CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)

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Project Number

S0621

Project Title

The Dance of the Calcium Cage

Abstract

Objectives/Goals

This aim of this project is to discover why EGTA-decalcified gastrolith solutions produce Nuclear Magnetic Resonance spectra with twice the number of expected peaks. My initial hypothesis was that EGTA, in the presence of calcium, is in chemical exchange between two different populations with chemically distinct environments

Methods/Materials

NMR Spectrometers: I used three different NMR spectrometers: Bruker DPX-200, AV-III 400, and AV-III 600. Between these three instruments, over 54 spectra were recorded, creating a large database from which to find patterns, assign the 1H and 13C spectra, and monitor the suspected dynamic equilibrium of EGTA-Ca++ binding and release over a variety of temperatures and concentrations. Samples: At the start of the project, four D2O solutions of different Ca:EGTA N,N,N′,N′-tetraacetic acid) ratios (0:1. 0.5:1, 1:1, and 2:1) were prepared by Ms. Anat Akiva. DSS (2,2-Dimethyl-2-silapentane-5-sulfonic acid) was subsequently added to the samples as a reference (δDSS = 0 ppm) to calibrate the chemical shifts (x-axis) of the NMR spectra.

Results

With a Ca:EGTA ratio of 0.5:1, I observe a combination of the two EGTA spectral populations previously observed ("free" and "bound"). The peak assignments for the 0.5:1 Ca;EGTA spectrum are shown as a combination of the 0:1 and the 1:1 ratio spectra. Next, several experiments were conducted on the 0.5:1 Ca:EGTA sample under various temperatures (304K, 320K, 330K, 336K, 340K, and 343K) in the 200MHz spectrometer. If the free and bound states are in dynamic equilibrium, by heating the solution, I expected to observe the classic textbook behavior of the bound and free resonance frequencies experienced by the same proton as it broadens and merges on the NMR timescale. The peaks on the spectra did merge together, revealing a dynamic equilibrium between the free and bound states.

Conclusions/Discussion

EGTA, in the presence of calcium, has been proven to exist in a dynamic equilibrium between free and caged states. When there is a non-stoichiometric ratio of EGTA to calcium, there is a combination of spectra from the free and caged states, representing a slow dance of the calcium cage. Also, the peak pairs merge as the temperature increases, indicating that the EGTA cage has begun a faster dance on the NMR timescale. Now that this has been settled, gastroliths can be sampled again, so bone regrowth research can be continued.

Summary Statement

Discovering the chemical exchange between calcium and EGTA so bone regrowth can be achieved.

Help Received

Used lab equipment and received all materials from Dr. Yael Balazs at the Technion University in Haifa, Israel.