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DESTRUCTION OF INDOLE-3-ACETIC ACID DURING THE AEROBIC OXIDATION OF SULFITE

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Abstract—Indole-3-acetic acid (IAA) was rapidly destroyed in the presence of Mn^{2+}, oxygen and sulfite ion. The optimal pH for the reaction was between 5 and 6. The destruction was dependent on the aerobic oxidation of sulfite, but was not inhibited by superoxide dismutase. Tracer studies indicate that IAA was converted into at least 3 compounds. Decarboxylation of IAA was not involved in the destruction.

INTRODUCTION

INDOLE-3-ACETIC ACID (IAA) is a natural hormone, and SO_2 is an atmospheric pollutant which causes serious damage to vegetation. The present study shows that IAA is rapidly destroyed aerobically in the presence of sulfite and Mn^{2+}. Meudt[1] has studied the interactions of sulfite and Mn^{2+} ions with peroxidase-mediated oxidation products of IAA and has noted that the absorption spectra of IAA changed when it was incubated in the presence of Mn^{2+} and sulfite ions.

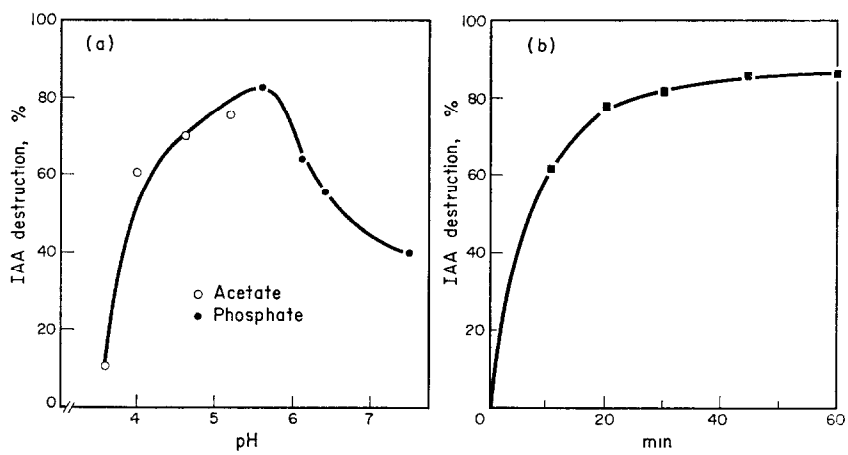

FIG. 1. DEPENDENCE OF IAA DESTRUCTION ON pH AND INCUBATION TIME.
The reaction conditions and mixtures were as described in the Experimental, except that in Fig. a, 20 μmol of acetate or phosphate were employed and the incubation time was 30 min.

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RESULTS

The optimal pH for the destruction of IAA was about 5·5 (Fig. 1a). The reaction rate was sharply reduced when the pH of the reaction medium was lower than 4. The time curve of the IAA destruction under standard conditions is shown in Fig. 1b. More than 75% of the IAA was destroyed during the first 20 min of the incubation. Table 1 illustrates the requirements for Mn^{2+}, SO_3^{2-} and oxygen; when any one of them was omitted, there was little or no destruction of IAA. The addition of catalase to the reaction mixture did not affect the rate of IAA destruction, suggesting that the formation of H_2O_2 is not involved in the reaction. Formaldehyde, which makes addition products with bisulfite ion, was found to be an effective inhibitor. Superoxide dismutase, which catalyzes the disproportionation of superoxide radical anion[2] was ineffective as an inhibitor. Superoxide dismutase has been shown to strongly inhibit the oxidation of methionine to methionine sulfoxide during the aerobic oxidation of sulfite.[3]

TABLE 1. DESTRUCTION OF IAA BY THE Mn^{2+}-SO_3^{2-}-O_2 SYSTEM

Components	IAA disappearance (%)	Components	IAA disappearance (%)
Complete	87	Under N_2	11
$-Mn^{2+}$-SO_3^{2-}	0	+Catalase (20 μg)	82
$-Mn^{2+}$	7	+HCHO (20 μmol)	26
$-SO_3^{2-}$	1	+Superoxide dismutase (2 μg)	85

The complete reaction mixture contained, in a total vol of 0·2 ml, 20 μmol of phosphate at pH 5·6, 0·15 μmol of IAA, 60 nmol of $MnSO_4$ and 0·5 μmol of Na_2SO_3. Incubation was at 25° for 30 min.

In order to elucidate the relationship between IAA destruction and sulfite oxidation, oxygen uptake was measured in reaction mixtures of the same compositions. In parallel with the data presented in Table 1, no oxygen uptake was observed when either Mn^{2+} or sulfite was omitted from the reaction mixture. The ability of metal ions to initiate the aerobic oxidation of sulfite to sulfate is well known.[3–7] When formaldehyde was added, oxygen uptake was greatly inhibited. The addition of superoxide dismutase did not significantly inhibit the oxygen uptake. Thus, the IAA destruction and the sulfite oxidation are closely interrelated. The inability of superoxide dismutase to inhibit the sulfite oxidation in the presence of IAA may be explained on the basis that the IAA·radical[8] was produced in the sulfite–oxygen chain system and was able to initiate sulfite oxidation.

