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Ethylene-enhanced Synthesis of Phenylalanine Ammonia-Lyase in Pea Seedlings[1]

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ABSTRACT

The effect of ethylene on the development of phenylalanine ammonia-lyase activity in segments excised from the epicotyl apex of pea seedling was studied. Although there was some increase in phenylalanine ammonia-lyase activity in segments not treated with ethylene, a marked increase in phenylalanine ammonia-lyase activity occurred in ethylene-treated tissues during the incubation. The induction period was estimated to be about 6 hours. The activity reached a maxmum at 30 hours and then declined. On withdrawal of ethylene, the increase was sustained for a short period and then stopped. After retreatment with ethylene, the increase was resumed. Addition of CO_2 reduced the effect of ethylene. Administration of cycloheximide or actinomycin D at an early period almost completely suppressed the increase in phenylalanine ammonia-lyase activity. However, if these inhibitors were administered at a later period, while phenylalanine ammonia-lyase activity was approaching a maximum, they not only failed to reduce but rather stimulated the activity. These results are consistent with the view that there exist both phenylalanine ammonia-lyase-synthesizing and -inactivating systems, and that the development of both systems may involve *de novo* synthesis of protein.

Ethylene is known to be a plant hormone initiating fruit ripening and regulating many aspects of plant growth (3, 28). Numerous reports on the biochemical effect of ethylene on protein and nucleic acid metabolism have appeared, although the mechanisms involved remain obscure (1, 18, 34, 39). It has been reported that ethylene enhances the formation of enzymes such as peroxidase (16, 20, 26, 37), cellulase (19), polyphenol oxidase (37), and PAL[3] (20, 31) and also the formation of phenolic compounds in plants (5, 20). PAL is known to be a key enzyme in the biosynthetic pathway of phenylpropanoids in plants (7, 41). In addition to ethylene, several factors are known to affect the development of PAL. They are light (2, 8–10, 24, 35, 44, 46), wounding (23, 44), disease (12, 14, 23, 29), gamma-ray irradiation (25, 30), germination (38), development and differentiation (17, 22, 32, 42), and the application of certain macromolecules (15).

One of the most spectacular effects produced by ethylene is the "triple response" seen in legume seedlings (28). Since certain phenylpropanoids, such as coumarins and flavonoids (27, 33), are known to act as regulators of plant growth, we have therefore examined the effects of applied ethylene on the development of PAL activity in intact etiolated pea seedlings as well as in excised epicotyl segments. The present paper describes the characteristics of PAL development in pea seedlings as affected by the application of ethylene.

MATERIALS AND METHODS

Etiolated pea seedlings (*Pisum sativum* L., cv. Alaska) were grown at 23 C in vermiculite in plastic pots. For the experiments with intact etiolated pea seedlings, two pots (nine plants per pot) of 5-day-old seedlings were placed in 18-liter glass jars fitted with rubber gaskets and metal plates. Water was added to the bottom of the jars to provide moisture. Each jar contained 50 ml of 20% KOH in a beaker to absorb CO_2 produced during the incubation. In the control jar, an additional beaker containing mercuric perchlorate solution (43) was provided to absorb evolved ethylene. For ethylene treatments a suitable amount of ethylene was injected into the jar by hypodermic syringe. The jars were kept in darkness. At the end of incubation 2.2 cm-long segments were excised from the epicotyls immediately below the plumular hook. These segments were used for assay of PAL activity.

