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Supporting Information

The Prone-to-Autoxidation Nature of Morin in Alkaline Media: Mechanism, Kinetics, and Implications for Cotton Bleaching Studies

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2. Materials and methods

2.2. General Method for Oxidizing Morin in Alkaline Solution

In experiments that require a deoxygenated environment, the deoxygenated solution is prepared by placing the alkaline solution in a three-necked flask and bubbling with nitrogen for 30 min.

In the experiment using the enzyme, 1U represents the amount of enzyme that can convert 1 μmol substrate in one minute. The 10^{-2}U, 10^{-1}U, 1U enzymes were added to 10 mL pH 9.0 morin solution to prepare the corresponding concentration of the reaction solution. The mechanism of the enzyme used in the experiment is as follows:

Superoxide dismutase (SOD) catalyzes the following reaction；

$$2O_2^- + 2H^+ \overset{SOD}{\rightarrow} O_2 + H_2O_2 \qquad \text{Equation S1}$$

Catalase (CAT) catalyzes the following reaction；

$$2H_2O_2 \overset{CAT}{\rightarrow} 2H_2O + O_2 \qquad \text{Equation S2}$$

The activity of catalase (CAT) under the actual reaction conditions (pH 9.0, 25 °C) was assessed and confirmed a partial inactivation (residual activity ~15%).

Superoxide dismutase (SOD) is reported to be stable under such alkaline conditions, and its efficacy in inhibiting the reaction (as shown in Results) functionally confirms its activity. All enzymatic experiments were conducted with this understanding.

In experiments using hydrogen peroxide, 1.02 g of 30% hydrogen peroxide was taken and diluted with water to a final volume of 10 mL to obtain a 1 $mol \cdot L^{-1}$ hydrogen peroxide solution. Then, 10 μL, 100 μL, and 1 mL of this solution were diluted to 100 mL with a pH 9.0 buffer solution to serve as the solvent for dissolving morin in the corresponding concentration hydrogen peroxide experimental groups, preparing the reaction solution.

Catalase activity was measured under reaction conditions (pH 9.0, 25℃) by monitoring the decomposition of H_2O_2 at 240 nm . The carbonate buffer (pH 9.0) was prepared by adjusting 0.01 $mol\,L^{-1}$ sodium bicarbonate with 0.01 $mol\,L^{-1}$ sodium carbonate. A catalase stock solution (100 $U\,mL^{-1}$) was prepared in deionized water, and the H_2O_2 working solution (50 mM) was prepared in the same carbonate buffer and its concentration verified spectrophotometrically. The assay mixture consisted of 4 mL buffer, 4 mL H_2O_2 solution, and 2 mL enzyme solution. Controls included Blank 1 (8 mL buffer + 2 mL enzyme solution) and Blank 2 (4 mL buffer + 4 mL H_2O_2 + 2 mL H_2O). All solutions were pre-incubated at 25℃, and absorbance was recorded 60 s after initiating the reaction by enzyme addition.

The actual activity of the enzyme solution was calculated using the following equation:

$$U = (A_{B1} + A_{B2}) \times V \times 1000/(A_T \times \varepsilon \times V_e \times L \times t) \qquad \text{Equation S3}$$

where U represents the specific enzyme activity ($U \cdot mL^{-1}$), A_{B1}, A_{B2}, and A_T are the absorbance values at 240 nm for Blank 1 (0.862), Blank 2 (0.021), and the test sample (0.755) respectively, V is the total reaction volume (10 mL), V_e is the volume of enzyme solution added (2 mL), ε is the molar extinction coefficient of H_2O_2 at 240 nm (43.6 $M^{-1}\cdot cm^{-1}$), L is the optical path length (1 cm), and t is the reaction time (1 min). Based on these parameters, the calculated enzyme activity was 14.6 $U\cdot mL^{-1}$.

