 | 4.96 | 3.04 | 0.0326 | 0.0099 | 0.0671 | 0.087 |
| 6 | 5.93 | 2.07 | 0.0571 | 0.0172 | 0.0662 | 0.090 |
| 7 | 6.85 | 1.15 | 0.119 | 0.0356 | 0.0653 | 0.093 |
| 8 | 7.56 | 0.443 | 0.341 | 0.101 | 0.0650 | 0.096 |
| 9 | 7.83 | 0.172 | 0.911 | 0.261 | 0.0656 | 0.099 |
| 10 | 7.90 | 0.096 | 1.65 | 0.451 | 0.0668 | 0.102 |
| 12 | 7.95 | 0.049 | 3.24 | 0.814 | 0.0697 | 0.108 |
| 14 | 7.97 | 0.033 | 4.90 | 1.13 | 0.0731 | 0.114 |
| 16 | 7.98 | 0.024 | 6.61 | 1.41 | 0.0768 | 0.120 |
| 18 | 7.98 | 0.019 | 8.36 | 1.66 | 0.0808 | 0.126 |
| 20 | 7.98 | 0.016 | 10.14 | 1.87 | 0.0851 | 0.132 |

All concentrations are mM; ionic strength as M. $[ATP]_T$ = 8 mM, $[Glycerophosphate]_T$ = 4.8 mM, pH 7.8. Caffeine (3 mM) and mercaptoethanol (30 mM) were also present. Stability constant for MgATP was taken as $5 \times 10^4$ $M^{-1}$.

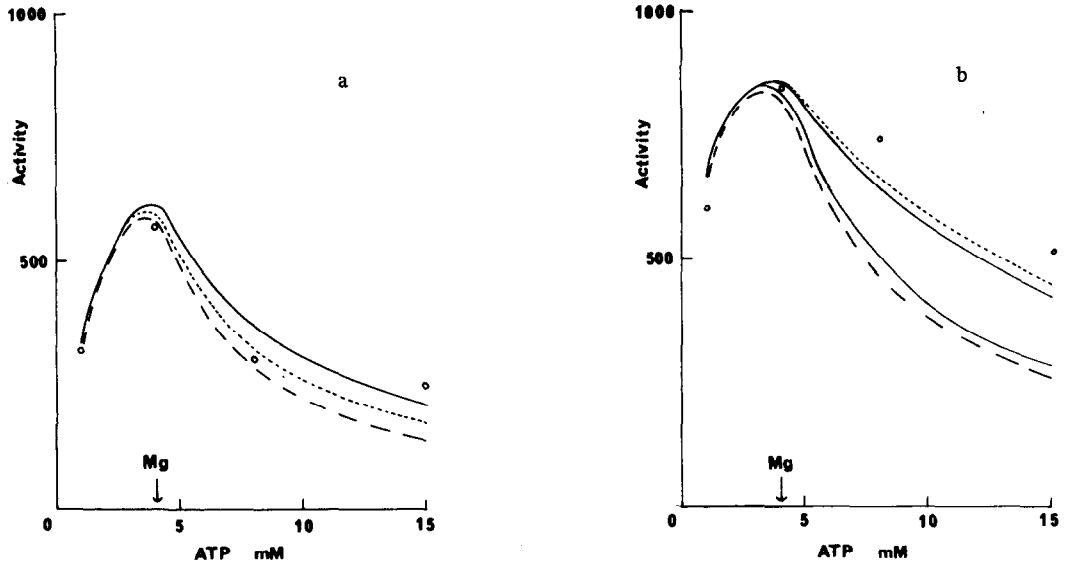


Fig. 1. Activity of muscle transferase kinase at different [ATP] with [Mg] maintained constant. (a) In the absence of cAMP, $K_m$ for MgATP = 2 mM, (b) in the presence of cAMP, $K_m$ for MgATP = 0.5 mM. $V_{max}$ is assumed to be 1000 arbitrary units (fig. 2, ref. [2]), the activities in the presence of cAMP being adjusted to this scale. (a) – – – Compulsory binding of $Mg^{2+}$ $K_a$ = 0.08 mM, ····· competitive inhibition by ATP $K_i$ = 1.28 mM, —— non-competitive inhibition by ATP $K_i$ = 5.12 mM. (b) – – – Compulsory binding of $Mg^{2+}$ $K_a$ = 0.16 mM, ····· competitive inhibition by ATP $K_i$ = 1.28 mM, —— non-competitive inhibition by ATP, upper line $K_i$ = 10.2 mM and lower line $K_i$ = 5.12 mM. Circles are results of Huijing and Larner [2].

