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Exploring a Role for Titanium in Bioinorganic Chemistry

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Abstract: Our research in bioinorganic chemistry focuses on the uptake, trafficking, and behavior of hydrolysis-prone metal ions in biology. We present here a three-pronged approach to the investigation of titanium bioinorganic chemistry. The first focuses on biologically and environmentally relevant titanium aqueous coordination chemistry. A second area probes titanium interactions with biomolecules, especially human biomolecules. A final area centers on biological interactions with titanium in mineral form, including titanium mineralization or demineralization.

Key Words: Titanium, Bioinorganic Chemistry, Coordination Chemistry, Mineralization, Demineralization.

BACKGROUND

The story of how I came to explore a possible biological role for titanium begins in my first year of graduate school, in 1993. Our bioinorganic textbook, then recently published by my PhD advisor Stephen Lippard along with Jeremy Berg [1], featured in the first chapter a partial periodic table with "Selected elements important in bioinorganic chemistry," designated either in red (for those important in essential roles, like iron and copper) or in gray (for those important as drugs or probes, like platinum and technetium). Titanium was blank, reflecting no important biological role. At the very end of that book, though, in the last chapter the authors wrote:

Moreover, it is likely that metal ions in addition to those listed in Table 1.1 will be found to be essential elements in biology, leading candidates among the transition metals being Ti, which has been identified in a number of marine organisms….[1]

The text went on, but I was hooked. For the rest of my graduate career and during my postdoctoral work, as I worked on other problems but planned and hoped to have my own academic lab, I read and thought about a possible role for titanium in biology. Where would we find it? What would it look like? What might titanium do?

If you ask a bioinorganic chemist why certain elements are used by biology, you might get a two-part answer. First, biology uses elements that facilitate useful chemistry (reversibly carrying oxygen in blood cells, for example, or activating substrates for transformation). In this regard it would be rather surprising if biology did not use titanium; humans use titanium in various forms for many applications including as a catalyst, as a structural material, and as a pigment. The second part of the two-part answer is the more serious problem with titanium: biology uses elements that are sufficiently abundant and sufficiently bioavailable. Titanium is certainly abundant. It is the ninth most abundant element in the Earth's crust and occurs at appreciable concentrations in the oceans and in biological systems like the human body. But Ti(IV), the predominant oxidation state near neutral pH in the presence of oxygen, is a strong Lewis acid. The metal ion is prone to hydrolysis and hydrolytic precipitation in the form of titanium dioxide and related materials. The relative insolubility of Ti(IV) may thus preclude any essential biological role. The reputation of the element is certainly that it is extremely insoluble and extremely inert.

But whether or not titanium is essential for any organism, it is quite bioactive [2]. Radioactive ^{45}Ti naturally accumulates in and can be used to image solid tumors. Some titanium compounds have anticancer properties, and two of them, budotitane and titanocene dichloride, have been used in human clinical trials. Their

early promise has not yet been fulfilled, but one motivation for our work has been to understand and exploit the aqueous coordination chemistry of titanium to make compounds that preserve the anticancer activities of these compounds, but do not suffer from their dose-limiting toxicities. Various titanium compounds inhibit enzymes, affect bacterial growth, and are transported around the human body. The latter phenomenon has been studied because of the use of titanium and its alloys in biomedical implants. These implants are highly biocompatible, but it is prudent to know what happens to titanium ions that may leach or wear away from them. Some titanium compounds, particularly the titanium ascorbate formulation known as Titavit, are patented and used as growth promoters for plants and mediate weight gain in some animals. And finally, just as my first year bioinorganic textbook promised, some organisms, particularly marine organisms, contain remarkably high concentrations of titanium. The current world record holder, the ascidian *Eudistoma ritteri*, sequesters titanium in its blood at levels thirty million fold higher than the water it filters for food. We do not yet know how or why these organisms get so much titanium.

A serious inquiry into the bioactivity of titanium rests on an understanding of the aqueous coordination chemistry of Ti(IV). The ion is very Lewis acidic, and it renders any coordinated water molecules Bronsted acidic. A bound water is deprotonated below pH 0. But, contrary to conventional expectation, that hydrolysis does not necessarily equate to extreme insolubility. This ion is not as insoluble as its reputation would suggest. The neutral species invoked at pH 7, $Ti(OH)_4$, is soluble in equilibrium with amorphous hydrated TiO_2 at about 1 micromolar concentration. Coordination by complexing ligands can further increase the solubility.

