

Computing reduction potential of Glucose Oxidase Enzyme

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Abstract. *Glucose oxidase (GOx) is an enzyme and it is an important molecule to understand the energy transfer system in our body. Flavin adenine dinucleotide (FAD) is co-enzyme and it is frequently combined with GOx. GOx helps reduction or oxidation of FAD. Redox would not happen naturally unless FAD is combined with GOx, therefore understanding the effect of electrostatic environment which is made by atoms of GOx is one of the most important factors to decide the reduction potential of GOx-FAD. In this research, we calculate some models which have different range of electrostatic environment of GOx, and examine the effect to the redox potential and compare with previous research [1]. Additionally, we propose a new scheme for calculation of Standard Hydrogen Electrode (SHE), which is employed to measure the reduction potential to the experimental definition.*

Keywords: Redox potential, GOx, FAD, SHE

1 Introduction

Oxidation-reduction reaction, which is usually called redox reaction, is the important reaction in biochemical system. For example, photosynthesis is a process to derive the energy for plants. CO₂ is reduced and H₂O is oxidized in that process to yield carbohydrates and O₂ [2]. In biological system, there are important redox reagents. The famous ones are oxygen, transition metal ions and a number of coenzymes such as Flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD) [1]. Coenzyme is one of the kinds of cofactor and it is an organic molecule but not a protein. It combines with protein, which is called enzyme, and assist in biochemical transformations. Glucose Oxidase (GOx, PDBIB:1CF3) is an enzyme and it is combined with FAD as a coenzyme in order to work as a catalyst. GOx-FAD is reduced to GOx-FADH₂ by some steps. The ring of Flavin is known as the active side of the reaction, so we calculate the reduction potential of lumichrome part of FAD with some "environment atoms".

The difference of Gibbs energy between reduced and oxidized products is observed as reduction or oxidation potential experimentally. Because the absolute redox potential of half-cell cannot be obtained experimentally, we can discuss the redox potential by relative value to some electrode such as Standard Hydrogen Electrode (SHE) or silver-silver chloride electrode. SHE, the half-cell of redox reaction of hydrogen ion and hydrogen gas, is normally used as a reference. The absolute redox potential of SHE is estimated by some researchers. The International Union of Pure and Applied Chemistry (IUPAC) recommends to use the value of 4.44 V reported by Trasatti in 1986 [3]. We can calculate the Gibbs energy for each product, therefore we are able to estimate the absolute redox potential by the change of Gibbs energy. It is impossible to compare the absolute value and relative value. To compare experimental and calculated data, the absolute value should be measured *versus* SHE. Many researchers generally employ the IUPAC recommendation value to measure it. However, there is difficulty to choose the value of absolute potential of SHE because the other researchers report different values ranging 4.05 V to 4.73V by different models [4]. It gives different results when we use

the different computational method such as calculation theory or basis sets. If we place importance on experimental results, we should consider the change of absolute potential of SHE because of choosing the computational method. Matsui and coworkers calculate the absolute reduction potential of SHE depended on calculation methods [4]. In this research we use that value to measure the reduction potentials and we also propose a new scheme to calculate reduction potential of SHE.

2 Methods and Models

The difference of Gibbs energy between before and after reaction has relation with redox potential as shown in equation (1):

$$E_0 = -\frac{\Delta G_{red}}{nF} \quad (1)$$

Where E_0 is the absolute reduction potential, ΔG_{red} is the difference of Gibbs energy between reduced and oxidized, n is the number of electrons transferred and F is the Faraday constant. We calculate the Gibbs energy for each side of reaction half-cell by DFT and 6-31+G(d) is used as the basis set. PCM is used for implicit solvent model. Generally the change of Gibbs energy by reaction in solute cannot be calculated directly because of fluctuation. Thermodynamic cycle is usually employed. In our case the calculation gives the minimum of Gibbs energy because of using PCM, therefore we can get the change of Gibbs energy directly.

