



# Redox reaction characteristics of riboflavin: A fluorescence spectroelectrochemical analysis and density functional theory calculation



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## ABSTRACT

Riboflavin (RF), the primary redox active component of flavin, is involved in many redox processes in biogeochemical systems. Despite of its wide distribution and important roles in environmental remediation, its redox behaviors and reaction mechanisms in hydrophobic sites remain unclear yet. In this study, spectroelectrochemical analysis and density functional theory (DFT) calculation were integrated to explore the redox behaviors of RF in dimethyl sulfoxide (DMSO), which was used to create a hydrophobic environment. Specifically, cyclic voltammetry (CV) and derivative cyclic voltammetry (DCV) were employed to track the RF concentration changing profiles. It was found that the reduction contained a series of proton-coupled electron transfers dependent of potential driving force. In addition to the electron transfer-chemical reaction-electron transfer process, a disproportionation (DISP1) process was also identified to be involved in the reduction. The redox potential and free energy of each step obtained from the DFT calculations further confirmed the mechanisms proposed based on the experimental results. The combination of experimental and theoretical approaches yields a deep insight into the characteristics of RF in environmental remediation and better understanding about the proton-coupled electron transfer mechanisms.

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## 1. Introduction

Electron transfer reactions are ubiquitous in environmental remediation of pollutants, geochemical cycles of Fe and Mn, and microbial fuel cells that produce electricity from wastes [1–3]. Flavins are redox active species widely distributed both in nature and in vivo, and play a key role in mediating electron transfer in biogeochemical systems [3–5]. Also, flavin derivatives can act as photosensitizers to produce reactive oxygen species [6] and molecular catalysts for water-oxidation reaction [7], and can be chelated with metals easily [8,9]. Riboflavin (RF) is the primary redox active component of flavin and possesses three accessible oxidation states similar to quinones, each with protonation and deprotonation forms (Scheme 1) [10,11]. The elucidation of electron transfer reactions and redox properties of RF is critical to understand its biochemical function.

It is well known that under hydrophilic conditions the redox behavior of RF is pH-dependent and RF is directly reduced to hydroquinonoid RF (RF<sub>red</sub>H<sub>2</sub>) in one step via a two-electron/two-proton transfer pathway [10–14]. For the flavoenzymes that function as hydrophobic sites [15], however, an aprotic organic environment is suitable for the

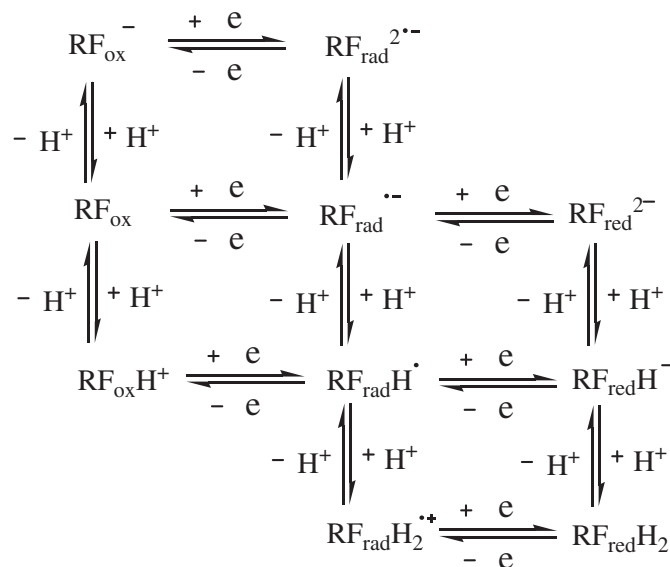
investigation into the electron transfer process. Cyclic voltammograms (CV) of RF in aprotic solvents have shown more complex redox behavior than those of simple quinonoid structures [16,17]. However, there are still controversies on the two deduced mechanisms involved in the reduction of RF in aprotic media, the electron transfer-chemical reaction-electron transfer (ECE) and the disproportionation (DISP1) mechanisms. In addition, the influence of potential driving force on the redox progress remains unclear yet. Thus, efforts should be made to elucidate the redox mechanisms involved and better understand its environmental behaviors.

