Title
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Specific Sequestering Agents for Iron and the Actinides

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Introduction

For several years I and my research group have been interested in the coordination chemistry of metal ions in biological systems and the extension of this chemistry into the preparation of new sequestering agents for both ferric ion and actinide(IV) ions. While it may not be obvious why the synthesis of metal-ion-specific complexing agents for iron in the +3 oxidation state and plutonium in the +4 oxidation state should be related, that is in fact a central thesis of our approach to the actinide coordination chemistry problem. I will describe in this paper the train of thought that has led to a number of compounds that we have prepared with the idea that these could be either used themselves or the precursors of compound that could be used in ameliorating the environmental problems presented by plutonium.

As a statement of the problem, it is clear that whether or not it is used as a part of a nuclear power program, plutonium is a major by-product of nuclear power reactions. The treatment and storage of the nuclear waste from power reactions remains a serious problem which has become in many countries a matter of substantial public concern. Because of its relative abundance and long half life, $^{239}$Pu is a major environmental hazard of long-term waste after the first 100 years of decay. In addition, the biological properties of plutonium make it a particular hazard once human contamination has occurred.

In this paper we will address a few of the following questions:

- What is the aqueous chemistry of Pu(IV) and what limits does this set for us in the design of plutonium-selective complexing agents?
- What are the biological properties of plutonium? Where is it stored in mammals and what damage does it cause?
- How is plutonium transported once incorporated in higher animals?
- What chemical reagents presently are used in the treatment of plutonium contamination in human beings?
- How can we design better complexing agents given the answers to these and related questions?

In Table I are some hydrolysis and dissolution kinetics data for plutonium and its polymeric hydroxides. These data (from Ref. 1 and the literature cited therein) dramatically illustrate the profound insolubility of plutonium, even at low pH. Furthermore the redissolution of the hydroxide, even in strong acid, can take a very long period of time once extensive polymerization occurs — either through high temperature treatment or long standing. Other structural and thermodynamic aspects of these questions\(^2-4\) and the biological properties of plutonium and its decorporation from higher animals\(^5\) have recently been reviewed. In the case of plutonium contamination of beagle dogs (see Ref. 5 and the literature cited therein) it is found that plutonium(IV) in blood plasma decreases by a factor of 2 during the first ten hours. Very little of this plutonium is subsequently excreted by the animal (approximately 90% is retained). A large fraction of the plutonium(IV) is complexed by transferrin, the serum protein used to transport iron in higher animals. Like the coordination of iron(III), binding of Pu(IV) requires bicarbonate anion. Formation of the Pu(IV) complex is blocked by prior coordination of ferric ion and transferrin-bound Pu(IV) can be replaced by Fe(III), indicating that both are bound at the same site. The Pu(IV)-transferrin complex can be dissociated by excess citrate at pH 7.5. It is the Pu(IV) transferrin complex which acts as the transfer
agents in carrying Pu(IV) from the blood to its eventual deposition sites such as bone marrow, bone surface and the liver. In cells of organs such as the liver, Pu(IV) eventually is concentrated in iron storage sites such as the protein ferritin. The ferritin complex of plutonium is highly stable.

In contrast, the problems posed by americium are quite different because of the uniform presence of americium only in the trivalent state. This makes for much more rapid transfer and relatively more rapid excretion of americium. It also makes the design of a metal-ion-specific complexing agent for americium a very different problem from that posed by plutonium.

