QnAs with Lia Addadi

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Lia Addadi has spent her career studying biomineralization: the formation of crystals in organisms. She has explored a wide variety of questions about the formation and structure of crystals in organisms in both physiological and pathological contexts. Her research on crystal formation in gout, atherosclerosis, and osteoporosis could lead to a better understanding of how these diseases occur. A professor of chemistry at the Weizmann Institute of Science, Addadi was elected a foreign associate to the National Academy of Sciences in 2017. In her Inaugural Article, Addadi and colleagues investigated the formation of cholesterol crystals in a cell-culture model of atherosclerosis (1). She recently spoke to PNAS about her findings.

PNAS: How did you become interested in studying biomineralization, particularly in a pathological context?

Addadi: During and immediately after my PhD, I started working with crystals, how crystals grow, and how their structure is related to their morphology. By chance I met a colleague, Steve Weiner, who was working on biomineralization on the crystals that form in organisms, and I just fell in love with biomineralization. After my postdoctoral research, I started collaborating with Steve Weiner on biomineralization, and we continue to do so to this day. From this work I developed an interest in also investigating pathological crystallizations that are much, much more complicated because they are not controlled by the organism; they are exactly what the organism doesn't want.

Lia Addadi. Image courtesy of Pupa Gilbert (University of Wisconsin–Madison, Madison, WI).

PNAS: What led you to the project described in your Inaugural Article (1)?

Addadi: We started to look at cholesterol crystals because they are related to pathological crystallization. I had the crazy idea that maybe an antibody could be isolated that recognizes crystal surfaces. So we isolated antibodies that interacted specifically with cholesterol crystals. Then at a meeting, I met with Howard Kruth, who works at the NIH in the unit for atherosclerosis, and I told him we had developed these antibodies and maybe he wanted to try them. We also started working on the formation of organized structures of lipids in cell membranes, called lipid microdomains. We studied those in synthetic bilayers in collaboration with Leslie Leiserowitz at the Weizmann Institute of Science. We found that there were special conditions in which cholesterol domains segregated inside membranes. Putting together the work with Howard Kruth on antibodies that detected the formation of organized cholesterol in cell membranes, and the information that cholesterol segregates in lipid bilayers under certain conditions, we formulated the hypothesis that cholesterol crystals in cells could originate from segregation of cholesterol from a membrane. We first proved that in one type of macrophage cells. With this work we wanted to test the generality of the crystal formation process in a different type of macrophages.

PNAS: What is the significance of the findings you report in your Inaugural Article (1)?

Addadi: Cholesterol crystals have for a long time been known to form in atherosclerotic plaques. The big conundrum was the fact that the crystals that are

This is a QnAs with a member of the National Academy of Sciences to accompany the member's Inaugural Article on page 7662.
Published online July 2, 2018.

observed in the plaques do not all look like the cholesterol crystals that you obtain if you crystallize them in the [laboratory]. Our work solves this problem, as we have now shown in cell culture that crystals that have a similar shape to those in plaques are also formed in cells. And we know why they form like that; it's because they are still cholesterol, but cholesterol with a different structure. The fact that these crystals look so similar to those formed in the plaques makes it possible that they are formed by the same mechanism. The real finishing touch was that they form helical or tubular crystals, and that is something that had been observed for cholesterol crystals in gallstone formation. So it all came together.

PNAS: What were some of the challenges in doing this work?

Addadi: A cell is such a complex environment. First we had to develop with Neta Varsano this correlative technique between soft X-ray tomography and STORM [stochastic optical reconstruction microscopy], where the antibody that we use in STORM identified the objects that we were seeing as cholesterol, and soft X-ray tomography told us where it was in a cell. This was something completely new that we developed, and now we could see the cells in three dimensions and also know exactly that these are crystals of cholesterol. We also had to figure out how to match the electron diffraction with the crystals that gave it. We had this diffraction that clearly showed cholesterol, but for the life of us we couldn't match it with the crystals. Then suddenly I had one of those crazy intuitions, and I looked at the crystals and said, "Just a moment, this crystal is a helix." We looked at it at higher magnifications, and we realized that the helix was really in many of these crystals, and not just in one. Then we could match it to the diffraction. At this point we connected this fact to the fact that in gallstones it had been reported that there were helical crystals. So it was really like a suspense story, every day we had a different hypothesis and it finally all came together.

PNAS: What are some of the potential applications of these findings?

Addadi: I think to contribute to the solution of a pathology you have to first of all understand what's happening there. I believe this may be the significance of this paper (1): that we know that these cholesterol crystals are formed in a certain manner and in a certain structure that is different from what was known and what was believed before. This can hopefully help us to relate it to the mechanism by which these crystals are formed. Then, maybe, if we know the mechanism of how they are formed, we also can test different ways to influence it in such a manner that it will change the result in the plaque formation.

1 Varsano N, et al. (2018) Two polymorphic cholesterol monohydrate crystal structures form in macrophage culture models of atherosclerosis. *Proc Natl Acad Sci USA* 115:7662–7669.