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PEROXIDASE-CATALYZED OXIDATION OF INDOLE-3-ACETALDEHYDE TO 4-HYDROXYQUINOLINE IN THE PRESENCE OF BISULFITE ION: ELIMINATION OF PYRROLE RING C_2 AS FORMIC ACID

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SUMMARY

Isotope labeling studies of the horseradish peroxidase (EC 1.11.1.7)-catalyzed oxidation of indole-3-acetaldehyde–$NaHSO_3$ adduct established that 4-hydroxyquinoline was formed from indole-3-acetaldehyde with the loss of the carbon at the ring-2 position. The carbon lost was recovered as formic acid. A plausible mechanism accounting for the reaction is presented, in which the pyrrole ring is first opened by oxidation, followed by the elimination of C_2 and ring closure.

INTRODUCTION

Yeh *et al.*[1] reported that horseradish peroxidase (EC 1.11.1.7)-catalyzed the oxidation of bisulfite adduct of indole-3-acetaldehyde to various products at different pH. At acidic pH, indole-3-carboxaldehyde was the major product and the reaction was not dependent on the presence of $NaHSO_3$. However, at pH near 7, horseradish peroxidase catalyzed the oxidation of indole-3-acetaldehyde–bisulfite adduct to 4-hydroxyquinoline. Two pathways are possible for this oxidative ring expansion reaction. One involves the migration of ring C_2 to side chain C_2 and the elimination of side chain C_1. The other involves the fission of pyrrole ring and the loss of C_2 followed by ring closure. The present report deals with the mechanism of the formation of 4-hydroxyquinoline from indole-3-acetaldehyde–bisulfite adduct catalyzed by horseradish peroxidase.

MATERIALS AND METHODS

Chemicals

[^{14}C]Indole-3-acetaldehyde labeled on ring-2, side chain-1 or side chain-2 was prepared from DL-[^{14}C]tryptophan labeled on ring-2, side chain-2 or side chain-3, respectively, by the method of Gray[2]. All radioactive tryptophans were the products of ICN. Indole-3-acetaldehyde–bisulfite adduct and horseradish peroxidase (Type II) were obtained from Sigma. 4-Hydroxyquinoline was purchased from Aldrich.

Method

A typical reaction mixture contained in a total volume of 1 ml 0.1 μmole of indole-3-acetaldehyde–$NaHSO_3$, 50 μmoles of phosphate buffer (pH = 7.8), 10 μg of horseradish peroxidase and 0.4 μmole of H_2O_2. For the cocrystallization experiments, the concentrations of the reaction components were the same as described above but with a total volume of 3 ml. The reaction was initiated by the addition of horseradish peroxidase or H_2O_2 and the rate of reaction was monitored by measuring absorbance change at 316 nm as described by Yeh *et al.*[1]. For the cocrystallization experiment, 0.5 mmole of unlabeled authentic 4-hydroxyquinoline was added to the reaction mixture which contained [^{14}C]indole-3-acetaldehyde and was incubated for 5 h. Successive recrystallization was carried out in hot and cold aqueous solution. The amount of 4-hydroxyquinoline was determined spectrophotometrically at 316 nm, and the radioactivity with a liquid scintillation counter. For paper chromatographic study, the reaction mixture was first reacted with 0.2 ml of saturated $NaHSO_3$, to allow formation of indole-3-acetaldehyde–$NaHSO_3$ adduct. The reaction mixture was then extracted three times with ethyl acetate. After concentration, the combined extracts were mixed with unlabeled 4-hydroxyquinoline and chromatographed on paper using 1-butanol–conc. NH_4OH–H_2O (10:1:9, by vol.), as developing solvent. The spot of 4-hydroxyquinoline was revealed by spraying with 2% $FeCl_3$ solution. The spots of indole-3-acetaldehyde and indole-3-carboxaldehyde were identified by their R_F values (0.93) with authentic samples labeled with ^{14}C. [^{14}C]Formic acid in the reaction mixture was determined by oxidation to CO_2 with $HgCl_2$ as described elsewhere[3].

RESULTS

The oxidation of indole-3-acetaldehyde–$NaHSO_3$ adduct was monitored spectrophotometrically at 316 nm. The conversion of indole-3-acetaldehyde to 4-hydroxyquinoline was estimated to be 20% in the presence of horseradish peroxidase, and 5% in the absence of the enzyme.