For the experiments with excised epicotyl segments, 1 cm-long segments were excised 1 cm below the plumular hook of 5-day-old etiolated pea seedlings. Ten to 15 segments were incubated in a 50-ml Erlenmeyer flask containing 10 ml of a medium consisting of 25 mM phosphate buffer, pH 6.0, 2% sucrose, and 0.5 mg of chloramphenicol. Sucrose was provided to serve as an energy source. Chloramphenicol was used to minimize growth of microorganisms. The flask was sealed with a rubber serum cap. A suitable amount of ethylene was injected into the flask with a syringe. In the control flasks, a polypropylene center well (Kontes Glass Company) containing a solution of mercuric perchlorate was provided to trap evolved ethylene. In both control and ethylene-treated flasks, a center well containing KOH solutions was provided to trap CO_2. At the end of incubation, the segments were blotted with filter paper, and the enzyme was extracted as subsequently described. All operations were performed in darkness except for the preparation of segments, which was performed under a dim green safe-light. Gas mixtures of known CO_2 concentrations were prepared by mixing CO_2 with air. These gases were introduced through a hypodermic needle into 50-ml Erlenmeyer flasks closed with rubber serum caps; another hypodermic needle was used as the outlet which was connected to a Beckman infrared analyzer to monitor CO_2 concentration. Ethylene was determined by gas chromatography using an alumina column and a flame ionization detector.

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[3] Abbreviation: PAL: phenylalanine ammonia-lyase.

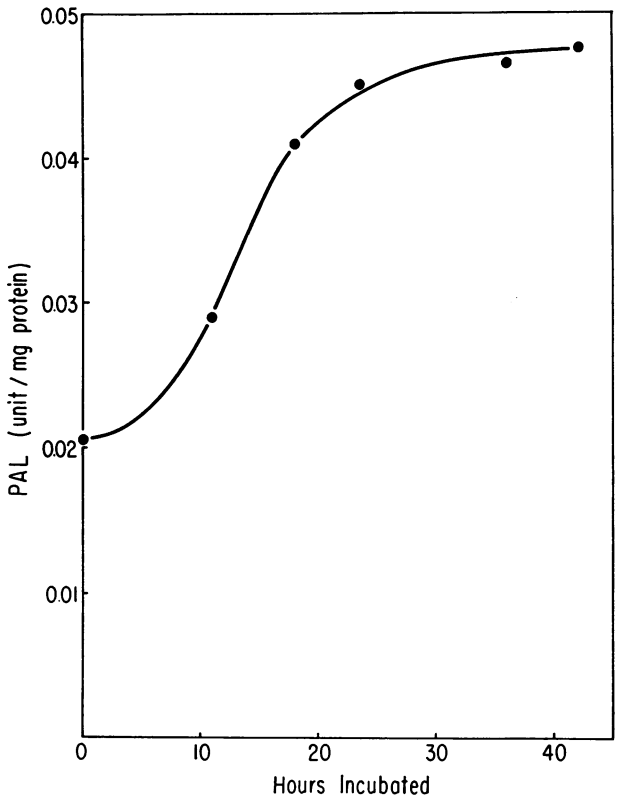

FIG. 1. Development of PAL activity in the apical region of the epicotyl of intact pea seedling. Five-day-old etiolated pea seedlings were transferred to a sealed glass jar containing 11 μl/liter ethylene. After growing for various periods of time, 2.2-cm long sections of the epicotyls were excised from immediately below the plumule hook and extracted for enzyme assay.

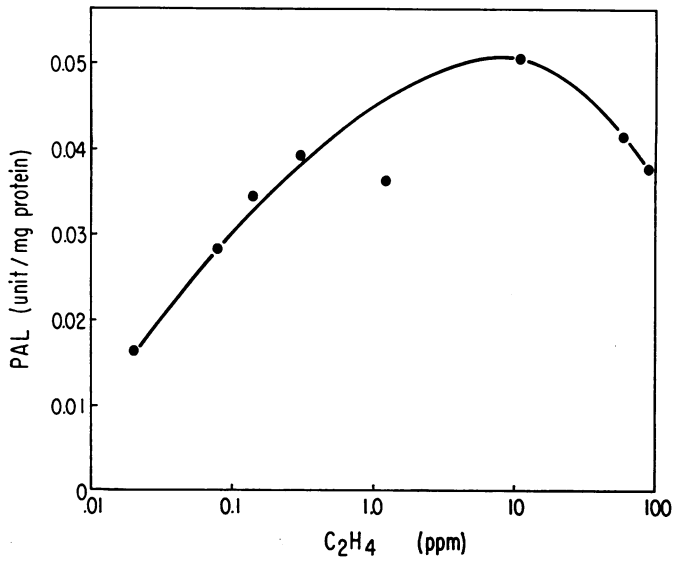

FIG. 2. Effect of ethylene concentration on development of PAL activity in the apical region of the epicotyl of intact pea seedlings. Experimental materials and methods were the same as in Figure 1, except that seedlings were grown for 24 hr and the concentration of ethylene was varied as indicated.

