Paya Lebar Methodist Girls' School (Secondary)

To investigate the effect of heavy metal ions on catalase in liver on the breaking down of hydrogen peroxide

By: Isabel Chin, Elena Povazanec, Mercia Yoong and Amanda Grace

Abstract

An investigation is performed to find out the impact of extended exposure of heavy metal ions on liver tissues. Common heavy metal ions such as iron(II), copper(II), cobalt(II) and zinc were chosen. Catalase is one of the liver enzymes which can break down toxic hydrogen peroxide into water and oxygen. The enzymatic functions of catalase could be inhibited after heavy metal ions exposure. Various experiments were performed using 4 different heavy metal ion solutions, from which a volume of oxygen gas was collected. Our datalogger showed that all but cobalt(II) ions competitively inhibit the catalase catalysis. Hence, lower percentage of oxygen gas was recorded. We advise monitoring of low exposure to heavy metal ions to safeguard healthy liver and human development.

Introduction

In this investigation, we investigated how the heavy metal ions affected the enzyme catalase in pig liver. We have chosen to use copper (II) sulfate, iron (II) sulfate, zinc nitrate, cobalt (II) chloride and distilled water as control. The metal ions we have chosen are heavy metal ions. While some heavy metal ions may be needed by our body for maintaining the metabolism rate, it can cause poisoning if there is an increase in concentration through accumulation. Heavy metal ions tend to bioaccumulate*, which posts a larger risk for heavy metal poisoning of living organisms. Heavy metal ions can negatively affect the human body as it acts as an enzyme inhibitor. Enzyme inhibitors can be competitive or noncompetitive inhibitors. To break up toxic substances, the liver uses specialised enzymes like catalase to help it break them down, making it safer for the body to process. Catalase breaks down hydrogen peroxide into water and oxygen. Heavy metal ions can enter our bodies via food, drinking water and air. As a chemical element that is only needed in little amounts in a particular environment, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations or when accumulated over time, they can lead to poisoning and health difficulties. Through investigating the effect of heavy metal ions on catalase in liver on the breaking down of hydrogen peroxide, we are able to conclude which heavy metal ions are the most dangerous to the human, particularly the liver, and provide research that may help in future investigations on heavy metal poisoning and its cure.

Method and Procedure

1. Cut six 2 cm by 2 cm cubes of liver (pig)
2. Place 1 liver cuboid in the different solutions (30 cm^3 , 0.5 mol/dm^3) for half an hour (in beaker)
   a. distilled water(control)
   b. iron(II) Sulfate [$FeSO_4$]
   c. cobalt(II) chloride [$CoCl_2$]
   d. copper(II) Sulfate [$CuSO_4$]
   e. zinc nitrate [$Zn(NO_3)_2$]
   f. distilled water(control) (liver was denatured by boiling)
3. Remove the liver from each solution and place it into a boiling tube of 25 ml hydrogen peroxide.
4. Immediately seal and attach data logger to record percentage of oxygen
5. Record results and compare.
6. Repeat Experiment another two times.