GOx-FAD is reduced to GOx-FADH₂. This reaction process is happen step by step as shown in equation (2) to (4):



We focus on the first step of this process shown in equation (2) and calculate the reduction potential. We make 2 models and we show them in figure 1 and 2. The model one is only lumichrome molecule which is known as the active side of the reaction [2]. This model contains only 31 atoms. It is enough small to be calculated by Quantum Mechanics. The second model is FAD molecule which has the lumichrome part and tail part. This model is little large therefore we use ONIOM method. The lumichrome part which is enclosed by circle in figure 2 is treated as QM region and the other part is MM region. QM region is treated by DFT/B3LYP 6-31+G(d). Universal Force Field, UFF, is used for MM region.

In this research, we calculate the Gibbs energy of each molecule on the half-cell for each model, and estimate the absolute value of reduction potential of GOx-FAD. The absolute potential is measured *versus* SHE to compare with experiment. We use 3 kinds of value for the absolute reduction potential of SHE, first is IUPAC recommendation value 4.44V, second is theory and basis set depend-

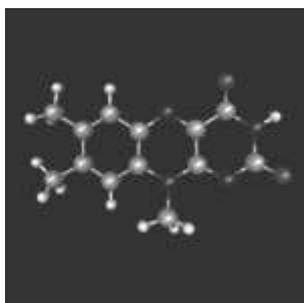


Figure.1 the structure of model 1
(lumichrome molecule model)

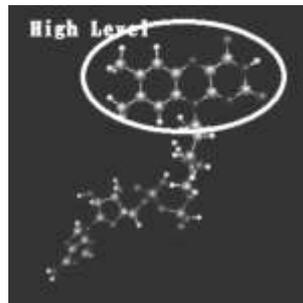


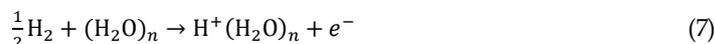
Figure.2 the structure of model 2
(FAD molecule model)

ence value 4.99V reported by Matsui and coworkers, the last one is calculated by our new scheme as mentioned below.

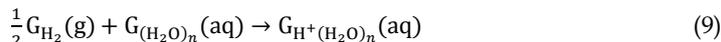
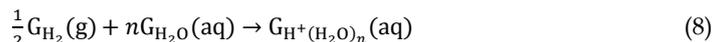
Experimentally SHE is the most common relative electrode. Even if silver-silver chloride electrode or the other electrode is used, the result is usually measured by SHE. The half-cell of SHE is shown in equation (5):



This reaction contains proton. If we were able to calculate a proton by quantum mechanics method, we can calculate the reduction potential of that half-cell. Unfortunately, a proton has no electron therefore we cannot use *ab initio* molecular orbital method. With focusing on the behavior of a proton in water solvent, we make new scheme to calculate the reduction potential of SHE. In the water solvent, a proton is not stable from the view point of free energy. Protons form oxonium ions by associating with one or more number of water molecules, like H_3O^+ , H_5O^+ , ..., $\text{H}^+(\text{H}_2\text{O})_n$, We approximate half-cell of SHE by equation (6) or (7):



The difference of these two equations is the form of water molecules on the left hand side. Both of equations (6) and (7) have n H₂O molecules. The Gibbs energy of water molecules is calculated by summation of an isolated water molecule for equation (6), on the other hand, it is defined as a cluster of n water molecules. To clarify this approximation, equations (6) and (7) are expressed by Gibbs Energy in equations (8) and (9):



Where $G_{\text{H}_2}(\text{g})$ is the Gibbs energy of hydrogen molecule in the gas, and $G_{\text{H}_2\text{O}}(\text{aq})$, $G_{\text{H}^+(\text{H}_2\text{O})_n}(\text{aq})$ and $G_{(\text{H}_2\text{O})_n}(\text{aq})$ are the Gibbs energy of a water molecule, oxonium ion and a cluster of n water molecules in water solvent. We estimate the absolute reduction potential of SHE from the difference of the Gibbs energy of this half-cells.

