Original Research Article

Investigation of the Conversion Process of Verdoheme Hydroxyl Iron (II) to Biliverdin Iron (II): Theoretical Study

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ABSTRACT

Heme oxygenase (HO) is an enzyme that breaks down heme and as a result iron is recycled in most organisms, including the human body. This enzyme produces bilirubin, divalent iron, and carbon monoxide. Heme Oxygenase was first described in the late 1960s [1-3]. Study of the reaction of the peripheral substituents on the heme ring with verdohemes offers models that are not very well known. In this project, a computational study of destruction reaction of verdoheme iron (ii) was carried out and the role of lateral substitutionson its hydrolysis process was investigated. Theoretical calculations show that the rate of hydrolysis reaction is slower for complexes containing peripheral substitutions. On the other hand, hydrolysis of verdohemes non-environmental is a convenient parameter for energy. To better understand this process, theoretical calculations were performed using the DFT method. Calculations of the stability energy of the compounds showed that the higher the intermolecular interactions of hydrogen, the greater the stability of the compound. Also, the highest stability energy is related to compound D, which has more intermolecular interactions from type of the hydrogen bonds.

Keywords: Hydrolysis verdohemes, DFT calculation, Hydrogen bond, Keq

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Introduction

Verdoheme in which can be achieved via the cyclization and dehydration of biliverdin iron (ii) complex in the presence of iron salts [4-8]. This enzyme produces bilirubin, divalent iron, and carbon monoxide [5, 9-13]. The biological activity of HO protein is also examined through the oxidation process. This protein has many biological uses, including its anti-cancer activity [14], as modulators of autophagy and inflammation[15] and also it is used as a way to develop methods for the development of selective reactions and medical and therapeutic applications. [16]. Safari et al. have newly reported the computational studies of the hydrolysis progress of verdoheme into biliverdin. To date, no studies have been reported to investigate the effects of peripheral substituent on the hydrolysis rate of verdoheme [5, 17-21]. In this study, the changes of verdoheme to biliverdin were studied by using the base set of 6-31G (d) and B3LYP method [22-29]. The geometric structure of the heme protein is shown in (Fig 1). The reaction of nucleophilic attack to verdoheme iron (II) complex was performed by G09 program. Calculations at this level are corrected for estimating a spin mode. Binding of nucleophiles to carbon exo in Fe (II) complexes reduces the bond strength of C-O in the intermediate. The failure of this bond is further exacerbated until it appears in the product as an open ring. The ring opening process in this complex is exothermic and the most stable spin state based on the energy of the products relative to the reactants in the final product production path is the ternary state. When the ring is opened, biliverdin is produced, which is an effective factor in the treatment of diabetes. Therefore, such a metal complex can be used as a drug in the treatment of jaundice. Biliverdin has a wide variety of applications, including in medicine and diabetes, hepatic disease [30-37]. In this project, the Iron(II) complex which consisted of two methyl groups, two vinyl groups and an axial ligand ($C_3N_2H_4$), Heme protein, were first optimized (A complex). In heme protein, the oxygen atom acts as a bridge between two pyridine rings. In the next step, the carboxylate groups were attached to the two upper pyridine rings and these structures were optimized under headings 1A-1E and 3A-3E complexes. These complexes were studied in three different spin states and their stability energies were studied. Then the mechanism of hydrolysis reaction was investigated and the structures were optimized in transition state. In the final stage, all clusters were optimized after the hydrolysis process and breaking of the carbon-oxygen bond,

which leads to the opening of the ring, and their stability energies and vibrational frequencies, were calculated.
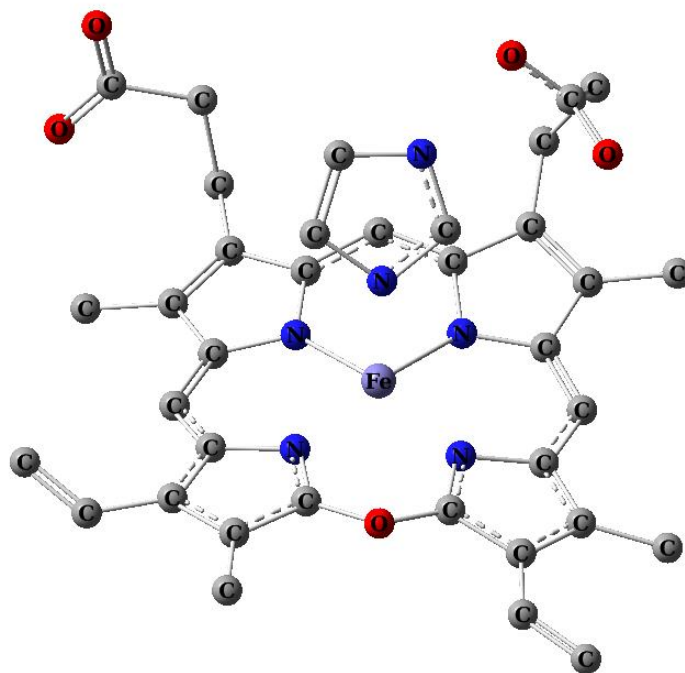