The present treatment of plutonium contamination in human beings still uses DTPA (diethylenetriaminepentaacetic acid) as either the calcium or zinc salts. The use of this chelating agent increases urinary output of plutonium (months or years after exposure) by 10 to 50 times but it is estimated that the total body burden of plutonium is still only decreased by no more than 25%. This treatment of the systemic contamination of plutonium is substantially the same as that of inhalation contamination. The treatment of plutonium contamination resulting from wounds is largely surgical [removal of tissue around the wound, plus DTPA or EDTA (ethylenediaminetetraacetic acid) washes]. The side effects of both EDTA and DTPA chelation therapy when injected at relatively high concentrations may include kidney damage, sometimes quite serious. In Table II are compared some of the chemical properties of Pu(IV) and iron(III).
The charge per radius ratio for Pu(IV) and Fe(III) are quite similar. This number, which corresponds to the electrostatic potential at the surface of a hard sphere model of the metal ion, correlates well with many acid-base and complex formation properties of metal ions. Note that the solubility product per hydroxide iron ion is approximately the same for Fe(III) and Pu(IV) and that the hydrolysis constants for the aquo ions are quite similar. However most important, as pointed out earlier, Pu(IV) is transported in the blood plasma of mammals as a complex of transferrin, the normal Fe(III) transport agent, and is bound at the same site that normally binds specifically iron. Thus in addressing the question of how to go about designing specific complexes for Pu(IV) we may start with this marked similarity between Pu(IV) and Fe(III).

We should further note that the required properties of specific sequestering agents include not only a high bonding constant for the metal ion to be sequestered but, equally important, a low affinity for most other metal ions. When one examines the properties of biological metal ion complexing agents it is found that their specificity is usually quite high and can generally be broken up into three requirements. First, the coordination number and geometry most favorable for the metal ion is achieved. Second, the cavity formed by the ligand exactly matches the size of the metal ion. Third, the electronic properties of the ligand atom and the metal match. In the case of iron(III) the siderophore ligands are hexadentate and capable of achieving an octahedral coordination in which the cavity size is just that of high-spin iron(III). The ligating oxygen atoms are strongly basic and compliment the high Lewis acidity of the hard ferric ion.
The siderophores are a good example of metal-ion specific complexing agents produced by living systems. These are low-molecular-weight complexing agents which are produced by bacteria, molds, yeast and other microbes to obtain the iron which is an essential element for microbial growth. These compounds form very stable six-coordination complexes of high-spin Fe(III). The iron binding characteristics of the siderophores are summarized in Figure 1. The specific example chosen is that of ferric enterobactin, which is produced by enteric bacteria such as E. coli. Notice that the cyclic triester backbone of enterobactin simply acts as a template to which are appended three catechol functional groups such that a hexadentate, octahedral cavity is formed.

Synthetic Iron(III) Chelating Agents Modeled After Enterobactin

The structure of enterobactin itself and two other representative siderophores are shown in Figure 2. These three compounds are three prototypes for the architecture of synthetic sequestering agents for iron. In the case of enterobactin we have already seen that the structure consists of a cyclic backbone to which are appended three catechol groups. The essential features of the structure of desferrichrome are very similar. There is a cyclic hexapeptide backbone to which are appended three hydroxamate groups. In contrast, the linear trihydroxamate desferrioxamine B has three hydroxamate groups arranged on a linear chain. In spite of these apparently large differences in ligand structure, the iron coordination sites of ferrichrome and ferrioxamine B are essentially identical and their vis-uv spectra are indistinguishable. Thus in the siderophores, where the major driving force of the reaction is the large
enthalpy change associated with the acid-base neutralization in the metal coordination, it is the ability to form the required coordination cavity rather than its pre-formation which is important.