3 Results and Discussion

Reduction potential of GOx-FAD

We calculate the Gibbs energy and reduction potential. Table 1 shows the calculated results of each model. To calculate the absolute potential, $n=1$ and $F=96.4853\text{kC/mol}$ are employed. The experimental data of this reduction potential is -0.200V [5]. We obtain the absolute potential of 3.69V by model 1. If we employ IUPAC recommendation value for SHE reduction potential 4.44V , the reduction potential is -0.748V . The model 2 gives -0.639V by the same way. The results show the model 2 gives loser value to the experimental data. A possibility reason is that the tail part of FAD molecule makes larger dipole moment on the reduced side. The dipole moments of oxidized side are 14.85 Debye and 14.30 Debye in model 1 and model 2. Those two values are almost the same but there is difference in the dipole moment of reduced side. The model 1 gives 17.41 Debye and model 2 gives 48.63 Debye. The model 2 has much larger dipole moment. Large dipole moment has effect to stabilize the molecule in the water solvent and give the lower Gibbs energy. This effect may give larger difference between reduced and oxidized species in model2. If FAD is combined with GOx, the dipole moment of the system becomes larger because of the effect of polarity residues of GOx. It may give the better result therefore we will report it in the future.

Table 1. The calculated results of GOx-FAD. The model 1 is calculated by full QM with DFT/B3LYP 6-31+G(d). ONIOM method is used for model 2. Same method with model 1 is used for high level layer of ONIOM, and UFF is used for the other mechanical part as the force field.

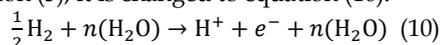
	G _{ox} (A)	G _{red} (B)	ΔG _{red} (B)-(A)	E _{abs} ^{red}	E ₀ ^{red} = E _{abs} ^{red} - E ₀ ^{SHE}	
					IUPAC ^[b]	Matsui ^[c]
Model 1	-871.975 ^[a]	-872.110 ^[a]	-0.135679 ^[a]	3.69V	-0.748V	-1.30V
Model 2	-832.227 ^[a]	-832.367 ^[a]	-0.139667 ^[a]	3.80V	-0.639V	-1.19V

[a] The unit is Hartree. [b] The absolute potential is measure by E₀^{SHE} = 4.44V which is recommended by IUPAC [3]. [c] E₀^{SHE} = 4.99V, reported by Matsui *et. al.* [4], is used to measure.

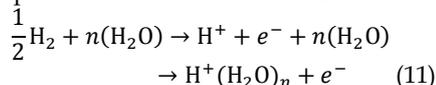
In case we employ Matsui and coworkers' value of SHE to measure, the reduction potential of FAD is -1.19V by model 2. This result is 0.55 V further from the result which is measured by IUPAC recommendation. It is surely caused by the difference of SHE value. This fact indicate that the choosing of the reduction potential of SHE cause important error.

Reduction potential of SHE

Figure 1 shows the calculated results in each number of water. We calculate the reduction potential of the half-cell shown in equation (6) and (7). Model 1, the equation (6), has the minimum with n=4 and this structure is known as the most stable [6]. The potential on the minimum is 5.04V. This value is very close to Matsui and coworkers' result 4.99V for DFT/B3LYP 6-31+G(d). On the other hand, model 2 which is the calculation of equation (7) is converged in the large n and the results have the range about 4.3 to 4.4V. The minimum in model 2 is 4.31V with n=6. In the equation (7), the water molecules on the left hand side is structured. The size of cavity when we use PCM is about 1.2 times larger than van der Waals surface. The half-cell of SHE is equation (5). If we add n water molecules in equation (5), it is changed to equation (10):



And a proton associate with water molecules therefore equation (10) is changed to equation (11):



Seeing this equation, model 1 is similar reaction to the SHE half-cell, but the model 2 leads to the better result because of the size of cavity. Here we consider the case of n=4. If we employ the model 1, the size of cavity is 4.8 times van der Waals surface. One water molecule volume of cavity with PCM is 37.6 Å³ therefore the size of cavity of model 1 is about 150 Å³. The cavity of model 2 is 126.6 Å³ from the result. We find the size of cavity of model 1 is much larger than model 2. Figure 2 shows the size of cavity in each n and each model. Figure 3 shows the size of cavity per one water molecule. Calculate from the density of liquid water, one water molecule has about 30 Å³ of volume. Now we calculate the water molecule in water solvent, so the volume should be around this value. The size of cavity per a water molecule is 29.5Å³ with n=6 in