The work that my lab has been doing over the past twelve years can be framed according to three key questions: What titanium chemistry can we do in water? What are important complexes, reactions, and processes related to titanium and human biology? And, because most of the titanium in the environment is in the form of metal oxide mineral materials, might organisms or biomolecules interact with mineralized titanium in the environment?

WHAT TITANIUM CHEMISTRY CAN WE DO IN WATER?

More and better aqueous- and oxygen-stable titanium complexes were required for this inquiry. We have taken inspiration from biology and stabilized Ti(IV) towards hydrolytic precipitation by using hard charged oxygen ligands like the ones nature uses to stabilize and solubilize the similarly Lewis acidic Fe(III). One of our favorite complexes is the one with citrate. Over most of its pH-dependent speciation, Ti(IV) coordinates three citrate ligands via their α hydroxy acids, making stable five-membered chelates, while two carboxylates on each ligand dangle in solution participating in protonation equilibria. Though the d^0 Ti(IV) does not offer many spectroscopic handles, methods including x-ray crystallography and electrospray mass spectrometry offer important characterization. Spectropotentiometric titrations can reveal the pH-dependent speciation. These ligands that stabilize Ti(IV) make the metal ion harder to reduce. The Ti(IV) citrate complex is very difficult to reduce electrochemically, but can be photoreduced in the ultraviolet by irradiating into the ligand to metal charge transfer band. The resulting d^1 Ti(III) is a lovely purple color and is stable if oxygen is not present in solution.

Over the years we have made Ti(IV) and, less often, Ti(III) complexes with a number of biological and bio-inspired ligands including citrate, oxalate, ascorbate, catechols and substituted catechols, and ligands that model the binding sites found in metal transport proteins like transferrin. In addition to the thermodynamic stabilities of these complexes and their properties, we have investigated the kinetics of ligand exchange under biologically or environmentally relevant conditions. In general, the multistep exchanges occur over minutes to hours.

WHAT ARE THE MEDICINAL APPLICATIONS? WHAT ABOUT INTERACTIONS OF TITANIUM WITH HUMAN BIOMOLECULES?

The anticancer activity of titanocene dichloride and butotitane was reported beginning in the late 1970s, in the wake of the tremendous success of the platinum anticancer drugs. Clinical trials began in the early 1980s, but those trials failed to progress by the late 1990s. Similar apparent mechanisms of action between titanocene dichloride and butotitane led to the proposal that these were really prodrugs, each of which served to deliver titanium, perhaps without its ligands, into a common biological pathway that mediated its anticancer activity. Hydrolysis was an undesired side reaction thought to lead to systemic dose-limiting toxicity. We and others thought that if we could figure out how to deliver titanium to whatever

biomolecule is binding it, while preventing the hydrolysis that seems to cause problems, then we could help fulfill the original promise of these compounds.

The leading contender in the early 2000s for that titanium-binding biomolecule was the Fe(III) transport protein transferrin. This protein occurs in human blood plasma. The simplest model was that the titanium compounds deliver titanium directly to transferrin, and transferrin delivers it selectively to tumor cells. In a series of papers, our lab used the well-characterized aqueous Ti(IV) coordination complexes mentioned above and showed that Ti(IV) binds to transferrin with a formal binding constant that is tighter for that for Fe(III), and that the cell surface receptor for transferrin binds titanium-carrying transferrin almost as tightly as it binds iron-carrying transferrin. These results supported the titanium-transferrin delivery model. But our work also showed that the protonation and reduction conditions that trigger Fe(III) release did not release Ti(IV) from transferrin or reduce it to Ti(III). So understanding how the titanium might get back out of transferrin and exert its action on a cancer cell was a problem. And, importantly, administering titanium-bound transferrin directly to cancer cell lines had no effect. So the original model is probably wrong, or perhaps incomplete. We are still working to understand the fate of Ti(IV) ions in the human body. We have looked at the interactions of titanium compounds with another human plasma protein, albumin, and found that the complexes bind that protein with ligands intact, and that hydrolysis and ligand loss are very much slower than in solution.

We studied a small-molecule model of transferrin called HBED (*N,N'*-di (o-hydroxybenzyl) ethylenediamine-*N,N'*-diacetic acid) which in complex with Ti(IV) has some promising activity against tumor cell lines. Moreover, this ligand provides a valuable experimental model system for titanium coordination in the metal binding site of transferrin, and gave us x-ray crystal structures related to species that we believe form in the transferrin protein as a function of pH.

