Computational simulation and theoretical comparative study for the complexes of heme-iron and heme-zinc

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Abstract: By using computer technology, molecular simulation and analysis of the coordination structure of heme iron and complex formed by the heme ligand and Zn are carried out under the same level of calculation. Studies have found that the ability of heme ligands to capture Fe is significantly stronger than that of Zn. However, the binding energy and the stability of the complex of heme ligand and Zn are stronger than those of the complex of heme ligand and Fe, and at the same concentration, the complex absorbs light in the near-ultraviolet region to a greater degree.

1. Introduction
Iron-porphyrins (i.e., hemes) exist throughout the biosphere and perform a wide range of functions[1-4], especially those involving complex multiple-electron redox processes[5-6]. The ligand-switch reactions of heme iron are common in biological systems[7]. When combined with hemoglobin, cytochrome c, cytochrome P450, and myoglobin, respectively, heme is an important cofactor for oxygen transfer, electron transfer, oxygen activation and oxygen storage. In addition, it has been shown that many functions, including translation, protein kinase activity, transcription, K⁺ channel function, DNA binding, protein degradation, microRNA splicing, heme degradation, two-component signal transduction and other functions are regulated by the association of heme and heme-responsive sensors. Studies have shown that heme can be used as a new chemical reaction center for N-N bond formation, aldoxime dehydratase, cis-trans isomerase, S-S formation and hydrazine formation[8]. Trace metals such as iron, zinc, manganese and copper play important roles in a variety of biochemical processes such as respiration and photosynthesis[9]. Studies have evaluated the stability of iron and zinc complexes in concentrated nutrient solutions and their reactivity with soil and soil components. Most complexes retain Fe and Zn in the concentrated nutrient solution of the irrigation system. Generally, the interactions of the compound with the soil and the soil components produce high retention. The results showed that the stability of the complexes under different conditions was related to the percentage of complex elements in the product. Although complexes can be used to maintain micronutrients in aqueous media, they should be considered for application in the soil to increase metal availability rather than solubility[10].
2. Research methods

By using Gaussian 16 B.01 program for density functional theory (DFT) [11-18] calculations, we used small nuclear relativistic pseudopotential ECP28MDF for Fe and Zn, and 6-311G (d, p) [19] basis set for C, H, O, N, all the stable configuration complexes obtained by optimization had no virtual frequency, and by using the same level of method, we calculated infrared spectrum, Ultraviolet-Visible (UV-Vis) absorption spectrum, binding energy and frontier orbital energy. The molecular models of heme iron and heme zinc synthesized by replacing the central metal ion were constructed with software. The infrared spectrum, ultraviolet-visible absorption spectrum, binding energy and frontier molecular orbital energy levels were compared to determine their characteristics and differences.

3. Results and discussions

Through simulation and calculation of heme iron and complex formed by the heme ligand and Zn, and comparing the Absorption Spectra, the Infrared Spectrum, the binding energy and the frontier molecular orbital energy levels of heme iron and complex of heme ligand and Zn, the characteristics and differences of the two complexes were identified.

3.1. Configurations analysis

The coordination geometry of the two complexes is shown in Figure 1. In Figure 1, complex a shows the optimized configuration of the heme iron, while complex b shows the optimized configuration of the complex formed by the heme ligand with Zn. Complex a and complex b are similar in coordination geometry.

![Figure 1 Optimized structure and main atom number of the complexes.](image)

The main coordinate bond lengths of the two complexes are shown in Table 1. Obviously, the bond length of Fe-N is shorter than that of Zn-N. It can be concluded that the ability of heme ligands to capture Fe is significantly stronger than that of Zn. Due to the difference in the coordination characteristics between ferric ion and divalent zinc ion, iron ion can bind to the heme cavity better than zinc ion.

### Table 1 Calculated bond lengths of the complexes (nm).

| Complexes | X-N1   | X-N2   | X-N3   | X-N4   |
|-----------|--------|--------|--------|--------|
| Complex a | 0.1928 | 0.1957 | 0.1959 | 0.1927 |
| Complex b | 0.2053 | 0.2051 | 0.2054 | 0.2049 |

3.2. Infrared spectrum

Using the same calculation level as the structural optimization, the infrared vibration spectrum of the complex of heme iron and zinc in the vacuum model is shown in Figure 2. Complex a and complex b correspond to the two complexes shown in Figure 1, the curve a and b in the figure represent the infrared vibration spectrum of complex a and complex b, respectively. As shown by the value of the spectrum, when the central atom Fe of heme is replaced by Zn atom, the peak of infrared vibration has a significant
red shift, and the carboxyl C=O stretching vibration absorption peak migrated from 1776.51 and 1818.9 cm\(^{-1}\) to 1707.42 and 1740.02 cm\(^{-1}\).

