The Two–Level Model on Absorption Spectra of Oxyhemo- and Neuroglobin

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Abstract. The absorption spectra of deoxyhemoglobin and oxyneuroglobin has been examined. The well–known two–level model has been used to study the absorption spectra. The model has been verified by experemental data for heomoglobin and neuroglobin, that gives good fittings. The discussion on the obtained results leads to further works

1. Introduction

The globin family evolved from a common ancestor. Its classical representatives, hemoglobin (Hb) and myoglobin (Mb), have been widely investigated for more than four decades[2], while neuroglobin was just identified in the nervous system of vertebrates[1] for more than ten years. Globin family has been studied for many years under different ways of view. Among which absorption spectra is one of the most effective method to touch various physical and chemical aspects of the members of globin family. Different physiological responses can be emphasized by imaging at different wavelengths[7]. Globins are heme–containing proteins involved in binding and/or transporting oxygen. There exist several stable states of globins characterized by the oxidation states of the heme iron, the heme orientation and the nature of the sixth ligand of the iron[3]. Oxygen binds in an "end–on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron. In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) while oxygen temporarily turns into superoxide, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable of binding oxygen[9]. It has been observed[10][4] that the visible absorption spectrum of deoxyhemoglobin has one pick, while the visible absorption spectra of oxyhemoglobin has two picks. It is also observed that for a sample of oxygenated neuroglobin, the distribution has only one pick. The phenomenon seems to contradict to the observation on hemoglobin. But theoretically it could be modeled by a two–state system, which solves the problem and draws a line through out the absorption spectra of globin family.

In theory, the two–level model is widely applied to consider a number of physical and chemical systems, such as $\text{spin} - 1/2$ particles are two–state quantum systems when only the spin degree of freedom is considered. It also used to study the "inverting" degree of freedom in an ammonia molecule; the nitrogen at the vertex of an ammonia molecule exhibits two molecular states – "up" and "down", corresponding to opposite positions with respect to the plane of the three hydrogen atoms. In an electric field, these two states are non-degenerate.

This work uses two–level model in studying globin group, builds a model which works well with the absorption spectrum of hemoglobin.

2. Materials and methods

The deoxyhemoglobin and oxyneuroglobin samples have been investigated, using two–level model. A sample of bovin Hgb was prepared, absorbance measurements were made at room temperature $(20–24°C)$ in the spectral range of $450–700nm$. A sample contains pure 100% Ngb and hemin was examined in the spectral range of $280 - 700nm$, in different conditions of $pH = 2.3, 5.1, \text{and } 8.1$.

In general, the absorption intensity varies in function of the wavelength. It has been already indicated[8] that absorption intensity of deoxyhemoglobin is proportional to the wavelength when wavelength approaches to $555nm$ and inversely proportional to the wavelength when it is longer than $555nm$. In the case of oxyhemglobin, the absorption intensity reaches its maxima at $\lambda_1 \cong 540nm$ and $\lambda_2 \cong 580nm$. The energy diagram of hemogblobin has been illustrated in Fig. 1..
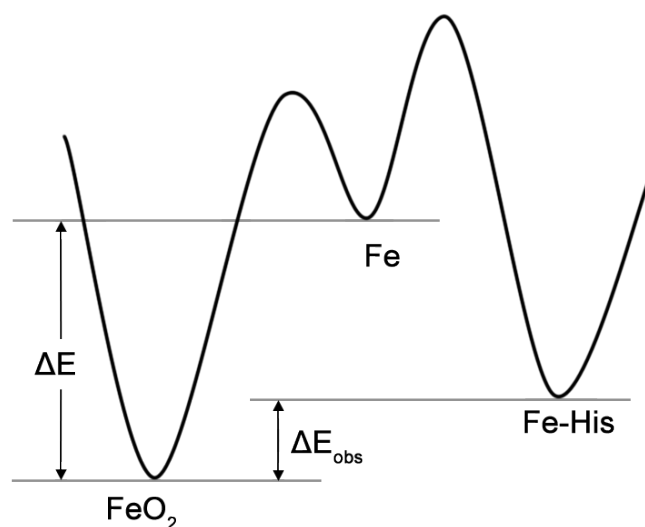

Figure 1. *Energy diagram of hemoglobin group*

The energy diagram of hemogobin group leads to an idea of using the two–level model to describe physics of heme, in which the protein would be considered as a system with only two quantum

states [5, 6]. At the relaxed state, the system has only one pick, at excited state, the system has two pick. It is clear that with the experimental data[11], the distribution of deoxyhemoglobin absorption intensity has the gaussian–like shape. The two–level description would be useful to investigate the protein and to compare the theoretical results with this experimental data set.

