RESULTS AND DISCUSSION

1. Optimized structure and geometry study

In order to determine the position of added hydrogen atoms in the structure of OAA-PEPCK complex, we fully optimized the starting geometry which was directly taken from the X-ray structure by PM3 semiempirical method. All heavy atoms of the OAA-PEPCK binding site complex were fixed at the positions taken from the X-ray structure during optimization. The optimized structure was used as the starting geometry for interaction calculation and showed in the Figure 10.

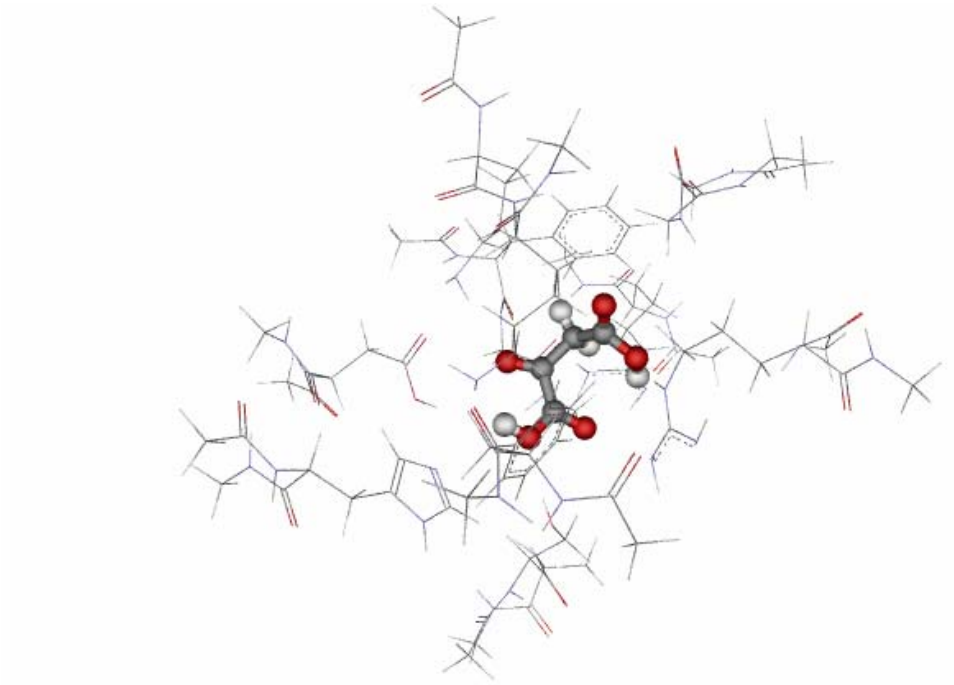

Figure 10 Show the optimized binding pocket of OAA-PEPCK complex, heavy atom fixed based on X-ray structure in order to determine the position of hydrogen atoms in the PEPCK pocket, and optimized structure by PM3 semiempirical method, a ball-stick structure represented oxaloacetic in the binding pocket and oxaloacetic is shown with the following atom colours: carbon, dark; oxygen, red and hydrogen, white.

The structural properties are the important factors to determine the nature of complex so fully geometry optimization of the isolated oxaloacetate were performed with MP2/6-31G(d,p) level of theory. Furthermore, the optimized geometry of oxaloacetate was checked by comparing with the X-ray crystallographic data of oxaloacetate in the complex with PEPCK. In order to compare the calculated results with the experimental data, the structural parameters such as bond length, bond angle and dihedral angle of optimized isolated oxaloacetate were compared with the X-ray data as shown in Table 2 and the atomic numbering of oxaloacetate for this investigation is given in figure 11.