Fig. 1. Heme molecule in the heme oxygenase enzyme

Theoretical calculations

To examine the vibrational properties, structural characteristics, and also achieve a more identification of the interaction between the molecules, All structures were studied, theoretically [24]. The Gaussian view and Gaussian 09 software have been used as applications to optimize the desired compounds [38]. [FeOP (L)]$^+$ Complex (OP=5-oxaporphyrin and L= peripheral substituent) as an originator with considering the side groups, in five diverse states, have been optimized and stable energies, changes in bond lengths and their vibrational frequencies computed using DFT method ‹B3LYP/6-31G (d) level and using Gaussian 09 program. Geometric structures and computational parameters for all complexes were studied in three spin states: singlet, triplet and quintet. In this project, side substituent roles on the process of

hydrolysis and opening of the ring were studied. To better understand this process, the following suggested mechanism was considered (Fig. 2).
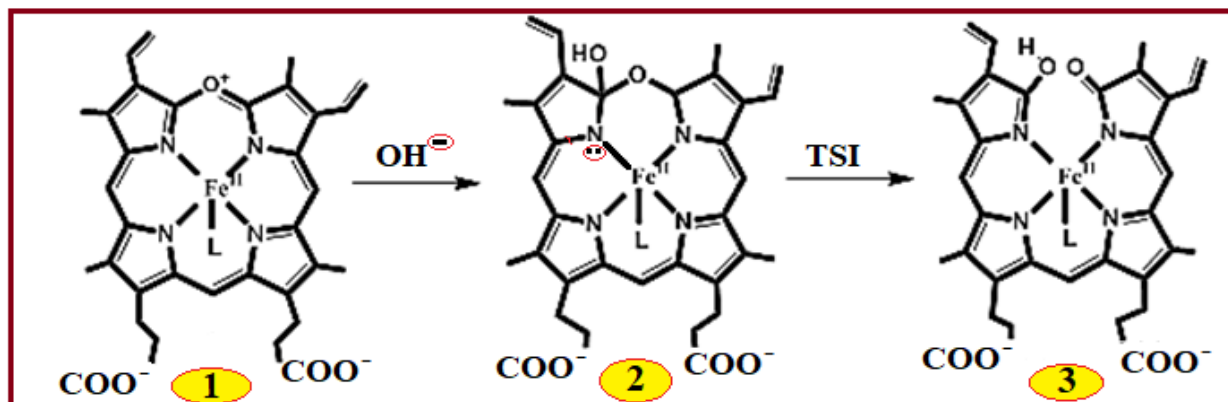

