Distinguishing Metal-Binding Sites in GPI 12P/PIG-L Proteins under Molecular Dynamics and Simulation

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Abstract: A dramatic increase in the number of solved metallo protein structures and recent breakthroughs in structural analysis have provided a sufficiently detailed understanding of the structural chemistry of some metal-binding sites to allow successful design through homology modeling. As a result, metal-binding site design is now one of the most powerful and promising approaches for influencing protein folding, assembly, stability and catalysis studied under simulation process. Furthermore, these analyses clearly demonstrated how the metal ions cause the structural rearrangements that are required to exhibit proper function.

Keywords: Metal binding sites, GPI 12P, PIG-L, Dynamics, Molecular Simulation

Introduction

Eukaryotic cell surface proteins are bound to the cell membrane by a glycosyl phosphatidylinositol (GPI) anchor. The conserved core of this glycolipid consists of a lipid containing inositol (usually phosphatidylinositol (PI)), a glucosamine, three mannose residues and phosphoethanolamine. At least 50 GPI-anchored proteins with a wide variety of functions have been identified in Sacchromyces cervasia, including cell-surface hydrolytic enzymes, receptors, adhesion molecules, complement inhibitors, and antigens of unknown functions. GPI anchor synthesis occurs in the endoplasmic reticulum (ER) and essentially consists of the sequential addition of sugar components and phosphoethanolamine to PI [1]. Gpi12p/PIG-L proteins are ER resident membrane proteins which catalyse the removal of the acetyl moiety from GlcNAc-PI to yield glucosamine-PI (GlcN-PI), a crucial intermediate in GPI biosynthesis. Although, PIG-L protein does not have a known ER retention signal in its sequence, it is localized in the ER membrane facing the cytosolic side of the membrane [1]. Moreover, over expression of PIG-L resulted in the over expression of GlcNAc-PI de-N-acetylase activity. These characteristics are consistent with the idea that PIG-L is GlcNAc-PI de-N-acetylace; however, because PIG-L has no similarity with proteins with known functions, its function could not be predicted.

The yeast PIG-L homologue termed GPI12 was essential for growth of the yeast, suggesting that it is the only gene that encodes GlcNAc-PI de-N-acetylase in yeast and that de-N-acetylation of GlcNAc-PI is essential for GPI synthesis in yeast, as it is in mammals and protozoa. Mature GPI anchor precursors are post-translationally linked to proteins in the ER. Proteins that are to be GPI anchored have at their carboxyl terminus a signal sequence that directs GPI anchor addition. The signal peptide is exchanged with the GPI anchor by transamidation, forming an amide linkage between the new carboxyl terminus and ethanolamine [2]. Metal-ion binding proteins play a vital role in biological processes. Identifying putative metal-ion binding proteins is through knowledge-based methods. These involve the identification of specific motifs [3] that characterize a specific class of metal-ion binding protein. Metal-ion binding motifs [3] have been identified for the common metal ions. A robust global fingerprint that is useful in identifying a metal-ion binding protein from a non-metal-ion binding protein has not been devised. Such a method will help in identifying novel metal-ion binding proteins and proteins that do not possess a canonical metal-ion binding motif. Metal ion binding proteins leading us to believe that metal-ion binding proteins have a global fingerprint, which cannot be pinned down to a single feature of the protein sequence.

Materials and Methods

Identification of GPI12p/PIG-L protein sequences

GPI12p/PIG-L protein sequences were retrieved from NCBI protein database. Target sequence is submitted to PSIPRED for Secondary
Structure Prediction and modeling: The query sequence structure was compared after modeling based on PSIPRED results for satisfactory model validation.

Comparative Modeling:

Building a homology model comprises four main steps: Identification of structural template(s), alignment of target sequence and template structure(s), model building, and model quality evaluation. These steps can be repeated until a satisfying modeling result is achieved. The MODELLER is used for homology or comparative modeling of protein three-dimensional structure prediction [4].

Preparation of the Modeled structure for Simulation: PIG-L model structure (Figure 1A) modeled structure undergo Molecular Dynamics simulation for getting metalloprotein interactions.

Molecular dynamics setup:

The GROMACS molecular dynamics package was used [5-6] with the Gromacs force field. Assigning of the protons to PIG-L 3D modeled protein was performed automatically using the program pdb2gmx within the GROMACS package. Protonation is for correct hydrogen bonds within the catalytic triad. The protonations of PIG-L 3D modeled protein was based on the optimal hydrogen-bonding conformation. All atoms in the aromatic rings and the amino group in side chains were converted into virtual sites in order to eliminate fast improper dihedral fluctuations [7]. This modified model of PIG-L with Zn metal ion was solvated using the TIP3P CHARMM TIP 3-point with LJ on H’s water molecules [8-9] in a cubic box with a 1 nm solute-wall minimum distance. After a first steepest descent energy minimization with positional restraints on the solute, 2 chloride ions and 2 sodium ions were introduced by replacing water molecules at the highest electrostatic potential to compensate for the net positive charge on the protein. This was summed to a total of 34,047 atoms for the resulting system. A second energy minimization was performed until no significant energy change could be detected. Subsequently, the system was simulated by one successive run with 45 ps dynamics runs with decreasing harmonic positional restraint force constants on all protein atoms and followed by the production MD run. Protein, solvent and counter-ions were coupled independently to a reference temperature bath at 300 K with a coupling constant $\tau_t$ of 0.1 ps [8-9]. The pressure was maintained by weakly coupling the system to an external pressure bath at 1 atmosphere with a coupling constant $\tau_p$ of 1.0 ps. Long-range electrostatic interactions beyond the cutoff were treated with the generalized reaction field model using a BORN ELECTROSTATICS [10-11]. The LINCS algorithm [12] was used to constrain the bond lengths to their equilibrium positions, in conjunction with the virtual atom for aromatic rings and amino group in side chains, allowing a time step of 2 fs. The production simulation was performed for 20 ns, and coordinates were saved every 8 ps.

