

## Electrocatalytic Oxidation of Vitamin B<sub>6</sub> by 4-Hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl

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The electrochemistry of pyridoxine (vitamin B<sub>6</sub>, VB<sub>6</sub>) was studied by cyclic voltammetry at a glassy carbon electrode. The electrochemical response of VB<sub>6</sub> could be significantly enhanced by using 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (HO-TEMPO) as a mediator via an electrocatalytic EC' mechanism with the oxoammonium ion of HO-TEMPO as the active oxidant. The catalytic rate constant was determined to be  $5.4 \times 10^3$  (mol·L<sup>-1</sup>)<sup>-1</sup>·s<sup>-1</sup> by using chronoamperometry.

**Keywords**    cyclic voltammetry, catalytic electrooxidation, vitamin B<sub>6</sub>, nitroxide

### Introduction

Nitroxides such as 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) are well-known stable free radicals which have been extensively used in spin labeling,<sup>1</sup> spin trapping<sup>2</sup> and as antioxidants.<sup>3</sup> Nitroxides are also easy to undergo reversible one-electron oxidation to form the corresponding oxoammonium ions.<sup>4</sup> Oxoammonium ions are mild one-electron oxidants which have been used in organic synthesis<sup>5-8</sup> and to generate radical cations.<sup>9</sup> We<sup>10</sup> found recently that electrochemically generated oxoammonium ion of 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (HO-TEMPO) could catalyze the electrochemical oxidation of *L*-tyrosine, and hence dramatically enhanced the electrochemical response of the substrate.

Pyridoxine (vitamin B<sub>6</sub>, VB<sub>6</sub>) is an important biologically active compound which has been determined by micellar electrokinetic capillary chromatography,<sup>11</sup> capillary electrophoresis,<sup>12</sup> differential pulse polarography<sup>13</sup> and single sweep voltammetry at a carbon paste electrode.<sup>14</sup> However, the electrochemical response of VB<sub>6</sub> is generally low.<sup>13,14</sup> We wish to report herein that electrooxidation of VB<sub>6</sub> can be catalyzed by HO-TEMPO, providing a basis for a facile and sensitive determination of VB<sub>6</sub>.

### Experimental

A conventional single-compartment, three-electrode cell thermostatted at 20 °C and kept under nitrogen atmosphere was used for all experiments. The electrochemical instrumentation consisted of a PAR model 173 potentiostat coupled with a PAR model 175 universal programmer, and a Houston Instruments model 2000

X-Y recorder. A glassy carbon electrode (4.5 mm in diameter) employed as a working electrode was carefully polished with 0.05 nm alumina slurry on a flat surface and sonicated immediately before use. A platinum wire was employed as an auxiliary electrode. All potentials were recorded relative to a saturated calomel electrode (SCE) reference electrode.

Vitamin B<sub>6</sub> and HO-TEMPO were from Sigma and Shanghai Chemicals respectively and used as received. All solutions were prepared by doubly distilled water and thoroughly flushed with nitrogen before use. Other chemicals were of analytical grade and used as received.

### Results and discussion

#### Catalytic oxidation of vitamin B<sub>6</sub> by HO-TEMPO

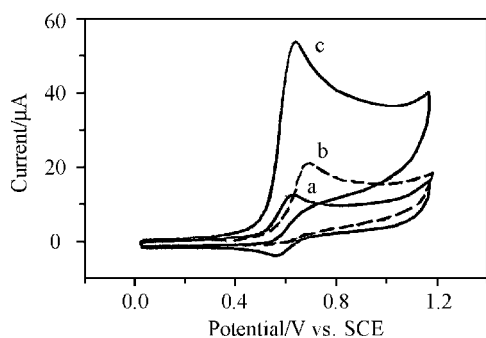
HO-TEMPO is water-soluble and showed a nice cyclic voltammogram at a glassy carbon electrode (GCE) in aqueous phosphate buffered solutions, *e.g.*, Figure 1a. The quasi-reversible one electron redox behavior at the GCE was indicated by a couple of peaks corresponding to the oxidation of HO-TEMPO and the reduction of its oxoammonium ion as reported previously.<sup>4</sup> The  $E_{pa}$  and  $E_{1/2}$  are 0.624 and 0.590 V vs. SCE respectively with the peak potential difference,  $\Delta E_p = 68$  mV, in agreement with literature.<sup>15,16</sup> However, the cathodic peak was smaller than the anodic peak because the experiment was conducted at pH=9.4 and the intermediate oxoammonium ion could react with water in alkaline media.<sup>17</sup> On the other hand, direct electrooxidation of VB<sub>6</sub> at the GCE gave a weak irreversible voltammogram with an anodic peak ( $E_{pa}$ ) located at *ca.* 0.670 V (Figure 1b), demonstrating a slow oxidation process at the electrode

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surface. However, in the presence of a small amount of HO-TEMPO the anodic peak current was significantly enhanced accompanied by the disappearance of the cationic peak of the nitroxide (Figure 1c). The  $E_{pa}$  was 0.636 V which is very close to the anodic peak potential of HO-TEMPO. This suggests clearly a catalytic EC process, similar to the electrocatalytic oxidation of *L*-tyrosine<sup>10</sup> and hydrazine<sup>16</sup> by HO-TEMPO reported previously.