Extraction and assay of PAL were carried out according to the method described by Zucker (45) with a minor modification. A sample consisting of several hundred milligrams of pea epicotyl tissue was homogenized in the cold in 6 ml of 25 mM borate buffer, pH 8.8, containing 5 mM β-mercaptoethanol, using a glass homogenizer. The homogenate was centrifuged at 14,000g for 10 min at 2 C and the resulting supernatant fraction was dialyzed against the same buffer for 20 hr at 2 C. The dialyzed extract was used directly for PAL assay. The enzyme activity was found to be greater after dialysis, indicating the existence of an inhibitory substance in the crude extract. The assay mixture contained 0.2 mmole of borate buffer, pH 8.8, 60 μmoles of L-phenylalanine, and the enzyme preparation in a total volume of 6 ml. The incubation was at 40 C. Enzyme activity was determined spectrophotometrically by the increase in absorbance at 290 nm in a 60-min interval. The increase in absorbance was found to be proportional to the amount of enzyme added to the assay medium. A unit of activity was defined as that amount of enzyme which produces an increase in absorbance at 290 nm of 1.0 per hour with a 1-cm light path. Specific enzyme activity was expressed as units per milligram protein of the enzyme extract or as units per segment. Soluble protein content was determined with phenol reagent (21). Most of the experiments were carried out in duplicate. The actinomycin D was a gift from Merck Sharp & Dohme Research Laboratories, Rahway, N. J. All other chemicals and materials were commercially available.

RESULTS

Effect of Ethylene on Development of PAL Activity in Intact Etiolated Pea Seedlings. When etiolated pea seedlings were exposed to ethylene, the PAL activity extracted from the epicotyl apices increased in a sigmoidal fashion. The activity reached a plateau after treatment with 11 μl/liter ethylene for about 25 hr (Fig. 1). The dependence of the development of PAL activity on concentration of ethylene is shown in Figure 2. Treatment with about 10 μl/liter ethylene gave the maximal effect. The activity declined gradually with a greater ethylene concentration. A comparison of the PAL activity in the epicotyl of intact seedlings following 21-hr growth in the absence or in the presence of 11 μl/liter ethylene is shown in Figure 3. It is seen that the lower epicotyl region (X region) did not increase in length during the 21-hr treatments, but the PAL activity increased considerably. In the presence of ethylene, the upper epicotyl region (Y region) increases only 30% in length but increases considerably in diameter. In the absence of ethylene, this same region of epicotyl increases 230% in length. A new region (Z) of 1.3 cm is formed during the 21-hr period. In all cases examined, the PAL activity was found to increase with the age of the tissue. Although PAL activity in the apical region of the epicotyl of the ethylene-treated seedling (Y region) is much higher than that of the air control seedling (Z region), this higher activity is at least partly due to the difference in age of the tissues, since the Y region of the ethylene-treated tissues contains both old and newly formed tissues. In order to avoid this complexity, we have examined the effect of ethylene on development of PAL activity by the excised apical segments of epicotyls.

