Biomimetic Nitration of Phenols Using Metalloporphyrins/H₂O₂/NO₂[−]

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Abstract An efficient metalloporphyrins/H₂O₂/NO₂[−] nitration of phenols has been developed. The total yield of nitrophenol could reach up to 55.1 %, which is about 4 and sixfold higher than that of horse radish peroxidase catalysis and peroxynitrite nitration, respectively. Furthermore, the nitration system attained an enhanced regioselectivity of 1.0 *o/p* ratio, and exhibited a good substrate scope of monophenols. This protocol is environmentally friendly compared with HNO₃/H₂SO₄ nitration, and stable, inexpensive and organic solvent tolerant compared with enzyme catalytic nitration and peroxynitrite nitration reaction.

Graphical Abstract

Keywords Phenol · Nitration · Metalloporphyrin · Mimic enzyme

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1 Introduction

Nitrophenols represents an important class of compounds and useful building blocks, in particular of various pharmaceuticals, energetic materials, dyes, chemical fibers and rubber products [1, 2]. The traditional nitration method is performed using nitric acid and sulfuric acid, which results in serious pollution, corrosion resistance, by-products formation.

In recent years, enzymatic methods have become increasingly important in nitration of phenolic compounds, which are well known for their high efficiency and selectivity under mild reaction conditions, especially for peroxidases. Peroxidases have been used in a broad range of reactions including oxidation, nitration halogenation, hydroxylation, demethylation, sulfoxidation, epoxidation and polymerization [3–5]. In vivo, peroxidases can lead to nitration of tyrosine and tryptophan [6–8]. Inspired by natural metabolism, some kinds of peroxidases have been researched for the nitration of phenols in the H₂O₂/NO₂[−] system in vitro, such as horse radish peroxidase [9, 10], soybean peroxidase [11], myeloperoxidase [12], microperoxidase [13, 14] and hemin–peptide mimic enzyme (Fe^{III}–mimochrome VI). However, the yields are usually low. For example, the highest yield of nitrophenol catalyzed by horseradish peroxidase was only 25 % [10]. Furthermore, the thermo sensitivity, poor stability, and scarce resources of enzyme limit the application.

It is generally known that iron porphyrin is the active center of peroxidase (Scheme 1). Actually, several researches showed that metalloporphyrins could catalyze nitration of phenols in the presence of peroxynitrite (ONOO[−]), which could be prepared by H₂O₂ and NaNO₂ [16–18]. Thus, metalloporphyrins may have the potential to catalyze nitration reaction in the H₂O₂/NO₂[−] system. Compared to

Scheme 1 Mechanism for the nitration of protein with peroxidase [15]

enzymes, these “mimic enzymes” are thermal-insensitive, stable and inexpensive. So in this study, we try to develop an efficient and economical metalloporphyrins/ $\text{H}_2\text{O}_2/\text{NO}_2^-$ system for nitration of phenols.

Here, we have synthesized four series of metalloporphyrins as “mimic enzymes”: metal-tetraphenylporphyrin (MTPP), metal-tetra (*p*-nitrophenyl) porphyrin (MTNPP), metal-tetra (*p*-carboxyphenyl) porphyrin (MTCPP) and metal-tetra (*p*-sulfohenyl) porphyrin (MTSPP), to catalyze nitration of phenols in $\text{H}_2\text{O}_2/\text{NO}_2^-$ system (Fig. 1). Then a study of the catalytic ability under various reaction conditions has been done. The nitration effects of hemin, horse radish peroxidase and laccase have also been investigated as controls.

2 Experimental Section

All of the products and metalloporphyrins synthesized in this research were known compounds and the data accorded with those given in the references. All metalloporphyrins were synthesized in our laboratory by Alder reaction according to the literature [19–22]. Other reactants, solvents and standard samples were purchased from commercial sources, and used without further purification.