Fig. 2. The studied mechanism in verdoheme hydrolysis

Results and Discussion

Optimizing the verdoheme Iron (II) complexes with peripheral substituents

In this paper, we studied the cationic complex of verdoheme Iron (II) of five coordinated in three different spin states. Verdoheme Iron (II) was studies along with two groups of propionate ($CH_2CH_2COO^-$), two groups of methyl (CH_3), and two groups of vinyl ($CH_2=CH_2$). This complex with peripheral substituents in four different states and each state was calculated in three spin states (Fig. 3). Also, Safari et al. have indicated that the direct nucleophilic attack to the verdoheme iron ion is a practicable path for the transformation of verdoheme to biliverdin [17, 18]. All these structures were first optimized in terms of energy and then examined in terms of vibrational frequencies. Positive frequencies are a sign of stable structures. And the presence of negative frequency indicates that the optimized structure is a transition state.
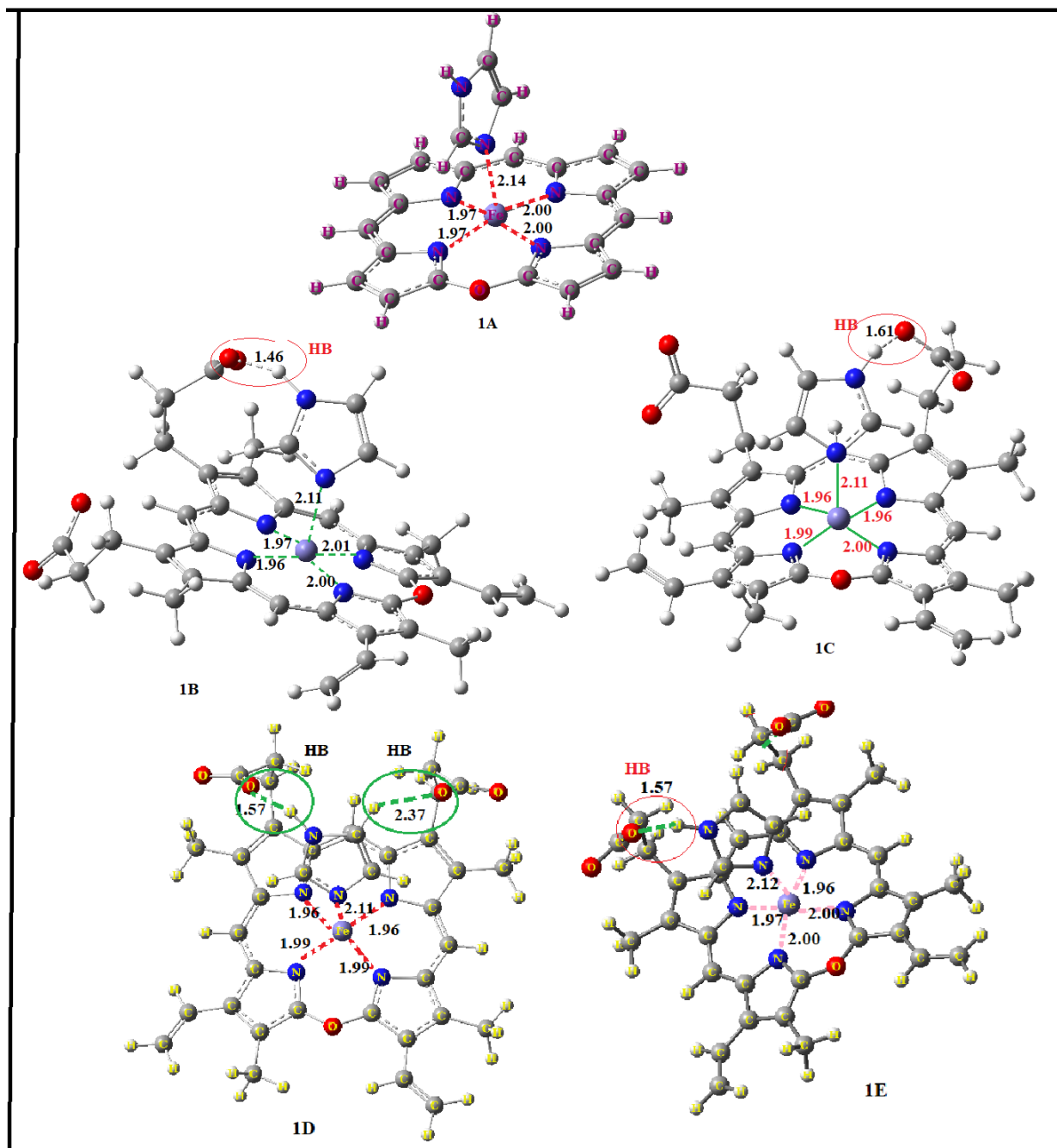
Fig. 3. Optimized Structure of verdoheme Iron (II).

Stability energy data for all studied complexes in different spin modes are presented in Table 1. The data in this Table show that isomer 1D is the most stable isomer and this indicates that the presence of hydrogen interactions increases the stability of the system, as well as the greater the

number of hydrogen intermolecular interactions (hydrogen bond=HB), the greater the stability of the complex. Also, the resulting data confirmed that for all isomers, the triplet spin and singlet spin states are the most stable and unstable form, respectively. Thus, all subsequent steps of the calculations were performed in triple spin mode.

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Table 1. Calculated Stabilization Energy (SE) for of verdoheme Iron (II) complexes in Singlet, Triplet and Quintet spin states

	SE		
	Singlet	**Triplet**	**Quintet**
1B	-210810**5**.23	-210812**2**.10	-210811**9**.41
1C	-210810**6**.97	-210812**3**.84	-210812**1**.15
1D	-210810**6**.05	-210812**4**.84	-210812**2**.62
1E	-210810**2**.53	-210812**0**.51	-210811**8**.84