While the formal stability constants [coordination of a fully deprotonated ligand with Fe(III)] of ferric enterobactin, ferrioxamine B and ferrichrome are $10^{52}$, $10^{30.6}$, and $10^{29.1}$, respectively, these numbers do not give an adequate description of the relative stability of these complexes under physiological conditions. This is because the very weak acid nature of the ligands makes their reactions highly pH dependent. If instead one establishes an assumed concentration of total metal ion of $10^{-6}$ M, a total ligand concentration 10 times that and a physiological pH of 7.4, knowledge of all of the associated formation and acid-dissociation constants allows one to calculate the equilibrium concentration of free ferric ion in aqueous solution. While this calculated value may be so low as to have no real chemical meaning, it is well defined in a thermochemical sense and gives an absolute ranking of the relative free energies of the iron complexes. When defined in this way, the $-\log[Fe(H_2O)_6^{3+}]$ values (pM) are 35.5, 26.6 and 25.2 for enterobactin, ferrioxamine B and ferrichrome, respectively. The much higher relative stability of the enterobactin complex led to our use of it as a model for ligand synthesis. Some representative examples of the synthesized ligands are shown in Figure 3. These are all catechoyl amides, hence the acronym CAM. The prefix (ME for mesitylene, LI for linear, and CY for cyclic) indicates the chemical backbone used. Shown in this figure are the sulfonated derivatives in which a 5-sulfo group has been attached to each catechol ring. Further details about the synthesis of these compounds has been reviewed elsewhere.2
The deprotonation of the catechoyl amides occur at relatively high pH. Shown in Figure 4 are the deprotonation constants for the simple model N,N-dimethyl-2,3-dihydroxybenzamide. It has been found that all catechoyl amides, including the multidentate ligands, have pK\textsubscript{a} values which cluster around the first and second pK\textsubscript{a}'s of this ligand. If one examines a titration curve of the free ligand for the sulfonated tricatechoyl amide called MECAMS (Figure 5) two plateaus are seen in the titration of the free ligand. The first corresponds to the removal of three protons, the first of each of the more acidic catechol protons. The second corresponds to titration of the remaining three catechol protons. The presence of divalent metal cations such as Ca(II), Mg(II) causes a relatively small depression of the pH titration curve, indicating only weak complexation of these metal ions. The most strongly complexed of the divalent metal ions is copper(II), which still is complexed by only two of the three catechol groups. In contrast there is a very strong depression of the titration curve of MECAMS by Fe(III), indicating its very strong, and relatively specific complexation.

**Synthesis of Actinide(IV)-Specific Complexing Agents**

We have seen that, while there is a need for strong complexing agents which are relatively specific for plutonium(IV) and the other actinide ions, the presently used complexing agents such as DTPA are relatively nonspecific and thus do not satisfy this criterion. A rational approach to the synthesis of complexing agents specific for Pu(IV) has been based on the following points:
1. Plutonium(IV) is chemically similar to iron(III) and has similar biological transport and distribution properties in mammals.

2. The most powerful naturally-occurring sequestering agents which are specific for iron(III) are produced by microbes. The strongest of these is the tricatecholate ligand called enterobactin.

3. The higher coordination number requirement of Pu(IV) compared to iron(III) means that four catechol groups should be incorporated into a Pu(IV)-specific complexing agent to make it octadentate.

In order to determine the preferred coordination geometry for tetra-catecholato complexes of actinide(IV) ions when no geometric constraints are placed on the ligand structure, a series of $M(\text{cat})_4^{4-}$ complexes (cat = catecholate dianion) were prepared and structurally characterized. These structures showed a roughly tetrahedral distribution of the catecholate groups around the metal ion with the eight-coordinate geometry very close to the $D_{2d}$ symmetry of the trigonal-faced dodecahedron. From molecular models a series of compounds which contained four catecholate ligands appended to a backbone molecular framework were prepared. The synthesis of one series of these compounds is shown in Figure 6. The sulfonated derivatives were found, through animal tests, to be effective plutonium removal agents but also had a significant toxicity at the administered dose rates. Substitution of the catecholate rings by groups such as sulfonate is required, since the neutral catecholate groups of the 2,3-dihydroxybenzoyl containing ligands tend to have very low water solubility, are relatively unstable to air
oxidation, and have an acidity too low to maintain metal complexation under weak acid conditions. As an alternative to sulfonate substitution, carboxylate derivatives were prepared as shown in Figure 7. These compounds have eliminated the toxic side effects observed with the sulfonated derivatives while maintaining their effectiveness. The effectiveness of 3,4,3-LICAMC (see Table III and Figure 7) have been measured using mice as test animals and a protocol in which a small amount of $^{238}\text{Pu(IV)}$ citrate is injected, followed one hour later (for the non-control group) by injection of the chelating agent in saline solution and sacrifice of the animals after 24 hours for an evaluation of plutonium removal. Under these conditions only 6.4% of the administered plutonium would naturally be eliminated by the animal in urine feces. In contrast, 75% of the plutonium was eliminated when the chelating agent was administered. This is a substantially larger amount of plutonium removal for these ligand concentrations than has been seen in other chelating agents heretofore.