2.1. An introduction to the two–level model

The two–level system which is also known as two–state system (TLS), is the simplest quantum system that can exist. Two–state formalism can be used to describe simple mixing of states which leads to phenomenon such as resonance stabilization and other level crossing related symmetries, so that it has a wide variety of application in physics, chemistry and biology.
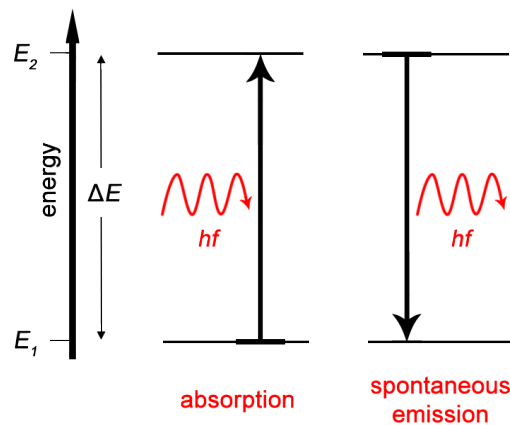
Figure 2. *Energy diagram of two-state quantum system*

The most well–known application of two–state system is of the spin of a spin $1/2$ particle such as an electron, whose spin can take value $+\hbar/2$ or $-\hbar/2$, where $\hbar$ is the reduced Plank constant. Another example, frequently studied in atomic physics, is the transition of an atom to or from an excited state, where the TLS formalism is used to quantitatively explain stimulated and spontaneous emission of photons from excited atoms.

The Hilbert space describing such a system fills up with only two degrees of freedom. Therefore, a complete basis spanning the space will consist of two independent states. The physics of a quantum mechanical two–state system is trivial if both states are degenerate. That is, if the states have the same energy. However, if there is an energy difference between the two states, then nontrivial dynamics can occur.

2.2. Dynamics for a time–independent Hamiltonian of the two–level system

Let H denote the time–independent Hamiltonian, and let $|\psi_1\rangle$ and $|\psi_2\rangle$ denote the two energy eigenstates of the system, with respective eigenvalues E_1 and E_2. Any state $|\psi(t)\rangle$ of the two–level system can be written as a superposition of the given energy eigenstates. In particular, at time $t = 0$, the state $|\psi(0)\rangle$ would read

$$|\psi(0)\rangle = c_1 |\psi_1\rangle + c_2 |\psi_2\rangle, \tag{1}$$

where c_1 and c_2 are complex coefficients with normalized condition $|c_1|^2 + |c_2|^2 = 1$.

At some later time t, the energy eigenstates $|\psi_1\rangle$ and $|\psi_2\rangle$ will have evolved into $|\psi_1\rangle\, e^{-iE_1 t/\hbar}$ and $|\psi_2\rangle\, e^{-iE_2 t/\hbar}$, respectively, and so

$$|\psi(t)\rangle = c_1\,|\psi_1\rangle\, e^{-iE_1 t/\hbar} + c_2\,|\psi_2\rangle\, e^{-iE_2 t/\hbar}. \tag{2}$$

Every two–level system has a characteristic angular frequency given by

$$\omega = \frac{E_2 - E_1}{\hbar}, \tag{3}$$

where it has been assumed that $E_2 > E_1$.

The physics of two–state systems can be usefully applied to multi–state systems where the system is known to have only enough energy available to excite the lowest two states, thus effectively creating a two–state system. In fact, in the nature, it is difficult to identify any true two–state systems, merely systems where the energetics of the circumstances isolate two particular states.

In other way, the set of all possible states in a two–level system can be mapped onto a representation known as the Bloch sphere (Fig. 3.).
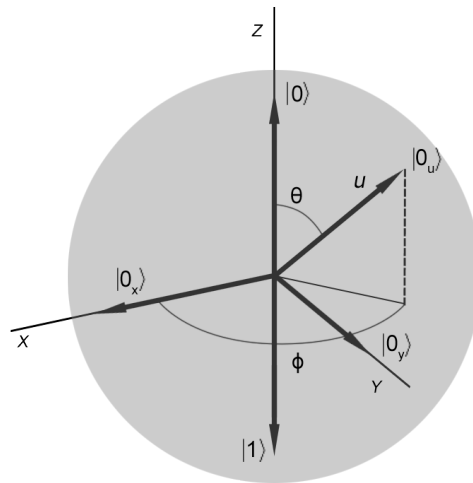

Figure 3. *Bloch's sphere for all possible states of a two–state system*

In this formalism, each state is represented as a point on the unit sphere. The spherical coordinates are usually chosen so that the energy eigenstate $|\psi_1\rangle$ has colatitude $\vartheta = 0$ and the energy eigenstate $|\psi_2\rangle$ has $\vartheta = \pi$, that is, $|\psi_1\rangle$ sits at the north pole and $|\psi_2\rangle$ at the south pole. A general state $|\psi\rangle$ has colatitude ϑ and azimuth φ determined by the coefficients c_1 and c_2.