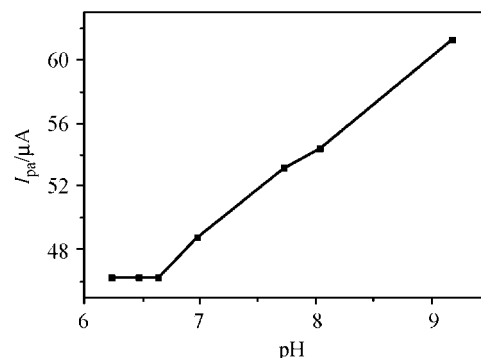
**Figure 1** Cyclic voltammograms of HO-TEMPO and VB<sub>6</sub> recorded at a glass carbon electrode in 0.10 mmol·L<sup>-1</sup> phosphate buffer (pH=9.4) at room temperature with a scan rate of 100 mV/s. (a) 0.30 mmol·L<sup>-1</sup> HO-TEMPO; (b) 1.00 mmol·L<sup>-1</sup> VB<sub>6</sub>; (c) 1.00 mmol·L<sup>-1</sup> VB<sub>6</sub> in the presence of 0.30 mmol·L<sup>-1</sup> HO-TEMPO.

### pH dependence of the catalytic oxidation current and potential

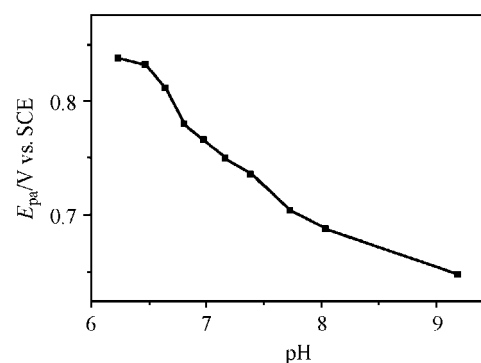
It has been reported that the solution acidity exerts little effect on the redox potentials of HO-TEMPO in the pH range of 1.8–9.2.<sup>17</sup> It was found, however, that the catalytic electrochemical oxidation of VB<sub>6</sub> in the presence of HO-TEMPO remarkably depended on the solution acidity. The anodic peak current,  $I_{pa}$ , increased significantly with the increase of pH above pH=6.7 (Figure 2), while the peak potential,  $E_{pa}$ , decreased with the increase of pH (Figure 3). In addition, the cathodic peak of the oxoammonium ion reappeared in case of pH<6.8, demonstrating that the oxidation of VB<sub>6</sub> by the oxoammonium ion of HO-TEMPO goes slowly in acidic solutions. This pH dependence is understandable because it would be easier for the positively charged oxoammonium ion to react with the negatively charged phenoxide of VB<sub>6</sub> in alkaline solutions. In case of pH>9.4 the electrooxidation of HO-TEMPO became irreversible, making the catalytic electron transfer unfavorable.<sup>17</sup> Since VB<sub>6</sub> is easy to dissolve in alkaline solution and the catalytic electrochemical oxidation is more efficient at higher pH, pH=9.4 was chosen for the following experiments.

### Quantitative determination of VB<sub>6</sub>

The catalytic oxidation peak current,  $I_{pa}$ , of VB<sub>6</sub> in the presence of HO-TEMPO increased with increase of the concentration of HO-TEMPO. At a definite concentration of the catalyst the catalytic current was proportional to the concentration of VB<sub>6</sub> in the concentration

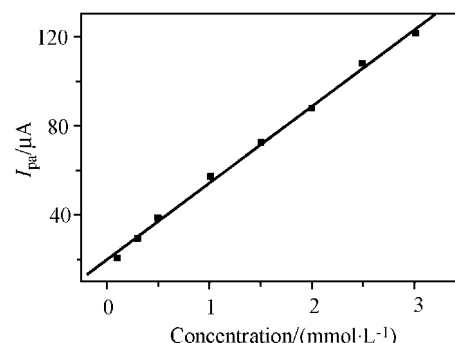


**Figure 2** Dependence of the catalytic peak current on pH. The experimental conditions were the same as those described in the legend of Figure 1 except the pH. Concentrations of HO-TEMPO and VB<sub>6</sub> were 0.30 and 1.00 mmol·L<sup>-1</sup>, respectively.