Spectroelectrochemical methods have been widely used to explore the redox properties of compounds analogous to quinonoid structure [18–20]. A combination of electrochemical regulation and spectroscopic monitoring helps to correlate the electron transfer processes in electrode redox reactions to spectroscopic observations. Some spectroelectro-chemistry-based analytical methods have been developed. Among them, cyclic voltammetry (CVA) and derivative cyclic voltammetry (DCVA) are useful tools to track the changes of reactants, intermediates and products in the electrochemical process [21,22]. With high sensitivity and salient structural information fluorescence can provide, spectrofluorochemistry offers an efficient and sensitive approach to investigate redox properties of species [23,24]. On the other hand, the density functional theory (DFT) calculations can also provide molecular level insights into the change of RF

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**Scheme 1.** Possible species of riboflavin in the steps of sequential electron and proton addition reactions.

reduction in aprotic organic environment to further interpret the experimental results. However, so far information regarding this respect is limited.

With strong fluorescence and variable intensity under distinctive redox states, RF is an appropriate compound for fluorescence spectroelectrochemical analysis. In this contribution, we report our UV–Vis and fluorescence spectroelectrochemical studies of electron transfer of RF in DMSO. Cyclic voltammetry (CV) and derivative cyclic voltammetry (DCV), similar to CVA and DCVA respectively, were employed to elucidate the mechanisms of the redox reactions and to find out whether the reduction process was reversible. With a combination of spectroelectrochemical analysis with the DFT calculation, the compounds formed in the reduction and their correlations with the peaks in CVs were identified, and thus the mechanisms of the electrochemical redox reaction of RF in aprotic media were elucidated.

## 2. Experiments and computations

### 2.1. Reagents

*n*-Bu<sub>4</sub>NPF<sub>6</sub> was purchased from J&K Tech. Ltd., China, while RF was purchased from Sigma Co., USA. DMSO (Sinopharm Chemical Reagent Co., China) was used as solvent. All chemicals were reagent grade and were used without further purification.

### 2.2. Spectroelectrochemical instruments and analytical procedures

CVs were recorded on a CHI760D electrochemical workstation (CHI Instruments Co., China). Working electrodes were a 1-mm diameter planar platinum disk for electrochemical study and a piece of 6 mm × 7 mm Pt mesh for spectroelectrochemical experiments. In addition, a Pt wire counter electrode and an Ag/Ag<sup>+</sup> reference electrode (a silver wire as quasi reference electrode for spectroelectrochemical experiments) were used. Before each experiment, the Pt disk electrode was polished (alumina polishing paste), washed with water, sonicated and activated by H<sub>2</sub>SO<sub>4</sub>. The Pt mesh electrode was cleaned by immersing it in 0.1 M HNO<sub>3</sub> for 3 min and rinsed extensively, followed by H<sub>2</sub>SO<sub>4</sub> activation. Accurate potentials were obtained using ferrocene as an internal standard. All solutions were deoxygenated by

nitrogen purging before the tests and all experiments were conducted under ambient temperature (22 ± 2 °C). A thin-layer quartz glass spectroelectrochemical cell (CHI Instruments Co., China) was used for complete electrolysis in a short time and eliminating the influence of diffusion at very low scan rates (*v*). 300 μL of the solution was added into the thin-layer cell for all spectroelectrochemical experiments. The configuration of the three-electrode arrangement in the thin-layer cell for spectroelectrochemical measurements is shown in Scheme S1.

