Lead Substitution in Synaptotagmin: A Case Study

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Quantum chemistry computations have been used to investigate the possibility of a Pb\textsuperscript{2+}/Ca\textsuperscript{2+} substitution in the three calcium sites of the synaptotagmin enzyme. Provided explicit cation solvation is taken into account, it is shown that the substitution is energetically feasible and induces a strong reorganization of the Ca\textsuperscript{2+}-coordinating sites, which may preclude the enzyme for any efficient role when lead poisoning occurs.

I. Introduction and Structural Modeling

Synaptotagmin is an antigen calcium protein first described by Matthew,\textsuperscript{1} and identified by Perin.\textsuperscript{2,3} It expresses at the outer part of synaptic vesicles and plays an essential role in supporting neurotransmitters as communication signals relying on tuned Ca\textsuperscript{2+} fluxes.

It is now well established that saturnism (lead poisoning) can induce neurological dysfunctions, the intimate molecular origins of which still have not been fully characterized. As Godwin\textsuperscript{4} and Suszkiw\textsuperscript{5} have recently shown that synaptotagmin can be perturbed by lead, thus providing another example of a metalloprotein targeted by Pb\textsuperscript{2+}, we have found of interest to perform some molecular modeling of this system. For that purpose we here follow and refine a procedure initially established to investigate commonly used lead chelators interacting with metallic oligoelements, or lead substitution in both calmodulin and the ALAD ([\(\delta\)-aminolevulinic acid dehydratase) enzymes.\textsuperscript{6,7}

A simple view of synaptotagmin is provided in Figure 1: it reveals that the three calcium sites are located on the surface of the protein between two external loops that connect three \(\beta\)-sheets: this suggests that cation/cation exchanges with the outer medium can be envisioned. These three calcium sites have been modeled according to the description given in Figure 1: only their first coordination spheres have been retained, which led to the consideration of about 85 atoms per site. On the basis of the 1UOV X-ray structure of synaptotagmin,\textsuperscript{8} these models will thereafter be referred to as 1UOV\_M\textsubscript{n} where M stands for the dication involved (Ca in the native form, or Pb after substitution), and index n allows differentiating the three sites. From the 1UOV crystallographic data, the calcium–calcium distances amount to: (Ca\textsubscript{1},Ca\textsubscript{2}) = 3.71 Å, (Ca\textsubscript{2},Ca\textsubscript{3}) = 4.43 Å, and (Ca\textsubscript{1},Ca\textsubscript{3}) = 7.41 Å. Each site can be described using its nomenclature:

\begin{itemize}
  \item 1UOV\_Ca\textsubscript{1}: three water molecules (O1, O2, and O3), two \(\eta^1\)-coordinated formate anions (O5 and O6), and one \(\eta^2\)-coordinated amide function (O7)
  \item 1UOV\_Ca\textsubscript{2}: four water molecules (O2, O5, O6, and O7), one \(\eta^2\)-coordinated formate anion (O3 and O4), and the carbonyl group of an amide function (O1).
\end{itemize}

When referring to a substitution, we will shortly use the notation 1UOV\_Ca\textsubscript{n}/Pb\textsubscript{n}.

II. Computational Protocol

II.1. Methodology. The calculations have been performed using the GAUSSIAN03 package\textsuperscript{6} within the B3LYP formalism. This functional\textsuperscript{10,11} was chosen as it has proven to provide geometries and energies close to the CCSD(T) approach.\textsuperscript{12–16} The standard 6-31+G** basis set was used to describe the H, C, N, and O atoms. Scalar relativistic pseudopotentials\textsuperscript{17} were used for Pb\textsuperscript{2+}: for the final results reported, the large-core relativistic SDD pseudopotentials by Dolg et al.\textsuperscript{18} (78 electrons pseudized for neutral Pb) coupled to a (4s4p1d)/[2s2p1d] contraction to describe the valence electrons. These functional, basis set, and pseudopotential have been successfully employed in our previous works devoted to Pb\textsuperscript{2+} or to other heavy cations and have proven to reproduce 4-component relativistic computations.\textsuperscript{19–21}

Several levels of geometry optimization have been considered. First, starting from the atomic PDB coordinates, only hydrogen atoms have been optimized (thereafter, H\textsubscript{opt}). Second, the metallic cation also is optimized (M\textsubscript{opt}). Then all atoms are relaxed, except those directly linked to the proteic skeleton (L\textsubscript{opt}). Finally, all atomic positions are optimized (Topt) and the nature of the stationary points encountered has been characterized by a vibrational analysis performed within the harmonic approximation, which allowed us to estimate free enthalpy \(\Delta G\) values at \(T = 298\) K.