Study of the bond length in 1B -1E complexes

The C-O (in the COO$^-$ group) bond length in the 1E complex was 1.29 Å indicating 0.02Å elongation in 1B–1D clusters and the highest increase in bond length was observed for cluster 1D (1.30, 1.31 Å). N-H (in the peripheral ligand) bond length in the 1A complex was 1.00 Å indicating 0.07 Å elongations in 1B and 1C clusters (1.07 Å). While the amount of this elongation in the 1D cluster is 0.08 Å (1.08 Å length bond). In cluster 1D, in addition to the hydrogen bond with the N-H bond, the carboxylate group also participates in the hydrogen interaction with the C-H bond in the peripheral ligand. For this reason, the bond length in this cluster shows a relatively good elongation compared to other clusters (1.10 Å). The lowest elongation of the N-H bond length was observed for cluster 1E, which has the weakest hydrogen interaction. These variations are the result of charge transfer from the σ(C-O) to σ*(H-N, C-H) bond. That is to say, in 1B-1E clusters, there were small interactions in carboxylate group and peripheral ligand. These results indicate that the presence of carboxylate substitutions and the

formation of intermolecular hydrogen bonds have enhanced the interactions of iron with side atoms (Table 2) and this factor increases the stability of the clusters.

Table 2. Bond lengths (Å) complexes of the 1B-1E at B3LYP/6-31-G(d) level

complex	bond length(Å)		
	C-O (COO$^-$)	N-H $_{peripheral}$	H-C $_{peripheral}$
1A	-	1.00	1.07
1B	1.30	1.07	1.07
1C	1.30	1.07	1.08
1D	1.30, 1.31	1.08	1.10
1E	1.29	1.05	1.07

Investigation of the Hydrogen interactions (HB) in 1A -1E complexes

According to Fig 3, it can be said that the presence of carboxylate substituent groups (COO$^-$) causes new interactions that are of the type of hydrogen intermolecular interactions**.** The greater the number of these interactions, the greater the stability of the cluster. According to these results and according to the data presented in Tables 1, 3 and Fig. 3, it can be said that cluster 1D is the most stable cluster, because it has the highest number of hydrogen intermolecular interactions. On the other hand, complex 1A, which lacks hydrogen interaction, has the least stable energy.

Table 3. Hydrogen Bond lengths (Å) complexes of the 1A-1E at B3LYP/6-31-G(d) level

complex	bond length(Å)	
	HB(COO…H-N)	HB(COO…H-C)
1A	-	-
1B	1.46	-
1C	1.61	-
1D	1.57	2.37
1E	1.57	-

Study of the stretching frequencies of in 1A -1E complexes

In Table 4 are arranged the stretch frequencies of the 1A -1E complexes, that all of these values were achieved at the B3LYP/6-31-G(d) level. The O-C-O stretching frequency in the 1D complex was 1584 cm^{-1} showing -28 cm^{-1} red shift relative to 1E cluster and -25 to -45 cm^{-1} red shift relative to 1C and 1B clusters. Additionally, the H-N stretching frequency in the 1A cluster was 2564 cm^{-1} indicating -120 cm^{-1} red shift in 1D and -15 to -41 cm^{-1} red shift in the 1B-1E clusters. Also, a similar trend is observed for changes in the stretching frequency of C-H bond in clusters, and most of the changes are related to 1D cluster (-77 cm^{-1}). The changes observed in the vibrational frequencies show a good correlation with the changes in the bond length.

Table 4. Vibrational frequencies (cm^{-1}) for the complexes of the 1B-1E

complex	$\upsilon(cm^{-1})$		
	O-C-O(carboxylat)	N-H(peripheral)	C-H(peripheral)
1A	-	2564	3394
1B	1559	2549	3356
1C	1579	2523	3343
1D	1584	2484	3317
1E	1556	2524	3351

