Development of a Method to Quantitate Divalent Metal Ion Dependence of Retinal Phosphodiesterase Enzyme

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Background

Phosphodiesterases make up a superfamily of enzymes that degrade cyclic nucleotides in cells and are important in many cellular signaling mechanisms in the body. Phosphodiesterase enzyme 6 (PDE6) is involved in visual signal transduction, or turning light energy into electrical signals to be sent to the brain and processed. PDE6 has divalent cations involved in its catalytic processes.[1,2]

The type and concentration of metal ion can impact the enzyme's catalytic function. For example, lead ($Pb^{2+}_{(aq)}$) in the retina binds to PDE6 in such a way as to prevent PDE6's catalysis.[3] Picomolar lead ion concentration was sufficient to outcompete a millimolar concentration of magnesium ion, which led to PDE6 inhibition.[3] Other metals are necessary for the catalytic functioning of the enzyme.[2] PDE6αβ binds two zinc ions tightly per catalytic subunit, though activity of the enzyme requires additional metals to be present.[2] The effect of cadmium concentrations on PDE6 activity has not been investigated.

Our research is targeted to quantitate the relationship between free divalent cation concentration and enzyme activity to characterize PDE6's catalytic mechanism for the purpose of aiding future medical developments by enhancing knowledge regarding mechanisms of signal transduction in the eye.

Initial Work

Quantitative zinc solutions were prepared with metal and pH buffers to model a human cellular environment. The buffered solutions were intended to supply precise concentrations of $Zn^{2+}_{(aq)}$ to PDE6 during activity assays

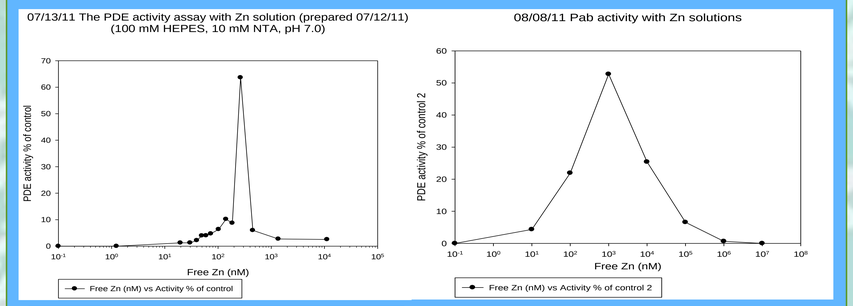


Figure 1a- PDE6 activity in **Zn-NTA** buffer at pH 7 using HEPES. Image courtesy of Wei Yao, buffer soln. prepared by Wei Yao

Figure 1b- PDE6 activity in **Zn-Histidine** buffer at pH 7 using HEPES. Image courtesy of Wei Yao, buffer soln. prepared by Rose Hadley

The enzyme shows maximum activity at ~$10^{2.2}$ nM $Zn^{2+}_{(aq)}$ concentration for the NTA buffer solution (**Figure 1a**) and at ~10^{3} nM $Zn^{2+}_{(aq)}$ concentration for the histidine buffer solution (**Figure 1b**). Different $Zn^{2+}_{(aq)}$ concentrations appear to produce maximum activity with different metal ion buffering systems, which does not make sense.

Results

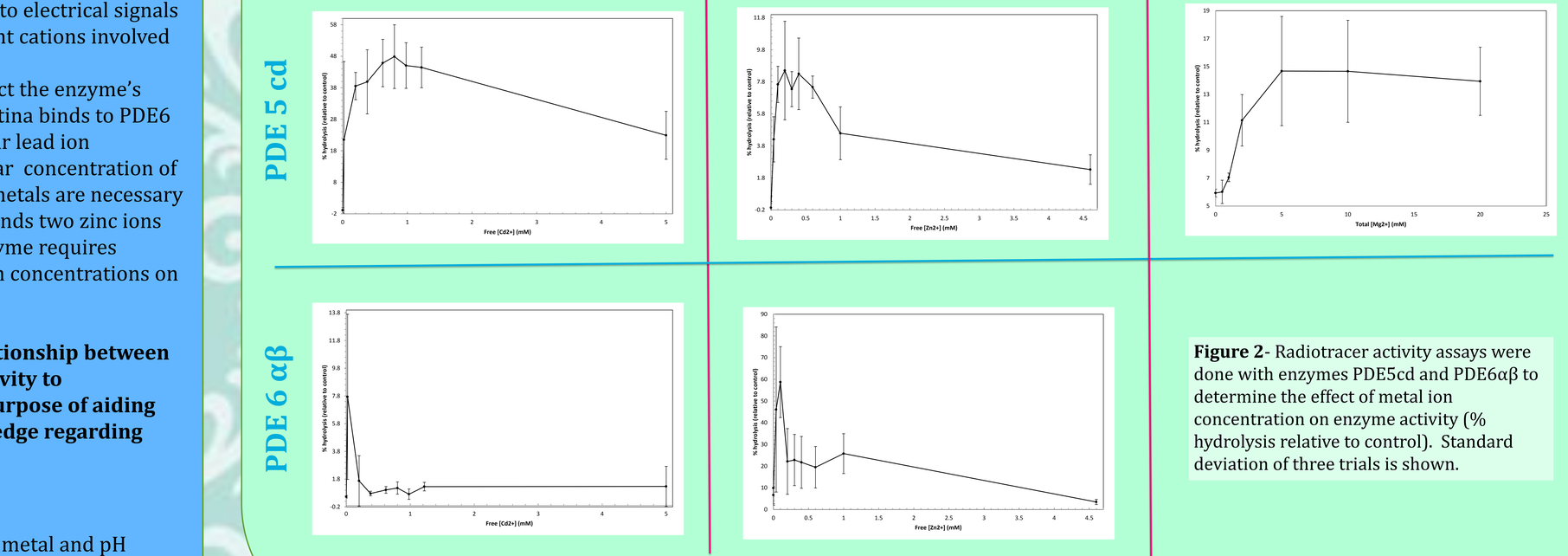
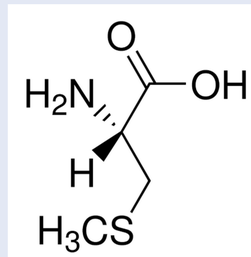


Figure 2- Radiotracer activity assays were done with enzymes PDE5cd and PDE6αβ to determine the effect of metal ion concentration on enzyme activity (% hydrolysis relative to control). Standard deviation of three trials is shown.

Future Work

- Cadmium buffer
- Cadmium ISE to test buffer
- Fluorescence quantitation of buffer system



S-methylcysteine

$log\beta_1$	$log\beta_1$	$log\beta_1$
3.78	7.06	9.63

Acknowledgments

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References

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