Figure 1

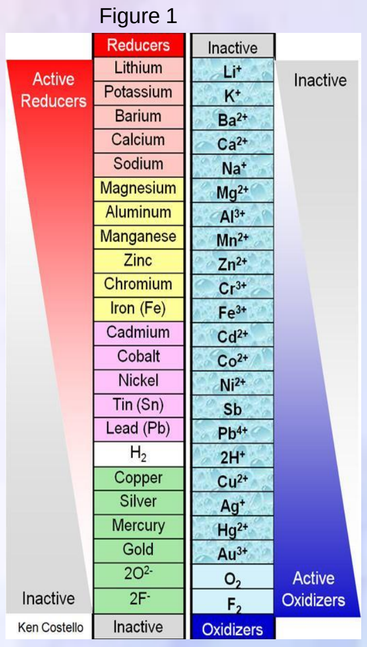

Figure 2

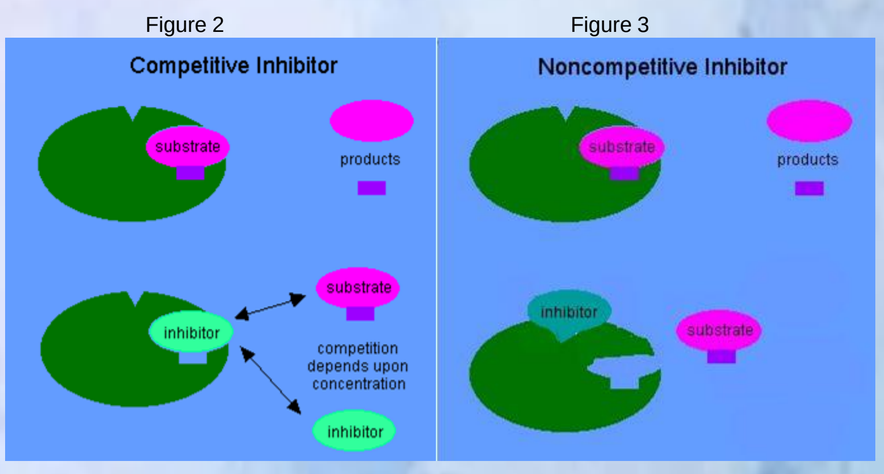

Figure 3

Keywords and definitions:

Competitive inhibitor-blocks the action of an enzyme by binding to its active site and prevents binding of the substrate

Noncompetitive inhibitor-reduces the activity of the enzyme and binds equally well to the enzyme whether or not it has already bound the substrate.

Free radical-Groups and atoms that are formed at oxygen's interaction with specific molecules, having an odd number of electrons

bioaccumulate-Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted.

References

1. PUB (2016). *Our Drinking Water Quality.* Retrieved from https://www.pub.gov.sg/Documents/WQ2016.pdf.
2. Lenntech (n.d.) *Heavy Metals.* Retrieved from http://www.lenntech.com/processes/heavy/heavy-metals/heavy-metals.htm
3. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). *Heavy Metals Toxicity and the Environment. EXS, 101,* 133–164.
4. Edward Group (March 18, 2013). *The Effects of Toxic Metals. Retrieved from http://www.globalhealingcenter.com/natural-health/effects-of-toxic-metals/*
5. Science Buddies (March 8, 2012). *The Liver: Helping Enzymes Help You!.* Retrieved from https://www.scientificamerican.com/article/bring-science-home-liver-helping-enzymes/
6. Chemguide (n.d). *Enzyme Inhibitors.* Retrieved from http://www.chemguide.co.uk/organicprops/aminoacids/enzymes3.html
7. MadiScience Network. (Sept 27, 1999). Where Does Hydrogen Peroxide Come From in the Body? Retrieved from http://www.madsci.org/posts/archives/1999-09/938519528.Bc.r.html
8. L Mizrahi . (July, 1989). *Effect of heavy metals ions on enzyme activity in the Mediterranean mussel, Donax trunculus* Retrieved from https://www.researchgate.net/publication/20611449_Effect_of_heavy_metals_ions_on_enzyme_activity_in_the_Mediterranean_mussel_Donax_trunculus