Study of verdoheme hydrolysis process

During the hydrolysis process, a nucleophilic attack by the hydroxyl group occurs, which results in the opening of the verdoheme ring. There are two different types of carbon-oxygen bonds on the ring, close to the methyl group (2D'')) and (close to vinyl group (2D'). Thus, both states were studied (Fig. 4). Studies showed that in terms of energy, the hydroxyl attack on carbon-oxide is more favorable. So, in this project, hydroxyl (O-H) group was added to the carbon-oxygen bond.
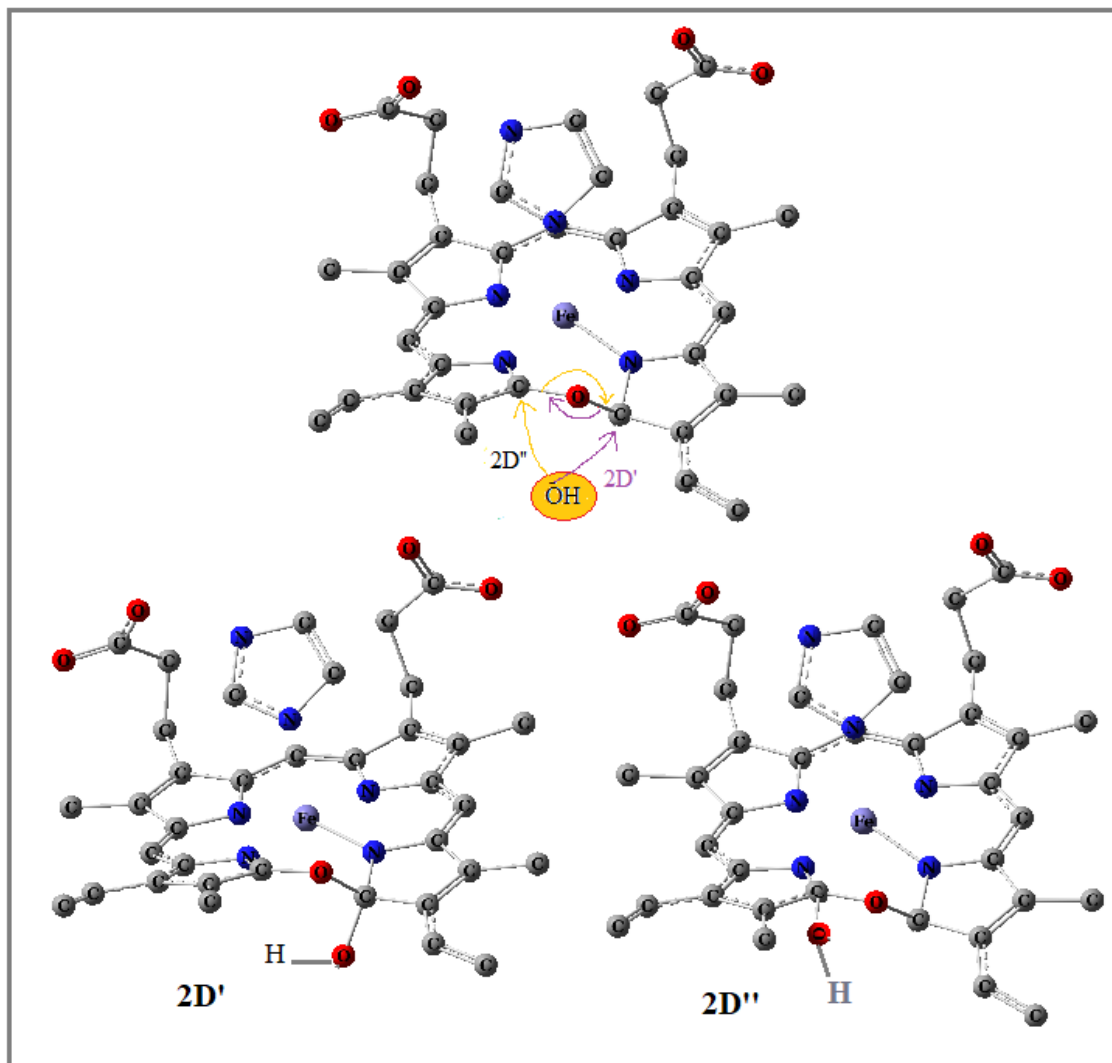
Fig. 4. Optimized various isomeric structure of verdoheme Hydroxyl Iron (II).

O-H group can be added to the ring in two states, Trans (2D") and Cis (2D'). The studies show that the verdoheme in Trans mode (2D") is more unstable than Cis mode (2D'). Because in the cis case, it is possible to form a hydrogen bond between the hydroxyl group and the nitrogen atom of the pyridine ring, and this factor increases the stability of the cluster. Therefore, nucleophilic (hydroxyl group) was added to the ring in Cis state and the stability of the

complexes was studied. Complex (2B) is the most stable and (2E) is the most unstable structure among the four isomers (Table 5).

Table 5. Obtained absolute and relative energy (E_{ab} and E_{re}) for optimization of verdoheme Hydroxyl Iron (II) in triple spin states

	E_{ab}	E_{re}
2E	**-2155690.60**	2.38
2D	-2155692.**48**	0.50
2B	-2155692.**98**	0.00
2C	-2155692.**41**	0.57

Optimization of biliverdin Iron (II) complexes

Biliverdin Iron (II) complexes with side substituent (peripheral) were optimized in triple spin state and four diverse structures. The stability energy for these isomers is described in Table 6. Results indication that the **3B** cluster is the most stable cluster (SE=-2155709.69 kcal.mol^{-1}). According to Fig 5 and the results shown in Table 6, it can be said that the higher the number of hydrogen interactions in a structure, the greater the stability of that structure. In addition, the strength of hydrogen bond N-H$^{+\sigma}$…$^-$OOC is higher than bond C-H$^{+\sigma}$…$^-$OOC, and this bond creates more stability for the clusters. According to the data in Table 6, the order of stability of the clusters is as follows**.**   **3B > 3C > 3D > 3E**
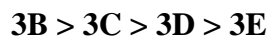
Table 6. Obtained stability energy for of biliverdin Iron (II) complexes in triple spin state

