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DEGRADATION OF MUTAGENS FROM PYROLYSATES OF TRYPTOPHAN, GLUTAMIC ACID AND GLOBULIN BY MYELOPEROXIDASE[1]

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SUMMARY: Mutagenic compounds isolated from pyrolysates of tryptophan, glutamic acid and globulin were broken down by myeloperoxidase and hydrogen peroxide with loss of their mutagenicity toward *Salmonella typhimurium* TA98. Lactoperoxidase and horseradish peroxidase were as effective as myeloperoxidase in degradation of the mutagens.

INTRODUCTION

It has been reported that Trp-P-1 and Trp-P-2, Glu-P-1, and 2-amino-α-carboline, isolated from pyrolysates of tryptophan, glutamic acid, and soybean globulin, respectively, are mutagenic towards *Salmonella typhimurium* TA98 and TA100 (1-3). The structures of these compounds are given in Fig. 1. Activation of these compounds by the postmitochondrial fraction of rat liver was essential for demonstrating their mutagenicities. During studies on the mechanism of activation of mutagens, we found that peroxidase inactivates these mutagenic compounds. This paper reports that myeloperoxidase, lactoperoxidase, and horseradish peroxidase degrade mutagenic compounds isolated from amino acid and protein pyrolysates.

MATERIALS AND METHODS

Chemicals. Trp-P-1, Trp-P-2, and 2-amino-α-carboline were purchased from Nard Institute. Glu-P-1 was from Katsura Chemical Co.. [1-^{14}C]Labeled Trp-P-2 was synthesized at the Radiochemical Centre, Amersham. Lactoperoxidase and horseradish peroxidase were from Sigma Chemical Co..

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Abbreviations used: Trp-P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole; 2-amino-α-carboline, 2-amino-9*H*-pyrido[2,3-*b*]indole; norharman, 9*H*-pyrido[3,4-*b*]indole.

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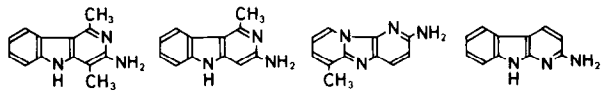

Trp-P-1 Trp-P-2 Glu-P-1 2-Amino-α-carboline

Fig. 1 Structures of Trp-P-1, Trp-P-2, Glu-P-1, and 2-amino-α-carboline.

Measurement of Trp-P-1 degradation. The reaction mixture (0.5 ml) contained 33 mM sodium phosphate (pH 7.3), 15 μM Trp-P-1, 0.4 mM H_2O_2, and myeloperoxidase. The reaction was started by adding H_2O_2 and the decrease in absorbance of Trp-P-1 at 263 nm was followed in a Hitachi Spectrophotometer, Model 200.

Preparation of myeloperoxidase from human promyelocytic leukemia HL-60 cells. The human HL-60 cell line was established from leukocytes of a patient with acute promyelocytic leukemia by Collins *et al.* (4). HL-60 cells were transplanted subcutaneously into the back of nude mice and solid tumors were obtained from the animals 1.5 month later. Myeloperoxidase was extracted from these tumors with 1% cetyltrimethylammonium bromide containing 0.1 M potassium phosphate (pH 7.8), and purified by chromatographies on CM-Sepharose CL-6B and Sephacryl-S-200. The final preparation was homogeneous as judged by the facts that the ratio of the absorbances at 430 nm and 280 nm was 0.8 and that on sodium dodecyl sulfate polyacrylamide gel electrophoresis the preparation gave only two protein bands of light and heavy chains of myeloperloxidase, as found in canine myeloperoxidase (5). Details of the purification of the enzyme will be published elsewhere.

The concentrations of myeloperoxidase, lactoperoxidase, and horseradish peroxidase were estimated taking their millimolar absorptivities as 95 mM^{-1} cm^{-1} at 430 nm, 112.2 mM^{-1} cm^{-1} at 412 nm, and 102 mM^{-1} cm^{-1} at 403 nm, respectively (6-8).

Measurement of mutagenicity. Mutagenicity to *Salmonella typhimurium* TA98 was tested by Ames' method with the modifications described previously (9,10). Test compounds were incubated with myeloperoxidase and then diluted to 1.0 ml with 50% dimethylsulfoxide. Various amounts of the diluted solution were then mixed with 2×10^8 bacteria and 0.5 ml of S-9 mix in a final volume of 0.7 ml and incubated for 20 min at 37°C. Then, the mixture was placed on a minimal agar plate. After 2-days incubation, histidine prototroph colonies were counted. S-9 mix (0.5 ml) contained 30 μl of postmitochondrial fraction (S-9), 2 μmol of NADPH, and 2.5 μmol of glucose-6-phosphate. S-9 was prepared from the liver of rats given polychlorinated biphenyls (500 mg/kg body weight) 5 days before sacrifice.

