Permeation of Fe2+ ions through three-fold pores of the human H-chain ferritin

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I. Background information and summary of the proposed research

Ferritin is a spherical protein that regulates the concentration of oxygen atom free radicals by keeping a sufficient amount of iron ions in the cytosol, thus reducing the possibility of oxidative stress that results in various life-threatening diseases such as diabetes, cancer, Alzheimer's syndrome and others. [1,2,3] Ferritin conducts Fe^{+2} ions from the cytoplasm, oxidizes them to Fe^{+3} form, stores and then releases them during periods of iron deficiency. The goal of this project is to study the Fe^{+2} ion induction mechanism, which is currently not well understood.

Human ferritin consists of 24 subunits, comprised of about 4500 atoms that form a hollow spherical shell. The outer diameter of the shell is about 120 Å, the internal diameter is about 80 Å and the thickness of the shell is about 20 Å. The influx of Fe^{+2} ions into the protein occurs through 3-fold symmetry pores that pergorate the shell. [4,5,6] Although ferritin is not technically an ion channel protein, the process of ion flow through the pores is very similar to that of the flow through the ion channels and therefore the same computational techniques can be utilized.

The time scale of the permetation process observed in experimental studies makes direct all-atom MD simulation of Fe^{+2} influx computationally expensive. [7] Therefore, we propose to study the transport of the Fe^{+2} ions through the 3-fold pore by simulating hopping events between discrete binding sites inside the pore that arise due to the location of rings of negatively charged amino acids inside the pore and that were observed in the previous studies done in this group. [5] In order to quantitatively define the binding sites we will calculate the Potential of Mean Force (PMF) for an Fe^{+2} ion inside a 3-fold pore via two methods, namely ABF and Umbrella Sampling (US) at three different concentrations of Fe^{+2} and Cl^{-} in the cytosol: $10^{-4}M$, $10^{-5}M$ and $10^{-6}M$, which are within physiological range. [8,9] We will verify that both ABF and US methods produce the same outcome, i.e., the US method will be implemented as a consistency check on the ABF results.

In preliminary studies done by [redacted] the MD simulations were performed on the SaM cluster using NAMD employing the CHARMM36 force field. The crystal structure of the human heavy-chain protein $2FHA$, consisting of 66,456 atoms was solvated in a water cube consisting of 253,600 water molecules. The total charge of the protein was computed to be -216 and was neutralized by adding 108 Fe^{+2} ions. The system was then re-solvated in the 0.15 M/L $NaCl$. The "tagged" Fe^{+2} ion was placed at the entrance of a three-fold channel. The molecule then was energy-minimized and pre-equilibrated for 5 ns before production runs. In all simulations that followed the backbone atoms of the protein were harmonically restrained with k=3 $kcal/mol$. Of the 108 Fe^{+2} ions, 1 Fe^{+2} ion was unrestrained and 107 Fe^{+2} ions were harmonically restrained with k = 1 $kcal/mol$ in order to prevent them from entering the pore occupied by the tagged ion. The restrained Fe^{+2} ions were distributed randomly throughout the simulation box.

First, an SMD [10] run with the force constant set to k=5 $kcal/mol$ was performed in order to explore possible locations of the binding sites and provide starting structures for the ABF calculations that followed. Two binding sites were identified: the first binding site at ~60 Å is located near the GLU 134 residue and the second binding site at ~53 Å is located near ASP 127 which is consistent with observations made by R. Laghaei for the case of high concentration of Fe2+ ions in the previous studies done in this group. [5]

Second, preliminary ABF calculations were performed in order to estimate the PMF of one Fe^{+2} ion along the channel axis. The reaction coordinate was chosen to be the distance between the center of mass of the tagged ion and the center of mass of the protein along the central axis of the three-fold pore. The distance over which the PMF trajectory was estimated was divided into five windows, each ~4-6 Å long, and each containing one Fe^{+2} ion. The pdb snapshots for each window were obtained from the previously performed SMD simulations as the ion progressed through the pore. Each window was equilibrated for ~6 ns. Finally, the trajectories obtained from each ABF simulation window were assembled to produce the PMF curve. The same binding sites as in the SMD simulation above were identified by the ABF method, namely ASP 127 and GLU 134, both having escape barriers of ~15 $kcal/mol$.

Funding: NSF grant, "Molecular Modeling of Protein Nano-Pores" ($470,000, 2015 - 2018).

II. Justification of resources requested

We will start by pre-equilibrating the system for 10 ns, followed by 6 ns Steered Molecular Dynamics (SMD) run that will provide the trajectory napshots subsequently used to define the windows in the ABF simulations. Both pre-equilibration and SMD runs will be performed for each of the three proposed Fe^{+2} concentrations resulting in 3 x (10 + 6) = 48 ns. Then the ABF method will be used to calculate the single ion PMF for transporting Fe^{+2} through the 3-fold pore over a distance of approximately 25 Å along the central axis of the channel at three different concentrations of Fe^{+2} in the cytosol. For each case, ABF calculations of the PMF will be performed in 5 windows of length 5 Å each. For each window, we will perform a 6 ns equilibration simulation, resulting in 3 x 6 x 5 = 90 ns. In addition, we will verify the energy barriers associated with the binding sites by computing the same PMF via Umbrella Sampling. In this case a 3 ns run will be performed in each of the twelve 2 A windows at three different concentrations, resulting in 3 x 12 x 3 = 108 ns.

The ABF and Umbrella Sampling simulations combined will take ca. 48 + 90 + 108 = 246 ns of MD simulation. All simulations will be implemented in NAMD. [11]

We have experience running MD simulations for the MD setup described above on the SaM cluster. Using the definition of a System Unit (SU): SU = (walltime)x(cpu scale)x(number of cpus), and considering that a 1ns MD run takes ~20hr to complete according to our preliminary simulation results, for 1 ns MD simulation we will need (1 ns x 20.0 hours) x (1.5) x (64) = 1920 SU. A total of 246 ns simulation will thus require 1920 x 246 = 472,320 SU. The breakdown of estimated computer time is as follows:

Pre-equilibration 57,600 SU; SMD 34,560 SU; ABF 172,800 SU; US 207,360 SU.

Total: 472,320 SU.

References:

1. [redacted]