Is Magnesium good for the gut?

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Abstract

Alkaline phosphatase is a phosphomonoesterase that produces a free inorganic phosphate group, this enzyme is found in many organisms, both prokaryotes and eukaryotes. Calf Intestinal alkaline phosphatase (CIAP) regulates bicarbonate secretion, detoxifies lipopolysaccharides, regulates gut microbes and dephosphorylates proinflammatory nucleotides by removing phosphates from the 5' and 3' positions from molecules such as nucleotides, proteins and alkaloids (Singh et al., 2020). It was suggested that Magnesium may play a role in enhancing the activity of CIAP however that has not been confirmed. Therefore, this project aims to identify the effect of Magnesium ions on the activity of CIAP . We hypothesize that CIAP activity in a 5mM of $MgCl_2$ solution will increase with increasing concentration of pNPP substrate, as measured by the Vmax (velocity and efficiency) and Km (affinity) constants through enzyme kinetics analysis. The conclusion obtained from this project is that Magnesium does behave as an activator by increasing the Vmax from 0.105 uM/min to 0.164 uM/min.

Introduction

Alkaline Phosphatase in the Calf Intestines

Alkaline phosphatase is a phosphomonoesterase that produces a free inorganic phosphate group, this enzyme is found in many organisms, both prokaryotes and eukaryotes.

Calf Intestinal alkaline phosphatase removes phosphates from the 5' and 3' positions from molecules such as nucleotides, proteins and alkaloids (Vincent, 2021). In intestines, alkaline phosphatase regulates bicarbonate secretion, detoxifies lipopolysaccharides, regulates gut microbes and dephosphorylates proinflammatory nucleotides (Singh et al., 2020). Alkaline phosphatase has two active sites which are highly conserved and found in every AP regardless of the organism. Each active site accommodates one magnesium ion and two zinc ions. The role of zinc ions in the hydrolysis of phosphate groups is well understood as it attacks oxygen in water and the Ser active site (Behera et al., 2017).

However, the role of magnesium is not well defined. What is known is that it controls the occupancy of the structural and catalytic binding sites to provide the appropriate environment for the reaction mechanism to occur as well as stabilize the dynamic structural properties, therefore it is expected to increase the catalytic activity (Anderson, 1975).

Aim of the study

It has been observed that AP has the highest activity at 45°C and pH of 9.0 pH without the presence of an activator, thus such conditions have been deemed optimal (Behera et al., 2017). Therefore, the aim of the study is to understand the activation effect, if any, of magnesium on the calf intestinal alkaline phosphatase.

This study is important as it will enable a better understanding of enzyme kinetics of CIAP and ways to enhance kinetics of CIAP, in order to utilize CIAP in bioremediation. It is also very useful in molecular biology applications as it non-specifically dephosphorylates substrates.

Hypothesis

We hypothesize that magnesium ions will behave as an activator to alkaline phosphatase under optimal conditions such that it would decrease the Km value and increase the Vmax value. In other words, the presence of magnesium ions will enhance and maximize the CIAP's activity.

Figure A: Magnesium supplements, a source of magnesium that can be ingested. Magnesium is a natural occurring mineral. Magnesium is essential mineral for a healthy functioning body. It is important in many processes such as regulating muscle contraction, maintaining blood glucose levels, bone and DNA formation etc. While magnesium as a mineral cannot be directly ingested by humans, people with magnesium deficiency are prescribed magnesium supplements to boost their magnesium levels for a healthier body.

