Notes

Catalytic Determination of Hydrogen Peroxide by Using the Molybdenum-Porphyrin Complex as a Mimetic Enzyme of Peroxidase

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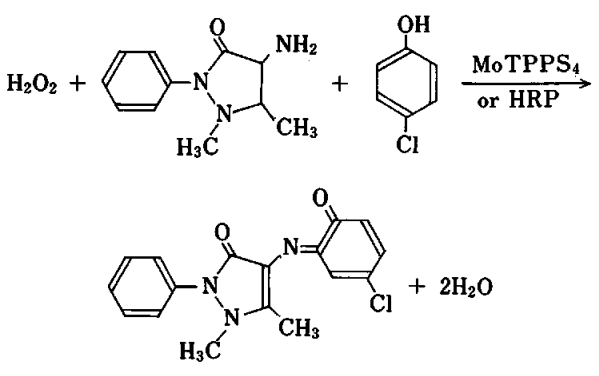
With the development of bionics, chemists attempt to mimic the biological properties of natural enzymes using stable mimetic enzymes.[1]

$MnTPPS_4$ [manganese-5,10,15,20-tetrakis(4-sulfophenyl)-21*H*,23*H*-porphine] was synthesized by Saito *et al.*[2], immobilized on anion exchange resins, and used to mimic horse radish peroxidase (HRP). Mimic behaviors of metalloporphyrins in aqueous solution were studied by Ci *et al.*[3] As the mimetic enzyme was not so good as the natural enzyme in catalytic selectivity, different sorts of peroxidase were mimicked only through synthesizing metalloporphyrins with different possible metals and different possible substitutents.[1] On the other hand, myoglobin (Mb) and HRP substituted by molybdenum and tungsten were studied by Shiro *et al.*[4] to mimic the heme environmental structures of compounds I and II for hemoproteins with high valent iron porphyrin moieties.

In this paper, $MoTPPS_4$ was described as the mimetic enzyme for peroxidase to determine H_2O_2 in the system of 4-aminoantipyrine (4-AAP) and *p*-chlorophenol. HRP and various metalloporphyrins were investigated in different catalytic activity situations. It was coupled with glucose oxidation reaction to determine glucose in human sera.



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Experimental

Reagents and apparatus

Doubly distilled water and analytically pure reagents were used. Clark–Lubs buffer solutions were prepared. Glucose peroxidase (GOD) and HRP were purchased from Sino-American Biotechnology Company in Beijing. Metalloporphyrins were prepared by a known method.[5]

Absorbances were measured with a Shimadzu UV-240 Spectrophotometer with a 10 mm cell.

Procedure

Synthesis of $MoTPPS_4$. Molybdenum powder (2.5 mg) was dissolved in aqua regia, and the pH of the solution was adjusted to 7. Then 40.0 mg $TPPS_4$ ($TPPS_4$ was prepared by a known method[5]) was added and the mixture was refluxed under argon until the Soret band of $TPPS_4$ disappeared (about 4 h). The solution was diluted to 2.0×10^{-4} M as used.

Determination of H_2O_2. In a 10 cm^3 colorimetric tube, 3.0 cm^3 of the buffer (pH 10.0), 0.5 cm^3 of 2.0×10^{-4} M $MoTPPS_4$, 0.5 cm^3 of 5.0×10^{-3} M 4-AAP, 0.5 cm^3 of 8.0×10^{-2} M *p*-chlorophenol and various amounts of the H_2O_2 standard solution were added and diluted to the mark. After 30 min the absorbance was measured at 510 nm against a reagent blank in a 10 mm cell.

Results and Discussion

Absorption spectra of $MoTPPS_4$ and $TPPS_4$

In Fig. 1, the maximum absorption of $MoTPPS_4$ was located at 392 nm, whereas that of $TPPS_4$ was at 410 nm (pH=7.0). It was shown that excess molybdenum ions did not catalyze this reaction. The result obtained by a molar ratio method indicated that $TPPS_4$ was complexed with molybdenum ion in the ratio of 1 : 1.

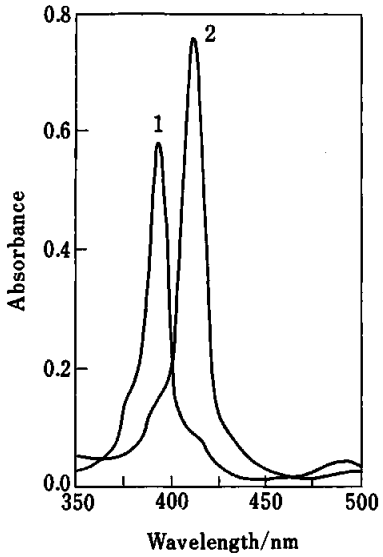

Fig. 1 Absorption spectra of $MoTPPS_4$ (1) and $TPPS_4$ (2): [$MoTPPS_4$], 2.5×10^{-6} M; [$TPPS_4$], 1.5×10^{-6} M.

