Expression of ACC Synthase and ACC Oxidase Genes in Different Tissues of Immature and Mature Cucumber Fruits

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Summary

Ethylene biosynthesis and expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS) and ACC oxidase (ACO) genes were investigated in immature [harvested at 10 days after anthesis (DAA)] and mature (harvested at 35 DAA) cucumber fruits. During storage or in response to exogenous ethylene, ACS activity in fruit harvested at 35 DAA remained at low levels resulting in very little ethylene production, although ACO activity was markedly enhanced by the treatment. Northern blot analysis revealed that transcripts for *CS-ACS1*, a cucumber ACS gene highly homologous to *CMe-ACS1* which is expressed during melon fruit ripening, did not accumulate in any mature cucumber tissues. Exogenous ethylene did not induce accumulation of *CS-ACS1* transcripts but suppressed *CS-ACS2* mRNA accumulation in the peel and mesocarp tissues. *CS-ACO1* transcripts were increased by exogenously applied ethylene, but *CS-ACO2* was constitutively expressed regardless of the treatment. The results confirm that cucumber belongs to non-climacteric fruits because *CS-ACS1* expression was not induced during fruit ripening or in response to exogenous ethylene.

Key Words: *Cucumis sativus* L., ethylene biosynthesis, non-climacteric fruits.

Introduction

We previously, cloned three cDNA homologs of 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS; *CS-ACS1*, *CS-ACS2* and *CS-ACS3*) and two of ACC oxidase (ACO; *CS-ACO1* and *CS-ACO2*) from cucumber, a non-climacteric fruit. These genes are expressed in immature cucumber fruit slices in response to wounding and auxin treatments (Shiomi et al., 1998). Melons which also belong to the *Cucurbitaceae* are climacteric fruits, and *CMe-ACS1* and *CMe-ACO1* are 96% and 97% identical to *CS-ACS1* and *CS-ACO1* at the amino acid level, respectively (Shiomi et al., 1998). Their transcripts accumulate during ripening (Yamamoto et al., 1995; Shiomi et al., 1999) or in response to wounding or exposure of preclimacteric fruits to exogenous ethylene (Shiomi et al., 1999). We have, therefore, investigated ethylene biosynthesis in cucumber fruit during development at both the biochemical and molecular levels in the peel, mesocarp and placenta tissues.

Materials and Methods

Parthenocarpic cucumber (*Cucumis sativus* L. cvs. Hokushin and Honor) fruit for the fresh market were grown in a plastic house. Fruit were harvested at 10

(commercial harvest maturity, immature stage) and 35 (mature stage) days after anthesis (DAA). Since the peel of the fruit harvested at 35 DAA was more yellow than green, the fruit were considered to have attained physiological maturation stage. Fruit harvested at 35 DAA were stored for 2 weeks at 25°C and treated with exogenous ethylene (1000 ppm) at 25°C for 24 h.

Ethylene production was measured by the head-space method (Shiomi et al., 1998). Ethylene-treated fruit were exposed to air for 8 h when ethylene measurement was begun. ACC was assayed as described (Shiomi et al., 1999). ACS and *in vivo* ACO activities were determined as previously described (Shiomi et al., 1999).

Total RNA was extracted by the hot borate method (Wan and Wilkins, 1994). Isolation of poly(A)⁺ RNA and northern blot analysis were performed as described by Shiomi et al. (1998, 1999).

Results and Discussion

Since data for ethylene biosynthetic activity and northern blot analysis for both 'Hokushin' and 'Honor' were similar, only data for 'Hokushin' are presented.

Immature and mature intact cucumber fruits produced 0 and 0.023 $\mu\text{l g}^{-1} \text{h}^{-1}$ ethylene, respectively. During storage for 2 weeks and after treatment with exogenous ethylene for 24 h following 2 weeks of storage period, ethylene was undetectable in mature fruit (data not shown).

Although ACS activity (Fig. 1A) in the peel tissue

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from fruit harvested at 10 DAA was $0.8 \text{ nmol ACC mg protein}^{-1} \text{ h}^{-1}$, ethylene production from fruit at this stage was not induced. ACS activity in other tissues at different stages of maturity was equally low, and no increase in its activity occurred during ripening and by exogenous ethylene treatment (Fig. 1A). ACC content was also low (Fig. 1B). When the mesocarp tissue of mature 'Andes' melon, a typical climacteric fruit, commenced to produce ethylene, ACS activity was $1.93 \text{ nmol ACC mg protein}^{-1} \text{ h}^{-1}$ and ACC accumulated to 75.9 nmol g^{-1} (Shiomi et al., 1999), 7- and 200-folds higher, respectively, than those of mature cucumbers. In contrast, ACO activity in the cucumber fruit was enhanced by exogenous ethylene as observed in many other plant tissues (Yang and Hoffman, 1984); the activity increased from the placenta towards the peel (Fig. 1C).

Among the ACS genes studied, transcripts for CS-

Fig. 1. ACC synthase activity (A), ACC content (B) and in vivo ACC oxidase activity (C) in the peel, mesocarp and placenta tissues of 'Hokushin' cucumber fruit harvested at 10 (■) and 35 (▨) days after anthesis (DAA). Fruit harvested at 35 DAA were further stored for 15 days (□) or treated with ethylene (1000 ppm for 24 h) 2 weeks after storage (▨).

