Title: Understanding protein transport of certain metals through molecular dynamics
simulations

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Author's note

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Introduction

Protein-metal interactions (PMI), are very important in the understanding of the impact of certain metals on our organism, not only in the form of their organic relationship with us, but also in the form of cognizing how metal transportation occurs within the human body, and especially in the process of elimination. Some researchers have been fascinated by the design of drug transporters that emulate metals, or how metalloproteins work, but nobody has ever asked how metals are transported throughout our organism to date, and particularly in the context of exploring the microphysical sector through molecular dynamics (MD) simulations, which can give enough hints at what happens at that level, thus leading us to actually improve not just the design of drugs, but proposing an alternative to chelating agents too. Moreover, the study of metal transportation of proteins, which can reveal how metals actually interact with biorganic materials, such as the living, is still unascertained. This approach can be used not just in the case of the heavy metal lead (Pb), which we have here explored, due to the large extent of the damages that this heavy metal can cause on our body, both as a proto-oncogen, and as a poison (Singh, 2018), but also how all other heavy metals affect our body, which is still not fully known.

It is why we propose a preliminary molecular dynamics (MD) study which can be exploited in the understanding of PMI in any setting. In this regard, we analyse the impact of “atomic” Lead (Pb) onto a protein, Calmodulin. The same analysis can be performed in a reproducible manner using different proteins or different metals.

Materials and Methods

Firstly, the crystal structure of Calmodulin bound to lead (Pb) was retrieved from the protein database (PDB code = 2v01; Kursula, 2007). Then, a very basic short molecular dynamics (MD) simulation was performed for the whole *ensemble* with a conventional molecular dynamics package (Brooks, 2009); irrespectively, the parameters employed were a standard of 1.5 fs per timestep (TS), periodic boundary conditions with a periodic cell of 30x32x45. In reality, minimisation is achieved after only 0.175 ps (100 TS, timesteps), but the simulation was run for about 2.25 ps (1500 TS), thus for a very short time. The *Charmm* force fields (topology and parameters) were added, of note, the *par_all24_prot_lipid.inp* parameters which include some metals. The latter were adjusted to include lead (Pb) data too (Tabaee Damavandi, 2016), particularly after using a portable structure file package (*psfgen* 2.0 version), since the weight of lead (Pb) is larger than the ones included in the *Charmm* force fields (of over 32 amu). The quick convergence was potentially due to the small size of the box in terms of short-term interactions (Tabaee Damavandi, 2016). Langevin Dynamics and Particle Mesh Ewald (PME) “constraints” were also applied, with standard pairlist distances (12 Å). Pressure control was group based, and the temperature for the simulation was physiological (310 K). 5675 waters were included and the *wrapall waters* algorithm was used.

Discussion

The initial and final structure of the protein wrapped in lead (Pb) give us preliminary hints at how this metal behaves around Calmodulin and as a consequence on other proteic structures in general. Firstly, we can clearly observe that the weight of lead has a perpetual effect on the protein, and it does also have an impact on the surrounding waters, regardless of the simulation timestep or minimisation status (Figure 1). In fact, the protein conformation is destabilised by the presence of Calmodulin and a pseudo-flattening of the β -sheet loops wrapping the lead atoms can be also viewed.

In particular, the terminal aminic group of Asn42, Asp58, Asn60, Thr62, and Ile63, thus the polar hydrophilic and hydrophobic amino acids namely, are impacted, thus inducing a beta-sheet flattening from the bond stretches and thus a proteic breakdown, in a process very similar to denaturation and unfolding, which might potentially lead to a mutation of the protein itself, in those regions involved with the lead atoms, as well as the death of the protein itself, due to the breakage of the beta-sheet conformation, which denote an unfolding process. Unfolding processes indicate the inability of the protein to function properly. The MD shows this reality (Figure 2), therefore, it can be deduced that the impact of Lead (Pb) on enzymatic death can be quite important. It can also be guessed that the lead is transported by proteins thanks to its persistent interaction once it becomes trapped in a loop, truthfully, it is the lead that entraps the protein, possibly due to its large weight compared to the atoms of amino acids which atomic weight is tendentially below 16 g/mol. Additionally, the simulation can make us grasp how the amount of heavy metals, such as lead, affects our “biomolecular world”.

Conclusion

Molecular dynamics (MD) simulations can demonstrate the impact of lead atoms on proteins, and other guesses based on the study. Potentially this can lead to the design of protein-like structures that could chelate lead, however, this is not why this study was done. The hereby analysis attempts at demonstrating that molecular dynamics can be used to understand even the effect of metals on our organism, and also how the transport, deposition and potentially removal (through chelation) might occur. Lead was just an example of metal used, in reality, a lot of other metals and proteins can be investigated with this approach, which is actually pretty simple.

No conflicts of interest to disclose.

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Figure 1.

Figure 1. Crystal structure of recombinant calmodulin interacting with lead and wrapped in water (red and white). Lead (green) demonstrates to have a highly attractive influence on both water molecules and the amino acids within the Calmodulin protein in cyan (*Pymol v4.5TM*).

Figure 2. (left) Initial structure prior to beginning the molecular dynamics simulation, in contrast with *(right)* final structure, after 1500 TS (2.25 ps). We can see how the structural conformation is destabilised by the lead atoms, throughout the molecular dynamics simulation, as a flattening of the β -sheet is achieved to the point of the loss of its ordinary neat structure. It can also be observed the permanent connotation of lead atoms within the β -loops (in the asparagine, threonine, isoleucine and aspartate, chelating the aminic and hydroxy groups of the β -involved amino acids (Pymol v4.5TM). Calmodulin (yellow) and Pb atoms (grey),