Effect of Ethylene on Development of PAL Activity in Excised Pea Epicotyl Segments. Epicotyl segments 1 cm in length were excised from 1 cm below the hook of pea seedlings grown for 5 days in darkness. The segments were incubated in darkness in the sucrose medium with or without ethylene. The changes in the enzyme activity are shown in Figure 4. Marked increase in PAL activity following ethylene treatment was observed. The maximal activity appeared to occur at about 30 hr after treatment with ethylene. During the first 30-hr incubation, the activity increased nearly 15-fold with ethylene and about 5-fold in the absence of ethylene. Mercuric perchlorate solutions were included in all of the control flasks to trap endogenously produced ethylene. The ethylene content in the

control flasks was found to be 0.044, 0.020, 0.009, 0.008, and 0.004 μl/liter, respectively, at 7.5, 18, 24.5, 31, and 43 hr after commencement of incubation. These concentrations of ethylene may be too low to bring about any significant bio-

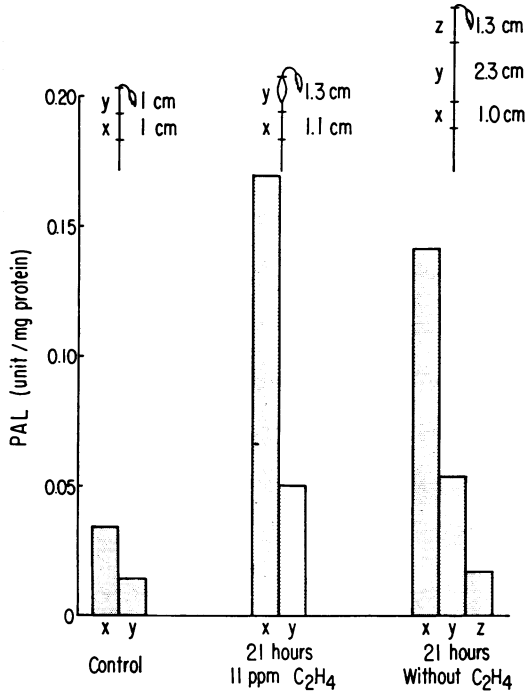

FIG. 3. Effect of ethylene on elongation and PAL activity of the apical region of the epicotyl of intact pea seedlings. Experimental materials and methods were the same as described in Figure 1, except that the apical region of each epicotyl was marked in 1-cm intervals before transferring to air or ethylene treatment. Following 21 hr of growth, the marked sections were excised and extracted for enzyme assay. The length of each section shown in the figure was for an average of 20 seedlings. Z represents the newly formed epicotyl portion following the 21-hr growth.

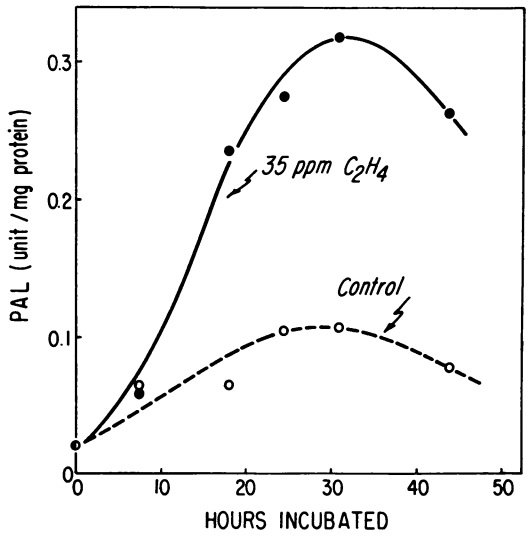

FIG. 4. Effect of ethylene on development of PAL activity by excised epicotyl segments. Epicotyl segments, 1 cm in length, were excised from 1 cm below the hook of 5-day-old etiolated pea seedlings and were incubated in a medium containing phosphate buffer, sucrose, and chloramphenicol with (35 μl/liter) or without ethylene. After various incubation times, segments were extracted for enzyme assay. In control flasks mercuric perchlorate solution was provided to absorb endogenously produced ethylene.

