CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

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Project Number

S0414

Project Title

Investigative Study on Pigmented Gallstones: Is Cu(II)-Induced Oxidation of Bilirubin Responsible for Their Formation?

Abstract

Objectives/Goals

The goals of this project were to determine a) whether Copper-induced oxidation of Bilirubin leads to the bluish black pigment noted in Pigmented Gallstones in patients with hemolysis, b) the structures of the species involved, and c) what other metals participate in the formation of the stones.

Methods/Materials

Materials: Bilirubin (BR), Copper, Zinc and Calcium salts, Potassium superoxide, cholesterol, deoxycholic acid, Varian 500 MHz NMR spectrometer, Bruker ELEXYS EPR spectrometer, Cary 50 UV/Vis Spectrophotometer.

Methods: By varying the ratio of Zinc acetate and BR in DMSO, the formulation of the Zn(II) complex of BR was determined by spectrophotometry and Nuclear Magnetic Resonance Spectroscopy. The structure of the Cu(II)-BR was investigated with the aid of spectrophotometry and Electron Paramagnetic Resonance (EPR) spectroscopy. The formation of bluish purple pigment by the Copper-BR complex at various pH was determined by spectrophotometry. Formation of pigmented solid by the pigment forming Cu-BR solution in presence of cholesterol, calcium salts and bile acid was carefully monitored over time. Finally, Electron Emission Spectroscopy was employed to confirm the presence of Copper and Calcium in both synthetic and authentic gallstones.

Results

A 1:2 BR:Zn complex was identified in aprotic solvents, that readily decomposed in water. In contrast, the 1:1 BR:Cu complex (determined by spectrophotometry and EPR Spectroscopy) was found to be stable in aqueous solution. EPR measurements indicated the formation of radicals in such solution, presumably via redox cycling of the Cu(II) ion. This complex readily turned dark purple in air. The darkening reaction was accelerated by the addition of Potassium superoxide. In presence of cholesterol, bile acid, and calcium salts, the Copper-BR complex gave rise to bluish black granules over days. In aprotic solvents, the Cu-BR complex was stable and did not give rise to radicals (as indicated by EPR data). Close examination of the solid pigmented granules as well as authentic pigmented gallstones indicated the presence of Copper and Calcium ion.

Conclusions/Discussion

Among the biologically relevant metal ions (Zn, Cu, Ca), only copper initiates formation of O-based radicals in aqueous solution. A radical based polymerization of BR leads to bluish black pigment which imparts the signature color of pigmented gallstones from patients with hemolytic episodes.

Summary Statement

My project aims to determine the role of Copper in the oxidative polymerization of Bilirubin to form Pigmented Gall Stones.

Help Received

Nicole Fry, a graduate student at UCSC helped me in the NMR experiments; Christopher Dudzik, a graduate student in the Millhauser lab, provided assistance in the EPR measurements; The Electron Emission measurements were performed by Mr. Rob Frank of the UCSC analytical lab.