

生物酶HRP催化H<sub>2</sub>O<sub>2</sub>氧化间苯二胺反应的研究

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摘要 应用电化学分析,高效液相色谱(HPLC),紫外-可见光谱(UV-vis),红外光谱(IR)和核磁共振(NMR)等技术对辣根过氧化物酶(HRP)催化H<sub>2</sub>O<sub>2</sub>氧化间苯二胺(MPD)

的反应进行了研究。伏安法和高效液相色谱实验说明,在所选择的酶催化反应条件下,

酶催化反应生成一种产物。用化学方法制得了HRP酶催化H<sub>2</sub>O<sub>2</sub>氧化MPD的产物纯品。经UV-vis,

IR和<sup>1</sup>H NMR谱鉴定,产物为2,7-二氨基吩嗪。写出了酶催化反应过程,

同时对酶催化反应产物的电极还原过程也进行了研究。

关键词 [苯二胺](#) [氧化](#) [过氧化氢](#) [辣根过氧化物酶](#) [红外分光光度法](#) [高速液体色谱](#) [核磁共振谱法](#)

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## Studies on the oxidation of m-phenylene diamine by H<sub>2</sub>O<sub>2</sub> catalyzed by horseradish peroxidase

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**Abstract** The oxidation of m-phenylene Diamine (MPD) by H<sub>2</sub>O<sub>2</sub> catalyzed by horseradish peroxidase (HRP) has been investigated using electrochemical analysis, high performance liquid chromatography (HPLC), UV-vis, IR and NMR spectroscopy. The experiments of voltammetry and HPLC indicate that only one enzymatic product is present under the selected reaction conditions. The best results for the enzymatic reaction were obtained with  $2.0 \times 10^{-3}$  mol/L MPD and  $9.0 \times 10^{-4}$  mol/L H<sub>2</sub>O<sub>2</sub> when reacting in the presence of HRP in 0.02 mol/L BR buffer solution for 60 min at room temperature. The authentic sample of 2,7-diaminophenazine, the product of the enzymatic reaction, was prepared by chemical means and characterized by UV-vis, IR and <sup>1</sup>H NMR spectroscopy. The processes of the enzymatic reaction are described. The enzymatic product shows a pair of reversible redox peaks in the cyclic voltammogram. The reduction peak and the oxidation peak are completely symmetric, which demonstrates that the enzymatic product behaves as a complete adsorption reversible redox process on the mercury electrode. The product, 2,7-diaminophenazine, undergoes a reversible two-electron reduction to give N,N'-dihydro-2,7-diaminophenazine, which can be reoxidized reversibly back to 2,7-diaminophenazine. This enzymatic reaction can be used in the voltammetric enzyme-linked immunoassay.

**Key words** [BENZENEDIAMINE](#) [OXIDATION](#) [HYDROGEN PEROXIDE](#) [INFRARED](#) [SPECTROPHOTOMETRY](#) [HIGH SPEED LIQUID CHROMATOGRAPHY](#) [NMR SPECTROMETRY](#)

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