

Analysis of the Crystal Structure of Oxalate Kidney Stones

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Introduction

This report will summarize the work performed under this Sigma Pi Sigma Undergraduate Research Award. As described in our original proposal¹, our work is divided into two distinct parts: computer modeling and microscope work. However, both of these approaches seek to answer the central question of this field: how do the kidney stones “stick” together?

Most kidney stones consist of sub-micron calcium oxalate crystals which we believe are “glued” together with an organic matrix. Opinion amongst researchers is divided into two camps: those who believe that proteins are the main component of this organic matrix², and those who believe phospholipids play this role^{3,4,5}. The immediate goal of our research is to settle this question.

Experimental Work

Our experimental work follows, in turn, two complementary paths. We use a Field Emission Scanning Electron Microscope (“FESEM” or simply “SEM”) to study stone topography and composition, and an Atomic Force Microscope (“AFM”) in liquid to study the change in stone topography during immersion in a protease.

SEM Microscopy with Backscatter Imaging

Merely observing kidney stones using an SEM is not trivial, as kidney stones do not conduct electricity. Typically, an SEM sample must be conductive and grounded in order to prevent incoming electrons from building up on the surface of the sample – a phenomenon known as “charging”. However, the mechanism of SEM imaging in itself creates a small outgoing current (consisting of secondary and backscattered electrons) whose magnitude is dependent

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upon the material being imaged. Since the incoming current is a function of accelerator voltage, we program the SEM to use a voltage that allows us to image the material clearly and without charging; for most kidney stones, this occurs in the range 2.5 kV to 3.0 kV.

Our early sessions on the SEM were to investigate the topography of the stones. We did this in part as practice, and in part as a test of a hypothesis tangential to our main inquiry. In the kidney is a nipple-like protrusion known in medical terminology as the renal papilla. Many stones exhibit a dimple (see Figure 1) in the surface which would fit the papilla. A.P. Evans⁶ suggests that oxalate stones nucleate on calcium phosphate deposits on the papilla known as Randall's plaques. Thus we hypothesized that dimpled stones should contain characteristic phosphate formations in the dimple. We examined several dimpled stones from our collection but were unable to find any trace of these formations.

Our later work revolved around the backscatter detection capacity of our SEM. As described in the SEM literature, backscattered electron intensity is correlated to the atomic number of the atom with which they interacted. Although we cannot exploit this fact to directly measure the presence (or absence) of phospholipids, there is a compound – osmium tetroxide – which binds to phospholipid, but not protein, calcium oxalate or any other major stone constituent. We attempted to map the phospholipid content of our sample stones through backscatter imaging. Unfortunately, the photomultiplier tubes our SEM was equipped with were unable to amplify the signal sufficiently to allow us to acquire usable data, unless we raised the accelerator voltage to levels that caused excessive charging. We believe our method is sound, but we have encountered an equipment limitation which the cost of overcoming is far too great for an undergraduate research award.

AFM Microscopy in Liquid

Our other empirical approach follows the suggestion of Rosemary Ryall⁷. We use a protease, Cathepsin-D, to dissolve the organic matrix while conducting rapid, continuous AFM scans over the surface of the stone. This will yield a series of pictures showing the gradual “peeling away” of the matrix. We cannot simply compare “before” and “after” images of the stone as the revealed topography is so different to the original that it is impossible to correlate locations between the two.

We have completed some preliminary work, as described in the interim report. Two problems confronted us at that point, and they remain with us now. Firstly, the AFM is not calibrated correctly – images appear skewed and length scales are distorted; thus we cannot collect publishable data. This problem has yet to be rectified by the university's support staff. Secondly, when the protease is applied to the surface of the stone, it causes the organic matter to dissolve, which lowers the scanned surface. Just as the LP on a record player must be at a certain height relative to the stylus base for the needle to make contact, so the scanned surface must stay within a certain height range for the AFM tip to make contact and give a reading. The challenge is to find the correct dilution of protease such that the stone dissolves slowly enough to get accurate AFM scans. We believe we could achieve this, but we are not going to spend time on it until the first AFM issue is fixed.

Theoretical Work

The focus of our theoretical work is on attempting to fit protein structures to calcium oxalate, rather like a jigsaw puzzle. The strength with which a given protein binds to calcium oxalate is roughly proportional to how close aspartic and glutamic residues in the protein can be brought to the calcium ion in calcium oxalate. We will build upon the work of Asiya Gul⁸, who evaluated various candidate proteins by visually attempting to achieve the matching described above. However, we shall take a more rigorous approach, namely, by using a freely available quantum chemistry program⁹, attempt to find the lowest energy configuration of such a macromolecular bond. Unfortunately, because the author (Bruce) was unable to attend school due this fall due to a personal situation, little work has been done on this since the interim report.

Conclusion

This Undergraduate Research Award has allowed us engage in genuine, practical research. In so doing, we have experienced the difficulties and tribulations of such research. We believe that our work has contributed to the science of this field; we expect some of our results to be incorporated into future published works. We would in closing like to express our gratitude to Sigma Pi Sigma and SPS, whose generosity made this possible.

References

¹B. Nourish, S. Sandersius et al. (2004), Analysis of the Crystal Structure of Oxalate Kidney Stones, unpublished grant proposal

²S.R. Khan, B. Finlayson, R.L. Hackett (1983), Stone matrix as proteins absorbed on crystal surfaces: A microscopic study, *Scanning Electron Microscopy* 1983:379-385

³S.R. Khan, P.A. Glenton (1995), Increased urinary excretion of lipids by patients with kidney stones, *British Journal of Urology* 77:506-511

⁴S.R. Khan, P.N. Shevock, R.L. Hackett (1988), Presence of lipids in urinary stones: Results of preliminary studies, *Calcified Tissue International* 42:91-96

⁵S.R. Khan, P.A. Glenton, R. Backov, D.R. Talham (2002), Presence of lipids in urine, crystals and stones: Implications for the formation of kidney stones, *Kidney International* 62:2062-2072

⁶A.P. Evans et al. (2003), Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle, *Journal of Clinical Investigation* 111:607-616

⁷Ryall, R.L., Fleming D.E., Doyle, I.R., Evans, N.A., Dean, C.J. and Marshall, V.R.: Intracrystalline Proteins and the hidden ultrastructure of calcium oxalate urinary crystals: Implications for kidney stone formation, *Journal of Structural Biology* 134:5-18

⁸Asiya Gul (2005), A modeling study of the role of proteins in calcium oxalate kidney stone formation, unpublished masters thesis

⁹HARLEM, by Igor Kurnikov, http://www.kurnikov.org/harlem_main.html