Studies on the Destruction of Indole-3-acetic Acid by a Species of *Arthrobacter* III. Effect of Carboxylic Acids

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Abstract

Effect of carboxylic acids on IAA oxidation by lyophilized *Arthrobacter* cells were examined. 1) Inhibitory action of unsubstituted aliphatic mono- and dicarboxylic acids increases with carbon chain length, monocarboxylic acids being much more inhibitory than dicarboxylic acids. Introduction of amino or hydroxyl group to α -carbon decreases the effectiveness of monocarboxylic acids, but not that of dicarboxylic acids. Amide derivatives and α -keto acids do not inhibit the oxidation. Some aromatic carboxylic acids are also inhibitory. 2) The inhibitory effect of carboxylic acids increases as pH of medium decreases, suggesting that unionized acids are responsible for the inhibition. 3) Degree of inhibition by cinnamic acid is scarcely altered by substrate (IAA) concentration, suggesting that the inhibition is not competitive. 4) Inhibitory action of cinnamic acid is not modified by CoA added.

Introduction

Although a number of inhibitors¹⁻¹¹⁾, including carboxylic acids, of plant IAA oxidases have been reported, little is known about inhibitors of bacterial IAA oxidases. It was found that IAA is hardly destroyed by *Arthrobacter* system in acetate buffer. The present paper describes the effect of carboxylic acids, especially fatty acids and cinnamic acid, an antiauxin¹²⁾, on the bacterial IAA oxidizing system.

Materials and Methods

Preparation of lyophilized cells

A species of *Arthrobacter* was used throughout this study. Preliminary culture and preparation of induced cells to oxidize IAA were carried out as described in a previous paper¹³⁾. Induced cells were washed with 0.9% NaCl solution, lyophilized for 2 hr, and stored at -20°C ¹⁵⁾.

Determination of IAA oxidation

IAA oxidation was determined by measurement of oxygen consumed or by IAA loss from reaction mixture. Oxygen consumption was measured by oxygen electrode

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Abbreviation: IAA, Indole-3-acetic acid; ATP, Adenosine-5'-triphosphate; CoA, Coenzyme A.

Table 1. Effect of carboxylic acids and their derivatives on the IAA oxidizing activity.

Compound tested (10^{-2} M)	% Inhibition
Acetic acid	62
Propionic acid	69
<i>n</i> -Butyric acid	75
<i>n</i> -Valeric acid	80
<i>n</i> -Caproic acid	95
<i>n</i> -Caprylic acid	100
Acrylic acid	81
Glycolic acid	0
α -Hydroxy- <i>iso</i> -butyric acid	50
D, L- α -Hydroxy- <i>n</i> -butyric acid	50
Acetamide	0
Acrylamide	0
Cyanoacetic acid	0
Glycine	0
Malonic acid	0
Succinic acid	0
Glutaric acid	0
Adipic acid	0
Pimelic acid	14
Sebacic acid	27
β -Alanine	0
L-Alanine	0
L-Arginine	0
L-Histidine	0
L-Lysine	0
L-Methionine	0
L-Serine	0
L-Isoleucine	0
L-Leucine	0
L-Threonine	0
L-Valine	0
L-Ornithine	0
L-Citrulline	0
L-Tyrosine	0
D, L-Norleucine	0
D, L-Asparagine	0
L-Phenylalanine	0
L-Hydroxyproline	0
L-Proline	0
L-Aspartic acid	64
L-Glutamic acid	85
γ -Aminobutyric acid	0
Lactic acid	0
Pyruvic acid	0
D, L-Malic acid	38
Fumaric acid	0
Oxalacetic acid	0
Tartaric acid	0
α -Ketoglutaric acid	0
D, L-Isocitric acid lactone	55
Citric acid	0
Benzoic acid	0
Homophthalic acid	14
<i>p</i> -Hydroxyphenylacetic acid	28
Phthalic acid	82
Cinnamic acid	92

Reaction mixture was shaken at 30° for 10 min, followed by colorimetric estimation of remaining IAA. See text for further explanation.

(Yanagimoto Co., Ltd.), 0.6 V, sensitivity 3, temperature 30° and chart speed 3 cm/min, and IAA was estimated colorimetrically as described elsewhere¹³⁾. CoA and ATP were purchased from Sigma Chemical Company, and IAA from Tokyo Kasei Co., Ltd.

Results

Effect of carboxylic acids and their derivatives on IAA oxidation.

Three mg of lyophilized cells were suspended in 10 ml of phosphate buffer (M/15, pH 6.0) containing 10^{-2} M carboxylic acid, 10^{-3} M chloramphenicol and 2×10^{-4} M IAA. Modification of pH due to the organic acid added was corrected with N HCl or N NaOH.