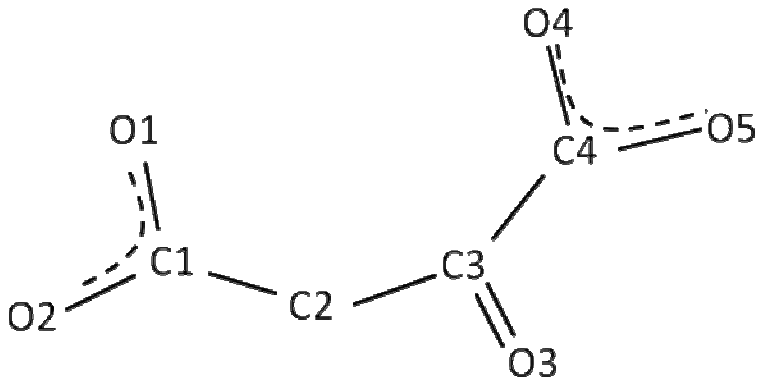

Figure 11 Chemical structure of oxaloacetate and atomic numbering that used in this study.

Table 2 Selected structural parameters of oxaloacetate, obtained by MP2/6-31G(d,p) level of theory and compared to the X-ray crystallographic data.

	MP2/6-31G(d,p)	X-ray
Bond length		
C1-O1	1.27	1.31
C1-O2	1.27	1.31
C1-C2	1.56	1.51
C2-C3	1.52	1.51
C3-O3	1.24	1.31
C3-C4	1.56	1.51
C4-O4	1.26	1.32
C4-O5	1.27	1.32
Angle		
∠C1-C2-C3	117.16	112.55
∠C2-C3-C4	115.72	117.30
∠O1-C1-O2	128.54	122.10
∠O4-C4-O5	129.52	122.40
Dihedral angle		
∠C1-C2-C3-C4	-177.45	-75.00

Comparison to the experimental data, the structural bond lengths obtained by calculation are not significantly different and the deviations are less than 0.07 Å. The angles and dihedral angles were partially different from X-ray geometric structure due to adaptation of structure for the prepared orientation in binding pocket of PEPCK active site. Superimposition of the optimized geometry of oxaloacetae, based on MP2/6-31G(d,p) and the X-ray structure is shown in Figure 12.

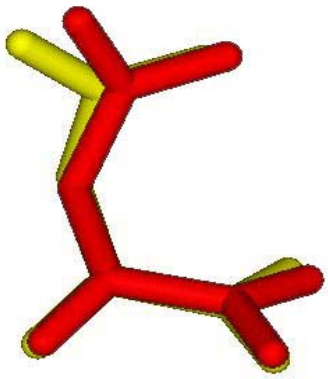

Figure 12 Superimposition of the optimized oxaloacetate by MP2/6-31G(d,p) (red) and the X-ray structure (yellow).

2. Interaction strength of oxaloacetic with individual amino acids (X_i) in the binding pocket

The interaction energies between oxaloacetic and the invidual amino acids (X_i) were calculated on the optimized complex structure obtained from the PM3 calculation in the previous step by single point calculation at MP2/6-31G(d,p) level with the basis set superposition error (BSSE) correction. The calculated interaction energy results are given in Table 3. These results explain the interaction strength between oxaloacetic to each amino acid surrounding the binding pocket. The interaction energy of oxaloacetic-X_i was calculated with the definition of the interaction energy (INT) as:

$$INT_{(OAA\text{-}Xi)} = E_{(OAA\text{-}Xi)} - [E_{(OAA)} + E_{(Xi)}]$$

The interaction energies can be presented by graphical representation, attractive interactions in red colour and repulsive interaction in blue colour, as shown in Figure 13. It is clearly seen that there are more attractive interactions between oxaloacetic and amino acids surrounding the binding pocket of PEPCK enzyme. These attractive interactions increase the stability of oxaloacetic upon binding in the PEPCK enzyme.

Table 3 Interaction energies of oxaloacetic with individual amino acids (X_i) (in kcal/mol), calculated at the MP2/6-31G(d,p) level of theory.