Fig. 1 Metalloporphyrins used in nitration of phenols

All of the products are known compounds, the identification of whom was determined by NMR, HRMS, HPLC and TLC. TLC was performed on a silica gel plate purchased from Merck Ltd. (Darmstadt, Germany). NMR data were obtained on a Bruker Avance III 600 MHz NMR Spectrometer. HRMS data were obtained on a Bruker Q-TOF maXis Spectrometer. HPLC analysis was performed on a Agilent 1100A chromatograph with a ODS C_{18} column (4.6×250 mm, KR100-5 C_{18}) and methanol/water (35:65) mixture as eluent (pH 3). The flow rate was 1 mL/min with a column temperature of 25 °C. Samples were monitored at 254 nm. The quantitative analytical method was external standard calibration curve method, using commercial standard samples as external standard.

2.1 Catalyst Preparation

2.1.1 General Procedure of the Synthesis of Porphyrins

Porphyrins were prepared using the typical Adler reaction. Pyrrole (30 mmol) was slowly added to a mixture of substituted benzaldehyde (30 mmol) and propionic acid (105 mL) through a dropping funnel. The mixture was heated to 120–140 °C for 1–3 h. After cooling, the reaction mixture was filtered, washed and dried under vacuum to obtain the crystal of porphyrins.

2.1.2 General Procedure of the Synthesis of Metalloporphyrins

Metalloporphyrins were prepared by refluxing porphyrins (0.33 mmol) with FeCl_3 , CoCl_2 , MnCl_2 , CuCl_2 , or $\text{Zn}(\text{OAc})_2$ (1.82 mmol) in *N,N'*-dimethylformamide (DMF, 70 mL) for 12 h. DMF was removed by distillation and the metalloporphyrins were precipitated by adding water. The precipitate was dissolved in 0.1 M NaOH solution and recrystallized by adding 1 M HCl solution. Finally, metalloporphyrins were filtered and dried under vacuum.

2.2 General Procedure of Nitration of Phenols

To a solution contained phosphate sodium buffer (50 mL, 0.1 M, pH 7.0), phenols (1 mmol) and NaNO_2 (50–60 mmol), H_2O_2 (30 % aqueous solution, 3 mmol) was added dropwise in 80 min. The reaction was initiated by adding metalloporphyrin (50–60 mg) at room temperature. After stirring for 80 min, the reaction products were extracted several times with chloroform or ethyl acetate, and the organic phase was combined, dried with sodium sulfate. The organic solvent was removed under vacuum. The residue was dissolved in methanol and quantitatively analyzed by HPLC.

3 Results and Discussion

3.1 Nitration of Phenol in Metalloporphyrins/H₂O₂/NO₂[−] System

We began the study by screening and evaluating catalysts for phenol nitration. Nitration of phenol in H₂O₂/NO₂[−] system was carried out using series of porphyrins combined with different metals. Products and residual substrates were quantitatively analyzed by external standard calibration curve method. Reaction of phenol in the presence of FeTSPP, H₂O₂ and NO₂[−] resulted in the formation of 4-nitrophenol and 2-nitrophenol (Fig. 2). The products were analyzed by HPLC and confirmed by comparing with the commercial samples.

The effect of different metalloporphyrins on nitration was investigated under the conditions: phenol (1 mmol), NaNO₂ (20 mmol), H₂O₂ (30 % aqueous solution, 2 mmol), phosphate sodium buffer (PBS, 50 mL, 0.1 M, pH 7.0), 80 min at 25 °C. The results are given in Table 1.

As expected, the blank experiment generated only a trace of nitration products (Table 1, entry 1), and metalloporphyrins could increase the conversion of phenol (Table 1, entries 2–23). Among the catalyst screening, iron porphyrins (FeTPP, FeTNPP, FeTCPP, FeTSPP) performed the best catalytic activity (Table 1, entries 4, 9, 14, 19). FeTCPP and FeTSPP showed better yields than FeTPP and FeTNPP which could be attributed to the water solubility. The best yield was 14.6 % and *o/p* ratio was 0.62. As a control, corresponding chlorinated metal salts were also investigated (Table 1, entries 24–28). No significant change in phenol conversion was observed. This result proved the nitration activity of metalloporphyrins.

