A COMPARATIVE STUDY OF THE IMPACT OF Cd(II) AND As(III) ON THE STRUCTURAL STABILITY OF THE HUMAN URACIL DNA GLYCOSYLASE ENZYME; AN *IN-SILICO* APPROACH

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INTRODUCTION

Most organisms employ DNA repair pathways to recognize and correct damages in DNA molecules (Li et al., 2021). DNA glycosylases initiate the base excision repair pathway by recognizing and removing mutagenic bases from DNA (Parikh et al., 1998). However, the function of these enzymes can be affected by the accumulation of toxic heavy metals like cadmium, arsenic, and copper. Experimental studies have been performed on the heavy metals accumulation on glycosylase enzymes such as methyl-purine DNA glycosylase (MPG) and have shown that Cd(II), Ni(II), Pb(II), and Zn(II) have moderate to strong inhibitory effects on MPG (Gokey et al., 2016). Gokey et al. have also tested the ability of Cd(II) to inhibit the activity of the hUNG enzyme using both experimental and theoretical methods. The structural investigation by Gokey et al., using computational techniques, has demonstrated the inhibition of the activity of the hUNG enzyme by the Cd(II). The computational analysis based on steered molecular dynamics (SMD) and QM/MM MD simulations determined the entering of Cd(II) into the active site of the enzyme by removing the catalytic water and forming close contacts with some residues of the enzyme (Gokey et al., 2016). As a further study, a computational analysis on structural stability of hUNG in the presence and absence of Cd(II) was performed and the results supported the idea reported by Gokey et al on Cd(II) inhibition of hUNG (Paligaspe et al., 2021). The computational-based study done on As(III) accumulation on the hUNG enzyme has shown the feasibility of As(III) to bind with the enzyme and the reduction of activity of the enzyme by forming a stable enzyme-metal ion system (Paligaspe et al., 2022). However, the current work focused to study on structural changes and stability of the enzyme in the presence of toxic heavy metal ions; Cd(II) and As(III). The work was performed using computational techniques based on molecular dynamics simulations to identify the toxic heavy metal ion among Cd(II) and As(III) with high impact on the stability of the hUNG enzyme and hence activity.

METHODOLOGY

All molecular dynamics simulations of the hUNG enzyme with metal ions Cd(II) and As(III) were performed using the SPC/E water model at 300K temperature and 1 bar pressure as implemented in the GROMACS program (Abraham et al., 2018). The enzyme was modelled using the Kirkwood Buff force field (Ploetz & Smith, 2011). The Lennard-Jones parameters 0.27 nm and 0.025 kJ/mol for van der Waals diameter (σ) and potential well depth (ε) respectively, were taken for Cd(II) while 0.42 nm and 1.292 kJ/mol were taken for σ and ε of As(III) respectively (Kommu et al., 2016; Srivastava et al., 2017). Two enzyme systems were prepared for the simulation placing the enzyme in the middle of the cubical-shaped simulation box with a distance of 1.5 nm from the enzyme to the edge of the simulation box. For each simulation, the metal ion was placed in the position of the catalytic water found in the active site of the enzyme. The simulation box was solvated with the appropriate number of SPC/E water molecules. To maintain the electro-neutrality of the system, seven and eight chloride ions were added to each system of the enzyme with Cd(II) and As(III), respectively. Each system was then subjected to 1000 steps of steepest descent energy minimization followed by a 100 ps long molecular dynamics equilibration run. Finally, 150 ns long MD

simulations were conducted for each system. All the coordinates of the systems were stored at one ps interval for the analysis.

RESULTS AND DISCUSSION

Root mean square deviation (RMSD) is the most commonly used standard tool to quantitatively measure the similarity between two molecular structures (Kufareva & Abagyan, 2012). The backbone RMSDs of the enzyme in the two simulation systems in Figure 1 show how the enzyme conformation varies with simulation time. During the first 120 ns of the simulation, the RMSDs of the enzyme in both systems do not show large fluctuations. The gradual increase of the RMSD variation of the enzyme in the presence of As(III) continues till the end of the simulation, indicating that the enzyme has not reached the stable structural conformation. In contrast, the enzyme with Cd(II) obtains a stable structural conformation bringing firm variation around 2.22 nm at the end of 150 ns. However, when comparing the RMSD variations during the last nano-seconds of the simulation systems, the enzyme in Cd(II) presence shows a lower RMSD than the RMSD of the enzyme with As(III). This RMSD change indicates that the enzyme in the presence of Cd(II) has obtained a stable structural arrangement compared to the structural arrangement of hUNG with As(III). The RMSD result suggests that the enzyme stability is more affected by the Cd(II) presence than the As(III).

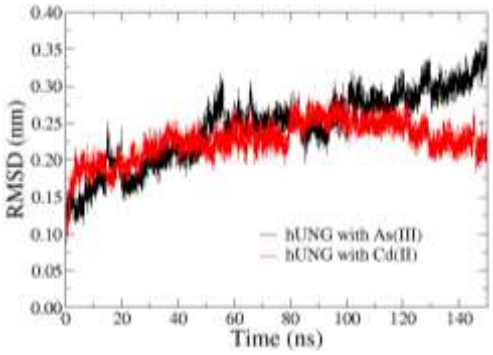

Figure 1: Backbone RMSDs of hUNG enzyme in the presence of Cd(II) and As(III) as a function of simulation time.