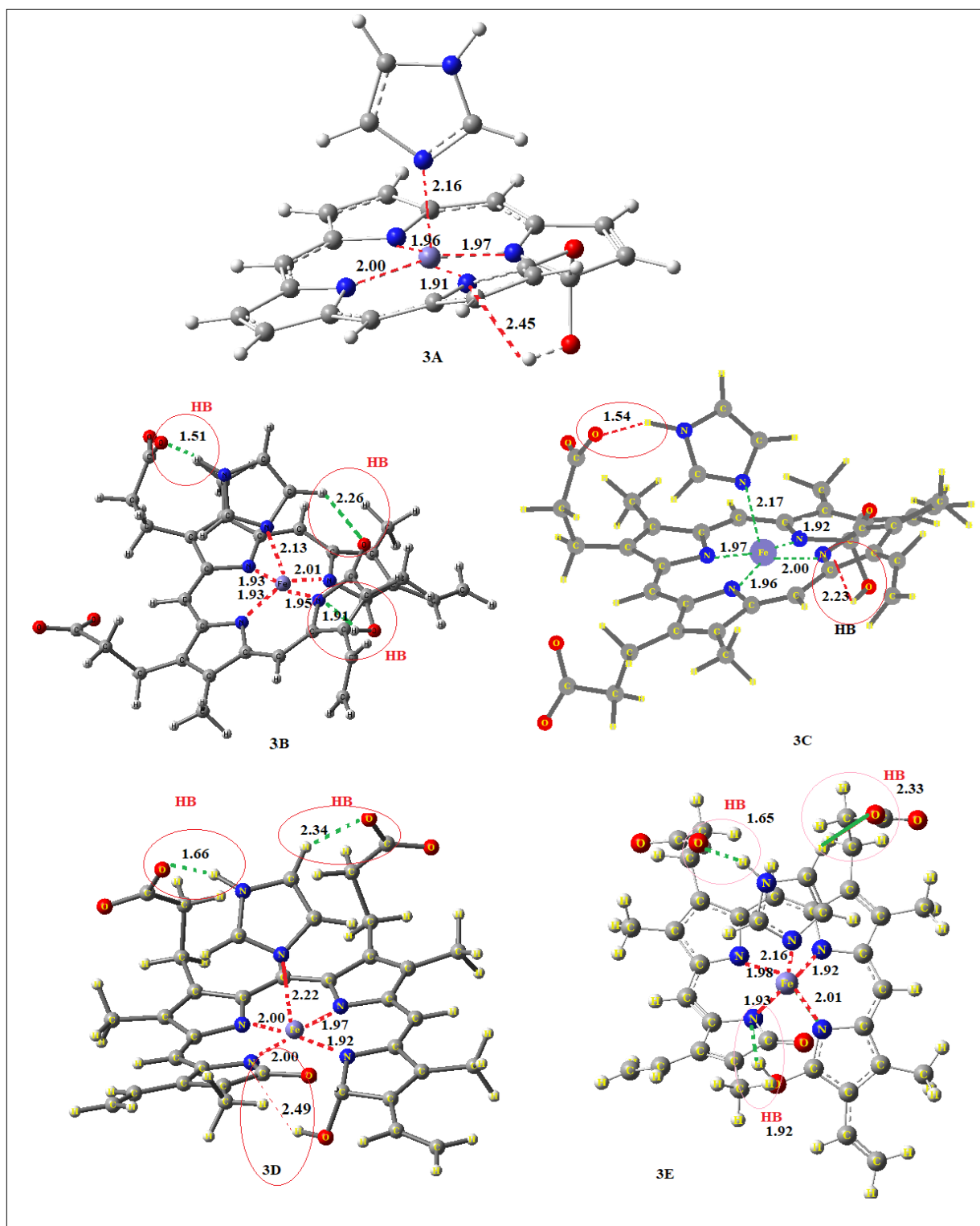| Complex	SE
3D	-2155706.36
3C	-2155708.61
3E	-2155706.20
3B	-2155709.69

Fig. 5. Optimized different isomeric structures of biliverdin Iron (II).

Investigation of the Hydrogen interactions (HB) in 3A -3E complexes

As mentioned earlier, the greater the number of hydrogen molecular interactions, the greater the stability of the system. According to the data in Table 7, it can be said that the strongest intermolecular hydrogen interaction is for the 3B cluster. According to the bond length data in the table below, it can be said that carboxylate groups form stronger intermolecular interactions than other linkages on the ring. The shorter the bond length, the stronger the bond, it can be said that the most stable and strong interactions are related to 3B cluster and the weakest interactions were observed for 3E cluster.