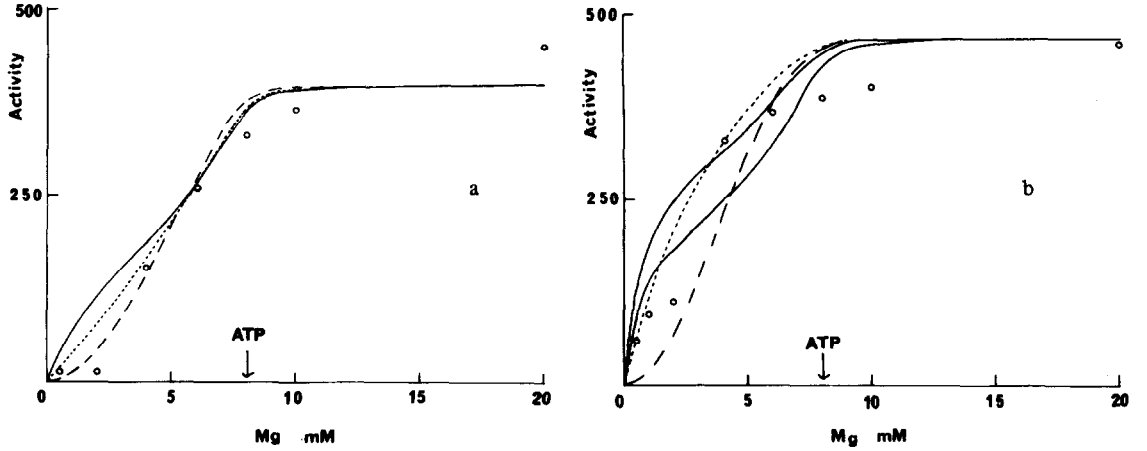


Fig. 2. Activity of muscle transferase kinase at different [Mg] with [ATP] maintained constant. (a) In the absence of cAMP, $K_m$ for MgATP = 2 mM, (b) in the presence of cAMP, $K_m$ for MgATP = 0.5 mM. $V_{max}$ is assumed to be 500 arbitrary units (fig. 3, ref. [2]). (a) – – – Compulsory binding of $Mg^{2+}$ $K_a$ = 0.08 mM, ····· competitive inhibition by ATP $K_i$ = 1.28 mM, —— non-competitive inhibition by ATP $K_i$ = 5.12 mM, (b) – – – compulsory binding of $Mg^{2+}$ $K_a$ = 0.16 mM, ····· competitive inhibition by ATP $K_i$ = 1.28 mM, —— non-competitive inhibition by ATP, line to left $K_i$ = 10.2 mM, line to right $K_i$ = 5.12 mM. Circles are results of Huijing and Larner [1, 2].

of $K_a$ when the compulsory order requiring $Mg^{2+}$ binding first, as opposed to random order, was assumed ; conversely in the plot varying $[Mg]_T$ of constant [ATP], activity was greater at lower [Mg] concentration. It was thus found easier to find a value of $K_a$ moderately consistent with the data in both figs. 1 and 2 when compulsory binding order was assumed than for random binding order. In the absence of cAMP, the best available values for $K_a$ seemed to be 0.32, 0.08 or 0.04 mM depending on whether $K_m$ for MgATP is 1-, 5-, or $8 \times 10^4$ $M^{-1}$, respectively. In the presence of cAMP figures of 0.32, 0.16 and 0.08 mM respectively were most satisfactory. Thus the presence of cAMP appeared to raise the $K_a$ towards $Mg^{2+}$ rather than lowering it 50 × as previously deduced. It must be admitted however, that for each of the values stated, when [ATP] reached levels greater than $[Mg]_T$, the activity in fig. 1 fell off faster than previously observed; with the same $K_a$ the activity reached a limiting value (fig. 2) at a faster rate than the observed rise in experimental values, and the slow rise in activity as $[Mg]_T$ was raised from 8- to 20 mM was not simulated. At first it was thought possible that the complexing capacity of glycerophosphate, which only becomes appreciable after virtual saturation of the ATP with magnesium, might be important in this context. However, although the presence of glycerophosphate does reduce (table 2) the rate of rise of $[Mg^{2+}]$, by this stage the enzyme, with a $K_a$ towards $Mg^{2+}$ of approx. 0.16 mM, will already be saturated.

