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Metal 2-Hydroxy-1-Naphthaldehyde Thiosemicarbazone (Me-HNT) Complexes–A New Kind of Biomimic Enzyme Catalyst

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Abstract: Metal (Me=Fe(III), Mo(VI), Mn(II), Co(II), Ni(II), Zn(II) and Cu(II)) 2-hydroxy-1-naphthaldehyde thiosemicarbazone complexes (MeHNT) were synthesized and used as mimic-enzyme catalysts to mimic the active group of horseradish peroxidase (HRP) . The results showed that Fe-HNT, Mo-HNT are effective catalysts, which have similar catalytic activity as HRP. The sequence of catalytic activities of tested biomimic peroxidas is Mo-HNT> Fe-HNT > Zn-HNT > Ni-HNT > Mn- HNT. Among them, Fe-HNT is used as a mimic-enzyme catalyst in determination of ascorbic acid and glucose by coupling the catalytic reaction of glucose oxidase.

Keywords: Biomimic enzyme, catalytic activity, metal 2-hydroxy-1-naphthaldehyde thiosemicarba-zone complexes, spectrophotometry.

Enzymes have been widely used in analytical biochemistry and have considerable advantages of rapidity and high selectivity[1-3], but they are expensive and their solutions are quite unstable. So study of biomimic enzyme become an important branch of enzyme analysis[4-5]. Metal complexes are important mimetic resources, *e.g.* the metalloporphyrins (MPs) system has been used as the model of mimic peroxidase[6]. As common ligands, Schiff bases are easy to be synthesized, dissolved and are widely used in metal analysis[7-8], but they are seldom used in mimic-enzyme. We have found that the complexes of metal 2-hydroxy -1-naphthaldehyde thiosemicarbazone complexes (Me-HNT; Me=Fe(III), Mo(VI), Zn(II), Ni(II), and Mn(II)) have similar catalytic activities as HRP. They can catalyze the redox reaction of ascorbic acid (AsA) with H_2O_2 .Also they can be used in determining glucose indirectly. In this paper, the catalytic behaviours of Me-HNT were studied. The kinetic characteristics of the Me-HNT were compared with HRP and Me-$TPPS_4$. The possibility as a new kind of mimic enzymes has been verified in determining ascorbic acid in normal human sera and glucose in diabetic urine.

Experrimental

Apparatus and Reagents

All absorbency measurements were carried out on a Shimadzu UV-265spectrophotometer. pH values were measured by employing a pHS-3C digital pH-meter (Shanghai, Lei Chi). The elemental analysis was carried out on a Yanaco (Japan) model. MF-3 elemental analyzer.Horseradish peroxidase (HRP), glucose oxidase (GOD) were purchased from Sino-American Biotechnology Company, Beijing. 2-hydroxy-1-naphthaldehyde thiosemicarbazone (HNT) was synthesized according to the literature[7], (mp 245 ℃, anal. Found: (calcd.) C, 58.85; N, (58.75), H, 4.58; (4.52)), dissolved in dimethylformamide (DMF). the resulting solution was diluted with distilled deionized water. Ascorbic acid (AsA) solution was prepared daily by diluting a stock solution kept in refrigerator. The stock solution was prepared every week. Tris(hydroxymethyl) aminomethane-HCl (Tris-HCl) buffer (0.2 mol/L, pH7.00) was used. Fe^{3+} standard solution was prepared by dissolving 99.99% pure iron wire in HNO_3. Na_2MoO and $MeCl_2$ (Me=Mn, Cu, Co, Ni and Zn) were of analytical reagent or higher grade.

Procedure (1) Ascorbic acid determination.

In a 10 ml colorimetric tube, 2.5 ml of Tris-HCl buffer, pH 7.00, 0.1 ml of Me, HNT (5.0×10^{-4} mol/L) solutions, 0.5 ml of hydrogen peroxide (0.003%) and suitable volume of AsA were added. The solution was then diluted with distilled deionized water. The initial absorbance (A_i) at 265 nm was recorded immediately, the absorbance was then recorded at various time intervals for kinetic calculation. The final absorbance (A_f) was recorded after 10 minute. The absorbance difference was defined as $\triangle A_{265} = A_i - A_f$. *(2) Glucose determination* In a 10 ml colorimetric tube, 2.5 ml of Tris-HCl buffer (pH=7.00), 0.2 ml of 94 U/ml GOD, appropriate amounts of glucose standard solution were added. After 5 min, the solutions were cooled to 0 ℃ immediately to stop the oxidation reaction. Then 0.1 ml of 5.0×10^{-4} mol/L Fe^{3+} , HNT, 0.8 ml of 5.0×10^{-4} mol/L AsA were added and the mixture was diluted with distilled deionized water. The initial absorbance (A_i) at 265 nm was recorded immediately. After 10 min, the final absorbance (A_f) was recorded and the absorbance difference ($\triangle A_{265}$) was calculated.