2.3. Two–level system approach to hemoglobin group

Several experiments on globin family have showed that globins are heme–containing proteins involved in binding and/or transporting oxygen. The stable states of globins are characterized by the oxidation states of the heme iron as illustrated in Fig. 4., the heme orientation and the nature

of the sixth ligand of the iron[3]. It has been observed[10][4] that the visible absorption spectrum of deoxyhemoglobin has one pick, while the visible absorption spectra of oxyhemoglobin has two picks. However, for a sample of oxygenated neuroglobin, the spectra has only single pick. The phenomenon seems to contradict to the observation on hemoglobin. But theoretically it could be modeled by a two–state system, which solves the problem and draws a line through out the absorption spectra of globin family.
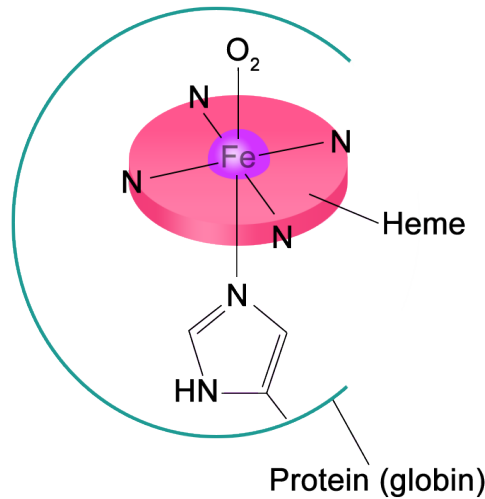
Figure 4. *Heme element in hemoglobin*

In the language of second quantization, the Hamiltonian of a two–state system reads

$$H = E_1 a^\dagger a + (E_2 + i\Gamma) b^\dagger b + \gamma \left(a^\dagger b + b^\dagger a \right), \tag{4}$$

where $a^\dagger$, a and $b^\dagger$, b are the creation and annihilation operators on the ground and excited states, $|\psi_1\rangle$ and $|\psi_2\rangle$, with energies E_1 and E_2, respectively, and γ is coupling coefficient between ground and excited states, which must be exponentially proportional to energy difference of two given states

$$\gamma \approx e^{-\beta(E_2 - E_1)}, \tag{5}$$

with $\beta = \frac{1}{k_B T}$ denotes the inverse of temperature.

Following standard analytical procedure which can be easily found in textbooks of quantum field theory, the cross-section of proposed model would be determined by the expression

$$\frac{\mathrm{d}\sigma}{\mathrm{d}\Omega} \quad \simeq \quad |M| \tag{6}$$

$$\approx \quad \delta \left(E_2 - E_1 + \hbar\omega + i\Gamma \right). \tag{7}$$

Since it is well–known that

$$\delta \left(E_2 - E_1 + \hbar\omega \right) = \lim_{\Gamma \to 0} \delta \left(E_2 - E_1 + \hbar\omega + i\Gamma \right), \tag{8}$$

and

$$\lim_{\Gamma \to 0} \exp\left\{ -\frac{E^2}{\Gamma^2} \right\} = \delta\left(E \right). \tag{9}$$

The last expression enlightens the signification of parameter Γ, the width of the Gaussian function.

In this work, firstly we examine the proposed model for the absorption of deoxyhemoglobin, or in other words, we are going to determine the coupling coefficient Γ from the absorption spectra of deoxyhemoglobin.
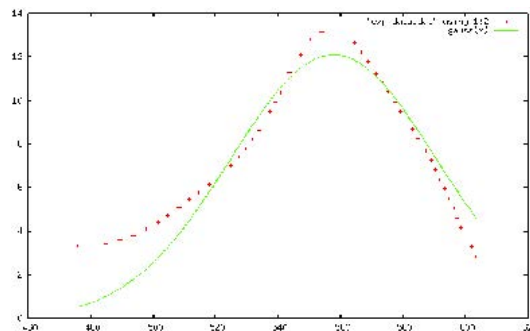
3. Numerical calculation and results

The Gaussian function is used

$$f\left(x \right) = \frac{a}{\sqrt{2\pi}\Gamma} \exp\left\{ -\frac{(x - \mu)^2}{\Gamma^2} \right\}, \tag{10}$$

to find the width Γ of the pick in the absorption spectra of deoxyhemoglobin.

For the deoxyhemoglobin sample, the deoxidized hemoglobin ($Fe(II)$) is considered as ground state. In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) and the hemoglobin goes to excited state. The absorption spectra of deoxidized hemoglobin corresponds to the relax line in energy diagram.