An alternative possibility is that the enzyme is subject to inhibition by $ATP^{4-}$. Thus the following additional models were considered: (4) competitive inhibition by $ATP^{4-}$ and (5) non-competitive inhibition by $ATP^{4-}$, assuming in each case no involvement of Mg other than as MgATP. Fits more or less as good as found with (1) could be obtained with either (4) or (5) (figs. 1 and 2). The system was more sensitive to competitive than non-competitive inhibition and $K_i$ was around 1.3 and 5 mM respectively, again both in the presence and absence of cAMP. Again the models were not able to simulate the rise in activity apparently occurring between 8 and 20 mM $Mg_T$ (fig. 2). Similar results were obtained with models assuming both a requirement for $Mg^{2+}$ and sensitivity to inhibition by $ATP^{4-}$.

Change in concentration of $ATP^{4-}$ has a large influence on ionic strength, but far less if ATP is present as $MgATP^{2-}$. This is shown in conjunction with the calculations of complexes (tables 1 and 2). When estimated in terms of nominal concentrations the ionic strength rises steadily as [Mg] or [ATP] is increased. When [Mg] is varied with [ATP] constant the true ionic strength at first decreases then rises when all ATP is in the form of MgATP, and at every point the true ionic strength is lower than nominal. If the activity of the transferase were to increase with increasing ionic strength (e.g. as seen with glucose 6-phosphate dehydrogenase [9]) then some of the present anomalies might be explicable – namely in the range 4–12 mM $Mg_T$ (fig. 2) there would be some flattening of the activity curve and conversely as [Mg] rises from 10–20 mM there would be a steady rise for this reason. The fact that the actual decline in activity with increasing [ATP] is slower than calculated would be consistent with this.

Though the system is a complex one both possibly because of the assay procedure used and the possibility of the enzyme existing in different forms [10], it is clear from the present analysis that there is insufficient evidence to justify the view that the enzyme either requires $Mg^{2+}$ *per se* (though this is not unlikely) or that cAMP lowers the $K_a$ towards magnesium. In the models studied the change in $K_m$ towards MgATP induced by the cyclic nucleotide seems sufficient to account for the changes in activity. The models obviously give no clue as to how the cAMP exerts this influence at concentrations as low as $10^{-7}$ M, though protein adenylation has recently been suggested [11]. If the concentration of $Mg^{2+}$ *in vivo* is several mM [12, 13] an enzyme with a $K_a$ of 0.32 mM or less would be fully active. If most intracellular ATP is MgATP, inhibition by $ATP^{4-}$ would be minimal – though Kerson et al. [12] calculate $[ATP^{4-}]$ and $[KATP^{3-}]$ to be one third of the total [ATP]. A change in $K_m$ towards MgATP from 2 to 0.5 mM might however be very significant.

An unexpected finding in the analysis was the greater resistance to "quenching" of activity by excess ATP of the model requiring compulsory binding of magnesium first as opposed to random order. This arises mathematically by virtue of an extra term in the denominator of the random order equation compared with compulsory order, but its physical

Table 3
Concentration of enzyme-substrate and activator complexes under conditions employed in figs. 1 and 2.