When [*side chain*-1-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ was employed as substrate, radioactive 4-hydroxyquinoline was obtained as revealed by paper radiochromatogram (Fig. 1). The formation of radioactive 4-hydroxyquinoline was further supported by the results of cocrystallization with authentic 4-hydroxyquinoline (Table I). The specific radioactivity remained constant after the third recrystallization. It is to be noted that the specific radioactivity of the 4-hydroxyquinoline was close to the theoretical value as would be expected if it retained the carbonyl carbon of indole-3-acetaldehyde as shown in Table I. Similar results were obtained when [*side chain*-2-^{14}C]indole-3-acetaldehyde–$NaHSO_3$, was used as the substrate. The yield of [^{14}C]formic acid was 26% when [*side chain*-1-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ was used as substrate, but none when [*side chain*-2-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ was used as substrate.

When [*ring*-2-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ was used as the substrate, no radioactive 4-hydroxyquinoline was detected as revealed by paper radiochromatogram (Fig. 1). This observation is consistent with the result of cocrystallization with authentic 4-hydroxyquinoline (Table I). The specific radioactivity of 4-hydroxyquinoline was less than one-fourth of the value as would be expected if it retained

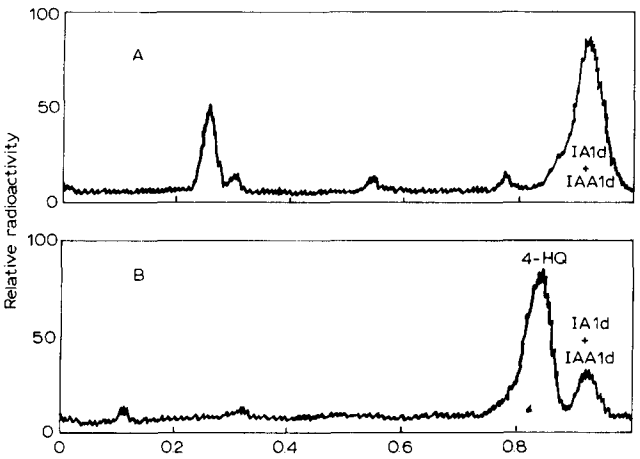


Fig. 1. Paper radiochromatogram of ethyl acetate extracts of reaction products. (A) Using [*ring*-2-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ as substrate. (B) Using [*side chain*-1-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ as substrate. Abbreviations used are IAld (indole-3-carboxaldehyde), IAAld (indole-3-acetaldehyde) and 4-HQ (4-hydroxyquinoline).

the C_2 of indole ring of indole-3-acetaldehyde. The yield of [^{14}C]formic acid was 12% which was close to the yield of 4-hydroxyquinoline (20%) as estimated by the spectrophotometric method. Chemical identification of the spot with R_F of 0.25 in Fig. 1A was not attempted.

TABLE I

COCRYSTALLIZATION OF RADIOACTIVE PRODUCT WITH AUTHENTIC 4-HYDROXYQUINOLINE

4-Hydroxyquinoline (0.5 mmole) was added to the radioactive reaction mixture containing 0.3 μmole and $6.4 \cdot 10^5$ cpm of [*side chain*-1-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ or 0.3 μmole and $8.8 \cdot 10^5$ cpm of [*ring*-2-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ at the end of 5 h incubation. Successive recrystallization was performed in hot and cold aqueous solution. Aliquots of hot solution were taken for measurement of radioactivity with a liquid scintillation counter and for total amount of 4-hydroxyquinoline with an ultraviolet spectrophotometer.

Substrate	*Specific radioactivity (cpm/μmole)*					
	Crystallization No.					
	1	*2*	*3*	*4*	*5*	*Theory**
[*side chain*-1-^{14}C]Indole-3-acetaldehyde	505	345	270	250	275	256
[*ring*-2-^{14}C]Indole-3-acetaldehyde	390	185	141	97	83	352

* The theoretical specific radioactivity was calculated on the basis that 4-hydroxyquinoline retained all of its radioactivity in the conversion and its yield was 20% as estimated spectrophotometrically.