Theoretical Background

Once heavy metal ions start to bioaccumulate in dangerous amounts after they enter our body, they cause damage at the cellular level by initiating oxidative stress, imbalancing the production of free radicals* and the body's ability to neutralize antioxidants to work against its harmful effects. Heavy metal ions are strongly bound by sulfhydryl (Made of a sulfur bonded to hydrogen atom) groups of proteins which changes the structure and enzyme related activities of proteins, causing toxic effects. Heavy metal ions with sufficiently high concentrations might kill organisms or result in other negative effects that change aquatic community structures. Heavy metals inhibit the activity of a wide range of enzymes like respiratory enzymes and digestive enzymes. This damage develops into many diseases and health problems as it is absorbed and accumulated in the liver. Research shows higher mortality rates of liver cancer in areas suffering from heavy metal overexposure. Catalase is found in nearly all living organisms that are regularly exposed to oxygen. This enzyme is the catalyst behind decomposition of hydrogen peroxide into water and oxygen, protecting it from oxidative danger/oxidative stress. Competitive inhibitors has a similar shape to the usual substrate for the enzyme, and competes with it for the active site [Figure 2]. However, once it is attached to the active site, it is rendered useless. A non-competitive inhibitor doesn't attach itself to the active site, but attaches somewhere else on the enzyme [Figure 3]. By attaching somewhere else, it affects the structure of the enzyme and the way the enzyme works. Because there isn't any competition involved between the inhibitor and the substrate, increasing the substrate concentration won't help.

Data collected

Solutions used	Average out of 3 experiments: (% of oxygen)
Distilled water (control)	30
iron(II) sulfate [$FeSo_4$]	22.1
cobalt(II) chloride [$CoCl_2$]	25.0
copper(II) sulfate [$CuSO_4$]	22.2
zinc nitrate [$Zn(NO_3)_2$]	22.7
Distilled water(control) (liver was denatured by boiling)	21.2

Discussion and Conclusion

The higher the percentage of oxygen in the test tube, the higher the amount of oxygen produced, the less effect on catalase in pig liver breaking down the hydrogen peroxide, and thus the less harmful it is to the liver. According to data collected, the heavy metal solution that resulted in the least harmful impact of inhibiting the enzyme catalase in the pig liver from breaking down hydrogen peroxide was cobalt (II) chloride. The most harmful solution was iron (II) sulfate.

The metallic solutions ranked in the order of least harmful to most harmful in inhibiting catalase: cobalt , zinc, copper and iron. We hypothesized that the reactivity of the metal had an effect on inhibiting the catalase in liver [Figure 1]. The most reactive metal would most probably be the most harmful, as it tends to react and bind to the active site of the catalase more readily and stops it from working. If this hypothesis was true, the trend would follow in the order of copper, cobalt, iron and zinc from least to most harmful, which proved otherwise based on our experimental data.. Hence, reactivity of the metals used in the solutions have no correlation to their inhibition on the catalase in liver.

Another factor we considered was the size(atomic number) of the metals used in the solutions. The smaller the size of the metal ion, the greater the harmful effect on the liver catalase. Based on this hypothesis, the trend would be iron, cobalt, copper and zinc, from the least to greatest inhibition effect. Though the data did not reveal a direct correlation between the size of the metal ion and their inhibition on the catalase in liver, it revealed that the smaller metallic ions [copper and zinc] had a greater harmful effect over the slightly larger metallic ions [iron and cobalt]. The type of inhibition of the metal ion on the catalase [competitive vs non-competitive] could have also affected our experimental results. Due to the limitation of equipment, we were not able to pinpoint the type of inhibition that each metal ion had on the catalase.

Other variables that were not within our control were the amount of enzymes in each liver cuboid even though we tried to use liver cuboids with the same size (surface area). The different negative ions in the metallic solutions could also have an effect on the inhibition ability as we could not have the same negative ions for all metal solutions due to the limited chemicals we had in school.

However, the effect of metal ions inhibition on the catalase in liver had a great harmful effect as the percentage of oxygen [22. 1 to 22.7 %] for iron, copper and zinc] was close to the percentage of oxygen for the totally denatured liver [21.2%]. Cobalt also significantly reduced the percentage of oxygen, by 5% , compared to the control where the liver was not denatured and by 3.8% for the denatured liver. Inhibition of catalase activity in liver is critical as the liver controls important cellular functions and the breaking down of toxic substances in our bodies. Hence, we advise the close monitoring of concentration of metal ions in our Singapore waters. In the quality of water report published by PUB [2016], the concentration of 9 types of transition metals and 4 non-transition metals have been monitored by PUB. Praise to PUB for safe guarding the quality of our Singapore waters!