3.2 Nitration of Phenol in FeTCPP (FeTSPP)/H₂O₂/NO₂[−] System

Then great efforts to optimize reaction conditions for FeTCPP or FeTSPP catalyzed nitration of phenol were conducted to increase the yields. The effect of metalloporphyrin content, NaNO₂ content, H₂O₂ content, pH, temperature, reaction time and solvent were investigated, respectively (Figs. 3, 4).

As shown in Fig. 3, experiments were set up under various conditions. It was observed that there was rapidly increase of the nitrophenol yield up to 0.05 g FeTCPP (5.9 mol% of phenol). Thereafter, no notable increase of the yield was noticed. This result suggested that the optimum content of FeTCPP was 5.9 mol% of reaction substrate (Fig. 3a). Similarly, the optimum content of NaNO₂ was 50 mmol and H₂O₂ was 3 mmol (Fig. 3b, c). The highest yields of 4- and 2-nitrophenol were 21.2 and 22.5 %, respectively.

Therefore, all the nitration experiments were performed under the conditions of 5.9 mol% FeTCPP, 50 mmol NaNO₂ and 3 mmol H₂O₂. The effect of pH on the activity was then examined (Fig. 3d). The pH profile of the yield for nitrophenol formation was characterized by a bell-shaped curve. The maximum activity was at pH 7, which was similar to the optimum pH of common peroxidases.

Next, the effect of reaction time and temperature were investigated (Fig. 3e). The yield of 4- and 2-nitrophenol in 40 min increased rapidly and then increased slowly. 80 min was found to be the optimum reaction time. Reaction temperature had relatively little effect on the yield of nitrophenol (Fig. 3f). The yield increased by raising temperature from 0 to 25 °C and then it decreased. In addition, the product distribution changed to some extent with temperature increasing. The *o/p* ratio decreased to below 1.0 when the temperature reached 45 °C or higher.

Fig. 2 HPLC data of products of nitration in FeTSPP/H₂O₂/NO₂[−] system

Table 1 Effects of metalloporphyrins on nitration of phenol

Entry	Catalyst ^a	Yield (%) ^b		<i>o/p</i> Ratio ^c
		4-Nitrophenol	2-Nitrophenol	
1	Blank	Trace	Trace	—
2	TCPP	<0.5	<0.5	—
3	TSPP	<0.5	<0.5	—
4	FeTCPP	5.1	8.0	1.57
5	CoTCPP	<0.5	<0.5	—
6	MnTCPP	<0.5	<0.5	—
7	CuTCPP	<0.5	<0.5	—
8	ZnTCPP	<0.5	<0.5	—
9	FeTSPP	9.0	5.6	0.62
10	CoTSPP	<0.5	<0.5	—
11	MnTSPP	<0.5	<0.5	—
12	CuTSPP	<0.5	<0.5	—
13	ZnTSPP	<0.5	<0.5	—
14	FeTPP	2.3	2.4	1.04
15	CoTPP	<0.5	<0.5	—
16	MnTPP	1.3	1.1	0.85
17	CuTPP	<0.5	<0.5	—
18	ZnTPP	<0.5	<0.5	—
19	FeTNPP	2.7	2.0	0.74
20	CoTNPP	<0.5	<0.5	—
21	MnTNPP	1.8	2.1	1.17
22	CuTNPP	<0.5	<0.5	—
23	ZnTNPP	<0.5	<0.5	—
24	FeCl ₃	Trace	Trace	—
25	CoCl ₂	Trace	Trace	—
26	MnCl ₂	Trace	Trace	—
27	CuCl ₂	Trace	Trace	—
28	ZnCl ₂	Trace	Trace	—

Reaction conditions: phenol (1 mmol), NaNO₂ (20 mmol), H₂O₂ (30 % aqueous solution, 2 mmol), phosphate sodium buffer (50 mL, 0.1 M, pH 7.0), 80 min at 25 °C

^a Catalyst content: 0.03 g

^b HPLC yields

^c Calculated from original HPLC data

The effect of solvent was also investigated (Fig. 3g). The yield of nitrophenol in pure aquatic system was only 23.5 %, obviously lower than that in PBS system. The result indicated the importance of pH stability. No reaction was observed in MeOH system. That could be attributed to the indissolubility of NaNO₂. So experiments in water/MeOH and water/PBS mixed solution system were

performed. The yields were almost equal those in water and PBS system proving the potential of this mimic enzyme nitration reaction in organic solvents.

