Wound-induced Ethylene Formation in Albedo Tissue of Citrus Fruit

HIROSHI HYODO AND TAKASHI NISHINO
Department of Horticulture, Faculty of Agriculture, Shizuoka University, Ohy, Shizuoka 422, Japan

ABSTRACT

Excised albedo tissue of citrus fruit (Citrus unshiu and Citrus hassaku) produced ethylene at an increasing rate in response to wounding and aging. The application of 1-aminoacyclopropane-1-carboxylic acid (ACC) enhanced ethylene production in both the fresh and aged tissues, but this increase was greater in the aged tissue than in the fresh tissue. ACC content was very low in fresh tissue but increased greatly in aging tissue, paralleling the rise in ethylene production. Aminoethoxyvinylglycine (AVG) strongly inhibited ethylene production in the aged tissue. In the presence of ACC, however, ethylene production was not inhibited by AVG. These results suggest that ACC is an intermediate in the pathway of ethylene biosynthesis in the albedo tissue and that both steps of ACC formation and ACC conversion to ethylene are enhanced by wounding and aging. Inhibitors of protein synthesis, cycloheximide and 2-(4-methyl-2,6-dinitroanilino)-N-methyl propionamide, strongly inhibited ethylene production in the albedo tissue, implying that protein synthesis is required to maintain the continuous evolution of ethylene. The stimulation of ethylene production by ACC was reduced by the addition of L-methionine, whereas D-methionine had very little inhibitory effect. Ethylene production in the albedo tissue was also inhibited by the addition of α-propyl gallate and 3,5-dibromo-4-hydroxybenzoic acid.

INTRODUCTION

Intact mature citrus fruits produce only a small amount of C2H4 (2, 8, 9). The young, immature fruits or the excised peel tissues, however, produce a large amount of C2H4 after harvest or during incubation after excision (2, 9, 10, 17). We have reported that C2H4 production is greatly enhanced in excised albedo discs of Satsuma mandarin (Citrus unshiu) during incubation of the tissue after slicing, and that 14C-labeled methionine is efficiently incorporated into C2H4 in parallel with the rise in C2H4 production (10, 11). These observations indicate that C2H4 is formed from methionine in wounded albedo tissue as in other plant tissues (13, 18).

Adams and Yang (1) have recently identified ACC2 as an intermediate of C2H4 biosynthesis in apple tissue and have established the following biosynthetic pathway: methionine → S-adenosylmethionine → ACC → C2H4. ACC has also been found to be an essential intermediate for C2H4 biosynthesis in other plant tissues (6, 7, 12, 15, 19-22). Here, we present evidence concerning the rise in C2H4 production in response to wounding and aging and the possible role of ACC as an intermediate of C2H4 production in wounded albedo tissue of citrus fruit.

1 This work was supported in part by the Agricultural Chemical Research Foundation and by the Matsushima Foundation.

2 Abbreviations: ACC, 1-aminoacyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; CHL, cycloheximide; MDMP, 2-(4-methyl-2,6-dinitroanilino)-N-methyl propionamide; PG, α-propyl gallate; DBHB, 3,5-dibromo-4-hydroxybenzoic acid.

RESULTS AND DISCUSSION

Intact albedo tissue produced only a trace amount of C2H4. After excision, albedo tissue prepared from either C. unshiu or C. hassaku began to produce C2H4 at an increasing rate (Fig. 1). There was a lag period of about 2 h before a rapid increase in C2H4 production became evident. The enhanced rate of C2H4 production was much greater in discs prepared from C. unshiu than in those from C. hassaku. C. unshiu usually ripens on the tree by the end of November, when the albedo layer becomes thin and the response to wounding is lower. In immature fruit, the albedo layer is thick and induced C2H4 production is greater.

Application of exogenous ACC caused a marked increase in C2H4 production in both the fresh and aged tissues of C. unshiu (Fig. 2). At a concentration of 0.01 mm, ACC significantly enhanced C2H4 production and saturation was reached at 1 mm ACC. The addition of 1 mm ACC enhanced C2H4 production by 571 and 589% in fresh and in aged tissue, respectively. The net increase caused by the application of ACC was much greater in aged tissue than in fresh tissue. ACC content in the fresh tissue of C. hassaku was very low (0.11 nmol/g), but it increased markedly during aging for 24 h (4.3 nmol/g). C2H4 production in the fresh
422

Plant incubation (aging) prepared 28. mandarin Satsuma Also, incubating decay curve presence of ACC found in the albedo h.

fold fresh (A) with MDMP. The tissue both control and ACC-enhanced C2H4 was found by Yu and Yang (20) in the albedo tissue of Valencia orange.

