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Deciphering the binding characteristics of DppA-dipeptide interaction by site directed mutagenesis

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This study was carried out to dissect how the dipeptide permeases protein A (DppA) responds to some changes of its binding site in order to decipher the mechanism governing the DppA-dipeptide interaction. Site-directed mutagenesis (SDM) was employed to understand the role of a specific residue at the molecular level for the structural or functional analysis of a protein two amino acid residues in wt-DppA, R355 and D408 were mutated separately as two single mutants and together as the corresponding double mutant. ITC titrations were performed with each mutant against each of the dipeptides (ala-ala, ala-phe, phe-ala and phe-phe) at 25°C. ITC analysis showed that all of the mutants bound tightly to the dipeptides except for phe-phe. In addition, even though the mutants bind to dipeptides they appeared to have low affinities compared to that of wt-DppA. This suggested that even with small modification in the binding pocket of DppA gave significant effect on its binding properties with clear differences visualized via heat outputs generated upon the binding process. This highlights the importance of particular amino residues in the wt-DppA binding site in controlling the binding preference between each dipeptide species. This study showed that the specificity of DppA-dipeptide interaction was significantly affected by the introduction of small mutations to its binding site especially when the polar negative residues that decreased in stability that led to lowering its binding affinity to dipeptides.

Biography

Mohamad Khairi Zainol has completed his PhD in Food Biophysics from Nottingham University, UK in 2012. He is currently working in Universiti Malaysia Terengganu, Malaysia as a Lecturer in the School of Food Science and Technology. His work is mainly based on food antioxidants and protein-ligand interactions.

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