To sum up, the optimum reaction parameters are: n(phenol) : n(FeTCPP) : n(NaNO₂) : n(H₂O₂) = 1 : 0.059 : 50 : 3, pH 7.0, 80 min and 25 °C. Under this condition, the total yield of 4- and 2-nitrophenol is 43.7 %. And compared with the 1.67 *o/p* ratio of the nitration of phenol in HNO₃/H₂SO₄, the nitration in FeTCPP/H₂O₂/NO₂[−] system shows enhanced regioselectivity, the *o/p* ratio of which is about 1.06.

As shown in Fig. 4, similar results were observed in the experiments in FeTSPP/H₂O₂/NO₂[−] system. The optimum parameters are: n(phenol) : n(FeTCPP) : n(NaNO₂) : n(H₂O₂) = 1:0.056:60:3, pH 7.0, 80 min and 25 °C. The total yield of 4- and 2-nitrophenol is 55.1 % under the optimum conditions. The 0.97 *o/p* ratio is even lower than that of FeTCPP catalyzed nitration, which shows better regioselectivity.

3.3 Nitration of Phenol in Different Systems

In order to evaluate the catalytic effect of metalloporphyrins/H₂O₂/NO₂[−] system, we compared this protocol with the nitration reaction of phenol catalyzed by different enzymes and performed by peroxynitrite reaction (Table 2).

As the active center of various enzymes, hemin is commonly used as a mimic enzyme of peroxidases. The result showed that hemin could also promote the nitration of phenol under the optimum conditions, albeit with lower yields (Table 2, entry 4).

In the presence of 1 mg horse radish peroxidase, the total yield of nitrophenol was only 14.8 %, about fourfold lower than that in TSPP/H₂O₂/NO₂[−] system (Table 2, entry 5). So we increased the content of horse radish peroxidase to 10 mg. The conversion was raised to 65 %, but the products were very complicated and almost no nitrophenol was detected (Table 2, entry 6). As described in the literatures, peroxidases can catalyze further reactions, such as oxidative decomposition. So too much peroxidase not only promoted various side reactions of phenol, but also resulted in the following reactions of nitrophenols. That's why peroxidase is less application in nitration reaction so far.

Another common metalloenzyme, laccase, was also used as a comparison. Laccase is copper-containing oxidase enzyme and requires oxygen as a second substrate for the enzymatic reaction. So oxygen was used instead of H₂O₂, and moreover, the pH was adjusted to 5, the optimum pH of laccase. However, no reaction was observed (Table 2, entries 7 and 8), which indicated the important roles of iron ion and porphyrins.

Fig. 3 Effects of various reaction conditions on nitration of phenol catalyzed by FeTCPP.**a** Effects of FeTCPP content.Conditions: phenol (1 mmol), NaNO₂ (20 mmol), H₂O₂ (2 mmol), pH 7.0, 80 min, 25 °C. **b** Effects of NaNO₂ content. Conditions: phenol (1 mmol), FeTCPP (50 mg, 59 μmol), H₂O₂ (2 mmol), pH 7.0, 80 min, 25 °C. **c** Effects of H₂O₂ content. Conditions: phenol (1 mmol), FeTCPP (50 mg, 59 μmol), NaNO₂ (50 mmol), pH 7.0, 80 min, 25 °C. **d** Effects of pH. Conditions: phenol (1 mmol), FeTCPP (50 mg, 59 μmol), NaNO₂ (50 mmol), H₂O₂ (3 mmol), 80 min, 25 °C. **e** Effect of reaction time. Conditions: phenol (1 mmol), FeTCPP (50 mg, 59 μmol), NaNO₂ (50 mmol), H₂O₂ (3 mmol), pH 7.0, 25 °C. **f** Effects of temperature. Conditions: phenol (1 mmol), FeTCPP (50 mg, 59 μmol), NaNO₂ (50 mmol), H₂O₂ (3 mmol), pH 7.0, 80 min.**g** Effect of solvent. Conditions: phenol (1 mmol), FeTCPP (50 mg, 59 μmol), NaNO₂ (50 mmol), H₂O₂ (3 mmol), pH 7.0, 80 min, 25 °C, $V_{\text{water}}:V_{\text{MeOH}} = 1:1$, $V_{\text{PBS}}:V_{\text{MeOH}} = 1:1$.