Amino acid	Interaction energy (BSSE), kcal/mol
Arg87	-3.06
Gly236	-0.32
Gly237	-0.41
Lys243	-0.79
Lys244	1.31
His264	-6.23
Ser286	-1.91
Ala287	0.08
Asp311	-3.40
Phe333	-0.23
Gly334	0.11
Arg405	0.82
Phe485	-0.52
Total	-14.55

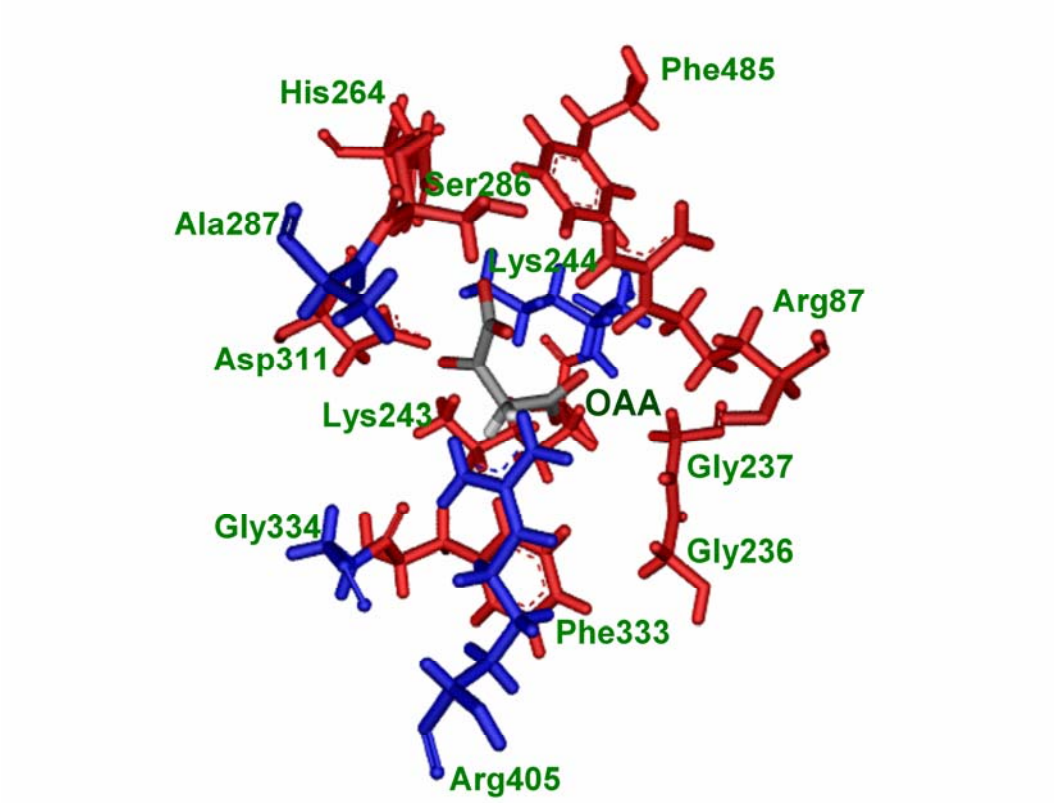

Figure 13 Graphical representation of the attractive (red) and repulsive (blue) interactions between oxaloacetic and individual amino acids (X_i) of PEPCK.

The obtained results in Table 2 show that three amino acids, Arg87, His264 and Asp311 are the main contributors in this system because they have the high interaction energies to oxaloacetic which are -3.06, -6.23 and -3.40 kcal/mol, respectively. The energetic results obtained from the interaction between these three amino acids and oxaloacetic show strong interaction than other amino acids because these three amino acids form weak hydrogen bond to oxaloacetic. The hydrogen bond distance between Arg87 and oxaloacetic which is defined in the range of H atom to heavy atom is 2.46 Å while His264 and oxaloacetic is 2.69 Å and Asp311 and oxaloacetic is 2.53 Å. The observable distances of hydrogen bond were demonstrated in Figure 14.