A more recent synthesis of an octadentate ligand based on the naturally-occurring chelating agent desferrioxamine B (presently used in the treatment of human iron overload under the trade name Desferal®) has been achieved by appending catechol groups to the free amine nitrogen of the ferrioxamine B ligand (Figure 8). While the unsubstituted 2,3-dihydroxybenzoyl-substituted DFO derivative is relatively insoluble, the carboxylate-substituted chelating agent has a usable aqueous solubility. Preliminary results for plutonium removal of these chelating agents are encouraging.

Another new approach to the synthesis of actinide-specific sequestering agents which addresses the problem posed by the complexation of
these metal ions under weakly acidic conditions has been undertaken using the chelating properties of hydroxypyridones. While catechol groups are both weakly acidic and confer a second-order dependence on hydrogen ion concentration per catechol group, the hydroxypyridonate ligands are much more acidic and are monoprotic ligands. Thus an octadentate chelating agent based on hydroxypyridonate ligands will show at most an inverse fourth order dependence on hydrogen concentration for the stability of the complex while the tetracatecholate ligands will show an inverse eighth order dependence. The hydroxypyridones can be viewed as derivatives of catechol in which a nitrogen has been substituted for a carbon of the catechol ring (see Figure 9). While Figure 9 summarizes the structural and acid-base properties of all the chelating isomers of the hydroxypyridonate [HOPO] ligands, to date only the 1,3-HOPO isomer has been incorporated into multidentate structures. The synthetic scheme for this procedure is shown in Figure 10. Use of the carbonate anhydride formed from phosgene as a general synthon allows the preparation of a wide range of multidentate chelating agents from either simple or polyamines. The coordination and biological properties of these compounds are presently under investigation.

Summary

In summary, the transuranium actinide ions represent one unique environmental hazard associated with the waste of the nuclear power industry. A major component associated with that waste and a potential hazard is plutonium, both because of its relative abundance and its chemical and biological properties. The synthesis of metal-ion-specific complexing agents for ions such as Pu(IV) potentially represents a
powerful new approach to many of the problems posed by waste treatment and represents a challenge to the synthetic chemist. What has been described here is a progress report of a rational approach to the synthesis of such chelating agents based on the similarities of Pu(IV) and Fe(III), the structures of naturally-occurring complexing agents which are highly specific for Fe(III), and the incorporation of the same kinds of ligating groups present in the iron complexes to make octadentate complexes highly specific for plutonium. Both thermodynamic and animal test results indicate that a relatively high degree of success has already been achieved in this aim.

Acknowledgments

I would like to acknowledge the many co-workers, past and present, whose work I have summarized in this short paper. These include Dr. Patricia Durbin, Dr. Frederick Weitl, Dr. Wesley Harris, Mr. Steven Rodgers, Mr. Rob Scarrow, Dr. David White, Dr. Mary Kappel, Dr. Carl Carrano, Dr. Vincent Pecoraro, Dr. Stephen Sofen, and Dr. Alex Avdeef.

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References


Table I. Hydrolysis and Complexes of Pu(IV)

<table>
<thead>
<tr>
<th>[Pu(^{4+})]</th>
<th>[Pu(OH)(_x)](_n)</th>
<th>dk. green colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH ~ 0</td>
<td>increasing pH +</td>
<td>MW 100 to &gt; 10(^6)</td>
</tr>
<tr>
<td>(irreversible)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ [\text{Pu}^{4+}], M (10^{-3}) \]

<table>
<thead>
<tr>
<th>Max. ([\text{H}^+]) for polymerization</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>62</td>
</tr>
</tbody>
</table>

Half-times for depolymerization of Pu(IV) polymer.