Fig. 2. Northern blot analysis in the peel, mesocarp and placenta tissues of 'Hokushin' cucumber fruit harvested at 10 and 35 days after anthesis (DAA). Each lane contains $10 \mu\text{g}$ of poly(A)⁺ RNA. Lanes 1, 5, 9, fruit harvested at 10 DAA; 2, 6, 10, fruit harvested at 35 DAA; 3, 7, 11, fruit harvested at 35 DAA were stored for 2 weeks and treated with ethylene (1000 ppm for 24 h); 4, 8, 12, fruit harvested at 35 DAA and stored for 15 days.

ACS1 accumulated only in the peel tissue of immature fruit (Fig. 2), coinciding with the relatively high ACS activity (Fig. 1A). Apart from that, signals for *CS-ACS1* mRNA were not detected in any other tissue at the two stages of maturity and in fruit treated with exogenous ethylene. Expression of *CS-ACS1* in immature cucumber fruit is induced by wounding, and the sequence shows 91% and 96% identity with *CMe-ACS1* at the nucleotide and amino acid levels, respectively (Shiomi et al., 1998). *CMe-ACS1* is the main ACS gene expressed in the mesocarp and placenta tissues of melon fruit at the mature stage when autocatalytic ethylene production has already commenced (Yamamoto et al., 1995; Shiomi et al., 1999). Moreover, exogenous ethylene applied to 'Andes' melon at the preclimacteric stage induces accumulation of *CMe-ACS1* transcripts (Shiomi et al., 1999); the expression pattern of *CMe-ACS1* coincides well with climacteric ethylene production and the ripening process (Yamamoto et al., 1995). Therefore, the failure of *CS-ACS1* gene expression and the lack of the enzyme activity in mature cucumber fruit and in response to ethylene treatment, may explain the difference in ethylene biosynthesis between cucumber and melon fruits.

The patterns of ACO gene expression (Fig. 2) were very similar to those demonstrated in melon fruit (Shiomi et al., 1999). Although *CS-ACO1* mRNA accumulated in all the tissues and was greatly enhanced by exogenous ethylene, *CS-ACO2* was almost constitutively expressed in all the tissues regardless of ethylene treatment (Fig. 2, lanes 3, 7, 11). Exogenous eth-

ylene promoted ACO activity (Fig. 1C) and enhanced the levels of *CS-ACO1* mRNA.

CS-ACS2 mRNA accumulated in the peel and mesocarp tissues and ethylene suppressed the transcript level in both tissues (Fig. 2, lanes 3, 7). However, the expression pattern of *CS-ACS2* did not coincide with ACS activity (Figs. 1 and 2), which suggests that the transcripts may not contribute much to the ACS activity because of the paucity of transcripts, post-transcriptional regulation, and/or other unknown factors. In melon fruit, mRNA accumulation of *CMe-ACS2*, which is nearly identical to *CS-ACS2*, is undetectable (Shiomi et al., 1999). The expression of *CS-ACS3*, a presumably auxin-inducible gene (Shiomi et al., 1998), was not detected in any of the tissues at the two stages of maturity (Fig. 2).

Intact parthenocarpic cucumber fruit for the fresh market produce very little ethylene, irrespective of the stage of maturity and the exogenous ethylene treatment. Since the conversion of SAM to ACC catalyzed by ACS is considered to be the rate-limiting step in ethylene biosynthesis in many plant species (Yang and Hoffman, 1984), the question is whether ACS activity is induced naturally during fruit ripening, by exogenous ethylene or its analogs, to distinguish climacteric fruits from non-climacteric ones (Biale and Young, 1981; Yang and Hoffman, 1984). We believe that the cucumber fruit in this study may belong to the non-climacteric class as previously reported by Biale and Young (1981). However, depending on the variety, cucumber may have different ripening characteristics as suggested by Kanelis et al. (1986) and as in melons (Shiomi et al., 1999).

The results from this study confirm that cucumber fruit belongs to non-climacteric fruits, and that the main difference in the ripening process of cucumber and

melon fruits may not result from ACO gene expression but from ACS1 gene expression, which was induced in the peel of immature fruit, but not in mature fruit tissues.

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キュウリの未熟果と成熟果の種々の組織における ACC 合成酵素および ACC 酸化酵素遺伝子の発現

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摘 要

キュウリの未熟果(開花後10日収穫)と成熟果(開花後35日収穫)を用いて、エチレン生合成活性と1-アミノシクロプロパン 1-カルボン酸(ACC)合成酵素(ACS)およびACC酸化酵素(ACO)遺伝子の発現を調べた。開花後35日収穫果のACS活性は、貯蔵中およびエチレン処理によって増加せず、低いレベルを維持したままで、成熟果からのエチレン生成はほとんど誘導されなかった。ACO活性はエチレン処理によって著しく増加した。ノーザンブロット分析を行った結果、メロン果実の追熟に伴って発現する*CMe-ACS1*と非常に高い相同性をもつキュウリのACS遺伝子である*CS-ACS1*の

mRNAは、成熟果のいずれの組織でも蓄積しなかった。エチレン処理によっても*CS-ACS1*の転写は誘導されなかった。*CS-ACS2*の転写産物は果皮と果肉で検出され、エチレン処理によって減少した。*CS-ACO1*の発現はエチレン処理によって強く促進されたが、*CS-ACO2*はエチレン処理に関係なくほぼ一定の発現を示した。以上の結果から、キュウリがノンクライマクテリック型果実に属することが確認され、そのことは、*CS-ACS1*の発現が成熟またはエチレン処理によって誘導されないことに起因すると考えられた。