**Figure 3** Dependence of the catalytic peak potential on pH. The experimental conditions were the same as those described in the legend of Figure 2.

range of  $1 \times 10^{-5}$ – $5 \times 10^{-3}$  mmol·L<sup>-1</sup> as exemplified in Figure 4. This makes a quantitative determination applicable and the sensitivity is over one order of magnitude higher than that reported previously.<sup>14</sup> In addition, the  $I_{pa}$  increased linearly with the square root of the sweep rate (figure not shown), demonstrating a diffusion-controlled reaction.



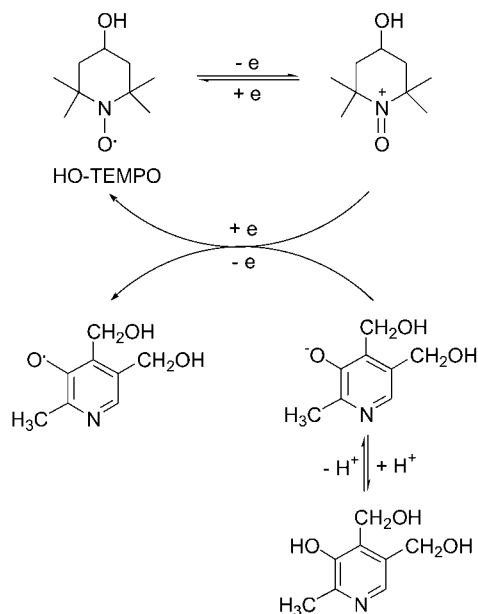
**Figure 4** Dependence of the catalytic peak current on the concentration of VB<sub>6</sub> in the presence of 0.30 mmol·L<sup>-1</sup> HO-TEMPO. The experimental conditions were the same as those described in the legend of Figure 1.

### Reaction mechanism

Based on the experimental results mentioned above

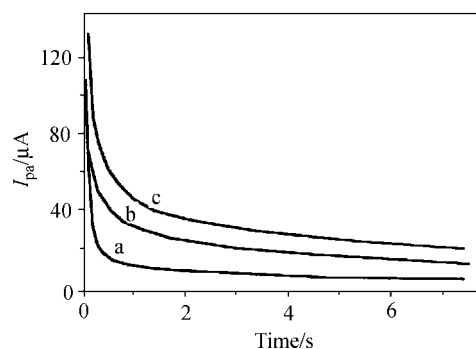
the HO-TEMPO catalyzed electrooxidation of VB<sub>6</sub> can be proposed as shown in Scheme 1. Since the oxidation potential of HO-TEMPO is lower than that of VB<sub>6</sub>, the former must be firstly electrooxidized, generating *in situ* its oxoammonium ion. The redox potential of the oxoammonium ion is more negative than VB<sub>6</sub>, making it able to oxidize VB<sub>6</sub> in solution to the corresponding phenoxyl radical via electron transfer. The oxoammonium ion is reduced back to the parent nitroxide, forming a redox cycle. This is a typical EC' process.

Scheme 1



### Chronoamperometric determination

The chronoamperograms of HO-TEMPO in the absence and presence of VB<sub>6</sub> are shown in Figure 5. The potential was stepped from 0 to 900 mV. It is seen that the current decayed slower in the presence of VB<sub>6</sub> owing to the reaction between the oxoammonium ion and vitamin B<sub>6</sub>. The catalytic rate constant,  $k'$ , of this reaction can be obtained by using Eq. (1).<sup>18</sup>



**Figure 5** Plots of chronoamperograms of 0.30 mmol·L<sup>-1</sup> HO-TEMPO in the absence (a) and presence of (b) 0.50 mmol·L<sup>-1</sup> VB<sub>6</sub>; (c) 1.00 mmol·L<sup>-1</sup> VB<sub>6</sub>. The experimental conditions were the same as those described in the legend of Figure 1.

$$\gamma = k'c^0t \quad (1)$$

where  $c^0$  is the initial concentration of VB<sub>6</sub> and  $\gamma$  can be calculated from Eq. (2) by measuring the current in the kinetic controlling region ( $\gamma > 1.5$ ).<sup>18</sup>

$$I_C / I_D = \pi^{1/2} \gamma^{1/2} \quad (2)$$

where  $I_C$  is the catalytic current in the presence of VB<sub>6</sub>,  $I_D$  is the diffusion-limited current in the absence of VB<sub>6</sub>. Taking  $t > 200$  ms ( $\gamma > 1.5$ ) the  $k'$  was calculated to be  $5.4 \times 10^3$  (mol·L<sup>-1</sup>)<sup>-1</sup>·s<sup>-1</sup>, demonstrating a pretty fast catalytic reaction.

In conclusion, this work demonstrates that the electrochemical response of vitamin B<sub>6</sub> can be significantly enhanced by using HO-TEMPO as a mediator via a catalytic EC' process that can be used for a sensitive determination of vitamin B<sub>6</sub>. This method is hopefully to be expanded to other electrocatalytic reactions.

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