3.3. Ultraviolet visible absorption spectrum
The Ultraviolet-Visible (UV-Vis) absorption spectrum of the two complexes is shown in Figure 3, the curve c and d in the figure represent the UV-Vis absorption spectrum of complex a and complex b, respectively. The maximum absorption peak of complex a is at 365 nm, while the absorption peak of complex b is at 370 nm, and its absorption intensity is stronger than that of complex a. Thus, due to the complex b has better absorbance, if complex a and complex b are detected by ultraviolet-visible absorption spectroscopy, the detection sensitivity of complex b will be higher, and the detection limit will be lower. The optimal absorption wavelength of complex b is 370 nm and that of complex a is 365 nm, indicating that the ultraviolet-visible absorption of complex b has a significant red shift compared with complex a.
3.4. Binding energy
Calculating the binding energy of the complexes at the same level, as shown in Table 2. In equation (1),
the term $\Delta W_{AB}(R)$ denotes the binding energy and $W_{AB}(R)$ denotes the total energy of the interacting
system, the terms $W_A(R)$ and $W_B(R)$ represent the energy of two independent molecules A and B,
respectively. All the binding energy values are calculated by the equation (1).

$$\Delta W_{AB}(R) = W_{AB}(R) - W_A(R) - W_B(R)$$

(1)

The results showed that the binding energy of the complex a and complex b were -1220.9744kJ/mol
and -708.7139kJ/mol, respectively, indicating that the binding capacity of heme ligand to iron is greater
than that to zinc. These changes are caused by the central atom of the complexes, since the radius of
iron ions are larger than those of zinc ions, the special cavity structure of the heme ligand is difficult to
capture zinc ions well. Thus, the binding ability of the heme ligand to iron is much greater than that of
zinc.

| complexes   | complex a               | complex b               |
|-------------|-------------------------|-------------------------|
| binding energy(kJ/mol) | -1220.9744             | -708.7139              |

3.5. Frontier orbital energy
The energy value of the lowest empty molecular orbital (ELUMO) and the highest occupied molecular
orbital (EHOMO) from the frontier molecular orbitals, and the energy gap ($\Delta E_{L-H}$) which is the energy
difference obtained by subtracting the highest occupied orbital from the lowest empty molecular orbital,
as shown in Table 3. The $E_{LUMO}$ and $E_{HOMO}$ of the complex a are -0.0859 and -0.1651 eV, respectively,
and the energy gap between $E_{LUMO}$ and $E_{HOMO}$ is equal to 0.0792 eV, that is, $\Delta E$=0.0792 eV. It shows
that in order to generate an excited state, 0.0792 eV will be absorbed by the ground state molecules of
complex a. However, the calculated $E_{LUMO}$ and $E_{HOMO}$ of complex b are -0.0881 eV and -0.1948 eV,
respectively, and the energy gap ($\Delta E_{L-H}$) is 0.1067 eV. From the above results, it can be shown that the
stability of complex b is significantly higher than that of complex a. At the same time, the energy gap
of complex a is lower than that of complex b. In summary, it can directly reflect that the stability of the
complex formed by the ligand and Fe is better than that formed by the ligand and Zn. It means that the
stability of the complex formed by Zn is stronger than that formed by Fe. Therefore, if zinc atoms are
used to replace the iron atoms in heme, the replaced complexes will have better stability.
Table 3  Energy value of $E_{\text{LUMO}}$, $E_{\text{HOMO}}$, and $\Delta E_{\text{L-H}}$ of the complexes (eV).

| complexes | complex a | complex b |
|-----------|-----------|-----------|
| $E_{\text{LUMO}}$ | -0.0859  | -0.0881  |
| $E_{\text{HOMO}}$ | -0.1651  | -0.1948  |
| $\Delta E_{\text{L-H}}$ | 0.0792   | 0.1067  |

4. Conclusion
The heme ligand binds more tightly to the iron atom than to the zinc atom. The bond length formed by the central iron atom and the N in the heme ligand is shorter than that formed by the central zinc atom and the N in the heme ligand, and the binding force is stronger. However, the binding energy and the stability of the complex of heme ligand and Zn are stronger than those of the complex of heme ligand and Fe, and at the same concentration, the complex absorbs light in the near-ultraviolet region to a greater degree. If the two complexes are detected by the ultraviolet-visible spectrophotometry, the detection sensitivity of the complex of heme ligand and Zn will be higher and the detection limit will be lower.

Acknowledgments
This work was supported by the Chenzhou City Heavy Metal Pollution Health Risk Assessment Technology Research and Development Center, and the National Natural Science Foundation and National Social Science Foundation Key cultivation projects of Xiannan University [grant number 2020XJ08].

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