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Analysis the UV-visible Spectra of Neuroglobin Based on Two-Level Model

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Abstract. Neuroglobin (Ngb), a novel member of the Globin Group, is recently discovered by Burmester et al. (2000). Its uncertain physiological function makes lots of interest. The existing of a six-coordination heme geometry with proximal and distal histidines directly creates an axis within the heme iron, while the sixth ligand coordination binds to small ligand reversibly. The analysis of UV-visible spectrum of Ngb by the well-known two-level model shows an agreement of the experiment data and theoretical results.

1. Introduction

Hemoglobin (Hb) and myoglbin (Mb) are types of globin family, having been studied for more than four decades. These proteins are characterized by a highly conserved α-helical fold and the ability to reversibly bind dioxygen and other small ligands at the central iron of a prosthetic group (protoheme IX). Neuroglobin (Ngb), namely the oxygen transporters/storage proteins, was recently discovered in the vertebrate globin family [1–3].

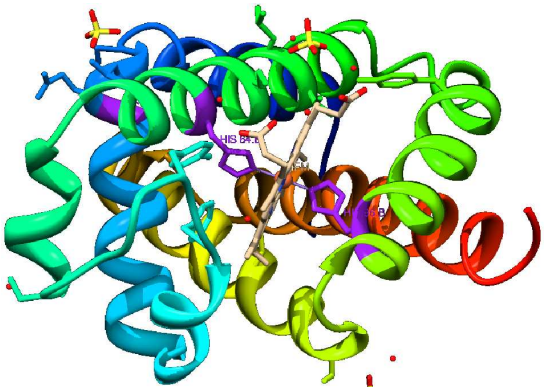

Figure 1. A model of the wild-type human neuroglobin (1OJ6.pdb file), in UCSF Chimera visualization.

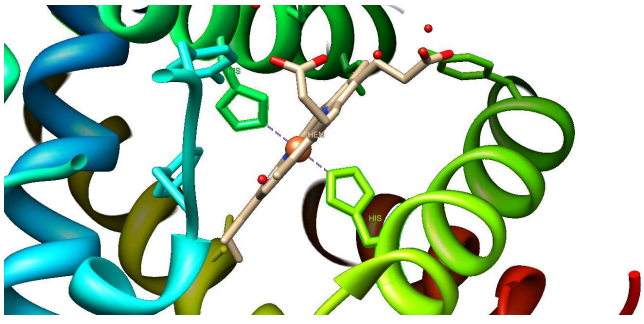

Figure 2. Zoom of active site of Ngb.

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Having a single chain of 151 amino acids, Ngb has less than 25% sequence identity with its more prominent cousins Hb and Mb, exhibit a classical three-over-three $\alpha-$ helix sandwich globin fold [3,4]. In the absence of an external ligand, the ferric (Fe^{+3}) and ferrous (Fe^{+2}) heme-iron of Ngb are both hexacoordinated, with histidyl imidazole of proximal His 96 (F8) and distal His 64 (E7) directly bound to the ion metal, whereas Hb and Mb are both pentacoordinated globins with only the proximal histidine bound to the iron. In the presence of external ligand (O_2, CO and NO), only the ferrous form is observed. This feature has been proposed as a novel mechanism for the regulation of ligand affinity in heme proteins [5, 6] but its functional significance is a problem of debate.

In Mb and Hb, the Fe^{2+} atom of the heme prosthetic group binds O_2 occurring on the distal side of a pentacoordinated heme, where O_2 establishes a sixth coordination bond to the heme Fe. While, in Ngb, the binding of O_2 is generally stabilized by interaction with distal residues. Often, in vertebrate globins, the main O_2 stabilizing interaction is provided by a hydrogen bond donated by residue HisE7.

Over the years, the bonding of O_2 to the iron-binding site of the heme protein makes a lots of interest [7–10], according to the strong electronic correlation effects associated with its localized Fe 3d electrons. It is well known that these electrons are energetically well-aligned with the π^* acceptor orbitals of CO and O_2, and that the molecules bound conformations seek to maximize intermolecular orbital overlap [11, 12]. While the spectra of oxygen binding to hemprotein is quite similarly to each other, the UV-vis spectra of wild-type neuroglobin (WT-Ngb) has also the same their feature. Therefore, in this report, we apply the two-level model to investigate the spectra of WT-Ngb.

2. Material and methods

The material and methods are detailed in the ref [13]. A sample contain WT-Ngb was examined in the spectral range of 280-700 nm, in condition of pH=2.3.

