ABSTRACT

In this study, we investigated the effect of each amino acid in the conserved linker “TGEKP” and one of its variants “TGQKP”, on the binding free energy of Zif268 to its optimal DNA binding site (5′ A GCG TGG GCG T 3′). Ten point mutants of Zif268 were created. The free binding energy for each mutant with the optimal binding site was estimated using Molecular Mechanics Generalized Born Surface Area Method (MM/GBSA).

Compared to the wild type protein Zif268, one mutant (T56Y) resulted in lower binding energy by 20.74 kcal/mol; Three mutants (Q30E, E58Q and P60A) produced considerably higher binding energy (by 25.5, 18.2, 27.6 kcal/mol, respectively); Six mutants (T28A, G29P, K31D, P32G, and G57V, and K59P) produced binding energy values within the standered deviation from the binding energy of the wild type , where T28A showed an increase in the free binding energy by 14.38 kcal/mol, whereas G29P, K31D, P32G, G57V, and K59P showed a decrease in the free binding energy by 13.44, 8, 11.42, 9.4, 15.83 kcal/mol , respectively. The free binding energy values were decomposed into their three major

components: electrostatic energy, van der Waals, and the electrostatic contribution to the solvation free energy. The binding free energy values for the ten mutants had the highest correlation with the total electrostatic energy (the sum of electrostatic energy as calculated by molecular mechanics and electrostatic contributions to the solvation free energy).

Having only one out of ten with considerably lower binding energy suggests that the main reason for the high conservation of these linkers could be due to their role in biological processes other than specific binding to the DNA. Hydrogen bond analysis revealed that each mutant affected the stability and bond lengths. The effect on hydrogen bond stability was not confined to the vicinity of the mutated amino acid, and was detected throughout the zinc finger protein.