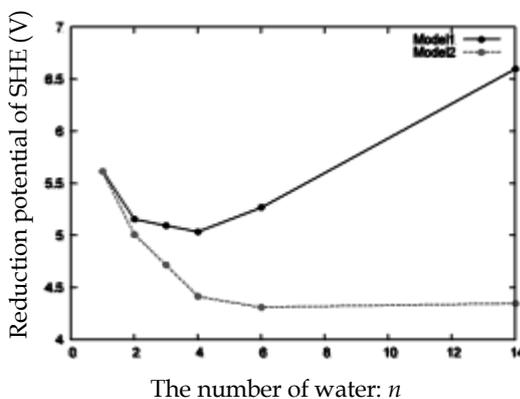


Figure 1. The number of water dependence of reduction potential of SHE. The solid line is the model 1 which is based on the equation (6). The dashed line is the model 2 which is based on the equation (7). The model 2 is converged in the large n, but model 1 has the minimum in n=4.

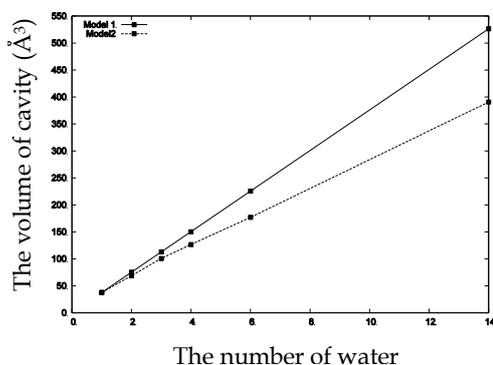


Figure 2. The volume of cavity. Model 1 is solid line and model 2 is dashed line. Model 1 has much larger cavity than model 2.

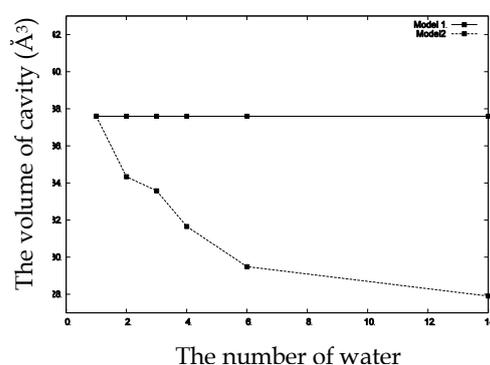


Figure 3. The volume of cavity per a water molecule. Model 1 is solid line and model 2 is dashed line.

model 2. We know $n=6$ in model gives the minimum and we consider this value is the suitable for reduction potential of SHE with DFT/B3LYP 6-31+G(d). this value is not o far from IUPAC recommendation value 4.44 V but little far from Matsui and coworkers' report 4.99 V. we need to calculate SHE potential with the other calculation theory or basissets and discuss the correctness.

Reduction potential of GOx-FAD measured by our SHE potential

We consider the reduction potential of SHE is 4.31 V and use this value to measure the reduction potential of GOx-FAD. The result is -0.500 V by FAD model (the model 2 of GOx-FAD). The experimental data is -0.200 V, consequently our result is 0.300 V further than experimental data. In this research, we consider only FAD molecule and just ignore the effect of GOx. If we calculate with a part of GOx, it may provide closer value. Kurniawan and coworkers report the reduction potential of GOx-FAD with similar calculation method [1]. According to their study, the reduction potential of FAD is -0.801 V. Our result is little close to the experimental data. We understand that our calculation find more stable structure.

4 Conclusions

We successfully calculated the reduction potential of FAD with ONIOM method. Additionally we make a new scheme to calculate the reduction potential of SHE. We estimate 4.31 V for SHE potential. This value is close to IUPAC recommendation. We calculate the FAD reduction potential by our SHE potential and get -0.500 V. This value is close to experimental data of -0.200 V.

In the future, we plan to calculate the reduction potential of FAD with some parts of GOx. Residues of GOx around the active site of reaction have effect to the reaction. This calculation may give better results.

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