Table 7. Hydrogen Bond lengths (Å) complexes of the 3A-3E at B3LYP/6-31-G(d) level

	bond length(Å)			
Complex	HB(COO…H-N)	HB(COO…H-C)	HB(C-N…HO)	HB(C-H…O=C)
3A	-	-	2.25	2.24
3B	1.51	-	1.91	2.26
3C	1.54	-	2.23	-
3D	1.66	2.34	2.49	-
3E	1.65	2.33	1.92	-

Study of the stretching frequencies of in 3A -3E complexes

Table 8 summarized the stretching frequencies (cm^{-1}) of 3A-3E complexes. The N-H stretching frequency in the 3A was 2561 cm^{-1} showing -75 cm^{-1} (2486 - 2561= -75) red shift in 3B and -51 to -40 cm^{-1} red shift in 3C-3E clusters. Additionally, the C=O stretching frequency in the 3A molecule was 1629 cm^{-1} indicating -37 cm^{-1} red shift(1592-1629= -37) in 3B and -5 to -12 cm^{-1} red shift in the 3C-3E clusters. Also, the O-H stretching frequency in the 3A was 3357 cm^{-1} showing -32 and -26 cm^{-1} red shift in 3B and 3E clusters, respectively. The vibrational frequency changes for C-H in all clusters are of decreasing type and the highest red shift was observed for complexes 3B and 3E complexes.

Table 8. Vibrational frequencies (cm^{-1}) for the 3A-3E complexes

Complex	O-C-O(carbonyl)	N-H(peripheral)	C-H(peripheral)	C=O	O-H
			υ(cm-1)		
3A	-	2561	3389	1629	3357
3B	1353	2484	3362	1592	3325
3C	1338	2491	3381	1617	3350
3D	1324, 1319	2886	3379	1623	3348
3E	1329	2521	3355	1624	3331

Study of carbon-oxygen bond length changes in verdoheme hydrolysis process

The verdoheme to biliverdin conversion process was carefully studied. For this reason, carbon-oxygen bond length changes and how to break that bond was examined. The data collected in Table 9 show that the bond length of the carbon-oxygen in final product (biliverdin) and transition state has increased rather than reactant (verdoheme Hydroxyl)

Table 9. Comparison of bond distances of C-O in reactant, TS and product for A-E isomers

Complex	Bond distance (C-O)		
	Reactant	TS	product
A	1.48	1.85	3.12
B	1.48	1.76	3.20
C	1.48	1.88	3.21
D	1.47	1.85	3.24
E	1.47	1.82	3.28

Role of side substituents on the verdoheme hydrolysis

According to the energy diagram of the verdoheme to biliverdin conversion process (Fig. 6) and also according to the data of the Table 10, The higher the carboxylate branching groups, the higher the stability of the system due to the formation of intermolecular hydrogen bonds, and the complex participates in the hydrolysis process with difficulty.direct attack of the hydroxyl group on the ring causes it to open. Therefore, the more secluded the ring, the easier and faster the

nucleophilic attack. For this reason, the hydrolysis of verdoheme without substitution (3A) is much better and faster than verdoheme with peripheral substitution (3B).

Table 10. Calculated stability energy (kcal.mol^{-1}) for verdoheme (reactant, 1), verdoheme Hydroxyl (Intermediate, 2), transition state (TS) and biliverdin (Product, 3) in the triple spin state.

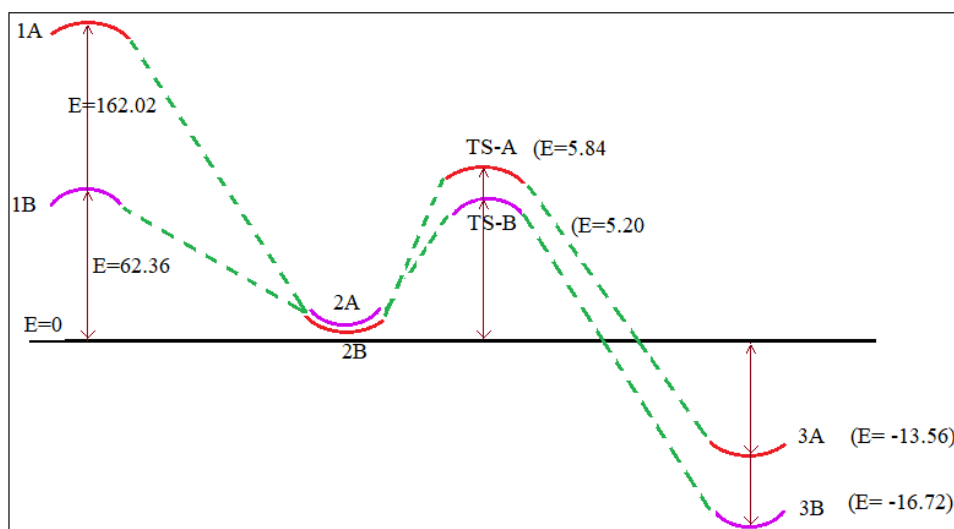| Complex	SE			
	Reactant	**Intermediate**	**transition state**	**Product**
A	167.02	0.00	5.84	-13.56
B	62.36	0.00	5.20	-16.72
C	63.53	0.00	5.43	-16.20
D	60.86	0.00	7.83	-13.89
E	63.31	0.00	6.48	-15.60