As we went back and looked carefully at the literature about the human clinical trials of the titanium anticancer compounds, we noticed that there was a change in pharmaceutical formulation between the preclinical testing against cancer cell lines (in which titanocene dichloride in dimethylsulfoxide was used) and the human clinical trials (in which titanocene dichloride was formulated in a malic acid buffer in the presence of a high concentration of mannitol). We tried to reconstruct what compound or mix of compounds must have been formed in those trials by using a combination of NMR, mass spectrometry, UV/vis-detected pH-dependent speciation, and computational methods. We found that a family of similar rapidly-interconverting complexes are formed, and that these complexes interact differently with potential protein targets than the parent titanocene dichloride, in both a thermodynamic and kinetic sense. The switch in formulation in the human clinical trials probably had unforeseen consequences for compound bioactivity.

WHAT ABOUT BIOLOGICAL INTERACTIONS WITH TITANIUM IN MINERAL FORM, INCLUDING TITANIUM MINERALIZATION OR DEMINERALIZATION?

Most of the titanium in the biosphere occurs in mineral form. Again, the reputation of these materials is that they are extremely inert, but after several years of learning to prevent the precipitation of titanium, we decided to do it on purpose. The earliest known example of "biotitanification" was reported in the form of TiO_2 needles embedded in the shell of a foraminiferan called *Bathysiphon argenteus*. Because fresh material could only be obtained by dredging the Irish Sea, we convinced the British Museum in London to send us samples from the original 1913 collection. We used scanning electron microscopy data to argue that the needles were TiO_2 in its rutile form, but that they were probably selectively collected from the organism's environment and not actively biomineralized by the organism.

Meanwhile, we were reading the literature about biosilicification in the siliceous frustules of diatoms, and were pleased to see that the biomolecules controlling that biomineralization were being isolated and studied. These biomolecules included both peptides and polyamines. Knowing that titanium concentrations can be quite high in the siliceous frustules of diatoms, we set out to see whether these molecules might also mediate biotitanification from an otherwise soluble titanium source. To be clear, making titanium precipitate from solution is not challenging, but doing so in a controlled way, and being able to tune the properties of the materials that are produced, is challenging. We found that peptides and both short- and long-chain polyamines did indeed mediate or catalyze the precipitation of titanium oxide or titanium phosphate materials with sizes between 2 nm and 5 μm. We could control the particle size and properties by varying pH and temperature. And finally, we found that we could

encapsulate proteins in the titanium phosphate material induced by poly(allylamine).

In later work, we achieved the non-photochemical biotitanification of 2-5 nm amorphous titanium oxide particles inside the protein nanocage of ferritin. We complemented the microscopy and used light scattering and analytical ultracentrifugation to demonstrate that, as biomineralization proceeds inside the nanocage, the particle gets heavier while the hydrodynamic radius is unchanged.

So biomolecules can induce mineralization of titanium, but more recent work is showing, to our surprise, that very avid titanium binding ligands can do just the opposite. Such ligands can demineralize titanium. They can scavenge quite high concentrations of titanium ions from the surfaces of crystalline titanium dioxide in its anatase or rutile forms. This result was a surprise because crystalline titanium dioxide is extremely thermodynamically stable as a bulk material. However, the soluble Ti species that can be scavenged from the surface can reach into the hundreds of micromolar concentration.

CONCLUSIONS

It has now been more than twenty years since my interest was piqued by the mention in my first year graduate textbook of a possible biological role for titanium. And it has been more than twelve years since, in my own independent career, I have been able to study this problem. We have made progress, but there is much more to do.

The most important thing I hope I have demonstrated is that titanium is not just impossibly inert, as its reputation would suggest. Whether or not it is essential, the element is certainly bioactive in a variety of settings. We hope to have demonstrated convincingly that the aqueous chemistry of titanium, though challenging, can be addressed by choosing ligands that stabilize the metal ion to hydrolytic precipitation. Titanium is bioactive in humans via mechanisms that may depend on protein interactions and that are sensitive to complex formation. Understanding these interactions may ultimately help revive the early promise of the titanium anticancer molecules. Finally, organisms and biomolecules interact with titanium in its mineral forms (precipitating it from soluble precursors, binding to it, and dissolving or demineralizing it).

I'd like to close with my heartfelt thanks to all my students and collaborators over the years, and to the funding agencies that have supported our work, especially the National Science Foundation, the American Chemical Society and the American Cancer Society.

REFERENCES

1. Lippard SJ, Berg JM in *Principles of Bioinorganic Chemistry*, University Science Books, Mill Valley, CA, 1994.
2. Buettner KM, Valentine AM. *Chem. Rev.*, 2012, 112, 1863.