II.2. Interpretative Tools: The Topological Analysis of the ELF Function. ELF (electron localization function) calculations and the topological analysis of the ELF function have been performed using the TopMod package.\textsuperscript{22–27} We here just recall that within the framework of the topological analysis of the ELF function, space is partitioned into basins, each of them having a chemical meaning. Such basins are classified as\textsuperscript{23}

\begin{itemize}
  \item (i) core basins surrounding nuclei,
  \item (ii) valence basins characterized by their synaptic order.
\end{itemize}

Further details can be found in the above-mentioned references and in a recent review dedicated to the applications of the ELF topological analysis to biological systems.\textsuperscript{28}
It has been shown possible to extend the ELF approach to pseudopotential approaches. Small-core pseudopotentials provide semiexternal cores and allow determining the synapticity of well-defined valence basins; large-core pseudopotentials preserve the number and the properties of valence basins.

In the present contribution, we will focus on V(Pb), the valence monosynaptic basin associated with the valence electrons of Pb$^{2+}$, which is chemically relevant to the electrons populating the 6s and 6p orbitals. For a given site, we will use the following notations: V(Pb) is the ELF basin defined previously, $N(Pb)$ and $\omega(Pb)$ are respectively the population and the volume associated with this basin. $F(Pb)$ is the corresponding mean charge density and is determined according to $F(Pb) = N(Pb)/\omega(Pb)$. 

### III. Results and Discussion

#### III.1. Structural Studies

Table 1 collects some characteristic metal–oxygen bond lengths for the three model active sites, using the atomic numbering depicted in Figure 1. Starting from the X-ray coordinates, no drastic changes are observed for $1UOV_{Ca}n$, $1UOV_{Ca}2$, and $1UOV_{Ca}3$ when going from the H$_{opt}$ level of optimization to the T$_{opt}$ level, which seems to indicate that the models considered are realistic from a structural viewpoint. Things are, however, different when the substitution of Ca$^{2+}$ by Pb$^{2+}$ is considered. At the M$_{opt}$ level, no strong distortions are observed between $1UOV_{Ca}n$ and $1UOV_{Pb}n$, which seems to indicate that Pb$^{2+}$ can effectively replace Ca$^{2+}$ in the three model active sites.

![Synaptotagmin](image)

**Figure 1.** View of synaptotagmin showing the three calcium cations (top left; from the X-ray structure reported in ref 8), and the three models retained after Pb$^{2+}$ substitution (before any structural relaxation), namely $1UOV_{Pb}1$, $1UOV_{Pb}2$, and $1UOV_{Pb}3$. Color convention: red for oxygen, green for calcium, purple for lead, gray for carbon, blue for nitrogen, and white for hydrogen.

**TABLE 1: Metal–Oxygen Bond Lengths (Å) for 1UOV$_{Ca}$/$Pb$ at Two Levels of Geometry Optimization (See Text for Details)**

<table>
<thead>
<tr>
<th>$M_{opt}$</th>
<th>1UOV$_{Ca}$/$Pb$</th>
<th>1UOV$_{Ca}$/$Pb$</th>
<th>1UOV$_{Ca}$/$Pb$</th>
<th>1UOV$_{Ca}$/$Pb$</th>
<th>1UOV$_{Ca}$/$Pb$</th>
<th>1UOV$_{Ca}$/$Pb$</th>
</tr>
</thead>
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<tr>
<td>$M_n$–O1</td>
<td>2.4/2.4</td>
<td>2.4/2.3</td>
<td>2.4/2.4</td>
<td>2.3/2.5</td>
<td>2.3/2.5</td>
<td>2.3/2.5</td>
</tr>
<tr>
<td>$M_n$–O2</td>
<td>2.4/2.4</td>
<td>2.3/2.3</td>
<td>2.3/2.3</td>
<td>2.3/2.7</td>
<td>2.3/2.7</td>
<td>2.3/2.7</td>
</tr>
<tr>
<td>$M_n$–O3</td>
<td>2.5/2.4</td>
<td>2.4/2.5</td>
<td>2.4/2.4</td>
<td>2.4/2.7</td>
<td>2.4/2.7</td>
<td>2.4/2.7</td>
</tr>
<tr>
<td>$M_n$–O4</td>
<td>2.5/2.5</td>
<td>2.5/2.6</td>
<td>2.5/2.6</td>
<td>2.5/2.6</td>
<td>2.5/2.6</td>
<td>2.5/2.6</td>
</tr>
<tr>
<td>$M_n$–O5</td>
<td>2.3/2.3</td>
<td>2.3/2.3</td>
<td>2.3/2.3</td>
<td>2.3/2.8</td>
<td>2.3/2.8</td>
<td>2.3/2.8</td>
</tr>
<tr>
<td>$M_n$–O6</td>
<td>2.3/2.3</td>
<td>2.3/2.3</td>
<td>2.3/2.3</td>
<td>2.3/2.9</td>
<td>2.3/2.9</td>
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<tr>
<td>$M_n$–O7</td>
<td>2.5/2.6</td>
<td>2.3/2.3</td>
<td>2.3/2.3</td>
<td>2.3/2.7</td>
<td>2.3/2.7</td>
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<tr>
<td>$M_n$–O8</td>
<td>2.4/2.4</td>
<td>2.5/2.5</td>
<td>2.5/2.5</td>
<td>2.4/3.4</td>
<td>2.4/3.4</td>
<td>2.4/3.4</td>
</tr>
</tbody>
</table>