Figure 5. *Absorption intensity of deoxyhemoglobin sample. The measurements have been done in the range of wavelength from 450nm to 700nm. Numerical calculation with ratio ¹/₂: $\Gamma = 32.9$; $a = 997.5$ and $\mu = 587$*

The numerical calculation for oxidized hemin of neuroglobin sample at different values of pH provides the clues of the influence of pH on the width parameter Γ. It seems that the width parameter depends strongly on the inverse of pH. The dependence of the width in the absorption spectra on pH as a function $\Gamma\left(x_{pH} \right)$ would be considered in a future works.

Moreover, absorption spectrum of neuroglobin at low pH presents only one pick and it tends to split in two picks while pH is increased. The observed spectra form of oxidized neuroglobin seems to be the same as for deoxihemoglobin for the very low pH, means for the acid environment. In acid environment, the protein has a strong binding with environment, the observed spectra could be considered as for the hemin alone. While increasing the pH of the environment, the binding
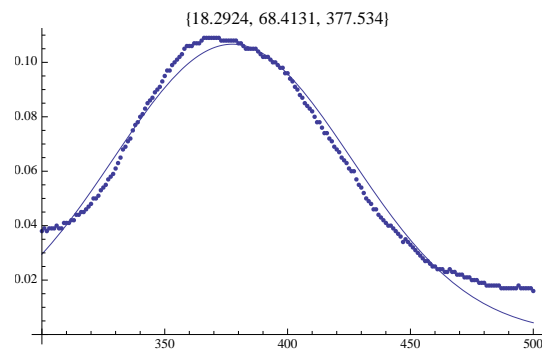
Figure 6. *Absorption intensity of Ngb and hemin sample. Wavelength varies from 280 to 700 nm. pH = 2.3. Numerical calculation with $\Gamma = 18.292$; $a = 68.413$ and $\mu = 377.534$*
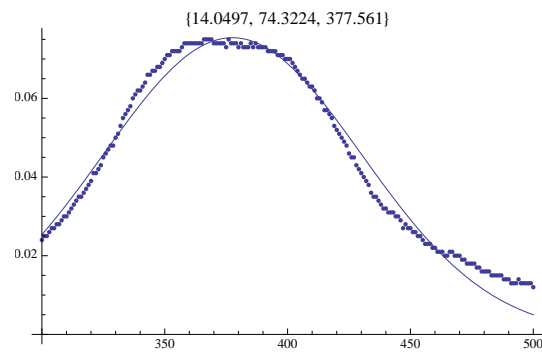
Figure 7. *Absorption intensity of Ngb and hemin sample. Wavelength varies from 280 to 700 nm, pH = 5.1. Numerical calculation with $\Gamma = 14.049$; $a = 74.322$ and $\mu = 377.561$*
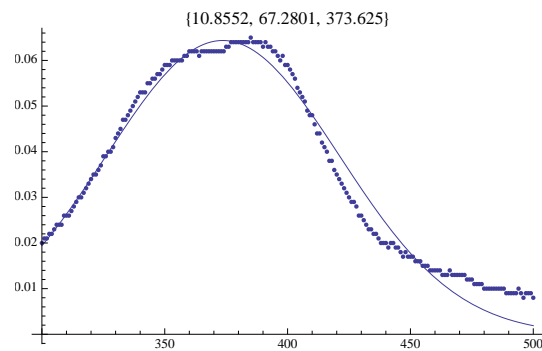
Figure 8. *Absorption intensity of Ngb and hemin sample. Wavelength varies from 280 to 700 nm, pH = 8.1. Numerical calculation with $\Gamma = 10.855$; $a = 67.280$ and $\mu = 373.625$*

between heme and protein becomes stronger, the spectra is affected by a large noise caused by the heme-protein binding.

In more general point of view, the absorption spectrum of oxidized neuroglobin could be considered as degenerated spectrum, in which two picks reach almost the same value, at which two resonance wavelengths quite close one to another, the change of environmental pH has an effect of external field causing splitting degenerated spectrum into two separated pick spectra.

The difference between hemoglobin and neuroglobin absorbtion spectrum is caused by the

difference of their symmetry.

4. Discussion

A model has been built for absorption spectrum of deoxihemoglobin and oxidized neuroglobin. The model gives a good agreement with experiment data, from which some parameters could be determined. A qualitative explanation of some hemin's behaviors is reasoned in the context of the two–level model.

The absorption spectrum of deoxihemoglobin is well fitted with the Gaussian distribution function, while the absorption spectrum of oxydized neuroglobin seems to be the superposition of two spectrum corresponding to two oxygen–iron binding, which reaches almost the same maximum value. This hypothesis will be the main subject of next work.

The model will be optimized and some other parameters will be determined in the next work, where further knowledge of the behaviors of oxihemoglobin and oxidized neuroglobin is presented.

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