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# Evidence for a free radical chain mechanism in the reaction between peroxidase and indole-3-acetic acid at neutral pH

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**Abstract**

The oxidation of indole-3-acetic acid (IAA) catalyzed by horseradish peroxidase (HRP) in the absence of added $H_2O_2$ was studied at pH 7.4 using spectral and kinetic approaches. Upon addition of a hundred-fold excess of IAA to HRP the native enzyme was rapidly transformed to compound II (HRP-II). HRP-II was the predominant catalytic enzyme species during the steady state. No compound III was observed. HRP-II was slowly transformed to the stable inactive verdohemoprotein, P-670. A precursor of P-670, so-called P-940 was not detected. After the cessation of IAA oxidation there was neither oxygen consumption nor P-670 formation; the remaining HRP-II was spontaneously reduced to native enzyme. Single exponential kinetics were observed in the steady state for IAA oxidation, oxygen consumption and P-670 formation yielding identical first order rate constants of about $6 \cdot 10^4$ $s^{-1}$. A comparison of the rate of IAA oxidation by HRP-II in the steady state and in the transient state indicated that more than 1/3 of the IAA was oxidized non-enzymatically during the steady state, confirming that a free radical chain reaction is involved in the peroxidase-catalyzed oxidation of IAA. IAA oxidation stopped before IAA was completely consumed, which cannot be ascribed to enzyme inactivation because 30–50% of the enzyme was still active after the end of the reaction. Instead, incomplete IAA oxidation is explained in terms of termination of the free radical chain reaction. Bimolecular rate constants of IAA oxidation by HRP-I and HRP-II determined under transient state conditions were $(2.2 \pm 0.1) \cdot 10^3$ $M^{-1}$ $s^{-1}$ and $(2.3 \pm 0.2) \cdot 10^2$ $M^{-1}$ $s^{-1}$.

*Keywords:* Horseradish peroxidase; Indole-3-acetic acid oxidation; Free radical chain reaction; Transient state kinetics; Oxygen consumption; P-670

## 1. Introduction

The aerobic oxidation of indole-3-acetic acid (IAA) catalyzed by peroxidase can occur in the absence of added $H_2O_2$ [1]. This reaction is a very complex process in which many intermediates and final products of IAA oxidation are formed [2–4]. Moreover, peroxidase is converted from the native enzyme into several catalytic forms (HRP (horseradish peroxidase)-I, HRP-II, HRP-III) and inactive forms (P-940, P-670, P-630, P-678) [5–12]. The observed behavior is highly dependent on experimental conditions.

Two main reaction mechanisms have been recog-

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