* The most important bond length increases upon substitution are underlined.
When all atoms (Topt) relax, some strong deformations are observed, however. In the 1UOV M1 models, both O1 and O2 are repelled upon the Ca2+/Pb2+ substitution: the two corresponding water molecules have been pushed out of the first coordination sphere. The same trends are observed for 1UOV M2 (O4 and O8 are strongly repelled), and for 1UOV M3 (O7 is repelled). In any case, there is also a quasi-systematic increase of the cation/oxygen bond lengths of 0.1–0.4 Å when the Ttop values for 1UOV Ca, and 1UOV Pb, are compared. It is expected that such distortions allow the valence pair of Pb2+ to expand, as will be seen by means of the topological analysis of the ELF function.

III.2. ELF Analysis. Our previous studies have established that both ω(Pb) and ρ(Pb), namely the volume and the mean charge density of the ELFic V(Pb) basin, reach plateaus for high coordination numbers: ω(Pb) cannot decrease below about 150 au3, and ρ(Pb) cannot exceed about 16.10−3 e−/au3. When all atoms (Topt) relax, some strong deformations are observed. 

Table 2 reveals that the three model sites of synaptotagmin follow these rules. If 1UOV Pb, Hopt structures are considered, namely those in which we have replaced Ca2+ by Pb2+ with no optimization (except for the hydrogen atoms), values of about 115 au3 are observed, and ρ(Pb) amounts to 21 × 10−3 e−/au3. These values show that the V(Pb) basin is too sterically constrained and that a reorganization must occur. Upon full relaxation (Topt), the volume of the V(Pb) basins increases to reach values above the threshold of 150 au3; concomitantly, ρ(Pb) decreases from 21 to about 15.10−3 e−/au3. These changes are driven by the expansion of V(Pb), which, consequently, induces reorganization of the ligands as described above. The emergence of V(Pb) is clearly illustrated by the ELF basins presented in Figure 2 for site 1UOV Pb1. Analogous trends are of course observed for the two other sites.

On the basis of their structural properties, lead complexes have been historically classified into two families, namely holodirected and hemidirected structures.31 Even if a third structural class, the so-called bisdirected structure, has been predicted very recently,32 we here seem to face such an holodirectionality competition, such as that encountered previously when either some commonly used chelators, the calmodulin enzyme, or model systems were investigated.30,21 Indeed, the Ca2+ enzyme site usually exhibits a holodirected character, but upon substitution by Pb2+, it tends to hemidirectionality by means of the expulsion of some ligands and the reduction of the coordination number in the first coordination sphere to allow the Pb2+ valence electrons to expand in space. This phenomenon is here clearly observed and can be quantified by both the volume and the electronic density of the V(Pb) basin.

III.3. Energetical Study. The energetic analysis related to the substitution is a difficult task as the exact nature of incoming Pb2+ and expelled Ca2+ are not known in biological conditions. We will thus consider several possibilities: the first of which, but the less probable, involves naked ions. The other ones involve hydrated cations. Of course, only Ttop structures will be considered to estimate ∆G values.