<table>
<thead>
<tr>
<th>Formation temp.</th>
<th>2M</th>
<th>4M</th>
<th>6M</th>
<th>10M</th>
<th>([\text{HNO}_3])</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>20 days</td>
<td>400 min</td>
<td>100 min</td>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>80°C</td>
<td>1 year</td>
<td>---</td>
<td>15 days</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

In 5M \(\text{HNO}_3\)

<table>
<thead>
<tr>
<th>at 25°C</th>
<th>20 hr</th>
<th>320 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>fresh PPT</td>
<td>aged months at RT</td>
<td></td>
</tr>
</tbody>
</table>
Table II. Similarities of Pu$^{4+}$ and Fe$^{3+}$

1) 

<table>
<thead>
<tr>
<th>Ionic radius$^a$</th>
<th>Pu$^{4+}$; $\frac{4}{0.96} = 4.2$</th>
<th>Fe$^{3+}$; $\frac{3}{0.65} = 4.6$</th>
</tr>
</thead>
</table>

2) 

- $\text{Fe(OH}_3\text{)} + \text{Fe}^{3+} + 3\text{OH}^- \rightarrow \text{K} \approx 10^{-38} (10^{-13} \text{ per OH}^{-1})$
- $\text{Fe}^{3+} + \text{H}_2\text{O} \rightarrow \text{Fe(OH)}^2+ + \text{H}^+ \quad \text{K} = 0.0009$
- $\text{Pu(OH}_4\text{)} \rightarrow \text{Pu}^{4+} + 4\text{OH}^- \quad \text{K} \approx 10^{-55} (10^{-14} \text{ per OH}^{-1})$
- $\text{Pu}^{4+} + \text{H}_2\text{O} \rightarrow \text{Pu(OH)}^3+ + \text{H} \quad \text{K} = 0.031 \text{ (in HC}_1\text{O}_4\text{)}$

3) Pu$^{4+}$ is transported in the blood plasma of mammals as a complex of transferrin, the normal Fe$^{3+}$ transport agent. The Pu$^{4+}$ binds at the same site as Fe$^{3+}$.

---

$^a$Ref. 2.
Table III. Removal of $^{238}\text{Pu(IV)}$ from Mice$^a$ by Polycatechoyl Carboxylate Ligands

<table>
<thead>
<tr>
<th>Ligand$^b$</th>
<th>Control</th>
<th>3,4,3-LICAMC</th>
<th>poly-LICAMC</th>
<th>dioctyl-LICAMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sacrifice interval</td>
<td>1 hr</td>
<td>24 hr</td>
<td>24 hr</td>
<td>24 hr</td>
</tr>
<tr>
<td>No. of mice</td>
<td>13</td>
<td>84</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>% of Injected $^{238}\text{Pu}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>31</td>
<td>49</td>
<td>8.4</td>
<td>29</td>
</tr>
<tr>
<td>Skeleton</td>
<td>24</td>
<td>32</td>
<td>9.6</td>
<td>10</td>
</tr>
<tr>
<td>Soft tissues</td>
<td>38</td>
<td>10</td>
<td>6.8</td>
<td>16</td>
</tr>
<tr>
<td>Urine</td>
<td>1.3</td>
<td>4.2</td>
<td>54</td>
<td>20</td>
</tr>
<tr>
<td>Feces and GI cont.</td>
<td>5.1</td>
<td>4.7</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$1.5 μCi/kg $^{238}\text{Pu(IV)}$ citrate i.v.

$^b$30 μmol/kg ligand in saline i.p. given 1 hr after $^{238}\text{Pu}$ injection.
Figure Captions

Figure 1. A schematic diagram of the coordination of ferric ion by the siderophore (microbial iron chelating agent) entero­bactin. Note that the octahedral coordination of the ion occurs through the oxygen atoms of three catecholate groups which are attached to the trigonally-symmetric tri-ester backbone composed of three serine residues.

