Supplementary information

Peroxidase-like activity of peroxotitanium complex and its inhibition by some hydroxyalkanoic acids

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As reagents, $TiCl_4$(>99.9%) and *o*-phenylenediamine(OPD, >98.5%), H_2O_2(30%), acetic acid, sodium acetate, dimethyl sulfoxide(DMSO), isopropanol(IPA), nitro-blue-tetrazolium(NBT), salicylic acid, citric acid, sodium L-tartrate, hemi-calcium DL-glycerate, sodium L-lactate, sodium pyruvate, oxalic acid, sodium succinate, glycine, L-cysteine, sodium L-glutamate, glucose, urea, uric acid, $Na_2HPO_4 \cdot H_2O$, $NaH_2PO_4 \cdot 2H_2O$, EDTA-2Na, KCl, $(NH_4)_2SO_4$, $MgSO_4$, $CaCl_2 \cdot 2H_2O$ were used and of analytical grade.

$TiCl_4$ and hemi-calcium DL-glycerate were purchased from Sigma-Aldrich, OPD from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and the other reagents from Sangon Biotech Co., Ltd (Shanghai, China). Sodium DL-glycerate was obtained by applying sodium carbonate to hemi-calcium DL-glycerate solution.

100mM $TiCl_4$ solution was prepared by diluting 1.0M $TiCl_4$ solution in 0.5M HCl with distilled water. The OPD 20mg/mL solution was prepared by dissolving100 mg OPD in 250μL DMSO and then diluting with 4.75mL distilled water. As the buffer solutions, 0.2M acetate buffer solution and phosphate buffer solution were used. And double distilled water was used through the experiment.

The PTC stock solution was prepared as the following.

First, 100μL of 100mM $TiCl_4$, 600μL of H_2O_2 solution at certain concentration, and 3.30mL of distilled water were mixed to produce the yellow PTC solutions. The absorption spectra of these PTC solutions are shown in Fig. S1a, where the maximum absorption peak appeared at 386nm. And the A_{386} values increased with increasing the H_2O_2 concentration before the molar ratio of H_2O_2:Ti^{4+}=1:1, but changed little above this molar ratio (Fig. S1b). Thus, the PTC stock solution was prepared with the molar ratio of H_2O_2:Ti^{4+}=2:1 in order to form PTC enough: 200μL of 100mM $TiCl_4$, 120μL of 333mM H_2O_2, and 3.68mL of distilled water were mixed to prepare the PTC stock solution, while the PTC concentration of the stock solution is 2.5mM because the PTC is present as dinuclear complex at pH>1. The stock solution was kept at 0~4℃.

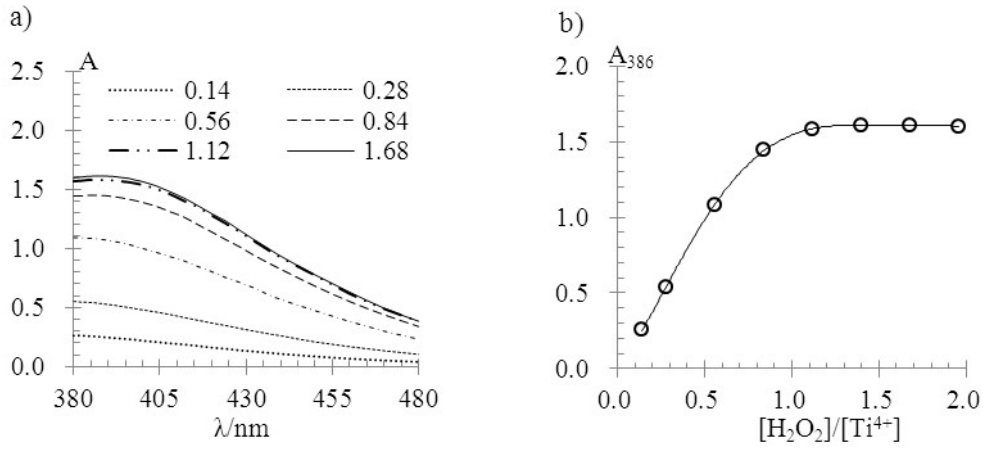

Fig. S1. The absorption spectra (a) and the A_{386} values (b) with varying the molar ratio of H_2O_2.

The effects of pH and temperature on the catalytic activity of PTC were investigated as below.