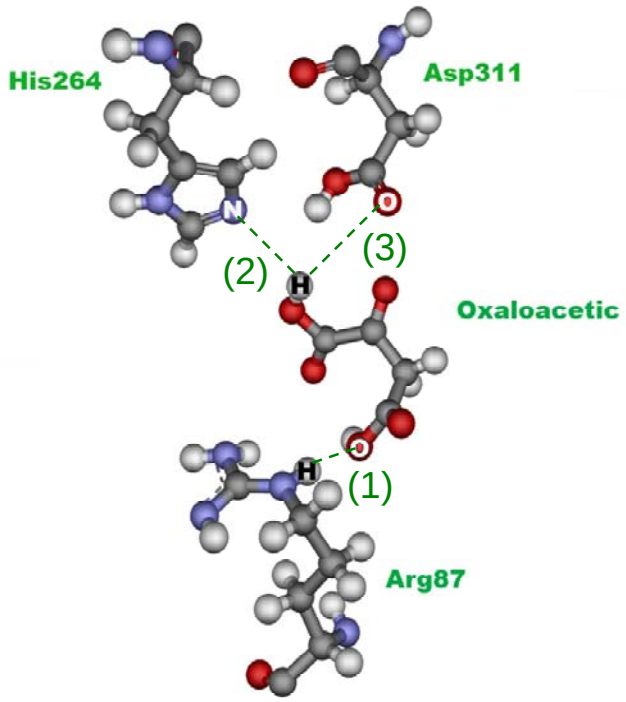

Figure 14 Graphical representation of the hydrogen bonds between oxaloacetate and three main amino acids, (1) = 2.46 Å, (2) = 2.69 Å and (3) = 2.53 Å.

Therefore, it is important to note that these hydrogen bond interactions play an important role in the binding of oxaloacetic in the bound PEPCK complex. If we change some properties of these interactions by using noncompetitive inhibitor, the enzymatic reaction may not occur.

3. Interaction strength of oxaloacetate with individual amino acids (X_i) in the binding pocket

In order to study the charge system that occurs in the nature, the interaction between oxaloacetate and individual amino acids in the binding pocket was performed at MP2/6-31G(d,p) level with BSSE correction. The calculated interaction energies are given in Table 4. These results showed that the amino acids having charge, Arg87, Lys243, Lys244, Asp311 and Arg405 strongly bind to oxaloacetate via electrostatic interaction.

Table 4 Interaction energies of oxaloacetate with individual amino acids (X_i) (in kcal/mol), calculated at the MP2/6-31G (d,p) level of theory.

Amino acid	Interaction energy (BSSE), kcal/mol
Arg87	-189.46
Gly236	-4.60
Gly237	-6.64
Lys243	-132.33
Lys244	-160.33
His264	6.89
Ser286	-9.52
Ala287	-1.72
Asp311	107.52
Phe333	1.97
Gly334	-2.77
Arg405	-154.34
Phe485	-9.55
Total	-554.88

According to the calculated interaction energies between oxaloacetate and amino acids surrounding the binding pocket, they showed that the charge residues, Arg87, Lys243, Lys244, Asp311 and Arg405, have the overestimate interaction energies ($INT_{(OAA-Xi)}$ > 100 kcal/mol). It is still the problem that I have to solve in the further work.

CONCLUSION

The structural properties such as bond length, bond angle and dihedral angle of isolated oxaloacetate were studied by MP2/6-31G(d,p) level of theory. These parameters were compared to the X-ray crystallographic data and it showed that the structural bond lengths obtained by calculation are not significantly different from the X-ray data and the deviations are less than 0.07 Å. However, the angles and dihedral angles were partially different from X-ray geometric structure due to adaptation of structure for the prepared orientation in binding pocket of PEPCK active site.

The total 13 piar-summed energies were used to describe the interaction energy of oxaloacetic and the PEPCK binding site, which is about -14.55 kcal/mol. The obtained results of interaction energies indicated that Arg87, His264 and Asp311 demonstrate stronger interactions than others (-3.06, -6.23 and -3.40 kcal/mol, respectively). These interaction energies were mainly contributed by the weak hydrogen bonding interaction between oxaloacetic and Arg87, His264, and Asp311. However, His264-oxaloacetic interaction is quite greater than others because His264 also forms H-π interaction to oxaloacetic. For the charge system, Arg87, Lys243, Lys244, Asp311 and Arg405 strongly bind to oxaloacetate via electrostatic interaction. A further work will be studied on hydrazine sulfate which is a noncompetitive inhibitor of PEPCK. This noncompetitive inhibitor should change some properties of the complex between PEPCK and oxaloacetate.