Analysis techniques:

Conventional structural and geometrical analyses such as energy calculations using the programs g_hbond, g_hdist and g_rms within the GROMACS software package, respectively. The non-bonded energies (Coulomb’s electrostatic energy) were calculated. The essential dynamics (ED) technique [13-14] was utilized to investigate large concerted motions in protonations of PIG-L Zn metalloprotein.

Results and Discussion

GPI12p/PIG-L protein sequences analysis:

GPI12p/PIG-L sequences retrieved from NCBI protein database consist of accession numbers were 392297454 and 45872616 and description of the function of N-acetylglucosamine-phosphatidylinositol de-N-acetylase precursor (GPI12p) and Phosphatidylinositol glycan anchor biosynthesis, class L (PIG-L), both shares common domain family “Phosphatidylinositol glycan”.

Homology and comparative modeling:

Homology modeling produced high-quality structural models when the target and templates are closely related. Structure modeling undertaken in present work could be a tool to study better structural characteristics of GPI12p/PIG-L proteins function community. The PIG-L protein structure was predicted by Swiss model automated mode. PIG_L protein 3D structure was satisfied with PSIPRED results. GPI12p three dimensional structures were built by modeller9v12 through multiple template modeling. It consists of 1UAN, 2IXD, 3DFF and 3DFI four X-ray crystallographic structure. Modeling studies manifested good stereo chemical placement of main chain parameters. Bond angles and bond lengths are under confined limits although side chain modeling introduced some levels of displacement of residues beyond most favored regions. Modeling process was repeated until model satisfied PSIPRED results.

Submission of the model to protein model database (PMDB):

The model generated for GPI12p was successfully submitted in Protein model database.
Large concerted protein motion with metal ion:

Essential dynamics was used to investigate the large concerted motions of metal ions in PIG-L protein structure. The covariance matrix built from atomic fluctuations in 45 ps. MD trajectory was diagonalized and the energies and their corresponding values were obtained. It has been shown that the overall internal motion of the protein with metal ion was described adequately by using only a few degrees of freedom. Energy files contain averages over all MD steps, or over many more points than the number of frames in energy file. When exact interactions between protein and metal atom has calculated in averages are present in the energy file are simply over the single, per-frame energy values (Figure 1, 2, & 3).

Figure 1: Zinc metallic compounds after lsq fit RMSD (Root Mean Square Deviation to protein showing interaction under simulation process.

Figure 2: Analyzed the interaction distance with respective time interval between Zinc atomic compound and protein.

Metallic Binding Sites:

Concerning with GPI12p covered with three-fourth part of PIG-L domain family. According to the survey PIG-L domain has more affinity towards metallic binding property. In this study mostly concentrated on the catalytic activity of the Zn metal ion plays a major role in activation of protein, was studied using two classical carbonic anhydrase assays: the hydration of acetaldehyde and the hydration of CO2. The complex displays high activity in the aldehyde hydration assay, comparable to that of carbonic anhydrase. With this hypothesis, experiments continued to identify metallic binding sites in GPI12p three dimensional structures. The simplest metal-binding sites that have been incorporated into proteins are those in which metal ions interact by the protein; the remaining coordination sites are filled by different metal ions. In this case, the goal has not been to mimic natural metallo proteins, but rather the metal-binding sites have been introduced with a view to applications in protein purification, stabilization, and the control of enzymatic activity. Due to this importance GPI12p under gone metallic binding sites prediction through auto dock vina. It stabilizes the assumption with introducing five metal ions such as Zn, Cu, Mg, Mn and Fe, to understand the interactive property with GPI12p amino acids (Figure.4). The designs realized to date are extremely simple and involve valine in appropriate position.
Figure 4: Image represents metallic binding amino acids from GPI12p protein

Conclusion
The present work addresses clearly that certain metal-sites designs have been extremely successful and the new proteins display the anticipated properties. A high resolution structures has been determined for the designed proteins (GPI12p/PIG-L), so the exact details awaited conformation of metallic binding sites of PIG-L zinc metallo protein laid a platform to analyze on GPI12p protein. PIG-L zinc metalloprotein studied under simulation process and retrieved effective metallic interaction as well as identified metallic sites for GPI 12p proteins to know whether it has stability to hold and maintain structure. GPI 12 was essential for growth of the cell.

References

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