First, 1000 μL of 0.2M buffer solution with certain pH, 50μL of 333mM H_2O_2, and 2.75mL of distilled water were added to test tube. Then 100μL of 20mg/mL OPD was added just before 100μL of the PTC stock solution was added to start the reaction at certain temperature. As for control, 5mM H_2O_2 (considering the total amount of free hydrogen peroxide in the PTC stock solution) was added instead of the PTC stock solution. Absorbance values were measured at the maximum absorption wavelength by UV-visible spectrophotometer (BECKMAN COULTER DU®730) and the change rate of absorbance at the linear velocity interval (usually from 2min to 4 min of reaction time) was determined as the initial reaction velocity.

The effect of initial concentration of H_2O_2 on the initial rate was investigated as below.

First, 1000 μL of 0.2M acetate buffer solution (pH4.8), 50μL of H_2O_2 with certain concentration, and 2.75mL of distilled water were added to test tube, which was kept in the water bath for about 10min at the temperature of 25℃. Then 100μL of 20mg/mL OPD was added just before 100μL of the PTC stock solution was added to start the reaction at certain temperature. As for control, 5mM H_2O_2 (considering the amount of free hydrogen peroxide in the PTC stock solution) was added instead of the PTC stock solution. And the change rates of A_{445} during the reaction period of 2~4min were measured as the initial rates.

The relationship between initial H_2O_2 concentrations and initial reaction rates were approximated according to the Michaelis-Menten equation (Fig. S2). Fig. S2 shows the apparent catalytic behavior of PTC does not follow Michaelis-Menten equation because the three plots were not linear at all.

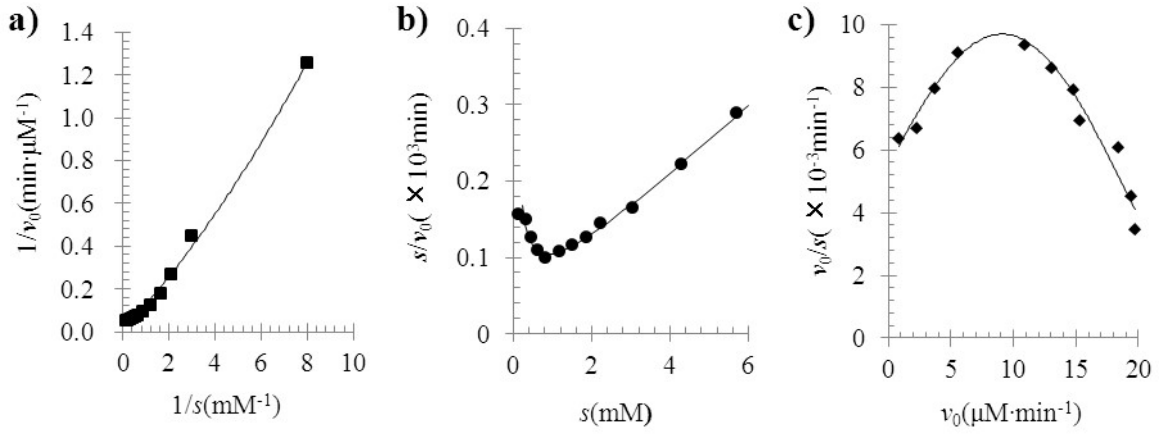

Fig. S2 The relationship between initial concentrations of H_2O_2(s) and initial reaction rates(v_0) according to the Michaelis-Menten kinetics, a)Lineweaver-Burk plot, b)Hanes plot, c)Eadie-Hofstee plot.

The curve of the initial rate vs. H_2O_2 concentration was approximated by the three linear transformations (Eqns. S1-S3) of the Hill equation :

$$\frac{1}{v_0}=\frac{{S_{0.5}}^n}{V_{max}}\cdot\frac{1}{S^n}+\frac{1}{V_{max}}$$ Lineweaver-Burk type (Eqn. S1),

$$\frac{S^n}{v_0}=\frac{{S_{0.5}}^n}{V_{max}}+\frac{1}{V_{max}}\cdot S^n$$ Hanes type (Eqn. S2),

$$v_0=V_{max}-{S_{0.5}}^n\cdot\frac{v_0}{S^n}$$ Eadie-Hofstee type (Eqn. S3),

where v_0 stands for initial velocity (μM min^{-1}), S for initial concentration of H_2O_2 (mM), V_{max} for the maximum velocity (μM min^{-1}), n for Hill coefficient, and $S_{0.5}$ for H_2O_2 concentration (mM) at $v_0= V_{max}/2$, respectively.

The catalytic stability of PTC was investigated as below.