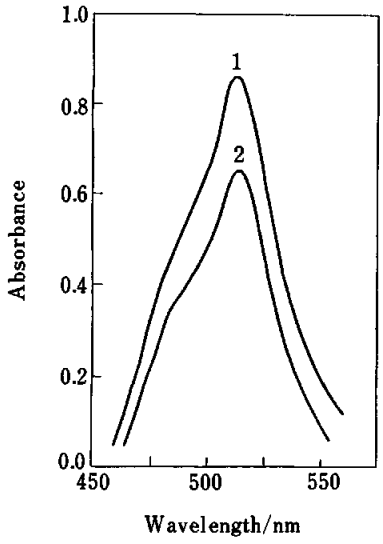

Fig. 2 Absorption spectra of chromogenic reaction catalyzed by: (1) 5×10^{-9} M HRP, 3.0 μg/cm³ H_2O_2; (2) 1×10^{-5} M $MoTPPS_4$, 3.0 μg/cm³ H_2O_2.

In Fig. 2, the shapes of the two spectra are very similar. The maximum absorption was 510 nm, showing the same product catalyzed by mimetic enzyme and natural enzyme. $MoTPPS_4$ reacted as a catalyst in this system because the absorption spectra of $MoTPPS_4$ before and after the reaction were the same.

Comparison of catalytic behavior of metalloporphyrins

Table 1 summarizes the absorbances of the quinonoid dye formed by the use of metalloporphyrins as a mimetic enzyme, together with the value obtained by HRP. The order of relative enzymetic activity was shown as: HRP≫$MnTPPS_4$>$FeTPPS_4$>$MoTPPS_4$>MnT(4-AP)P>FeT(4-TAP)P>$CuTPPS_4$>CuT(4-TAP)P in that concentrations and rates were considered simultaneously. $MnTPPS_4$ was used in strong basic (pH 11.4 - 11.6) solutions, and $FeTPPS_4$ aggregated easily and precipitated after a week's time. $MoTPPS_4$ was suggested as a mimetic enzyme for HRP because it had no such problems.

Selection of optimal conditions

It was shown that the optimal reaction pH for this system was at pH 9.6 - 10.0 and the reaction finished after 30 min at room temperature. There existed a maximum absorbance with the following quantities: ≥3.0 cm³ of pH 10.0 $NaOH-H_3BO_3$ buffer solution, 0.5 cm³ of 2.0×10^{-4} M $MoTPPS_4$ solution, ≥0.5 cm³ of 5.0×10^{-3} M 4-AAP solution and 0.5 cm³ of 8.0×10^{-2} M *p*-chlorophenol solution.

The calibration graph showed linearity, and its regression was given by

$$A = -0.002 + 7.24\times10^{3}C$$

when the concentration of H_2O_2 was $0-9.0\times10^{-5}$ M, where $r=0.9996$ and the absorptivity was 7.24×10^{3} dm³ mol⁻¹ cm⁻¹. Its recovery was in 100.6 - 103.5% and its relative standand deviation was 1.5% ($n=6$).

The effect of possible interference was examined at a concentration of 9.0×10^{-5} M H_2O_2. Cu(II), Mn(II), Ni(II), Co(II), Cd(II), Cl^-, CO_3^{2-}, SO_4^{2-} tested did not interfere.

Table 1 Comparisons of catalytic behavior of metalloporphyrins (H_2O_2: 9.0×10^{-5} M)

Enzyme	Concentration/M	pH	Rate/10^{-3} s⁻¹	ΔA	$\Delta A/\Delta A_{(HRP)}$, %
HRP	5×10^{-9}	5.5 - 7.0	1.24	0.992	100
$MoTPPS_4$	1×10^{-5}	9.6 - 10.0	0.47	0.638	64
$MnTPPS_4$	5×10^{-6}	11.4 - 11.6	0.86	0.687	69
$FeTPPS_4$	5×10^{-6}	9.2 - 9.5	0.60	0.689	69
$CuTPPS_4$	2.6×10^{-5}	10.0	0.076	0.261	26
MnT(4-TAP)P	2.6×10^{-5}	11.2 - 11.5	1.02	0.584	59
FeT(4-TAP)P	2.6×10^{-5}	8.2 - 8.6	0.74	0.589	59
CuT(4-TAP)P	2.6×10^{-5}	10.0	0.074	0.255	26

Table 2 Determination of glucose in human serum

Glucose added (mg/100 cm³)	Glucose found (mg/100 cm³)	Recovery (%)	Contents of glucose (mg/100 cm³)
0	76 73 74		74.3
50	127 119 125	98.6	
100	167 172 170	95.3	

Fe(III) and Zn(II), however, had serious inhibiting effects on the reaction.

Application

The chromogenic reaction coupled with oxidation of glucose was applied to the determination of glucose in human sera. The glucose contents in 100 cm³ of human sera were measured by the standard addition method, as summarized in Table 2.

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