RESULTS

Decompositions of Trp-P-1, Trp-P-2, Glu-P-1, and 2-amino-α-carboline by myeloperoxidase. Table I shows that degradation of Trp-P-1 depended on the presence of both myeloperoxidase and H_2O_2: no degradation of Trp-P-1 was observed with either myeloperoxidase or H_2O_2 alone. The rate of Trp-P-1 degradation was proportional to the amount of myeloperoxidase used with up to sev-

TABLE I

Degradations of Trp-P-1 and Related Aromatic Amines by Myeloperoxidase[a]

Compound	Amount degraded
	nmol / min
Trp-P-1	1.51
Trp-P-1, - H_2O_2	0.00
Trp-P-1, - MPO[b]	0.00
Trp-P-2	3.29
Glu-P-1	0.73
2-Amino-α-carboline	4.35
Norharman	0.00
L-Tryptophan	0.00

[a]The reaction mixture was as described in the Materials and Methods, except that 15 μM concentrations of the compounds indicated and 121 pmol of myeloperoxidase were used. H_2O_2 or myeloperoxidase was omitted as indicated. Decreases in the absorbances at 263 nm, 257 nm, 336 nm, 285 nm and 280 nm for Trp-P-2, Glu-P-1, 2-amino-α-carboline, norharman, and L-tryptophan, respectively, were followed for several minutes.

[b]MPO, myeloperoxidase

eral hundred pmol of enzyme, and the amount of Trp-P-1 decreases linearly with the incubation time under these conditions. Trp-P-2, Glu-P-1, and 2-amino-α-carboline were also degraded by myeloperoxidase (Table I). Of the compounds tested, 2-amino-α-carboline was broken down the most rapidly. Both myeloperoxidase and H_2O_2 were also essential for the degradation of these compounds. Norharman and L-tryptophan, which have no α-amino group in their aromatic rings, were not degraded significantly under these conditions. Trp-P-1 was also degraded by lactoperoxidase and horseradish peroxidase (Table II), and on the basis of the amount of Trp-P-1 degraded per mol enzyme, horseradish peroxidase was the most active of these peroxidases.

TABLE II

Degradations of Trp-P-1 by Myeloperoxidase, Lactoperoxidase and Horseradish Peroxidase[a]

Peroxidase	Trp-P-1 degraded
	nmol / min / nmol enzyme
Myeloperoxidase	13.5
Lactoperoxidase	3.3
Horseradish peroxidase	22.3

[a]The reaction mixture was as described in the Materials and Methods, except that myeloperoxidase, lactoperoxidase or horseradish peroxidase was used.

Loss of mutagenicity of Trp-P-1 and related compounds on treatment with myeloperoxidase. The mutagenicities of Trp-P-1, Trp-P-2, Glu-P-1 and 2-amino-α-carboline were almost completely lost on treatment of these compounds with myeloperoxidase and H_2O_2 (Table III). The mutagenicities of these compounds were not decreased by incubating the compounds with either myeloperoxidase or H_2O_2 alone, and myeloperoxidase and H_2O_2 did not affect bacterial growth under these conditions.

Analysis of reaction products. Decreases in the absorbances of Trp-P-1, Trp-P-2, Glu-P-1 and 2-amino-α-carboline with marked changes in the absorption spectra were observed on incubation of these compounds with myeloperoxidase. The reaction products of [1-^{14}C]Trp-P-2 gave two main bands on thin layer chromatography (Fig. 2). The intensity of the one located at the origin on the chromatograms increased with the incubation time, but the intensity of the other, which migrated faster than Trp-P-2, did not change during the incubation. Neither of these products was formed in the absence of myeloperoxidase or of H_2O_2. The same products were seen on thin layer chromatograms after treatment of [1-^{14}C]Trp-P-2 with horseradish peroxidase. Trp-P-1, Glu-P-1

TABLE III

Loss of Mutagenicities of Trp-P-1, Trp-P-2, Glu-P-1, and 2-Amino-α-carboline by Myeloperoxidase

Compound	Incubation[a]	
	None	Myeloperoxidase
	Number of colonies (% of control)	
Trp-P-1	100	20.0
Trp-P-2	100	1.3
Glu-P-1	100	23.9
2-Amino-α-carboline	100	26.7

[a]Trp-P-1 (36.9 nmol), Trp-P-2 (19.5 nmol), Glu-P-1 (35.8 nmol) or 2-amino-α-carboline (370 nmol) was incubated with or without myeloperoxidase (60 pmol) and H_2O_2 (200 nmol) in a final volume of 150 μl for 1 hr at 37°C and then diluted to 1.0 ml with 50% dimethylsulfoxide. The various amounts of aliquots were removed and subjected to mutagenesis assay as described in the Materials and Methods. The numbers of colonies without myeloperoxidase (7.25×10^4, 5.14×10^4, 1.29×10^5 and 1.62×10^4 colonies for Trp-P-1, Trp-P-2, Glu-P-1, and 2-amino-α-carboline, respectively) were taken as 100.

and 2-amino-α-carboline after incubation of these compounds with myeloperoxidase and H_2O_2 could no longer be detected on thin layer chromatograms under ultraviolet light (data not shown).