2.1. Crystal field theory for Fe^{2+} complex system

It is know that electron configuration of Fe is $1s^2 2s^2 2p^6 3s^2 3p^6 4s^2 3d^6$. Fe^{2+} has 6 d electrons so its configuration is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6$.

Neuroglobin contains a porphyrin ring with an iron at its center. Two histidines are the axial ligands of the Fe atom (Fig. 2), both in the Fe^{2+} and Fe^{3+} states, the heme is hexacoordinated. However, only ferrous state Fe^{2+} of heme-iron can binds to O_2.

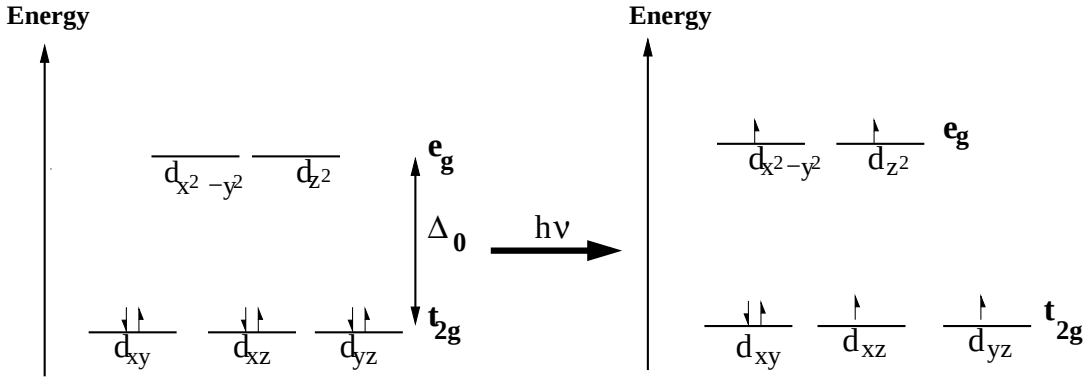

Figure 3. The energy level scheme for ferrous (Fe^{2+}) iron showing the occupation of the 3d orbitals (left) and d-d transitions when the electrons absorb the photons of light (right) [14, 15].

Following crystal field theory [14, 15], the Fe^{2+} complex has an octahedral shape. Fe^{2+} of neuroglobin has 6-coordination so its state is in low spin with weak ligand field. For the distorted octahedral geometry, the 5 degeneracy of d-orbitals split in sets of 2 (e_g) and 3 (t_{2g}) obitals (Fig. 3, left)

3 obitals of low energy: d_{xy}, d_{xz}, d_{yz}
2 obitals of high energy: d_{z^2}, $d_{x^2-y^2}$

The splitting between the t_{2g} and e_g orbitals stays constant at Δ_0 regardless of the nature of the distortion.

In the d–d transitions (Fig. 3, right), the electrons in the t_{2g} orbitals of Fe^{+2} ion in octahedral complex absorbs photons of light with energy equal to Δ_0, which causes the electron to move to an empty or singly occupied e_g orbit. The spectra of the absorbance is in UV-vis region. In this work, we apply a simple model to explain the peak of UV-vis absorbance spectra of WT-Ngb.

2.2. Two-level model

The figure 3 shows the model of structure of WT-Ngb and the energy level scheme for ferrous (Fe^{2+}) iron.

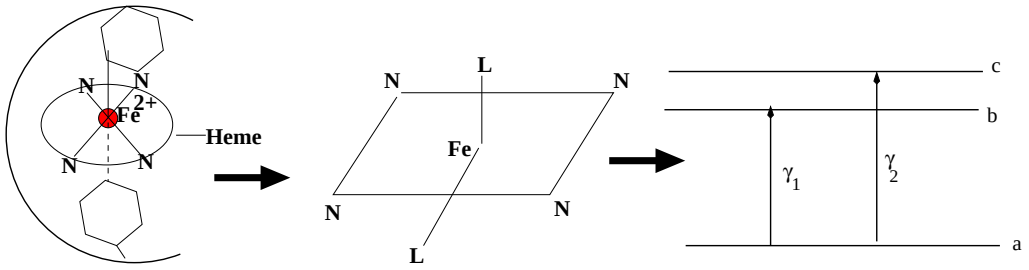

Figure 4. Two-level model diagram of Ngb.

