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MAP-H~2O~2-HPR伏安酶联免疫分析新体系和光谱及电化学研究

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摘要 提出了间氨基酸(MAP)-H~2O~2-辣根过氧化物酶(HRP)伏安酶联免疫分析新体系.
本方法以线性扫描二阶导数伏安法检测HRP催化H~2O~2氧化MAP的产物,
用于游离HRP和各种HRP标记物的测定,灵敏度均高于经典的ELISA显色光度法.测定游离HRP的线性范围为
1.0x10^-^8-1.0x10-6/L,检测限达3.8x10^-^9g/L.制备出了HRP催化H~2O~2氧化MAP的产物纯品并应用电化学分析,
高效液相色谱,元素分析,紫外-可见光谱,
红外光谱,^1H核磁共振谱,^1^3C核磁共振谱及质谱等技术对体系酶促反应进行了深入的研究.
在选择的酶促反应条件下,生成的产物为2-氨基-5-[(3-差苯基)]-2,5-环己烯基-1,4-二酮.
提出了酶催化反应机理及其产物的电极还原过程。

关键词 氨基酚 辣根过氧化物酶 伏安法 酶催化 过氧化氢 免疫测定 质子磁共振谱法 高速液体色谱 元素分析 紫外分光光度法 质子磁共振谱法 碳13核磁共振谱法 酶促反应

分类号 0657

Spetroscopic and electrochemical studies on the MAP-H~2O~2-HRP voltammetric enzyme-linked immunoassay system

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Abstract A voltammetric enzyme-linked immunoassay based on a new system of m- aminophenol(MAP)-H~2O~2-horseradish peroxidase (HRP) has firstly been developed and used for the detection of HRP and labelled HRP.HRP or labelled HRP catalyzes the oxidation reaction of MAP with H~2O~2,the product of which yields a sensitive voltammetric peak at potential of -0.46V(vs.SCE) in Britton-Robinson(B-R) buffer solution. By using this voltammetric peak, HRP can be measured with a detection limit of 3.0x10^-^9g/L and a linear range of 1.0x10^-^8-1.0x10^-^6g/L. The pure producd of H~2O~2 oxidizingMAP catalyzed by HRP was prepared with chemical method. The enzyme-catalyzed reaction has been investigated with electroanalytical chemistry,elemental analysis,UV- vis, IR ^1H NMR, ^1^3C NMR and MS spectroscopy. Under the selected enzyme-catalyzed reaction conditions,the oxidation product of MAP with H~2O~2 catalyzed by HRP is 2-amino-5-[(3-hydroxyphenyl) amino]-2, 5- cyclohexadiene-1,4-dione. The processes of the enzyme-catalyzed reaction and the electroreduction of the product of the enzyme- catalyzed reaction are described.

Key words AMINOPHENOL VOLTAMMETRY ENZYME CATALYSIS HYDROGEN PEROXIDE IMMUNOASSAY PROTON MAGNETIC RESONANCE SPECTROMETRY HIGH SPEED LIQUID CHROMATOGRAPHY ELEMENTAL ANALYSIS ULTRAVIOLET SPECTROPHOTOMETRY PROTON MAGNETIC RESONANCE SPECTROMETRY C13 NMR SPECTROMETRY

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