Peroxynitrite reaction was another common method in the research of protein and other aromatic compounds' nitration. We synthesized the ONOO[−] solution according to the literatures [23, 24], to perform the peroxynitrite

reaction with or without catalyst. As the result showed, ONOO[−] without catalyst could produce a small amount of nitrophenols (Table 2, entry 9). Catalyst FeTSPP and horse radish peroxidase could obviously improve the conversion

Fig. 4 Effects of various reaction conditions on nitration of phenol catalyzed by FeTSPP.

a Effect of FeTSPP content.

Conditions: phenol (1 mmol), NaNO_2 (20 mmol), H_2O_2 (2 mmol), pH 7.0, 80 min, 25 °C. **b** Effect of NaNO_2 content. Conditions: phenol (1 mmol), FeTSPP (50 mg, 56 μmol), H_2O_2 (2 mmol), pH 7.0, 80 min, 25 °C. **c** Effect of H_2O_2 content. Conditions: phenol (1 mmol), FeTSPP (60 mg, 56 μmol), NaNO_2 (60 mmol), pH 7.0, 80 min, 25 °C. **d** Effect of pH. Conditions: phenol (1 mmol), FeTSPP (60 mg, 56 μmol), NaNO_2 (60 mmol), H_2O_2 (3 mmol), 80 min, 25 °C. **e** Effect of reaction time. Conditions: phenol (1 mmol), FeTSPP (60 mg, 56 μmol), NaNO_2 (60 mmol), H_2O_2 (3 mmol), pH 7.0, 25 °C. **f** Effect of temperature. Conditions: phenol (1 mmol), FeTSPP (60 mg, 56 μmol), NaNO_2 (60 mmol), H_2O_2 (3 mmol), pH 7.0, 80 min. **g** Effect of solvent. Conditions: phenol (1 mmol), FeTSPP (60 mg, 56 μmol), NaNO_2 (60 mmol), H_2O_2 (3 mmol), pH = 7.0, 80 min, 25 °C, $V_{\text{water}}:V_{\text{MeOH}} = 1:1$, $V_{\text{PBS}}:V_{\text{MeOH}} = 1:1$.

of phenol and the yield of nitro-product. But the highest yield was only 9.9 %, about sixfold lower than that in TSPP/ H_2O_2 / NO_2^- system. This may be attributed to the instability of ONOO^- ion, and because of this, peroxynitrite reaction is unpractical in the synthesis of nitrophenols.

3.4 Nitration of Different Phenolic Compounds in FeTSPP/ H_2O_2 / NO_2^- System

At last, the general scope and versatility of this mimic enzyme nitration system were investigated. The catalytic

Table 2 Effects of different system on nitration of phenol

Entry	Catalyst (content)	Nitration system ^a	Conversion (%) ^b	Yield (%) ^c		<i>o/p</i> Ratio ^b
				4-Nitrophenol	2-Nitrophenol	
1	Blank	H ₂ O ₂ /NO ₂ [−]	<0.1	Trace	Trace	–
2	FeTCPP (50 mg, 5.9 mol%)	H ₂ O ₂ /NO ₂ [−]	45	21.2	22.5	1.06
3	FeTSPP (60 mg, 5.6 mol%) ^d	H ₂ O ₂ /NO ₂ [−]	57	28.0	27.1	0.97
4	Hemin (50 mg, 7.7 mol%)	H ₂ O ₂ /NO ₂ [−]	34	15.5	15.9	1.03
5	Horse radish peroxidase (1 mg) ^e	H ₂ O ₂ /NO ₂ [−]	41	6.7	8.1	1.21
6	Horse radish peroxidase (10 mg) ^e	H ₂ O ₂ /NO ₂ [−]	65	<0.1	<0.1	–
7	Laccase (1 mg) ^f	O ₂ /NO ₂ [−]	<0.1	<0.1	<0.1	–
8	Laccase (10 mg) ^f	O ₂ /NO ₂ [−]	<0.1	<0.1	<0.1	–
9	Blank	ONOO ^{−g}	<2	0.8	1.1	1.38
10	FeTSPP (60 mg, 5.6 mol%)	ONOO ^{−g}	7	2.4	2.8	1.17
11	Horse radish peroxidase (1 mg) ^e	ONOO ^{−g}	19	4.0	5.9	1.48