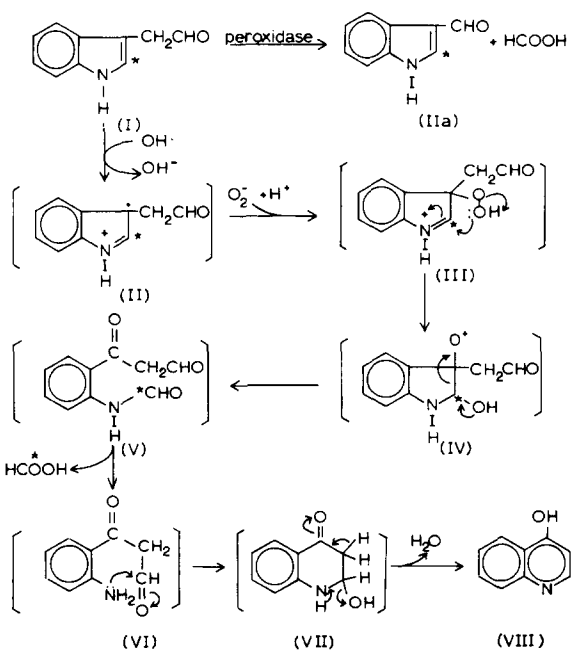
DISCUSSION

The results of the isotope labeling studies indicated clearly that 4-hydroxyquinoline was formed from indole-3-acetaldehyde with loss of the carbon at the ring-2 position. Moreover, the carbon lost was recovered as formic acid. The conversion of indole-3-acetaldehyde to 4-hydroxyquinoline was 20% as estimated by its absorbance

at 316 nm. However, the yield of [^{14}C]formic acid was only 12%. In this connection, it should be noted that peroxidase also catalyzes the oxidation of indole-3-acetaldehyde to indole-3-carboxaldehyde and formic acid[1,4]. The higher yield of 4-hydroxyquinoline as estimated by spectrophotometric method could well be in part due to the interference of the formation of indole-3-carboxaldehyde which also absorbs at 316 nm[4]. Under the present reaction condition, the yield of indole-3-carboxaldehyde was estimated to be about 26% based on the yield of [^{14}C]formic acid from [*side chain*-1-^{14}C]indole-3-acetaldehyde–$NaHSO_3$. When [*side chain*-2-^{14}C]indole-3-acetaldehyde–$NaHSO_3$ was employed, no radioactive formic acid was detected.

The existence of a free-radical chain mechanism for the aerobic oxidation of sulfite to sulfate has been well documented[5–8]. The available data are in good agreement with the view that O_2^-, OH· and HSO_3· radicals are generated during the aerobic oxidation of sulfite, and that these radicals are responsible in turn for the propagation of the sulfite–oxygen chain reaction. These oxidizing radicals, generated during the aerobic oxidation of sulfite, can function in a number of oxidative reactions of biological importance, including the following: Oxidation of NADH and NADPH[9], ethylene formation from 3-(methylthio)propionaldehyde[10] or from 2-oxo-4-(methylthio)butyric acid[11], oxidation of methionine or its thioether analogs to sulfoxide[8].

Based on the present finding that ring C_2 is eliminated as formic acid in the conversion of indole-3-acetaldehyde to 4-hydroxyquinoline, a possible mechanism accounting for the reaction is depicted in Scheme 1. The first step consists of the abstraction of an electron from C_3 probably by O_2^- or OH· radical which is generated during the oxidation of sulfite. Subsequent reaction of indole-3-acetaldehyde radical (II) with O_2^- yields (3-hydroperoxyindolenine)-3-acetaldehyde (III). The rearrange-



Scheme 1

ment of the hydroperoxide with indolenine double bond leads to ring opening and the formation of V. The sequence of the reactions leading to the formation of V is analogous to that proposed for the oxidation of tryptophan or its analogs by chemical or biological oxidation[12–14]. It is pertinent to note that O_2^- has been indicated as the active species of oxygen involved in the enzymic oxidation of tryptophan to *N*-formylkynurenine catalyzed by intestinal tryptophan 2,3-dioxygenase[15]. Compound V is subject to hydrolysis and yields VI, releasing formic acid. Once VI is formed, a nucleophilic attack of amino nitrogen on the aldehyde carbon would allow spontaneous ring closure, yielding 4-hydroxyquinoline. Such a spontaneous ring closure to form 4-hydroxyquinoline is analogous to the formation of kynurenic acid from the keto analog of kynurenine *in vivo*.

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