Conservation of conformational changes upon ligand binding in homologous lipocalins

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Protein molecules are dynamic, which facilitates its movements that results in conformational changes of the molecule in order to perform its function. These structural changes vary from movement of domains to small repositioning of side chains. Generally, the general nature of protein functions is preserved among the members of a protein family. This is achieved by retaining the fold, conservation of three-dimensional structural features at functional regions and conservation of functional residues at specific topologically equivalent positions. However small, but critical differences between them are seen which bring about finer variations in their specificity and/or affinity to a ligand. The structural changes as a consequence of function also need to be maintained as much as conservation of functional residues or the structure. Therefore, it is important to understand the conservation of these structural changes within a family of proteins. To do so, a set of homologous lipocalins has been chosen as the model family considering their sequential divergence, remarkable structural similarity and its ability to bind various small molecules. The objective of the present work is to compare the ligand bound and free forms of homologous lipocalins and explore if the nature and extent of conformational changes are similar among the homologues. Specific care has been taken to ensure that comparative analysis of structural changes in two lipocalins is performed by considering homologous lipocalins of known structure bound to the same ligand.

Lipocalin proteins are small extracellular proteins which vary from 160-180 amino acids in length. The lipocalin proteins are all-beta proteins which constitute of 8-stranded anti-parallel β-sheets closed back on itself to form a continuous hydrogen bonded β-barrel which encloses the ligand binding cavity. With growing knowledge on the number of ligands it binds to and the molecular recognition properties, a better understanding on the conformational changes upon ligand binding is important because this property adds to functional diversity of this family. Interestingly, in spite of large sequence divergence amongst the family members, the ability to

bind the same ligand or perform the same function is still retained amongst many proteins in the family. Therefore, the main objective of the current work is to determine the extent of conservation of conformational changes in lipocalin protein family when it binds to the same ligand. There are 35 lipocalin proteins binding to 9 different ligands from protein structure data bank which was considered for the analysis for which the three dimensional structures were determined using X-ray crystallography. The basic pre-requisite was to ensure that at least two protein structures binding to the same ligand are available in its ligand bound form for each of the 35 lipocalin proteins. The conservation of structural changes have been analyzed in three aspects such as, the gross structural deviation between the homologues ligand bound pairs, independent deviation between the bound and unbound pairs and local structural changes.

Firstly, 70 ligand-bound pairs obtained from 35 lipocalin proteins were compared in a pair-wise manner where, in each pair, both the homologues bind to the same ligand. It is evident from the 70 pairs that the amount of structural change among the homologues is not same, even when it binds to the same ligand. Secondly, in order to analyze the extent of conservation of conformational changes in detail, 10 out of the 70 ligand bound pairs of lipocalin proteins were compared with their respective ligand-unbound forms. It was observed that the overall structural deviation among the homologues does not vary much between the bound and unbound pairs. Thirdly, since the overall structural deviation is notvery high, the conformational deviations in the structurally equivalent and the ligand interacting regions were further analyzed between the homologues in each ligand bound pair. Interestingly, the extent of conformational difference in lipocalin proteins is not same either in the structurally equivalent regions or ligand interacting regions, even if they bind to identical ligand. So it is clear that in spite of conservation of nature of the interactions, the interacting regions, the conformational changes due to interactions are different among homologues.

Although there is predominant structural similarity in the lipocalin protein family, the extent of conformational change is not conserved among the homologues when it binds to the same ligand.