As shown in Table 1, inhibitory action of unsubstituted aliphatic mono- and di-carboxylic acids on the IAA oxidizing activity increased with carbon chain length, monocarboxylic acids being markedly more inhibitory than dicarboxylic acids. Inhibitory action of monocarboxylic acids was depressed by the substitution of hydrogen attached to α -carbon with amino or hydroxyl group, while the reverse was valid for di- and tri-carboxylic acids. Amides and α -keto acids tested were not inhibitory. Some aromatic carboxylic acids examined were also inhibitory.

Effect of carboxylic acids on IAA oxidation at different pH

Three mg of lyophilized cells were suspended in 10 ml of phosphate buffer (M/15) containing 10^{-3} M chloramphenicol, 10^{-2} M carboxylic acid and 2×10^{-4} M IAA. Buffers of different pH were prepared by mixing M/15 sodium phosphate (dibasic) and M/15 potassium phosphate (monobasic) at varied proportions. Modification of pH due to the addition of IAA and carboxylic acid was corrected with N HCl or N NaOH.

As shown in Table 2, the inhibitory effect of carboxylic acids tested increased as the pH of medium decreased, suggesting that unionized carboxylic acids are responsible for the inhibition.

Effect of cinnamic acid on IAA oxidation

Reaction mixture containing 7 ml of phosphate buffer (M/15, pH 6.0), 3 mg of lyophilized cells, 1 ml each of 10^{-2} M chloramphenicol, 2×10^{-3} M IAA and 5×10^{-4} M cinnamic acid, was shaken at 30°, and remaining IAA was measured at 10 min intervals. The data are not described here, but it was found that in this condition IAA oxidation proceeds almost linearly in the presence or absence of cinnamic acid at least during the first 30 min.

With a similar procedure, effect of various concentrations of cinnamic acid on IAA oxidation was investigated. In this case 2 mg of lyophilized cells were used instead of 3 mg in the preceding experiment and reaction time was 10 min.

As shown in Table 3, IAA destroyed in the presence of a given concentration of cinnamic acid was almost constant irrespective of IAA concentrations applied, suggesting that the inhibition by cinnamic acid is not competitive. In this experiment wider range of IAA concentration could not be investigated, since higher dose of IAA inhibit the IAA oxidizing activity¹⁵⁾.

Effect of cinnamic acid on IAA oxidizing activity in the presence of CoA

Bacterial concentration used was 6 mg per 10 ml of phosphate buffer (M/15, pH 6.0). Final concentrations of IAA, ATP, CoA and cinnamic acid were 2×10^{-3} M, 10^{-3}

Table 2. Effect of some carboxylic acids on IAA oxidizing activity at different pH.

Compound tested	pH			
	5	6	7	8
None	64	120	82	50
Acetic acid	14 (78)	46 (62)	48 (41)	50 (0)
Propionic acid	12 (81)	37 (69)	41 (50)	36 (28)
n-Caproic acid	0 (100)	6 (95)	14 (83)	19 (62)
Cinnamic acid	0 (100)	10 (92)	21 (74)	25 (50)

Reaction mixture was shaken at 30° for 10 min, followed by colorimetric estimation of remaining IAA. See text for further explanation. Figures indicate IAA (μ g) destroyed, and those in parentheses percentage inhibition.

Table 3. Inhibition of IAA oxidizing activity by various concentrations of cinnamic acid in the presence of varied amount of IAA.

Cinnamic acid (M)	IAA concentration (M)		
	$1/2 \times 10^{-4}$	$4/3 \times 10^{-4}$	2×10^{-4}
None	80	80	84
5×10^{-4}	64	68	68
2×10^{-3}	51	56	56

See text for explanation. Figures given are IAA (μ g) destroyed.

M, and 5×10^{-3} M, respectively. Oxygen consumption by each of seven reaction mixtures was almost constant during at least the first 5 min, and the consumption during the first 3 min was compared in Table 4.

It is clear that inhibitory effect of cinnamic acid is not affected by CoA and ATP added simultaneously.

Table 4. Effect of cinnamic acid on IAA oxidizing activity in the presence or absence of CoA.

	Reaction mixture						
	1	2	3	4	5	6	7
Bacterial suspension (ml)	1	1	1	1	1	1	1
IAA (ml)	—	0.5	0.5	0.5	0.5	—	—
Na ₂ -ATP(ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
CoA (ml)	—	—	0.5	0.5	—	0.5	—
Cinnamic acid (ml)	—	—	—	0.5	0.5	—	0.5
Phosphate buffer (ml)	1.5	1	0.5	—	0.5	1	1
Relative oxidizing activity	0	100	94	38	38	0	0

Velocity of IAA oxidation in control run (mixture 1) was subtracted from those of experimental runs (mixtures 1-7). Figures given are the relative velocities of IAA oxidation. See text for further explanation.

