Exchange of the Magnesium Ion in Chlorophyll by a Transition Metal

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Abstract

The purpose of this research is to determine the catalytic properties of chlorophyll when the central metal ion is exchanged for a different metal. The Chlorophyll molecule contains Magnesium as the central atom in its natural form and is used in plants, algae, and cyanobacteria as an electron transfer molecule in the process of photosynthesis. The two subclass molecules are a and b which only difference is that Chl-a has a methyl group and Chl-b has an aldehyde (formyl) group. The molecule is composed of a heterocyclic ring, known as a porphyrin or chlorin (in reduced form) with a Magnesium atom at its center and a long hydrocarbon (phytol) chain. The investigation focuses on the development of different heavy metal complexes that can be made using the chlorophyll molecule, and further analyze the catalytic properties of metalloporphyrins. Analysis of wavelength change in the complex will indicate a change in its property and the effect it may have on different reactions.

Introduction

Chlorophyll is a major class of macromolecules used in photosynthesis by plants, algae, and cyanobacteria. The reason for this is that the chlorophyll is involved in oxidation reduction reactions, by transferring absorbed energy and exciting electrons. The heterocyclic molecule in chlorophyll also known as a porphyrin has long been studied and it has shown to exhibits catalytic properties.

This investigation proposes to examine the ability to exchange the Mg2+ ion for a different heavy metal substituted chlorophylls (Chl-HMS) to further study their application as catalysts in organic reactions. The central atom coordinated in the porphyrin ring makes the complex absorb light of specific wavelength. When the magnesium of the chlorophyll is substituted for the Fe^{2+}, Cu^{2+}, or Zn^{2+} ions it is expected that the wavelength of light being absorbed shifts.

Methods

Sample preparation:

10 grams of frozen spinach was combined with 10 grams of anhydrous magnesium sulfate and 10 grams of sand. The mixture was grinded for 20 minutes in a mortar until a fine green powder was obtained. The green powder was transferred into a 500 mL beaker and 50 mL of acetone were added and mixed for 5 minutes. The mixture was allowed to sit still for 10 minutes. Then the sample was extracted using a syringe and placed in a 50 mL beaker. The solution extracted amounted to 45 mL. (Extract A).

Another sample was prepared using the same method except for transferring the extraction. The mixture was instead placed in vials for centrifugation, and the liquid was then removed by pipette and placed in two 250 mL flat bottom flasks. One of which was subjected to rotary evaporation in order to obtain a concentrated extract for later use. (Extract B (concentrated) and C)

TLC Preparation:

TLC Plates (2.5 cm x 7.5 cm). A solution of 70% hexane and 30% acetone was prepared for the elution in a closed chamber. The extract application was performed in the standard manner. Extract A was used for TLC analysis. The same solvent was used for pheophytins.

Column Chromatography:

A solution of 60% hexane and 40% acetone was prepared for column chromatography.

A column was dry packed with silica gel (40-60µm, 40A), and the concentrated extract was added for elution. The fragments that were collected and not discarded were chlorophyll a and b. Extract B was used for Column chromatography.

Methods

Demetallation of Complex:

- Dowex 50WX2-100 Strong cation exchanger was used to remove magnesium from chlorophyll fragments collected. 1 gram for every 1 mL was used in order to remove the Mg ion. This procedure was performed using extract A. TLC was performed to observe this change that resulted in the loss of the Mg ion and the appearance of pheophytin a and b.
- Treatment with Acid: A solution of 2M HCl was used to remove Mg from the extract solution, this was done by placing 15 mL of extract C in a 100 mL and adding 5 mL of acid dropwise under stirring. The stirring continued for one hour until TLC was performed and the results showed the appearance of pheophytins.

Synthesis of metal substituted complex:

Procedure 1 - Purchased Chlorophyll-a was used at a concentration of 1.44e-03 M/L. Two reactions by the following method were performed.

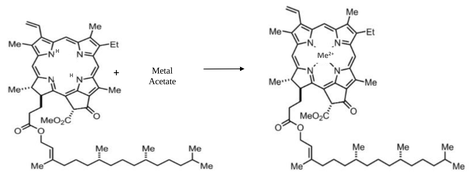
- Chlorophyll-a was dissolved in a 2:1 (v/v) chloroform/glacial acetic acid mixture.
- An excess of zinc acetate and copper acetate were added to each flask.
- 2 hours under magnetic stirring at room temperature.
- Mixture was then washed with DIH2O and were later used for characterization.