Methods

Experimental conditions for CIAP activity

The addition of *p*-NPP to alkaline phosphatase results in the dephosphorylation of p-NPP, a colorless substrate, to NPP, a yellow-colored product. Because of the yellow-colored product, the absorbance at 595 nm could be measured using *Thermo Fisher UV60* Spectrophotometer. The absorbance at 595 nm was measured at 30 seconds intervals for 2 minutes in order to determine the velocity of enzyme reaction. In addition to the *p*-NPP and the alkaline phosphatase, a 1.0 M Diethanolamine buffer was also added. The absorbance was carried out thrice for statistical significance. To determine the optimal alkaline phosphatase concentration, we first varied the concentration of alkaline phosphatase while keeping the concentration of the substrate, *p*-NPP, constant. Alkaline phosphatase concentrations of 0.5 units/µl, 1 unit/µl and 2 units/µl were tested. Then, to determine the optimal p-NPP concentration, the optimal alkaline phosphatase concentration was used and kept constant while the p-NPP concentrations were varied. The *p*-NPP concentrations of 0.05 mM, 0.1 mM, 0.2 mM and 0.5 mM. The absorbance at 595 nm were measured. Using these data, Michaelis Menten plot and Lineweaver -Burk plots were generated.

Determining the effect of magnesium ions on CIAP activity

Based on literature review that 1 mM of $MgCl_2$ has no activation effect on the alkaline phosphatase activity (Anderson et al., 1975), a higher concentration of $MgCl_2$ was tested. The 1 units/µl of calf intestinal alkaline phosphatase was used for this experiment along with varying concentrations of (0.05 mM, 0.1 mM, 0.2 mM and 0.5mM) *p*-NPP. A 5 mM $MgCl_2$ solution was added to the AP and *p*-NPP and the absorbance at 595 nm was measured. These scans were triplicates for significant significance. Using the results from the scans, Michaelis Menten plot and Lineweaver -Burk plots were generated.

Results

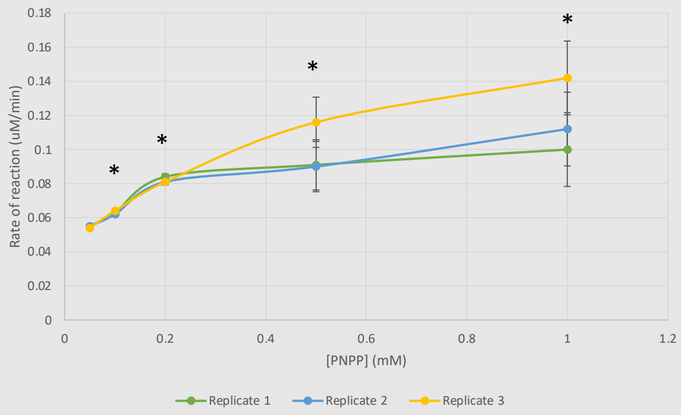

Figure 1. Micheales-Menten plot without magnesium:
The rate of reaction in mM/min plotted against the substrate concentration (pNPP) in mM. Three trials were done at each concentration of pNPP (0.05, 0.1, 0.2, 0.5, 1mM) using 1 unit of CIAP, without the use of Magnesium activator.

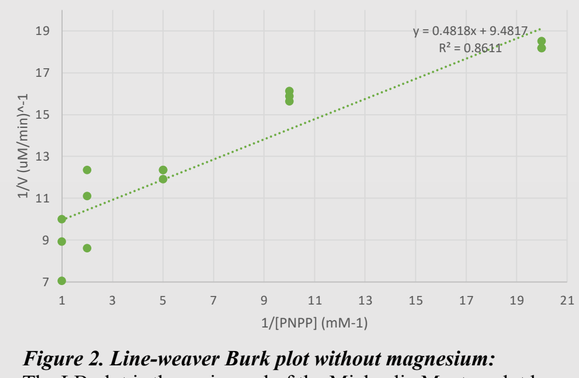

Figure 2. Line-weaver Burk plot without magnesium:
The LB plot is the reciprocal of the Michealis-Menten plot by use of 1 unit of CIAP enzyme all while varying the substrate concentration (pNPP). The rate of reaction in $(mM/min)^{-1}$ is plotted on the y-axis against the substrate concentration pNPP in mM^{-1}. The Km and Vmax values were calculated. The Km is the affinity of the enzyme for the substrate which was found to be 0.0508mM while the Vmax is the efficiency of CIAP which was found to be 0.105mM/min.
Note: Error bars are too small to be represented on the figure.