The level of compactness of a protein can be studied through radius of gyration (Rg) analysis and Figure 2 represents the Rg variation of hUNG in the presence of Cd(II) and As(III). During the last 70 ns of the simulation, the Rg of the enzyme in Cd(II) presence varies with a low value compared to the enzyme with As(III). This suggests that the enzyme has obtained a compact structure in the presence of Cd(II) which is more stable than the enzyme in As(III) presence.

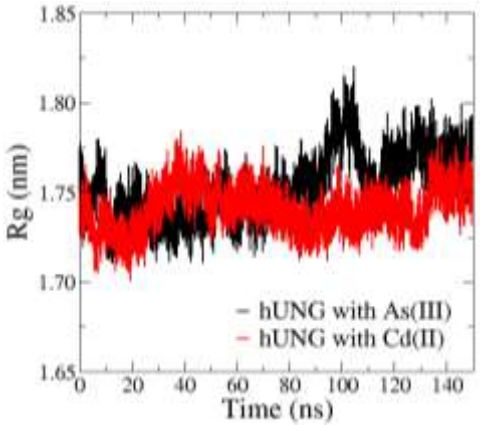

Figure 2: Radius of gyration of the hUNG enzyme in the presence of Cd(II) and As(III).

The number of hydrogen bonds formed within the enzyme during the simulation is given in Figure 3. Accordingly, for the enzyme with As(III), the number of hydrogen bonds formed has reduced at the end of the simulation compared to the beginning of the simulation. However, the enzyme in the presence of Cd(II) form more hydrogen bonds at the end of the simulation and is higher than in the enzyme with As(III). This highlights that at the end of the 150 ns simulation, the enzyme stabilizes by forming more hydrogen bonds than enzyme with As(III).

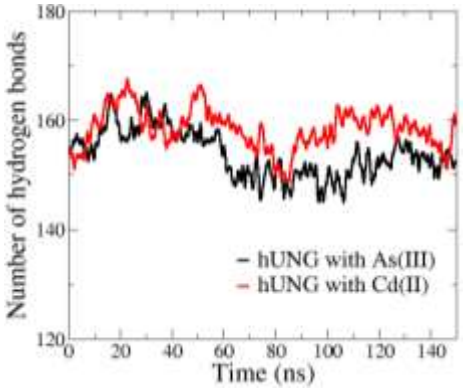

Figure 3: Number of hydrogen bonds formed within the enzyme during simulation time.

STRIDE is a software tool for secondary structure assignment that uses hydrogen bond energy and backbone torsional angle information (Heinig & Frishman, 2004). The STRIDE web portal was used to study the secondary structure variations of the enzyme hUNG in the two situations, and the server results are shown in Figure 4 for the final pdb structure obtained after the simulation. The alpha helices and beta sheets of a protein contribute to the most stable conformation of the protein by maximizing the pairing of hydrogen-bonding groups of the peptide backbone. According to the server results, the enzyme in the presence of Cd(II) has 72, and 24 amino acid residues that contributed to forming alpha helices and beta sheets respectively, while the enzyme with As(III) has only 63 and 26 amino acid residues. Therefore, the enzyme with Cd(II) has more residues in forming alpha helices and beta sheets when compared with the total number of amino acid residues forming alpha helices and beta sheets of the enzyme with As(III). This observation can suggest that the hUNG enzyme in the presence of Cd(II) has achieved a conformation that is more stabilized with a higher number of hydrogen bonds formed to hold the secondary structure of the enzyme in comparison to that of the enzyme in the presence of As(III). And this idea can be confirmed with the number of hydrogen bonds formed within the enzyme in the two situations at the 150 ns in Figure 3.

(a)

(b)

Figure 4: Cartoon representation of the secondary structure of hUNG; (a) in the presence of Cd(II), (b) in the presence of As(III). (Legend of secondary structure icons: Alpha-Helix Beta-Sheet Turn 3-10 Helix)

CONCLUSIONS

The RMSD and Rg analysis showed that the enzyme in the presence of Cd(II) has obtained a stable structure that is more compact than the enzyme structure in As(III) presence. This suggests that the Cd(II) has high impact on structural stability of hUNG enzyme than the As(III). The analysis on the number of hydrogen bonds formed within the protein also support the RMSD and Rg result showing more hydrogen bonds formed within the enzyme in Cd(II) presence indicating the stability of the enzyme. Therefore, the results of the stability analysis of the enzyme in the two systems show the possibility of toxic metals affecting the enzyme stability and indicates that Cd(II) has a higher impact than As(III). However, with the support of the idea reported on Cd(II) inhibition of hUNG enzyme by Gokey et al, we can suppose that Cd(II) has high possibility of inhibiting the hUNG activity than As(III).

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