The dependence of IAA destruction on the concentration of Mn^{2+} and sulfite is indicated by the data recorded in Table 2. The reaction rate increased progressively as the amount of Mn^{2+} was increased from 0 to 150 nmol. Under the standard reaction conditions, with a reaction time of 30 min, the optimal concentration of sulfite for maximum rate of IAA destruction was found to be 0·5 μmol. The destruction of IAA in this system is dependent on the availability of sulfite. In the reaction mixtures containing little sulfite (100 or 250

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nmol), there was no further IAA destruction when the reaction time was prolonged from 30 to 60 min. This was apparently due to the depletion of sulfite, because further destruction of IAA was observed when additional sulfite was provided. It can be estimated from Table 2 that approximately 0·5 mole of IAA was destroyed per mole of sulfite oxidized in a reaction mixture in which the sulfite concentration was less than that of IAA.

TABLE 2. DEPENDENCE OF IAA DESTRUCTION ON THE CONCENTRATION OF Mn^{2+} AND SULFITE

Compound	Amount (nmol)	IAA destruction (%) 30 min	Compound	Amount (nmol)	IAA destruction (%) 30 min	60 min
$MnSO_4$	0	7	Na_2SO_3	0	0	2
	6	44		100	34	32
	30	72		250	65	65
	60	88		500	83	85
	150	95		1000	73	97
				2000	55	90

The reaction mixture and conditions were as those described in Table 1, except that the concentration of $MnSO_4$ and Na_2SO_3 and incubation time were varied as indicated.

For the examination of reaction products, the reaction mixture containing IAA-2-^{14}C was incubated for 60 min. Paper radiochromatography of the reaction products revealed the presence of three radioactive products 1–3. The radioactivities of 1 (R_f, 0·05), 2 (R_f, 0·20), IAA (R_f, 0·30) and 3 (R_f, 0·92) were 36, 50, 5 and 9%, respectively, of the total radioactivity. Except for the remaining IAA, none of the products gave a positive test to Salkowski's reagent or to Ehrlich's reagent. When IAA-1-^{14}C was employed, a similar radiochromatogram was obtained, except that product 3 was absent. These data indicate that both 1 and 2 retained the carboxyl–carbon of IAA, while 3 is a decarboxylated product. Since 3 was also obtained in the reaction mixture containing no sulfite, the formation of 3 was not dependent on the cooxidation of sulfite. IAA has been known to be readily oxidized by various oxidizing agents, including enzymic reactions and to yield decarboxylated products.[9–11] The reaction described above is a rare example of IAA destruction in which decarboxylation did not occur. When product 1 was subjected to paper electrophoresis at pH 2·0 at 40 V/cm for 1 hr, the radioactivity was resolved into two spots: 1A remained at the origin as did IAA, and contained 55% of the radioactivity; and 1B had a mobility of 1·1 relative to dinitrophenyl (DNP)-cysteic acid, and contained 45% of the radioactivity. When 1A and 1B were subjected to paper electrophoresis with 0·05 M phosphate buffer at pH 7·0, their mobility was 1·2 and 0·72 relative to DNP-cysteic acid, respectively. In the same system, IAA had a mobility of 0·64. These data, coupled with the results showing that both 1A and 1B retained the carboxyl carbon of IAA, strongly suggest that 1A possesses a –COOH and a $-SO_3H$ group, while 1B possesses a –COOH group. This conclusion is in agreement with the observation that the percentage yield of 1A increased when the concentration of sulfite was increased. Product 2 did not move during paper electrophoresis at pH 2·0, but had a mobility of 1·1 relative to IAA at pH 7·0, suggesting that it is also a carboxylic acid. This conclusion is further supported by

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the observation that it can be methylated with diazomethane and yields a neutral compound on paper electrophoresis at pH 7·0. The chemical identities of these reaction products are under investigation.

DISCUSSION

The existence of a free-radical chain mechanism for the aerobic oxidation of sulfite to sulfate as been well documented.[3–7] 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 oxidize a number of biological molecules including the following: Oxidation of DPNH and TPNH,[12] ethylene formation from 3-(methylthio)propionaldehyde[13] or from 2-oxo-4-(methylthio)butyric acid,[14] oxidation of methionine or its thioether analogs to sulfoxide,[3] and the destruction of tryptophan.[15] It remains to be determined whether sulfite may inflict biological damage *in vivo* through such an oxidative mechanism.

EXPERIMENTAL

IAA-1-^{14}C and IAA-2-^{14}C were purchased fromA mersham/Searle. Superoxide dismutase prepared from bovine erythrocytes was the product of Truett Laboratories, Dallas, Texas A standard incubation mixture contained, in a total vol. of 0 2 ml, 150 nmol IAA, 20 μmol of phosphate (pH 5·6), 60 nmol $MnSO_4$ and 500 nmol of Na_2SO_3, in 12 × 75 mm test tubes The reaction was started by the addition of sulfite, and proceeded at 25°. At the end of incubation, 3 ml of Salkowski reagent (6·5 mM of $FeCl_3$ in 23% perchloric acid) was added to the reaction tubes and the IAA remaining in the reaction mixture was determined colorimetrically at 530 nm.[16] Oxidation of sulfite was determined by measuring the oxygen uptake with a Yellow Springs Clark oxygen electrode The concentrations of the reaction components were the same as listed above but with a total vol of 3 ml

When radioactive IAA was employed, the reaction products were first separated by PC using 1-butanol-3% NH_3 in H_2O (1:1) as the solvent. R_f values of products 1–3 were 0·05, 0 20 and 0 92 respectively, R_f of unchanged IAA was 0·30 After scanning the radioactive spots were eluted and their ionic properties of the compounds were characterized by paper electrophoresis at pH 2 0 and 7 0

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