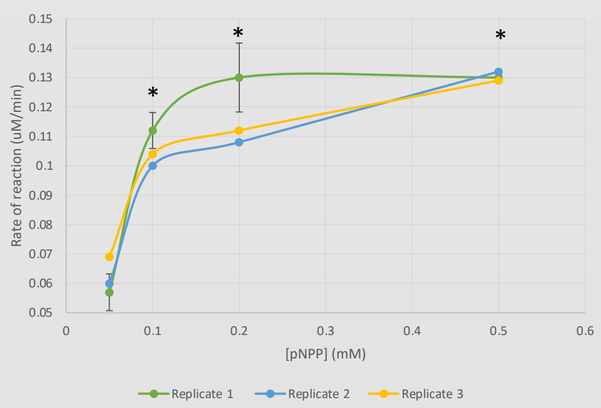

Figure 3. Micheales-Menten plot with magnesium:
The rate of reaction in mM/min plotted against the substrate concentration (pNPP) in mM. Three trials were done at each concentration of pNPP (0.05, 0.1, 0.2, 0.5mM) using 1 unit of CIAP, with the use of 5mM Magnesium chloride activator.

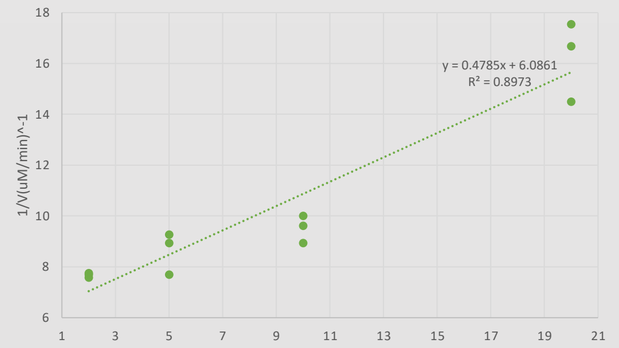

Figure 4. Line-weaver Burk plot with magnesium:
The LB plot is the reciprocal of the Michealis-Menten plot by use of 1 unit of CIAP and 5mM of Magnesium chloride all while varying the substrate concentration (pNPP). The rate of reaction in $(mM/min)^{-1}$ is plotted on the y-axis against the substrate concentration pNPP in mM^{-1}. The Km and Vmax values were calculated. The Km is the affinity of the enzyme for the substrate which was found to be 0.0786mM while the Vmax is the efficiency of CIAP which was found to be 0.164mM/min.
Note: Error bars are too small to be represented on the figure.

Table 1. The base-line Km and Vmax values with and without the addition of magnesium obtained from the equation of the line of figure 2 (without magnesium) and equation of line of figure 4 (with magnesium). The asterisks indicate significance.

Experimental conditions	Without $MgCl_2$	With 5 mM $MgCl_2$
V_{max} (uM/min)	0.105	0.164*
K_m (mM)	0.0508	0.0786*

Discussion & Conclusion

As seen in figures 2 and 4 and table 1, adding magnesium with concentration 5 mM increases the rate of activity of CIAP. This is because we observed a significant increase in Vmax from 0.105 uM/min (Table 1) to 0.164 uM/min (Table 1). This increase in activty is expected as magnesium is expected to behave an activator (Behera et al., 2017). Meanwhile, the Km increased which is not expected. We observed an increase from 0.0508 mM (Table 1) without magnesium to 0.0786 mM (Table 1) with 5 mM magnesium. This could be a result of $MgCl_2$ concentration being too high, as past research has suggested that a 1mM $MgCl_2$ concentration has no effect on the V_{max} or K_m (Behera et al., 2017). Therefore, since there was an increase in the V_{max} it can be said that Magnesium does behave as an activator, however the 5mM concentration of Magnesium may be due to the concentration being too high, which may have lead to decreasing affinity of CIAP since there was an increase in K_m. Perhaps if the Magnesium concentration were higher, it may have had an inhibitory effect on the activity and affinity of CIAP.

In conclusion, the 5mM $MgCl_2$ does behave as an activator due the increase in V_{max}, however in the future a lower $MgCl_2$ concentration could be used to ensure that the V_{max} increases while the K_m remains constant.

Reference:

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