Procedure 2 – Following demetallation of chlorophylls one by resin method and one by treatment with acid.

- Resin pheophytins were collected and then added 200 mL of 95% ethanol solution.
- 3g of $CuCl_2$ and $FeCl_2$ were added to solution and brought to a boil at 52º C.
- Placed under magnetic stirring and reflux for one hour.
- Removed from heat and remained under stirring for 24 hours.
- Mixture was then washed with DIH2O to remove excess copper, and 200 mL of 60% hexane and 40% acetone solution were added to extract complex by liquid-liquid extraction.
- Extract was placed under rotary evaporation until dry and collected from flask with acetone for later characterization.

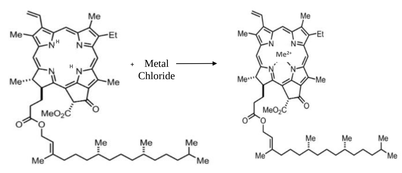
UV-Vis Spectroscopy: PerkinElmer Lambda 950

Several UV-Visible absorption spectroscopy samples were prepared from the extracts and analyzed under UV-Vis.



Metal Acetate: $Cu(CH_3COO)_2$, $Zn(CH_3CO_2)_2$

Figure 1: Procedure 1



Metal Acetate: CuCl2

Figure 2: Procedure 2

Results

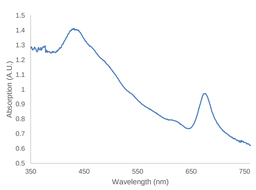


Figure 3: UV-Vis Spectra Chlorophyll a and b.

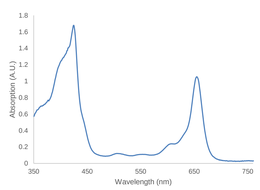


Figure 4: UV-Vis Spectra Chlorophyll a and b extracted from spinach.

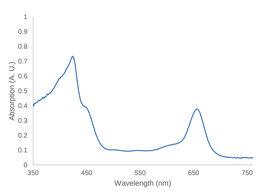


Figure 5: UV-Vis Spectra of the spinach extract.

Results

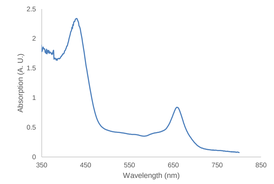


Figure 6: UV-Vis Spectra of Zn reaction.

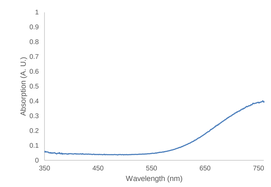


Figure 7: UV-Vis Spectra of Cu reaction.

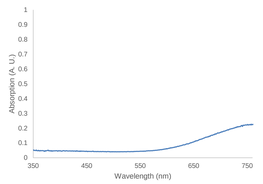


Figure 8: UV-Vis Spectra of Cu reaction (top layer).

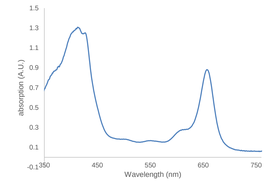


Figure 9: UV-Vis Spectra of Cu reaction (bottom layer).

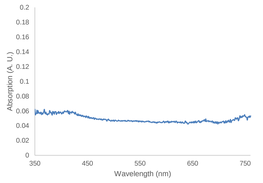


Figure 10: UV-Vis Spectra of Zn reaction (top layer).

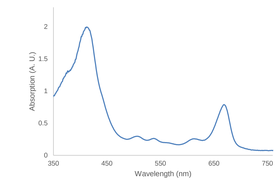


Figure 11: UV-Vis Spectra of Zn reaction (bottom layer).

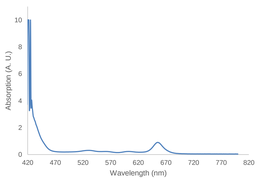


Figure 11: UV-Vis Spectra of Fe reaction.

Conclusion and Discussion

The Results show some coordination of the metal in the porphyrin ring. The shifts indicate binding of the new metal to the chlorophyll molecule, thus providing evidence that substitution took place and now instead of magnesium being bonded to the macrocycle the new metal has taken its place. The shift from normal chlorophyll UV spectra 430nm and 660nm, the new metal ion caused the shift in absorption. Further analysis can be done to confirm the present characterization. Coordination of these metals could provide insight into catalysis and the application in different areas.

Acknowledgements

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