2.3. Calculation of Reaction Kinetics and Activation Parameters

The pseudo-first-order rate constant (k) was determined by calculating the slope of the pseudo-first-order reaction kinetics curve ($\ln(C/C_0)$ vs. t). The fitted kinetic equation is presented in Equation S4.

$$ln(C/C_0) =- kt + B \qquad \text{Equation S4}$$

Wherein, C is the concentration of morin at time t, $\mu mol\cdot L^{-1}$; C_0 is the initial concentration, $\mu mol\cdot L^{-1}$; t is time, s; k is the pseudo-first-order rate constant, s^{-1}; B is the intercept.

Where C/C_0 is calculated according to Lambert-Beer 's law from the absorbance at the maximum absorption wavelength, see Equation S5.

$$C/C_0 = A/A_0 \qquad \text{Equation S5}$$

Wherein, A is the absorbance at the maximum absorption wavelength; A_0 is the initial absorbance at the maximum absorption wavelength.

The autoxidation process of morin is obviously a continuous reaction. For the continuous reaction, the overall reaction rate of the reaction is determined by the reaction rate of the rate-determining step. The activation enthalpy ($\Delta H^{\neq}$) and activation entropy ($\Delta S^{\neq}$) of the rate-determining step at 298 K can be calculated by the slope and intercept of the Eyring plot (ln (k/T) vs. 1/T). The equation is presented in Equation S6. Equation S6 is derived from the Eyring equation (Equation S7) and the reaction activation energy equation (Equation S8). The activation reaction activation energy ($\Delta G^{\neq}$) of the rate-determining step is calculated using Equation S8.

$$ln(k/T) = -\Delta H^{\neq}/RT + ln(k_B/h) + \Delta S^{\neq} \qquad \text{Equation S6}$$

$$k = k_B T/h \times exp(-\Delta G^{\neq}/RT) \qquad \text{Equation S7}$$

$$\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq} \qquad \text{Equation S8}$$

Wherein, $\Delta H^{\neq}$ is the The active enthalpy of the rate-determining step, J·mol^{-1}; $\Delta S^{\neq}$ is the activity entropy of the rate-determining step, J·mol^{-1}·K^{-1}; k_B is the Boltzmann constant, J·K^{-1}; h is the Planck constant, J·s; $\Delta G^{\neq}$ is the reaction activation energy of the rate-determining step, J·mol^{-1}.

2.4. Quantum chemical calculation methods and activation energy calculations based on Marcus theory.

All energy calculations, structural optimizations, and frequency analyses for the molecules were performed using the Gaussian 09W software based on Density Functional Theory (DFT) [S1-S4]. Unless otherwise specified, the calculations were conducted using the B3LYP/3-21+G basis set in an implicit solvent model representing an aqueous solution.

The activation energies of the reactions were calculated using Marcus theory, with the electron transfer reaction equation shown in Equation S9. The activation energies for each reaction were determined using the Marcus equation, which is presented as Equation S10.

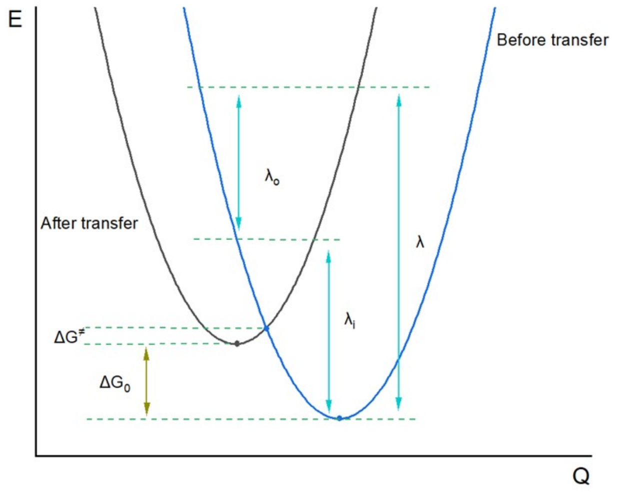
$$H2Mor(\,HMor, Mor\,) + O_2 \rightarrow H2Mor^*\,(\,HMor^*, Mor^*\,) + O_2^- \qquad \text{Equation S9}$$

$$\Delta G^{\neq} = \lambda/4(1 + \Delta G_0/\lambda)^2 \qquad \text{Equation S10}$$