III.3.a. Naked Cations. In that case, the substitution equation simply reads:

\[
Pb^{2+} + [1UOV_{Ca_n}] \rightarrow [1UOV_{Pb_n}] + Ca^{2+}
\]

The corresponding ∆E and ∆G values are gathered in Table 3 for the three sites. It can be seen that all ∆E values are positive, making the substitution unfavorable. Increasing the level of optimization decreases these positive values, but not enough to reverse their sign. ∆G values are also positive and amount to between 22 and 25 kcal/mol. Considering naked cations is thus not favorable for the substitution, but such a situation is anyway rather improbable in biological media.

III.3.b. Hydrated Cations. To better describe the state of the cations within a physiological environment, we have considered hydrated cations, and several states of hydration: [Ca(H2O)m]2+ with n = 6 or 7, coupled to [Pb(H2O)n]2+ with m = 5, 6, or 7. These coordination numbers have been chosen according to experimental and theoretical results. The sole available experimental value reported for the hydration number of Pb2+ in aqueous solution comes from NMR data and provides m = 5.7 ± 0.2.33 and Car–Parrinello dynamic simulations have provided a mean value of m equal to 7.0, which has been reduced to 6.4 by considering a longer simulation.34 This value corresponds to the time average of competitive and exchanging 5-, 6-, and 7-fold coordinations. The coordination number of hydrated Ca2+.
As revealed by Table 4, for $m = 7$, all energy values are positive, whatever the site and whatever the level of optimization considered: no substitution is expected to occur. For $m = 6$, the situation is still not encouraging. The most interesting substitution reactions still with the optimization level. The ∆E values vary between 7 and 8, depending on the simulations performed [see ref 38 and discussion therein].

Let us first consider $[\text{Ca(H}_2\text{O)}_6]^{2+}$, and, consequently, the following substitution reactions with $m = 5, 6, or 7$:

$$[\text{Pb(H}_2\text{O)}_5]^{2+} + (7-m)\cdot \text{H}_2\text{O} + [\text{1UOV_Ca}_n] \rightarrow [\text{1UOV_Pb}_n] + [\text{Ca(H}_2\text{O)}_7]^{2+}$$

As shown in Table 5, we now face many cases exhibiting negative reaction energies. For $m = 7$, ∆E values are positive, but entropy leads to negative ∆G values, except for the 1UOV_Pb3 site. For $m = 6$, the ∆G values are negative, and more favorable than when $[\text{Ca(H}_2\text{O)}_6]^{2+}$ is considered. These values are still more favorable for $m = 5$. In fact, for $m = 6$, the ∆E values become negative as soon as the Lopt optimization level is considered (and even from the Mopt level for the 1UOV_Ca1/Pb1 and 1UOV_Ca2/Pb2 substitutions).

We can thus conclude that the substitution reactions are favorable for the three model sites of synaptotagmin for the two following reactions:

$$[\text{Pb(H}_2\text{O)}_5]^{2+} + 2\text{H}_2\text{O} + [\text{1UOV_Ca}_n] \rightarrow [\text{1UOV_Pb}_n] + [\text{Ca(H}_2\text{O)}_7]^{2+}$$

Moreover, the following reaction is also favorable:

$$[\text{Pb(H}_2\text{O)}_5]^{2+} + \text{H}_2\text{O} + [\text{1UOV_Ca}_1] \rightarrow [\text{1UOV_Pb}_1] + [\text{Ca(H}_2\text{O)}_6]^{2+}$$

The rather large substitution ∆G values obtained for these cases show that cationic exchange can occur, with no hope for reversibility. Once Pb$^{2+}$ has replaced Ca$^{2+}$ in a given site, not only has this site lost its capability to deal with Ca$^{2+}$ cations but also, in view of the structural disorders induced, even the two other Ca$^{2+}$ sites might be perturbed and, maybe, unable to host other Ca$^{2+}$ cations. This could be especially true for sites 1 and 2, the calcium cations of which are only 3.71 Å far apart from one another.

### IV. Conclusions

In the present contribution, the quantum chemistry protocols previously applied to investigate Ca$^{2+}$ and Zn$^{2+}$ substitutions by Pb$^{2+}$ in the calmodulin and ALAD enzymes has been applied to model sites of synaptotagmin, an enzyme experimentally known to be targeted by lead.

It appears that such a cation exchange is thermodynamically allowed in any of the three Ca$^{2+}$-coordinating sites of synaptotagmin. Beyond favorable energetical values, such an exchange implies drastic structural reorganizations: even localized at a given site, such perturbations may preclude the enzyme to have any efficient role.

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References and Notes


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