^a Reaction conditions: phenol (1 mmol), NaNO₂ (50 mmol), H₂O₂ (3 mmol), PBS (50 mL, 0.1 M, pH 7.0), 80 min at 25 °C^b Calculated from original HPLC data^c HPLC yields^d Reaction conditions: NaNO₂ (60 mmol)^e Enzyme activity: >200 IU/mg^f Enzyme activity: >50 IU/mg. Reaction pH: 5^g Reaction conditions: phenol (1 mmol), NaONOO (9.6 mmol), PBS (20 mL, 0.1 M, pH 7.0), 30 min at 25 °C

performance of iron porphyrins FeTSPP to the nitration reaction of hydroquinol, resorcinol, phloroglucinol and *p*-cresol were tested under the optimum reaction conditions (Table 3).

As seen from Table 3, metalloporphyrins/H₂O₂/NO₂[−] system was an efficient nitration system for monophenols. But this protocol showed lower nitration efficiency and higher oxidation efficiency for polyphenols.

The results showed that the yields of nitration of diphenols in TSPP/H₂O₂/NO₂[−] system were only 20 % or so (Table 3, entries 2 and 3). Furthermore, the nitration of phloroglucinol obtained 68 % conversion but only 1.3 % yield of nitro product (Table 3, entry 4). Actually, phloroglucinol almost completely decomposed after 24 h in this system. It is noted that phloroglucinol was very easily oxidized and degraded, so this result proved that metalloporphyrins could catalyze both nitration and oxidation in H₂O₂/NO₂[−] system. By contrast, the nitration of more stable phenolic compound *p*-cresol showed a higher 69 % conversion and 66.8 % yield of 2-nitro-4-methylphenol (Table 3, entry 5). In addition, a small amount of 4-nitrophenol and dinitro-product 2,6-dinitro-4-

methylphenol were obtained, and no oxidative product was detected.

4 Conclusions

In summary, an efficient metalloporphyrins/H₂O₂/NO₂[−] nitration system under mild conditions has been developed. Under the optimum conditions, catalyzed by FeTSPP, the total yield of 4- and 2-nitrophenol can reach 55.1 %, and an enhanced regioselectivity of 1.0 *o/p* ratio was attained. This iron porphyrins/H₂O₂/NO₂[−] system can effectively promote nitration of phenols in the presence of organic co-solvent, such as MeOH. Meanwhile, the yield of this protocol is about fourfold higher than that of horse radish peroxidase catalysis, and sixfold higher than that of peroxynitrite nitration. Furthermore, this nitration system exhibits a good substrate scope of monophenols. In addition, this protocol is environmentally friendly avoiding the usage of HNO₃/H₂SO₄, and thermal-insensitive, stable, inexpensive and organic solvent tolerant compared with enzyme catalytic nitration reaction and peroxynitrite

Table 3 Nitration of different phenolic compounds in FeTSPP/H₂O₂/NO₂[−] system

Entry	Phenolic compound	Conversion (%) ^b	Nitration product and yield (%) ^c		
1		57	28.0	27.1	
2		47	20.3		
3		42	11.8	9.9	
4		68	1.3		
5		69	66.8	0.56	0.88

^a Reaction conditions: phenolic compounds (1 mmol), NaNO₂ (50 mmol), H₂O₂ (3 mmol), PBS (50 mL, 0.1 M, pH 7.0), 80 min at 25 °C

^b Calculated from original HPLC data

^c HPLC yields

reaction. Further explorations of “mimic enzymes” metalloporphyrins are currently undergoing in our laboratory.

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