The UV–Vis spectra obtained at different applied potentials were recorded on a UV-2450 spectrometer (Shimadzu Co., Japan). In situ electrochemical fluorescence was recorded on a fluorescence spectrometer (LS 55, Perkin-Elmer Co., USA) fitted with a pulsed Xenon excitation source. Unless otherwise stated, the excitation and emission slits were both 5 nm, and the scanning speed was set at 600 nm/min for all measurements.

### 2.3. DFT calculations

The structures of RF molecules in DMSO solution are studied by DFT computation. In the calculation, an all-electron method within the Perdew, Burke, and Ernzerhof (PBE) forms of generalized gradient approximation (GGA) [25,26] for the exchange–correlation term was employed, as implemented in the DMol<sup>3</sup> code [27,28]. The double precision numerical basis sets including p polarization (DNP) were adopted. The energy in each geometry optimization cycle was converged to within 1 × 10<sup>−5</sup> Hartree with a maximum displacement and force of 5 × 10<sup>−3</sup> Å and 2 × 10<sup>−3</sup> Hartree/Å, respectively. The solvent effect of the DMSO medium was described using the conductor-like screening model (COSMO) [29,30]. COSMO is a continuum solvent model where the solute molecule forms a cavity within the dielectric continuum of permittivity that represents the solvent.

## 3. Results and discussion

### 3.1. Electrochemical results compared with previous experiments

CVs of RF reduction in DMSO in different scan ranges are shown in Fig. 1a. Two oxidation peaks (Peaks 2 and 3) were observed upon reversal of the scan direction after the first reduction process (Peak 1). At more negative potential, other two reduction peaks (Peaks 4 and 5) appeared. Peak 2 decreased significantly when the forward scan was extended to pass Peak 5, compared with that when it just passed Peak 1. Meanwhile, the size of Peak 3 was enlarged when the forward scan direction was reversed after Peak 5.

The reduction process was diffusion-controlled as peak potential of Peak 1 was independent of the scan rate and the peak current increased linearly with the square root of scan rate (Fig. S1). Fig. 1b shows the CVs of 0.2 mM RF at different scan rates in a scan range from −0.4 to −1.4 V vs. Ag/Ag<sup>+</sup>. To clearly compare the peak change, the current data are scaled through multiplying them by *v*<sup>−0.5</sup>. With an increase in *v*, Peak 3 appeared and grew larger and then shrunk, while Peak 2 shifted conversely in magnitude. The changes in the relative magnitude of Peaks 2 and 3 at *v* ≥ 100 mV/s were similar to the results reported by Tan et al. [17], who failed to report the results at *v* lower than 100 mV/s. The reduction of RF<sub>ox</sub><sup>−</sup> produced the radical anion RF<sub>rad</sub><sup>•−</sup> (Eq. 1, Peak 1), followed by homogeneous protonation to form RF<sub>rad</sub>H<sup>•</sup> (Eq. 2). The protonated radical RF<sub>rad</sub>H<sup>•</sup> was further reduced to produce RF<sub>red</sub>H<sup>−</sup> and oxidized (Eq. 3, Peak 3) back when the CV scan was reversed.

The protonation step (Eq. 2) governs the ratio of RF<sub>rad</sub><sup>•−</sup> and RF<sub>rad</sub>H<sup>•</sup>, as well as the oxidation peak intensities of Peaks 2 and 3. At a higher scan rate (5000 mV/s), there was no sufficient time for RF<sub>rad</sub><sup>•−</sup> to be chemically protonated. Thus, the protonation of RF<sub>rad</sub><sup>•−</sup> was suppressed and the reoxidation of RF<sub>rad</sub><sup>•−</sup> (Peak 2) dominated when scanning was back. As *v* was decreased from 5000 to 100 mV/s, the protonation reaction began to dominate and more RF<sub>rad</sub><sup>•−</sup> radicals were turned into RF<sub>rad</sub>H<sup>•</sup>, which could be reduced into RF<sub>red</sub>H<sup>−</sup> immediately. When *v*

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