Fig 6. Energy Diagram verdoheme Iron (II) hydrolysis (kcal mol^{-1})

In the second stage of computational works, the vibrational frequencies were calculated for them. The presence of a negative frequency for the complexes shows of system instability and it show that of the broken the C-O bond at this stage. Data of the vibrational frequencies (cm^{-1}) and dipolar moment (μ. deby) for the A-E complexes show in Table.10. The lowest negative frequency is related to structure B, which indicates that this structure is more stable than the

others. Conversely, the highest negative frequency was observed for compound E, indicating that this isomer is the most unstable structure.

Table 10. Dipolar moment (deby) and vibrational frequencies (cm^{-1}) for TSA-TSE complexes

Transition State	freq	μ
TSA	-427.070	6.421
TSB	**-420.470**	21.626
TSC	-427.530	**23.306**
TSD	-428.920	18.571
TSE	-429.780	21.252

Study of thermodynamic parameters for the A-E complexes

Data related to thermodynamics parameters such as equilibrium constant (Keq), enthalpies (ΔH), entropy (ΔS) and Gibbs free energy (Δ**G**) are shown in Tables 11. The lower the free energy of Gibbs, the lower the stability of the composition and the easier it participates in the hydrolysis process. According to the data of the Tables 11, Gibbs free energy of verdoheme non-substitution (A) was decreases (-14.16) rather than verdoheme containing substitution (B-E complexes, -15.26 up to -18.32). This decrease is because the nucleophile attack to cationic ring is easier than to anionic ring (rings containing carboxylate groups, 2COO$^-$). According to the data in Table 11, Combination B has the most Gibbs free energy (ΔG, -18.32 kcal/mol). Thus, the isomer B is more stable than other isomers. Also calculated Gibbs free energy of TS (transition state) shows that the complex B has the lowest free Gibbs energy (3.99 kcal/mol). Therefore, isomer B is hydrolyzed faster. On the other hand, the higher the reaction equilibrium constant, the faster the reaction. Therefore, equilibrium constanst (Keq) show that the speed of the hydrolysis process in verdoheme non-peripheral substituent (A) is faster than other complexes. Also, among the verdoheme containing peripheral substituent structures, the highest equilibrium constant was observed for compound B, which has the highest stable energy.

Table 11. Thermodynamic parameters for the A-E complexes (kcal.mol^{-1}) in the triplet spin

Complex	ΔG, ΔH , ΔS		Keq
	TS	**3**	**Keq**
A	5.67, 4.75, 164.677	-14.16,- 13.56, -35.41	**6.861+122**
B	**3.99, 3.72, 266.877**	**-18.32, -17.10, -33.84**	**4.7683+48**
C	5.56, 4.73, 267.471	-17.47, -16.30, -32.68	2.7092+49
D	6.83, 6.50, 261.15	-15.26, -14.13, -33.83	2.6811+45
E	5.48, 5.23, 265.956	-16.90, -15.61, -33.85	2.7635+48

Conclusion

In this project, the process of converting verdoheme complexes (with and without peripheral substituent) to biliverdin was studied theoretically and various parameters such as stability energies, vibrational frequencies, Gibbs free energy, enthalpy changes, entropy and equilibrium constants of the complexes were calculated by Gaussian 09 software. The structures were studied in three different spin modes (singlet, triplet, and quintet) using the of B3LYP/6-31G(d) basis set and the DFT computational level. The results show that for all isomers, the triple mode has the most stability. The greater the number of intramolecular hydrogen interactions, the greater the strength of the interactions, resulting in a more stable complexion. For this reason, compound B has the most stable energy. The results of a constant study of the equilibrium of the compounds showed that the presence of substituent groups reduces the rate of hydrolysis reaction. The hydrolysis of verdohemes without substitution is much more desirable in terms of energy, because verdohemes has less space repulsion.

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