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How Cells Communicate: A Study of PKCdelta Phosphorylation Efficiency

Quamrul Hassan

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Introduction

Our bodies are complex systems that, at their root, rely on efficient inter- and intra-cellular communication. One such method of communication is the use of proteins- specifically enzymes- to phosphorylate. Enzymes are proteins that catalyze (speed up) reactions in the body. Phosphorylation is the act of removing a phosphate from ATP and attaching it to a substrate. This is a key biological activity, as many substrates require the addition of phosphate to work properly. One group of important kinases (enzymes that phosphorylate) is the Protein Kinase C group, which includes PKCdelta.

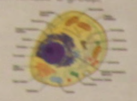
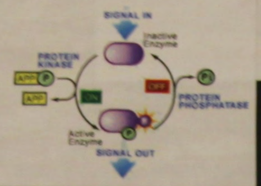


Diagram of Phosphorylation



PKCdelta

Procedure- Phosphate Standard

Add 40 microliter phosphate standard to 360 microliter Assay Buffer

Take 200 microliter of this solution, add to 200 microliter of buffer

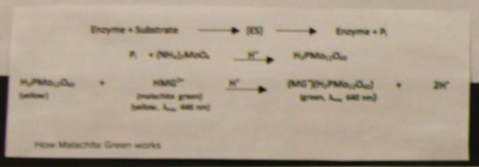
Take 100 microliter of this solution, add to 200 microliter of buffer, repeat this step 6 times to create a serial dilution

Add 15 microliter Malachite Green A, 50 microliter of DI, and 15 microliter of Malachite Green B, incubate for 20 minutes

Measure optical density at 620 nm using UV spectrophotometer

Abstract

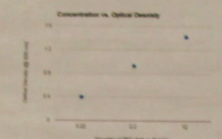
Communication is important for our bodies to function properly, especially at the cellular level. Our bodies utilize enzymes- specific proteins that catalyze chemical reactions- to effectively and quickly communicate many different kinds of messages. PKCδ is an enzyme that has been implicated in membrane transport, as well as the formation of bile in the liver. Any misregulation of PKCδ can lead to cholestasis and liver injury. Utilizing a coupled assay, we will determine the enzymatic activity of PKCδ as it phosphorylates ATP using the UV spectrophotometer. After we have established the standard curve, we will determine the peak enzymatic activity (using the Michaelis-Menton equation) with different variables, such as temperature and enzyme/substrate concentration.



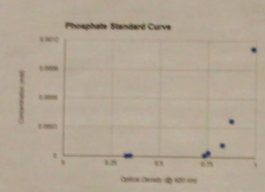
How Malachite Green works

Data

	Trial 1	Trial 2
Negative Control (buffer)	.240A	.478A
Low PKCdelta (.22 microliter)	0.57A	.397A
Medium PKCdelta (2.2 microliter)	0.882A	0.624A
High PKCdelta (12 microliter)	N/A	1.476A
Positive Control (ADP)	0.9A	2.50-3.00A



Phosphate Standard Curve



At this time, this standard curve is not usable for our experiment because it is not a linear correlation. The establishment of the curve was done twice, but the data has twice-given an exponential curve.

Procedure- Kinase Assay

Create substrate mix- 3.7 microliter ATP, 11.4 microliter peptide

Create enzyme mix- 2.2 microliter CP4, varying PKC delta volume (.22, 2.2, 12 microliter)

Positive Control- ADP instead of ATP

Negative Control- Buffer instead of kinase

Add substrate mix to each sample of enzyme mix, adding buffer until 114 microliter total volume is reached. Incubate for 10 minutes