Protein synthesis inhibitors CHI and MDMP strongly inhibited both control and ACC-enhanced C2H4 production in aged albedo tissue (Fig. 3). The half-life of the C2H4-forming system in the presence of ACC was calculated from the logarithmic plot of the decay curve to be 122 min for 0.144 mM CHI and 158 min for 0.5 mM MDMP. The d-isomer of MDMP has been reported to be an inhibitor which suppresses protein synthesis by interfering with the association of mRNA with ribosomes and preventing polyribosome formation (4, 5). Here, MDMP was added to the medium with dimethyl sulfoxide. Control discs were given only dimethyl sulfoxide. A concentration of 0.5 mM MDMP was not high enough to suppress protein synthesis completely, and this may have resulted in a longer half-life than that observed with CHI. Lieberman and Kunishi (14) reported that half-maximal rates were reached in approximately 100 min for pea epicotyl and 206 min for apple tissue in the presence of 0.1 mM CHI. Yu et al. (21) demonstrated that the addition of 0.02 mM CHI strongly inhibited ACC-stimulated and auxin-stimulated C2H4 production in mung bean hypocotyl tissue. The application of actinomycin D to the discs did not suppress C2H4 production. These data suggest that formation of the C2H4 producing system in albedo tissue requires protein synthesis, but that DNA-dependent RNA (mRNA) synthesis is no longer required once the system is formed.

AVG, a known inhibitor of pyridoxal phosphate-dependent enzymes, is a potent inhibitor of C2H4 synthesis in plant tissues (3, 16). It has been recently found that the specific site for AVG function is at the enzyme which converts S-adenosylmethionine to ACC (1, 6, 12, 20–22). AVG at a concentration of 0.2 mM inhibited C2H4 production almost completely in aged albedo tissue (Fig. 4A). In the presence of ACC (0.5 mM), however, the same concentration of AVG did not inhibit C2H4 production at all (Fig. 4B). These results are in agreement with those reported with other plant tissues in that ACC is an essential precursor of C2H4 and that its formation is arrested by AVG (1, 6, 12, 15, 19–22).

Enhancement of C2H4 production by ACC was reduced by the addition of L-methionine. L-Methionine at 10 mM inhibited ACC-enhanced C2H4 production by 77% (Fig. 5A). L-Alanine at 50 mM similarly inhibited ACC-enhanced C2H4 production by 89% (Fig. 5B). Other amino acids, including L-valine, cycloleucine (1-aminocyclopentane-1-carboxylic acid), and glycine, gave a similar but lesser inhibitory effect. Since L-methionine had very little effect (data not shown), the inhibition was stereospecific, assuming adequate entry of all the compounds tested. However, in the chemical system of C2H4 release from ACC in the presence of pyridoxal phosphate, MnCl2 and H2O2 as described by Boller et al. (6), both L- and d-methionine inhibited the conversion of ACC to C2H4. Lürssen et al. (15) reported that L-methionine strongly inhibited the conversion of ACC to C2H4 in soybean leaf tissue. They suggested that pyridoxal phosphate might be involved in

FIG. 1. Induction of C2H4 production in excised albedo tissue during incubation (aging) at 28 °C in the dark. A, albedo discs prepared from Satsuma mandarin (Citrus unshiu) fruit harvested August 6; B, albedo discs prepared from Hassaku (Citrus hassaku) fruit harvested November 28.

FIG. 2. Stimulation of C2H4 production by the application of ACC in fresh (A) and aged (B) albedo discs of C. unshiu. Discs were aged by incubating for 19 h at 28 °C before use.

FIG. 3. Inhibition by CHI and by d-MDMP of C2H4 production in aged albedo discs in the presence and absence of ACC. A, albedo discs prepared from C. unshiu and incubated for 21 h at 28 °C; B, albedo discs prepared from C. hassaku and incubated for 23 h at 28 °C. After incubation (aging), the albedo discs were placed in a medium containing the respective inhibitor and C2H4 production was measured periodically.

FIG. 4. Effect of AVG on C2H4 production in aged (24 h) albedo discs prepared from C. hassaku. A, inhibition by 0.2 mM AVG in the absence of exogenous ACC; B, effect of 0.2 mM AVG on C2H4 production in the presence of 0.5 mM ACC.
ACC conversion to C_{2}H_{4}. These amino acids may compete with ACC for an active site and reduce the amount of ACC available for C_{2}H_{4} formation. The addition of L-methionine to the tissue inhibited C_{2}H_{4} production even in the absence of exogenous ACC (Fig. 5A).

C_{2}H_{4} production in aged tissue in the presence of ACC was inhibited by PG and DBHB. Addition of PG at a concentration of 1 mM and DBHB at 0.2 mM to aged discs of C. hassaku in the presence of 1 mM ACC reduced C_{2}H_{4} production by 63 and 57%, respectively. Gallic acid (1 mM) was also inhibitory, but to a less extent. PG and sodium benzoate were reported by Baker et al. (3) to inhibit C_{2}H_{4} production in fruit tissues by serving as radical scavengers. DBHB may function similarly. However, in the chemical system of C_{2}H_{4} release from ACC with pyridoxal phosphate, MnCl_{2}, and H_{2}O_{2}, PG strongly inhibited C_{2}H_{4} production, but DBHB was inactive at either 0.1 or 1 mM. C_{2}H_{4} production by discs in aqueous solution was considerably reduced compared to those in air. Immersion in water or solution caused the tissue to produce less C_{2}H_{4}. The normal rate of C_{2}H_{4} evolution in air could not be restored by the addition of 0.4 to 0.6 M mannitol or sucrose to the solution. Thus, the reduction did not result from low tonicity. These observations may fit the assumption that radical reactions may be involved in C_{2}H_{4} formation in the albedo tissue.

Acknowledgements—We thank Drs. S. F. Yang and G. Von Abrams, Department of Vegetable Crops, University of California, Davis, for helpful suggestions and for reading the manuscript.

LITERATURE CITED