Among these, $\Delta G^{\neq}$ is the activation free energy of the reaction, ΔG^0 is the Gibbs free energy change between the reactants and products in the diabatic state, and λ is the reorganization energy during the electron transfer process.

The reorganization energy λ is a crucial parameter for calculating the rate of electron transfer reactions based on Marcus electron transfer theory. It represents the energy required to transform the nuclear configuration of the reactants and solvent into the product state. It consists of the inner reorganization energy λ_i and the outer reorganization energy λ_o.

$$\lambda = \lambda_i + \lambda_o \qquad \text{Equation S11}$$



SFig.1 Schematic diagram of the diabatic potential energy surfaces before and after electron transfer.

For the proposed electron transfer reaction between the morin anion and molecular oxygen, the inner reorganization energy should be calculated using the following steps:

(a) Optimize the structure of the morin anion before electron transfer.

(b) Calculate the energy E1 of the morin anion after electron transfer using the structure from step (a).

(c) Optimize the structure of the morin anion after electron transfer.

(d) Calculate the energy E2 of the morin anion after electron transfer using the structure from step (c).

(e) Optimize the structure of molecular oxygen before electron transfer.

(f) Calculate the energy E3 of molecular oxygen after electron transfer using the structure from step (e).

(g) Optimize the structure of molecular oxygen after electron transfer.

(h) Calculate the energy E4 of molecular oxygen after electron transfer using the structure from step (g).

The inner reorganization energy for morin during the electron transfer process is E1 - E2, and the inner reorganization energy for molecular oxygen is E3 - E4. When interactions are neglected, the total inner reorganization energy of the system λ_i is given by $\lambda_i = E1 - E2 + E3 - E4$.

For the proposed electron transfer reaction between the morin anion and molecular oxygen, the outer reorganization energy should be calculated using the following steps:

(i) Optimize the structure of the morin anion before electron transfer and write the solvent geometry information into the chk file.

(j) Calculate the energy E5 of the morin anion after electron transfer using the structure from step (i), where the solvent geometry remains as in (a) but the electronic structure is polarized.

(k) Calculate the energy E6 of the morin anion after electron transfer using the structure from step (i), where both the solvent geometry and electronic structure are fully relaxed.

(l) Optimize the structure of molecular oxygen before electron transfer and write the solvent geometry information into the chk file.

(m) Calculate the energy E7 of molecular oxygen after electron transfer using the structure from step (l), where the solvent geometry remains as in (l) but the electronic structure is polarized.

(n) Calculate the energy E8 of molecular oxygen after electron transfer using the structure from step (l), where both the solvent geometry and electronic structure are fully relaxed.

The outer reorganization energy of the system λ_o is calculated by freezing the solute molecules and is given by $\lambda_o = E5 - E6 + E7 - E8$.

According to the Marcus equation, another quantity required to calculate the activation energy of the reaction is the Gibbs free energy change ΔG^0. In this section, ΔG^0 is calculated using the following formula:

$$\Delta G_0 = \sum (\varepsilon_0 + G_{corr})_{products} - (\varepsilon_0 + G_{corr})_{reactants} \quad \text{Equation S12}$$

Here, the single-point energy ε_0 is the energy of the molecular structure fully optimized in the implicit solvent model of water, and G_{corr} is the Gibbs free energy correction. The single-point energy can be calculated simultaneously during the structural optimization of the molecule, while the Gibbs free energy of the corresponding structure will be obtained by performing frequency analysis on the optimized molecular structure.

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