Add 43 microliter of Malachite Green Reagent A

Add 100 microliter of DI

Add 43 microliter of Malachite Green Reagent B, incubate for 20 minutes

Load cuvette into UV spectrophotometer, measure optical density at 620 nm

Materials

R&D Systems Universal Kinase Assay Kit

SignalChem MARCKS Peptide

Enzo Life Sciences PKC delta

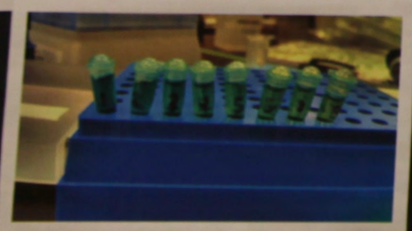
Deionized water

Micropipette

Cuvette

UV Spectrophotometer

Phosphate Standard developing with Malachite Green Reagents



Cuvette in the UV Spectrophotometer

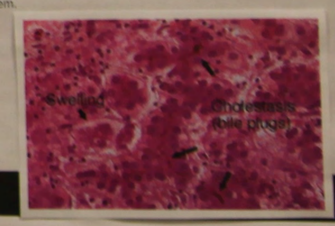
Discussion

While we are unable to utilize the data we have (as the phosphate curve is not ideal,) there are still many things to be taken away from this experiment. Primarily, the initial work-up of ideal molarity and amounts of each solution were important for my own development and understanding of the experimental process. It is also a good example of why almost all experiments rely on repeated trials, as more trials reduce the influence of extreme or bad data on the final averages. It is important to note that we were able to repeat the standard curve and the actual experiment only twice with our materials. Ideally, we would continue to repeat the experiment until the data were consistent, which would provide a better baseline to draw conclusions from.

Cuvette with a sample

Why does this matter?

PKC delta had been implicated in the development of cholestasis, which is any disorder where the liver either stops or severely reduces excreting bile. If a direct link between PKC delta and any aspect of bile formation can be established, then it may be possible to utilize some aspect of how PKC delta works in liver cells to treat the disorder. This targeted therapy would help to reduce the need for surgery, and could potentially reduce the negative side effects associated with the currently-used drugs by replacing them.



References

R&D Systems, Inc. Universal Kinase Activity Kit Manual

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http://bitesizebio.com/7214/ask-a-chemist-how-colorimetric-assays-work/

http://www.scq.ubc.ca/protein-phosphorylation-a-global-regulator-of-cellular-activity/

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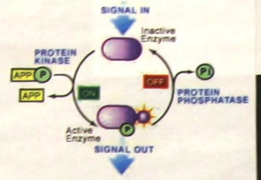
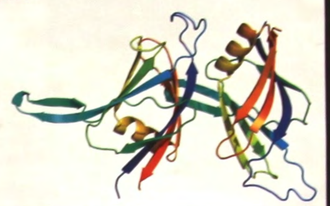


PKCdelta



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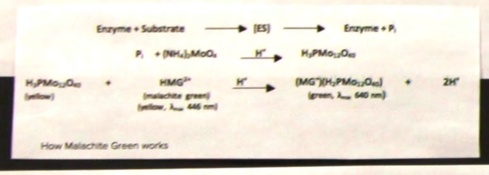

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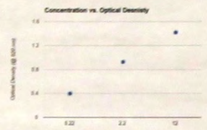

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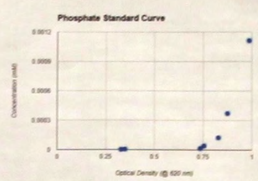

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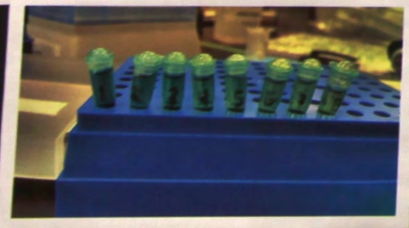

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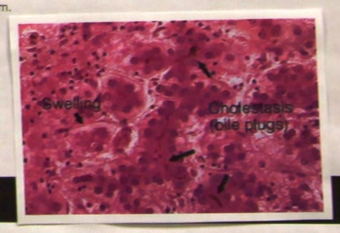

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