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Exploring human mitochondrial inorganic pyrophosphatase functionality: insights from molecular dynamics and effects of the deleterious Met94Val mutation

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Motivation and Aim: Mitochondrial inorganic pyrophosphatase (PPA2), encoded by the *ppa2* gene, is an essential yet poorly characterized enzyme [1]. Naturally occurring mutations in its human gene disrupt the regulation of mitochondrial membrane potential, leading to mitochondrial dysfunction and resulting in severe heart conditions [2, 3]. The main focus of our study revolves around two human PPA2 (hPPA2) proteins: the wild type (WT) and one of the pathogenic variants, Met94Val. To broaden our understanding during the research, we also incorporated two additional subjects, analogous PPA2 from yeast *O. parapolymorpha* (WT OpPPA2 and Met52Val OpPPA2). Our aim is to elucidate the effects of the mutation on the proteins' behavior and identify patterns of its conformational changes using molecular dynamics (MD).
Methods and Algorithms: For human proteins, we modelled structures using I-TASSER. Two cofactor Mg^{2+} ions were modelled using a crystal structure of homologous PPase. Subunit structure was duplicated to create a dimer. For *O. parapolymorpha* proteins, we used crystal structure of dimeric OpPPA2 obtained earlier. Mutations were introduced by altering the residues using UCSF Chimera. Molecular dynamics (MD) simulations of 100 ns duration were conducted using the AmberTools22 package. Each structure was embedded in a truncated octahedron unit containing 55.5 M water molecules and 0.15 M salt concentration. An energy cut-off parameter of 10 Å was set, with a buffer distance of 12 Å. After minimization the system underwent a heating process from 0 to 300 K. Production simulations were performed under constant pressure at 300 K.
Results: Although the methionine residue is conserved among homologs, it neither holds a known functional role nor directly relates to the active site. This residue is a center of PPA2 hydrophobic core. Methionine in such positions is responsible for sustaining protein stability and providing flexibility because of its long, sulfur-containing side-chain. To assess the proteins stability, we obtained RMSD values for all 4 objects backbone atoms. Throughout the simulation, RMSD consistently remained below ~3 Å. Additionally, RMSD of mutants and wild-type proteins showed similar levels, indicating comparable global system stability. To evaluate residue flexibility, we calculated backbone atoms RMSF. Both OpPPA2 and hPPA2 enzymes exhibited similar RMSF patterns across protein domains. As expected, a significant decrease in flexibility occurred at the hydrophobic beta-barrel region, where Met94 of hPPA2 (Met52 of OpPPA2) is located. Interestingly, the mean RMSF of the Met52Val OpPPA2 dropped by 2.1 Å compared to the wild type, while the RMSF of the Met94Val hPPA2 was only slightly lower. We supposed that Val52 in the OpPPA2 mutant might not offer enough

flexibility due to its branched aliphatic chain. Further research is needed to understand why the hPPA2 experiences fewer effects, despite structural alignment showing no spatial differences in hydrophobic cores between OpPPA2 and hPPA2. Most active site residues showed decreased RMSF values upon mutation in both proteins, with the difference being more significant in the OpPPA2. To check whether any atomic positions rearrangement happens in the active site of the mutants compared to WT, we aligned the respective structures of the representative MD frames. We found little difference, except for Glu97 in hPPA2 and analogous Glu55 in OpPPA1. As these residues are involved in Mg^{2+} binding, their displacement may impact all catalytic stages. Using the principal component method, we analyzed protein conformations reached during the 100 ns simulation. Projection of phase space on the first two components indicated two main conformations clusters for human enzymes and transitions between them. Similarly, for the Met52Val there are two clusters far from each other. However, WT clusters are close to each other. To evaluate the scope of atoms movement of each protein, we acquired the eigenvalues of the covariance matrices which correspond to the first principal component. While eigenvalues were comparable for human proteins, for the WT and Met52Val OpPPA2 their ratio was ~1:15 respectively which indicated a significant difference in conformational flexibility. Visualizing PCA modes trajectories revealed that the most pronounced mode is subunits' relative movement. Analyzing contacts of methionine with surrounding residues revealed minimal change for OpPPA2. Here and onwards, by residue contacts, we mean the presence of their atoms within a sphere with a radius of 7 Å, as within this distance, electrostatic and van der Waals interactions, as well as contacts driven by hydrophobic effects, may occur. However, the human Met94Val variant showed a threefold reduction in contacts with crucial Tyr142 compared to WT. The explanation may be that, while directed to the tyrosine 142 backbone atoms, methionine acts as its anchor, but valine cannot act the same because of its short chain. Tyr142 plays role in substrate and product binding and is a second cofactor ligand, therefore, mutation may lead to incorrect positioning of the residue within the active site. Moreover, number of contacts with the above-mentioned Glu97 decreases a lot, suggesting that its anchoring may be affected too.

Conclusion: RMSD and PCA results obtained from MD trajectories suggest that the global dynamics of hPPA2 does not significantly change upon Met94Val mutation. On the other hand, analogous mutation Met52Val significantly decreases residues' flexibility in *O. parapolymorpha* PPA2. Replacement of long sulfur-containing methionine chain by short valine in hPPA2 affects its interaction with the backbone of essential residues Tyr142 and Glu97, which may lead to the disruption of active site preorganization and catalysis.

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