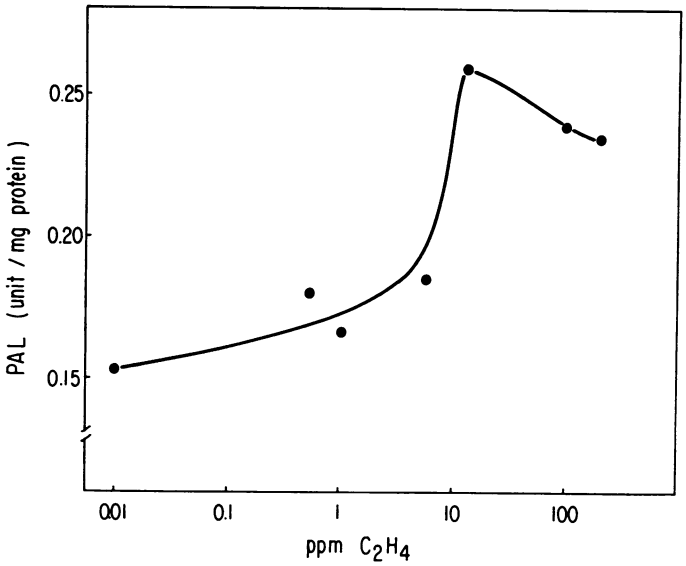

FIG. 5. Effect of ethylene concentration on development of PAL activity by excised epicotyl segments. Experimental materials and methods were the same as those described in Figure 4 except that various concentrations of ethylene were used as indicated. Incubation time was 25 hr.

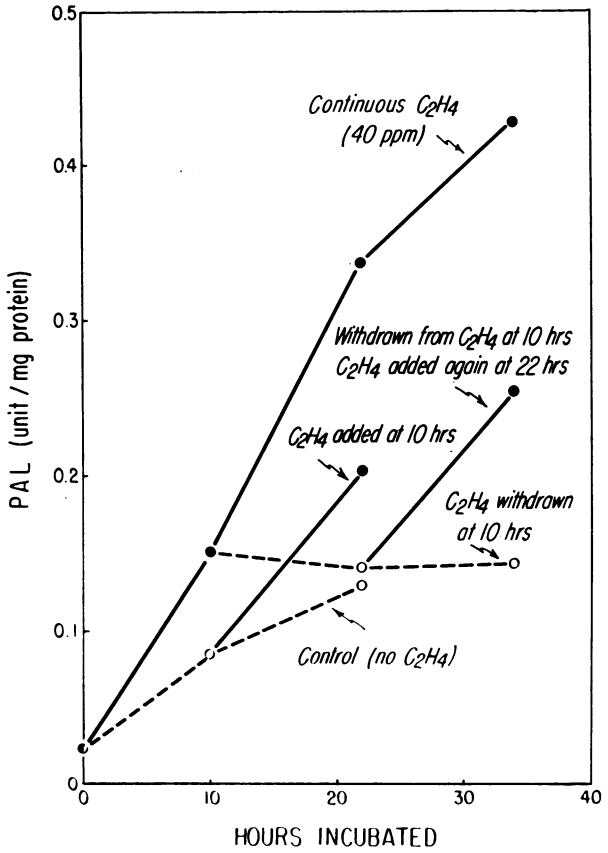

FIG. 6. Effect of addition or withdrawal of ethylene on development of PAL activity by excised epicotyl segments. Experimental materials and methods were the same as described in Figure 4, except that 40 μl/liter ethylene was added (●) or withdrawn (○) at various times.

