Investigating the use of molecular modeling in site-directed protein mutagenesis

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Site-directed protein mutagenesis aids in protein purification and the study of protein separation mechanisms. However, the ultimate question one faces before selecting the sites for mutagenesis is whether the side chains of the new amino acids would be able to participate in the desired interaction without altering the protein's tertiary structure. An *in silico* model consisting of copper ion complexed to iminodiacetic acid was built. The model protein chosen for our investigations was T4 lysozyme. From the 3D structure (Protein Data Bank) the solvent accessible surface of each amino acid was calculated. Amino acids with high surface accessible values were chosen for point mutations and homology modeling was used to incorporate the replacing amino acid into lysozyme. Energy minimization was performed (MMFF94 force field) on the protein-ligand complexes (homology models containing point mutations complexed with the *in sililco* copper ion model) to determine their stability. Sites for protein mutagenesis were selected from the results obtained from the energy minimization calculation. Two different experimental strategies were used to generate several point mutations (K19H, K83H etc., where K stand for Lysine, the number (19 and 83) indicate the position of the amino acid in the amino acid sequence, and H stands for Histidne). Wild type and all variants had comparable activity indicating that the global structure of all variants was not altered by the substitutions point mutation. The effectiveness of molecular modeling in site selection for site-directed protein mutagenesis was evaluated by experimentally quantifying the interaction between the variants and the immobilized copper ion column. A correlation between the surface accessibility of the amino acid and its affinity to immobilized metal copper ion was established.