DISCUSSION

Myeloperoxidase, lactoperoxidase and horseradish peroxidase degrade the mutagens Trp-P-1, Trp-P-2, Glu-P-1 and 2-amino-α-carboline. The degradations of these compounds were shown by changes in their absorption spectra, by thin layer chromatography of the reaction products, and by loss of the mutagenicities of these compounds. Kada *et al.* reported that extracts of vegetables, such as cabbage, radish or green pepper, could inactivate the mutagenicity of crude pyrolysis products of tryptophan and lysine in the absence of other additions and they named the factors responsible desmutagens (11,12). The preparations of myeloperoxidase and horseradish peroxidase used in the present work were homogeneous, and H_2O_2 was found to be essential for the degradations

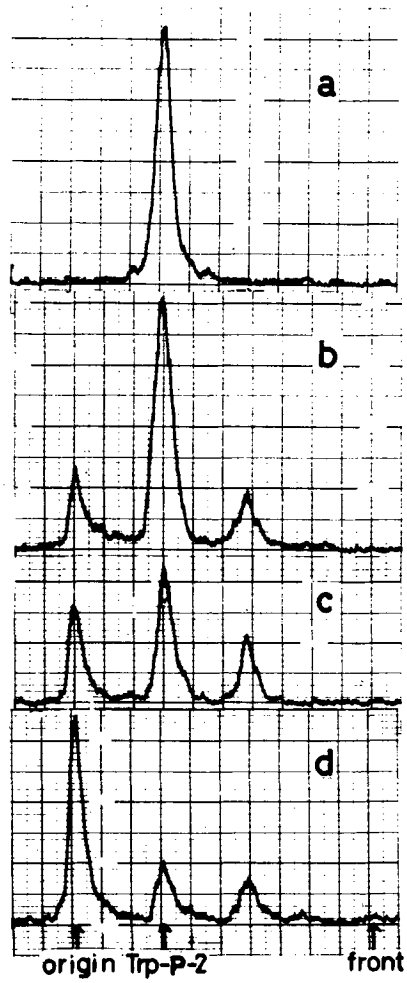

Fig. 2 Thin layer chromatograms of the reaction products of Trp-P-2 with myeloperoxidase. The reaction conditions were as described in the Materials and Methods, except that the reaction mixture (100 µl) contained 5 nmol of [1-^{14}C]Trp-P-2 (9.9 µCi/µmol) and 30 pmol of myeloperoxidase with or without H_2O_2. The reaction mixture was incubated at room temperature. 10 µl aliquots of the mixture were removed at various times, spotted on an aluminum oxide plates ($60F_{254}$), and developed with chloroform containing 3% methanol. Radioactivity was located by scanning the chromatograms with an Aloka chromatogram scanner. a, without H_2O_2, 2 min; b, with H_2O_2, 1 min; c, with H_2O_2, 5 min; d, with H_2O_2, 30 min.

of Trp-P-1, Trp-P-2, Glu-P-1 and 2-amino-α-carboline by these enzymes. Therefore, the breakdown of these compounds was due to the actions of myeloperoxidase, horseradish peroxidase and lactoperoxidase as peroxidases. The des-

mutagens present in plant extracts may be related to peroxidase. Peroxidase, which is present in a number of mammalian tissues (13), may be involved in activation (14-15) or inactivation of carcinogenic aromatic amines.

The degradations of various free amino acids and tryptophan and methionine residues in various proteins and killing of bacteria occur in the presence of myeloperoxidase, H_2O_2 and halide ions, and singlet molecular oxygen was reported to be involved in these reactions (16-18). The degradations of Trp-P-1 and related compounds by myeloperoxidase were not stimulated by increasing the concentration of NaCl to 0.3 M and also was not affected by β-carotene which is an effective quencher of singlet molecular oxygen (19). Therefore, singlet molecular oxygen may not be involved in the degradation of Trp-P-1 and related compounds by myeloperoxidase. To determine the mechanism of degradation of these compounds by myeloperoxidase, we are now attempting to isolate and identify the degradation products of Trp-P-2 by myeloperoxidase.

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