Results and discussion

Comparison of catalytic activities of Me-HNT, Me-$TPPS_4$ and HRP systems

Michaelis equation[9] was used to study the kinetics of enzymatic reaction. The maximum rate V_{max} were obtained based on Lineweaver-Burk plots[10] (1/V *vs.* 1/[substrate], **Table1**). K_{cat} (transformation constant) were obtained from $V_{max} = K_{cat}\times[C]_o$, where $[C]_o$ stood for the initial concentration of enzyme. The K_{cat} values represent the catalytic activities. The calculated results showed that the relative catalytic activity of Me-HNT systems attenuated in the following order: Mo, Fe, Zn, Ni, Mn. The catalytic activity of Fe-HNT is 75% of that of HRP, higher than that of $MeTPPS_4$. It can also be used as another substitute model for HRP besides metalloporphyrins complexes in enzymatic analysis (**Table 1**).

To examine the catalytic activities of Me-HNT, Fe-HNT was chosen as a catalyst to determine AsA contents in human sera or glucose contents in diabetic urine.

Optimisation of experimental variables

Experimental variables were optimised by the unvariate method. Optimisation of experimental variables of AsA and glucose are listed in **Table 2.**

Permitted interference Range: *AsA determination*: 25 times: D-fructose, D-galactose, lactose, arabinose, acanine, isoalanine; 10 times:D-glucose, sucrose, leucine, valine,

Table 1. Comparison of catalytic activities of Me-HNT, $MeTPPS_4$ and HRP[a]

Enzyme	$[C]_o$ (mol/L)	Km (Mm)	Vmax (S^{-1})	K_{cat}(10^6,L/mol •S)	Relative activity[c] (%)
HRP	1.0×10^{-7}	0.338	0.0124	2.48	100
Mo-HNT	5.0×10^{-7}	0.374	11.20	2.24	90
Fe-HNT	4.0×10^{-6}	0.176	9.32	1.86	75
Zn-HNT	4.0×10^{-6}	0.162	8.01	1.60	65
Ni-HNT	4.0×10^{-6}	0.148	6.78	1.36	55
Mn-HNT	4.0×10^{-6}	0.112	6.59	1.32	53
$MnTPPS_4$[b]	1.0×10^{-5}	_	8.60	1.72	69
$FeTPPS_4$[b]	1.0×10^{-5}	_	6.00	1.20	48
$MoTPPS_4$[b]	2.0×10^{-5}	_	4.70	0.47	19

a: Each sample was determined three times.
b: Ref. [11]
c: Relative activity is the ratio of K_{cat} of metal complexes to that of HRP

Table 2. Optimised experimental variables of AsA or glucose determination[a]

Experimental Variable	Determination	Condition
	AsA	Glucose
Acidity	PH 7.00	PH 7.00
HNT concentration (mol/L)	5.0×10^{-6}	4.0×10^{-6}
AsA concentration(mol/L)		4.0×10^{-5}
Fe^{3+} concentration (mol/L)	5.0×10^{-6}	4.0×10^{-6}
H_2O_2 concentration(%)	1.5×10^{-4}	
Reaction time (min)	10	10
Working line	ΔA_{265}= 0.0065C (μg/ml) +0.0167	ΔA_{265}= 0.00495C (μg/ml) +0.0144
Linear range (μg/ml)	0.60 ～100	2.0 ～40.0
Detection limit (μg/ml)	0.315	0.238

a: Each sample was analysed six times.

histidine, proline, threonine, arginine, phenylalanine; 5 times: isoleucine, lysine. *Glucose determination*: 20 times: D-fructose,D-galactose, phenylalanine, isoalanine, sucrise,

histidine, serine; 10 times: valine, arabinose, aspartate; 6times: lysine, arginine, alanine; 2 times:VB_1 ,VB_2 ,VB_6; 1 times: cysteine, glutamate.

Sample analysis

The organic interference in diluted human serum or human urine did not interfere the determination of AsA or glucose, so the AsA contents of human serum or the glucose contents of diabetic urine were determined with inner-standard addition method. The results were summarized in **Table 3.**

Table 3. Results and recovery of AsA contents in normal human serum and glucose contents in diabetic urine[a]

Sample	Contents	Recovery	RSD
Serum AsA (μg/ml)	6.23	98～103	3.3
	7.89	94～101	1.6
	5.37	103～104	2.7
Diabetic Urine Glucose	280.7	89～90	3.8
(mg/100ml)	503.5	95～103	5.2
	647.1	100～101	1.3

a: Each sample was analysed six times

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