Figure 2. Structural formulas for three representative siderophores. The linear compound, ferrioxamine B, is a tri-hydroxamate siderophore which is presently the chelating agent used in the clinical treatment of human iron overload. The other tri-hydroxamate, ferrichrome, has a general molecular architecture similar to that of entero­bactin (shown here and in Figure 1); each ligand consists of a cyclic structure to which are appended three bidentate chelating groups. Even though the geometries of ferrioxamine B and ferrichrome are very different, the coordination geometry of their iron complexes are essentially identical.

Figure 3. Synthetic tri-catecholate analogs of entero­bactin.

Figure 4. Deprotonation constants of N,N-dimethyl-2,3-dihydroxy­benzamide (a direct analog of the ligating groups in entero­bactin).
Figure 5. Titration curves (pH vs equivalence of base added) of MECAMS as the free ligand and in the presence of several metal ions.

Figure 6. A synthetic scheme for the linear tetra-catecholate ligand 3,4,3-LICAMS (m = 3 and n = 4) and related compounds.

Figure 7. A synthetic scheme for 3,4,3-LICAMC (compound #10) and related chelating agents.

Figure 8. The synthesis of catechol derivatives of ferrioxamine B. These are octadentate ligands which are capable of coordination through the appended catecholate and three hydroxamate groups.

Figure 9. Structural formulas for the three different isomers of the hydroxypyridones. Their relationship can be seen to the catecholate and hydroxamate ligand structures, which are shown at the bottom of this figure.

Figure 10. A general synthetic scheme for the incorporation of hydroxypyridonate functional groups into multidentate chelating agents.
IRON BINDING CHARACTERISTICS OF SIDEROPHORES

OXYGEN BINDING SITES

OVERALL HEXADENTATE

RIGID BIDENTATE UNITS W/ 5-MEMBERED CHELATE RINGS:

FLEXIBLE BACKBONE

FERRIC ENTEROBACTIN (M = Fe)

XBL 822-8077
Desferrichrome

Desferrioxamine B

Enterobactin

XBL 7910-4223
MECAM  \[ R = \text{CH}_2-\text{NH-} \]

MECAMS  \[ R = \text{CH}_2-\text{NH-} \]

TRIMCAMS  \[ R = \text{C-NH-CH}_2- \]

3,4-LICAMS

CYCAM  \[ R = \text{C-O-} \]

CYCAMs  \[ R = \text{C-O-} \]
DEPROTONATION EQUILIBRIA OF A CATECHOYL AMIDE

\[
\begin{align*}
N-(CH_3)_2 \quad & \quad \overset{\text{H}^+}{\leftrightarrow} \quad H^+ + \quad N-(CH_3)_2 \\
\text{pK} &= 8.4
\end{align*}
\]

\[
\begin{align*}
N-(CH_3)_2 \quad & \quad \overset{\text{H}^+}{\leftrightarrow} \quad H^+ + \quad N-(CH_3)_2 \\
\text{pK} &= 12.1
\end{align*}
\]
Titration Curves of MECAMS

pH

a (Moles Base per Mole Metal)
SYNTHESIS OF CATECHOYL DERIVATIVES OF DESFERRIOXAMINE B
THE CHELATING HYDROXYPYRIDONES

$\text{PK}_A$'s

1-HYDROXY-2(1H)-PYRIDINONE (1,2-HOPO)

3-HYDROXY-2(1H)-PYRIDINONE (3,2-HOPO)

3-HYDROXY-4(1H)-PYRIDINONE (3,4-HOPO)

CF. CATECHOL ($\text{PK}_A$'s 9.22, 13.0)

ACETOHYDROXAMIC ACID ($\text{PK}_A$ 9.36)

XBL 837-10490
Bri§lCOOH

SOCl₂

Acetone, trace H₂O

Acetone,
trace H₂O

HNMe₂

XBL 837-10678
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