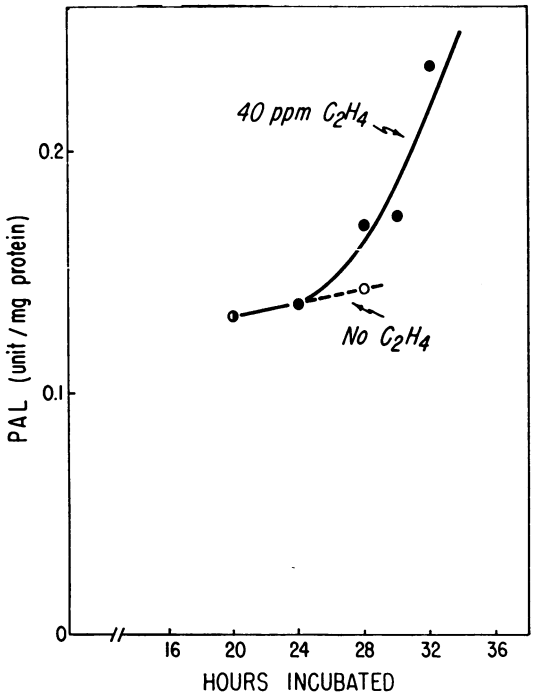

FIG. 7. Measurement of a lag phase period in the development of PAL activity. Ethylene, 40 μl/liter, was added to flasks containing epicotyl segments which had been incubated for 20 hr in the absence of ethylene. After incubation for various time periods PAL was extracted and assayed.

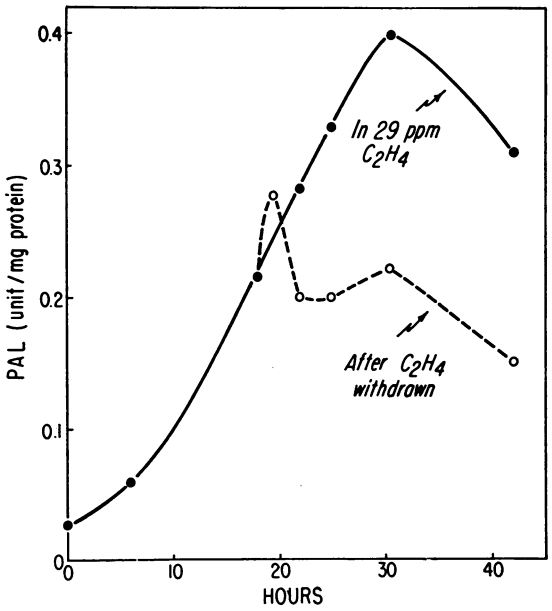

FIG. 8. Effect of withdrawal of ethylene on development of PAL activity. Segments were first incubated in the presence of 29 μl/liter ethylene for 18 hr. Ethylene was then withdrawn and PAL activity was measured at various times.

logical effect. It appears that two factors affect PAL activity in this system: ethylene and aging. More than 10 μl/liter ethylene was required to enhance PAL activity effectively (Fig. 5). On withdrawal of ethylene from the incubation flask, the increase in activity ceased. When ethylene was again added to the flask, the increase resumed as before (Fig. 6). It is illustrated in Figure 7 that after application of ethylene there was a lag period of about 6 hr before the steady rate of increase in PAL activity appeared. When ethylene was withdrawn, the increase in PAL activity was not stopped immediately but continued for a short while (about 2 hr), after which PAL activity decreased

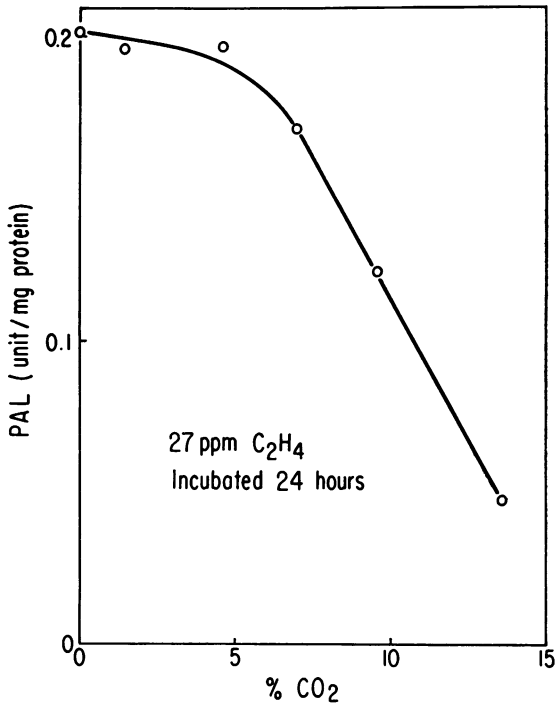

FIG. 9. Inhibition of the development of PAL activity by CO_2. Segments were incubated with 27 μl/liter ethylene and various concentrations of CO_2 for 24 hr in darkness.