| $[ATP]_T$ | Random order | | | | Compulsory order | | |
|---|---|---|---|---|---|---|---|
| | E | EM | ES | EMS | E | EM | EMS |
| 1 | 0.022 | 0.647 | 0.011 | 0.321 | 0.022 | 0.654 | 0.324 |
| 2 | 0.024 | 0.479 | 0.024 | 0.473 | 0.025 | 0.491 | 0.485 |
| 4 | 0.088 | 0.263 | 0.163 | 0.486 | 0.106 | 0.314 | 0.580 |
| 6 | 0.227 | 0.109 | 0.448 | 0.216 | 0.411 | 0.198 | 0.391 |
| 8 | 0.269 | 0.066 | 0.534 | 0.132 | 0.576 | 0.142 | 0.282 |
| 10 | 0.287 | 0.047 | 0.571 | 0.094 | 0.669 | 0.111 | 0.220 |
| 12.5 | 0.299 | 0.035 | 0.594 | 0.070 | 0.740 | 0.087 | 0.173 |
| 15 | 0.306 | 0.028 | 0.611 | 0.055 | 0.786 | 0.071 | 0.142 |
| $[Mg]_T$ | | | | | | | |
| 1 | 0.568 | 0.099 | 0.283 | 0.049 | 0.792 | 0.139 | 0.069 |
| 2 | 0.357 | 0.144 | 0.355 | 0.144 | 0.553 | 0.224 | 0.223 |
| 3 | 0.233 | 0.168 | 0.348 | 0.251 | 0.358 | 0.258 | 0.385 |
| 4 | 0.154 | 0.181 | 0.306 | 0.359 | 0.222 | 0.261 | 0.518 |
| 6 | 0.062 | 0.190 | 0.184 | 0.564 | 0.076 | 0.233 | 0.691 |
| 8 | 0.020 | 0.189 | 0.077 | 0.714 | 0.022 | 0.205 | 0.773 |
| 10 | 0.008 | 0.194 | 0.032 | 0.766 | 0.008 | 0.200 | 0.791 |
| 15 | 0.003 | 0.198 | 0.011 | 0.788 | 0.003 | 0.200 | 0.797 |
| 20 | 0.002 | 0.199 | 0.006 | 0.793 | 0.002 | 0.200 | 0.798 |

$[Mg]_T$ was held constant at 4 mM when $[ATP]_T$ was raised; $[ATP]_T$ was 8 mM when $[Mg]_T$ was changed. $K_a$ towards $Mg^{2+}$ was 0.08 mM and $K_m$ towards MgATP 2 mM. An essentially similar picture resulted when $K_m$ was 0.5 mM and/or other values of $K_a$ were substituted. Figures are the fractional proportion of the total enzyme concentration in the particular form.

significance is less obvious. If we write the two systems:

$$E + M \rightleftharpoons EM + S \rightleftharpoons EMS \rightarrow E + P + M \qquad (1)$$

and

$$E \begin{array}{l} \swarrow EM \searrow \\ \searrow ES \swarrow \end{array} EMS \rightarrow E + P + M \qquad (2)$$

we can write for (1)

$$\frac{EM}{E \times M} = \frac{1}{K_a} \text{ and } \frac{EMS}{EMS \times S} = \frac{1}{K_m}$$

and for (2)

$$\frac{EM}{E \times M} = \frac{EMS}{ES \times M} = \frac{1}{K_a} \text{ and } \frac{ES}{E \times S} = \frac{EMS}{EM \times S} = \frac{1}{K_m}.$$

(These equations on re-arrangement of course become the same as those used for models (1) and (2) and do not include for (1) the possibility of *ES* as a dead-end complex). Table 3 shows the results of calculation of *E, EM, EMS,* and with random order *ES*, for a value of $K_a$ that would fit some of the data. The more rapid decline of *EMS* in the random order model as $[Mg^{2+}]$ goes down and [MgATP] reaches a limiting value can be seen to result from increasing sequestration of *E* as *ES*. The steady rise in [*E*] as [ATP] increases and fall of [*E*] with increasing [Mg] is also of note. Magnesium is an activator for numerous enzymes. If intracellularly there is ever a "squeeze" on the availability of $Mg^{2+}$, enzymes employing a compulsory order would presumably be less affected than those where the mechanism is random.

## Acknowledgement

I am very grateful to Dr. J. McD. Blair for many helpful discussions.

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