First, 1000 μL of 0.2M acetate buffer solution with certain pH (typically pH4.8), 50μL of H_2O_2 with certain concentration, and 2.75mL of distilled water were added to test tube, which was kept in the water bath for about 10min at certain temperature(typically 25℃). Then 100μL of the PTC stock solution was added and kept for certain period before 100μL of 20mg/mL OPD was added to start the reaction. As for control, 5mM H_2O_2 (considering the amount of free hydrogen peroxide in the PTC stock solution) was added instead of the PTC stock solution. And the change rates of A_{445} during the reaction period of 2~4min were measured as the initial reaction rates.

The effects of dimethyl sulfoxide (DMSO) and isopropanol (IPA) on the catalytic activity of PTC were investigated as below.

First, 1000 μL of 0.2M acetate buffer solution (pH4.8), 50μL of 333mM H_2O_2, and 2.75mL of

DMSO or PTC at certain concentration were added to test tube, which was kept in the water bath for about 10min at the temperature of 25℃. Then 100μL of 20mg/mL OPD was added just before 100μL of the PTC stock solution was added to start the reaction. As for control, 5mM H_2O_2 was added instead of the PTC stock solution. And the change rates of A_{445} in the period of 2~4min were measured as the initial reaction rates. The effects of the concentrations of DMSO or IPA were relatively evaluated. (Fig. S3).

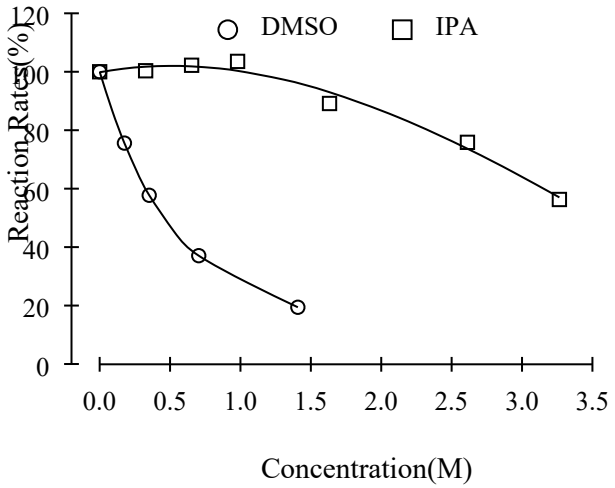

Fig. S3 The effects of DMSO and IPA on the initial reaction rates.

The effects of various organic compounds on the catalytic activity of PTC were investigated as below.

First, 1000 μL of 0.2M acetate buffer solution (pH4.8), 50μL of 333mM H_2O_2, and 100μL of an organic compound at certain concentration, 2.65mL of distilled water were added to test tube, which was kept in the water bath for about 10min at the temperature of 25℃. Then 100μL of 20mg/mL OPD was added just before 100μL of the PTC stock solution was added to start the reaction. As for control, 5mM H_2O_2 was added instead of the PTC stock solution. And the change rates of A_{445} in the period of 2~4min were measured as the initial rates. The effects of the organic compounds were evaluated as the inhibition rates, based on the initial velocity without them in the reaction medium.

The inhibitory effects of citric acid, EDTA-2Na, oxalic acid and lactate on the catalytic reaction rate of PTC at 4.28mM and 8.45mM of hydrogen peroxide respectively are shown in Figure S4.

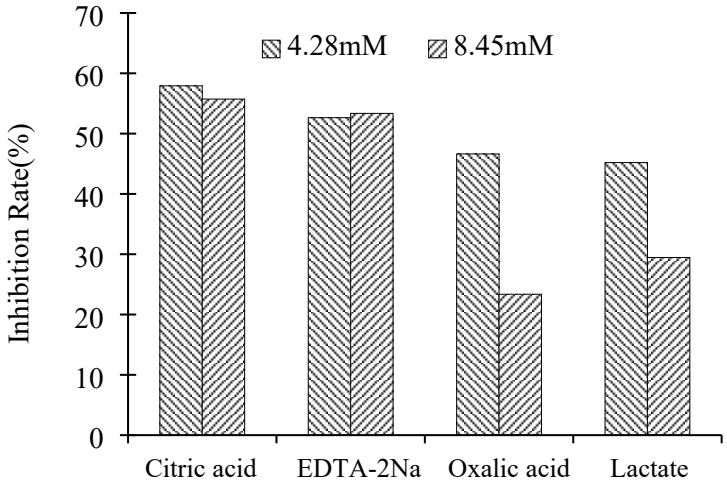

Fig. S4 Variation of inhibition rate with concentration of hydrogen peroxide (concentration of inhibitors in the reaction solution: citrate 35μM, EDTA-2Na 70μM, oxalic acid 125μM, and lactate 250μM)