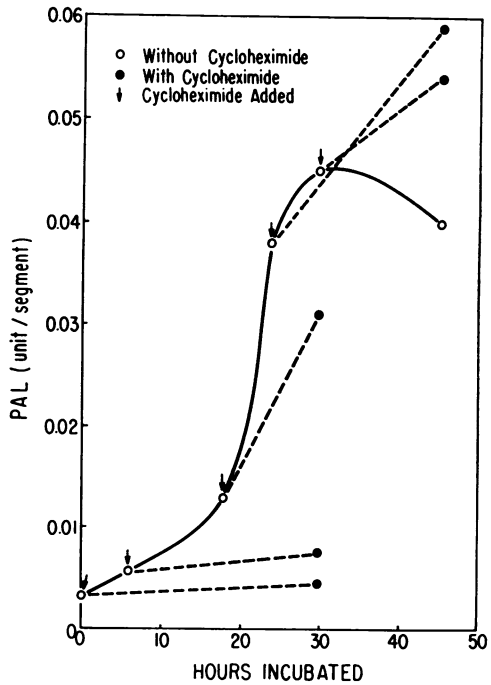

FIG. 10. Effect of cycloheximide on development of PAL activity. Segments were incubated with 27 μl/liter ethylene in darkness. At various times (indicated by arrows) samples of segments were transferred to medium containing 17.5 μM cycloheximide and incubated further in the presence of 27 μl/liter ethylene. Enzyme activity was expressed as units per segment.

(Fig. 8). Carbon dioxide has been known to act as a competitive inhibitor of ethylene (4). Figure 9 shows the effect of CO_2 concentration on the development of PAL activity in the presence of ethylene. An inhibition of such development was observed when the concentration of CO_2 exceeded 5%. The increase in PAL activity was inhibited 50% by 10% CO_2, and the inhibition was greater with a higher concentration of CO_2. Cycloheximide (5 μg/ml), if administered during the first 6 hr of incubation, almost completely suppressed the increase in PAL activity caused by treatment with ethylene (Fig. 10). Application of the same concentration of cycloheximide at a later phase when PAL activity was approaching maximum, however, failed to reduce the activity and even stimulated the increase. Since cycloheximide is known as an inhibitor of protein synthesis, presumably interfering with the transfer of amino acids from transfer RNA to protein (36), it may be tentatively inferred that the increase in PAL activity is derived from *de novo* synthesis of enzyme protein. Stimulation of PAL activity by the application of this inhibitor of protein synthesis during the later stages of PAL development has been observed by Engelsma (10) in gherkin seedlings and by Zucker (45) in potato tuber disks. They have postulated that there exists a PAL-inactivating system, the development of which was also blocked by cycloheximide. Actinomycin D at a concentration of 20 μg/ml or higher was also found to be effective in inhibiting the PAL development induced by ethylene (Fig. 11). When actinomycin D (50 μg/ml) was applied at the later stage, it prevented the reduction of PAL activity as in the case of cycloheximide.