Considering living time of excited state of electron in Fe^{+2} heme complex is much sorter than its living time of ground state, from uncertainty principle we can write for WT-Ngb complex system as follows

$$H = E_0 a^+ a + (E_1 + i\Gamma_1) b^+ b + (E_2 + i\Gamma_2) c^+ c + \gamma_1 (a^+ b + b^+ a) + \gamma_2 (a^+ c + c^+ a). \quad (1)$$

Now we have 2 levels:

- Level 0, ground state energy E_0:
  - $|0\rangle$ is the ground state of Fe^{+2} heme complex
  - a and a^+ are the annihilation and creation operators of Fe^{+2} heme complex in ground state.
- Level 1, excited state energy E_1 and E_2:
  - $|1\rangle$ is the excited state of Fe^{+2} heme complex,
  - b and b^+; c and c^+ are the annihilation and creation operators of Fe^{+2} heme complex in excited states E_1 and E_2, respectively.

This simple two-level model has a set of 4 parameters, which can be taken from experiments:

$\Delta E_1 = E_1 - E_0 = P_1$ and $\Delta E_2 = E_2 - E_0 = P_2$ are the pairing energies of the absorption peaks,

γ is the transition matrix element between the two levels (effective coupling coefficient with photon) characterized by the height of absorption peak,

Γ is the effective width of the excited state (or damping constant) characterized by the broaden of absorption peak from delta form.

So, absorption coefficient

$$A_{abs} \sim g^2 \delta(E_i - E_0 - \hbar\omega + i\Gamma), \tag{2}$$

where ω is photon frequency, $\delta(x+iy)$ is the general delta Dirac function with Gaussian form

$$A_{abs} \sim exp\left[-\left(\frac{E_i - E_0 - \hbar\omega}{\Gamma_i}\right)^2\right] = exp\left[-\left(\frac{P_i - \hbar\omega}{\Gamma_i}\right)^2\right], \tag{3}$$

where $i = 1, 2$ denote the excited states. By fitting theory with experiment data, we get numerical values of the parameters.

3. Results and discussion

In this work, we use the Gaussian functions

$$f(x) = \frac{cf_1}{\Gamma_1\sqrt{2\pi}} exp(-\frac{(x-\mu_1)^2}{\Gamma_1^2}) + \frac{cf_2}{\Gamma_2\sqrt{2\pi}} exp(-\frac{(x-\mu_2)^2}{\Gamma_2^2}). \tag{4}$$

in order to find the position μ and the width Γ of the peaks in the absorption spectra of WT-Ngb.

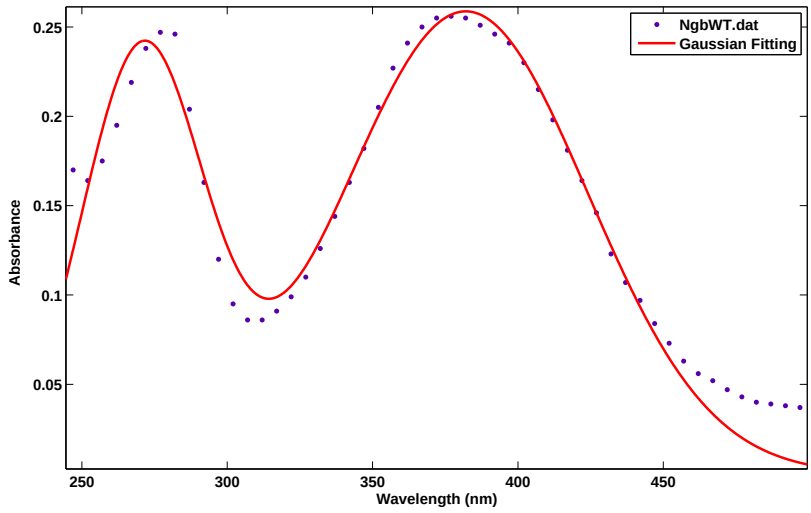

Figure 5. Fitting curve of spectra of WT-Ngb with pH = 2.3. Numerical calculation with ration 1/2: μ_1 =270.7 nm, $\Gamma_1 = 17.47, cf_1 = 17.47, \mu_2 = 382nm, \Gamma_2 = 59.39, cf_2 = 38.52$.

The UV-vis absorption spectra of WT-Ngb (Fig. 5) shows that there are two peaks at positions $\sim 270nm$ and $\sim 382nm$, which displays the same feature of oxyheme protein spectrum. It means that, the heme complex structure of Ngb differs from Hb or Mb one (which presents the typical penta-coordinated geometry). Moreover, we see that two peaks are not similar. Therefore, we suppose that the octahedral geometry of Fe^{2+} heme complex of Ngb is distorted: two bonds of axial distal are not equidistant with the bonds of Fe-N.

There is an agreement between experimental data and numerical calculation. The simple two-level model can be present quantum chemical calculation and can be used for investigating the properties of big molecules.

4. Acknowledgments

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