Discussion

All fatty acids tested inhibited IAA oxidation by lyophilized *Arthrobacter* cells. Several authors have pointed the inhibition of plant IAA oxidases by rather complex carboxylic acids such as chlorogenic^{2,3,6)}, caffeic^{2,4,6)}, gibberellic⁶⁾ and ferulic acids⁶⁾, but none has reported the inhibition by fatty acids. Ohno¹⁴⁾ indicated that glutamic acid decarboxylase of squash is inhibited by various mono- and di-carboxylic acids and the degree of inhibition increases with the chain length just as was demonstrated for bacterial IAA oxidation in the present study. He also showed that carboxylic acids with hydroxyl, carbonyl or amino group are less inhibitory than those without any such groups. This agrees with the present findings for monocarboxylic acids, but not with those for dicarboxylic acid. Thus our bacterial system was sensitive to malic and aspartic acids, but not to succinic acid. Bacterial IAA oxidation has shown to be inhibited by indole-3-carboxylic, -3-propionic and -3-butyric acids, but not by indole-3-lactic acid¹⁵⁾. This relation of chemical structure with inhibitory action agrees well with that disclosed for aliphatic monocarboxylic acids.

Inhibition of IAA oxidation by fatty acids was shown to be increased with decreasing pH (Table 2), indicating that the acids at unionized state are responsible for the inhibition.

It is of interest to cite Bull and Breese who reported that egg albumin is denatured by binding of fatty acids, and that the extent of denaturation increases with the length of carbon chain of the acids and also with decreasing pH. This binding might be due to van der Waals interaction between hydrocarbon chains of the acids and hydrophobic amino acid residues of the protein molecule¹⁶⁻¹⁷⁾.

Many aromatic¹⁸⁻²²⁾ and heterocyclic acids²³⁾ have been found to decrease the

activities of varied enzymes. The present study indicated that aromatic carboxylic acids such as cinnamic, homophthalic, phthalic and *p*-hydroxyphenylacetic acids have an inhibitory action on IAA oxidation. According to Hartman *et al*^[20], 3,4-dihydroxyphenylalanine decarboxylase is competitively inhibited by cinnamic acid and its derivatives. However, the inhibition of IAA oxidation by cinnamic acid appears to be non-competitive (Table 3).

Certain carboxylic acids were reported to act as metabolic poisons only after they have formed esters with CoA^[24-25]. As shown in Table 4, the inhibition by cinnamic acid is almost the same both in the presence and absence of CoA added. This suggests resistibility and sensitivity of bacterial IAA oxidizing system to the ester and free cinnamic acid, respectively, if the ester is actually formed in the cell.

In this study, cells were used without being disrupted. So there remains a possibility that the observed inhibition by carboxylic acids of IAA oxidation depends wholly upon changed permeability by the acids of the cells to IAA. And the effectiveness of added CoA, too, might be due to non-permeation of CoA into the cells. Therefore, precise mechanism of inhibition by carboxylic acids of IAA oxidizing ability of *Arthrobacter* should be studied with cell-free enzyme system.

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摘 要

美濃羊輔：アースロバクターの一種によるインドール酢酸の
分解に関する研究 III. カルボン酸の影響

凍結乾燥したアースロバクターの一種によるインドール酢酸 (IAA) 酸化におよぼす種々のカルボン酸の影響をしらべた. 1) 置換されていない鎖状のモノおよびジカルボン酸の阻害作用は炭素鎖が長くなるにつれて増大し, モノカルボン酸の方がジカルボン酸よりはるかに阻害作用が大きい. α -炭素にアミノ基または水酸基を導入すると, モノカルボン酸の阻害作用は低下するが, ジカルボン酸の場合は逆の傾向を示した. アミド誘導体およびケト酸は阻害作用を示さなかった. いくつかの芳香族カルボン酸にも阻害作用のあることが認められた. 2) カルボン酸による阻害効果は, pH の低下につれて増大するので, 電離していない酸が阻害に関与しているものと思われる. 3) 桂皮酸による阻害度は IAA の濃度により殆んど変化しなかったことから, 桂皮酸による阻害は基質 (IAA) との競争的なものではない. 4) 桂皮酸による阻害は CoA 添加によって影響されなかった. (*帯広畜産大学昆虫学・植物生理学教室)