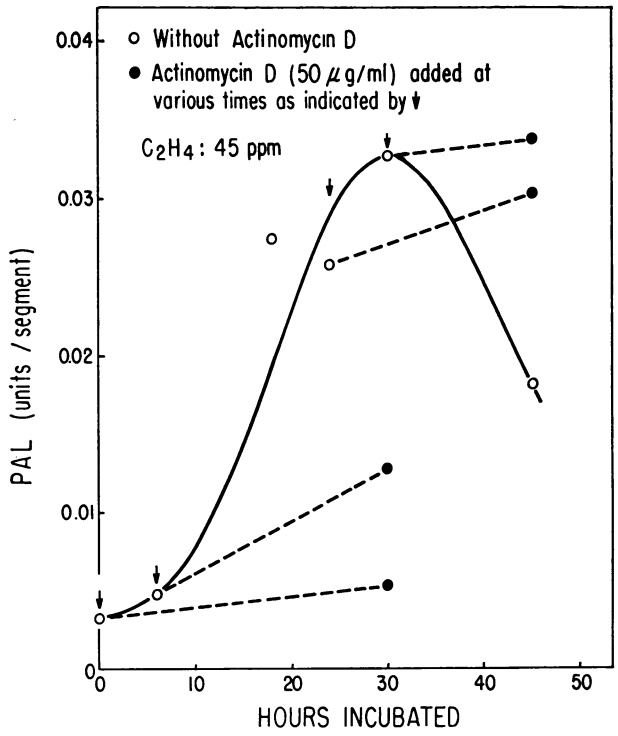

FIG. 11. Effect of actinomycin D on development of PAL activity. Segments were incubated with 45 μl/liter ethylene in darkness. At various times (indicated by arrows) samples of segments were transferred to the medium containing actinomycin D (50 μg/ml) and incubated further with 45 μl/liter ethylene.

DISCUSSION

Ethylene caused a marked rise in PAL activity in pea epicotyl segments. The lag period was estimated to be about 6 hr, while the lag periods required for α-amylase production in barley aleurone layers by gibberellic acid and for peroxidase in sugarcane stalk tissue were determined to be 7 to 9 hr and 4 to 5 hr, respectively (6, 13). The increase in PAL activity was almost completely suppressed by applying cycloheximide or actinomycin D. These results suggest that the increase in PAL activity by ethylene involves *de novo* synthesis of enzyme protein. Since the crude enzyme extract was dialyzed before the enzyme assay, it is not likely that the increase was due to an appearance or disappearance of a low molecular weight activator or inhibitor during the incubation period with ethylene. On withdrawal of ethylene, the increase in PAL activity continued for about 2 hr and then ceased, suggesting that the level of PAL mRNA persists for some time after withdrawal of ethylene. During the course of incubation in the presence of ethylene, PAL activity reached a peak at about 30 hr, after which the activity declined. The decrease in activity after 30 hr could be accounted for by assuming that there existed both PAL-synthesizing and -inactivating systems, and that the PAL-synthesizing system which was predominant in the exponential phase was compensated by a PAL-inactivating system which became active in the later stage and finally became dominant. Such views have been advanced by several investigators. Zucker (45) has reported that an inactivating system capable of degrading or inactivating PAL is formed in potato tuber disks. This system counteracts PAL synthesis and causes the eventual loss of PAL activity. To explain the development and subsequent decline of PAL activity in mustard seedlings exposed to far red light, Weidner *et al.* (40) have considered three processes through which regulation may be expressed: phytochrome-mediated PAL synthesis, inactivation of PAL, and eventual repression of PAL synthesis. Engelsma (11) suggested that the induction of a PAL-inactivating system may involve a protein inhibitor which might play a role either in repressing the synthesis of PAL mRNA or in associating directly with PAL.

A high concentration of CO_2 strongly inhibited PAL formation. It is possible that CO_2 interferes with ethylene by hindering its binding to an active site within the cell, as postulated by Burg and Burg (4).

In addition to the regulation by ethylene, wounding or aging affects PAL development in the present system. Whether some other plant hormone is involved in the wounding or aging process remains to be shown. The development of PAL by sweet potato roots and by potato tuber disks is well known (23, 44). Since ethylene may be evolved from plant tissues as a result of wounding (3, 28), we have examined the possible role of ethylene in the development of PAL in potato tuber disks. Based on our observation that the removal of ethylene from the potato tuber disks did not reduce the rate of PAL development and that the addition of ethylene did not enhance the rate of PAL development, we conclude that ethylene was not involved in this process (H. Hyodo and S. F. Yang, unpublished results).

The present experiments clearly demonstrate that ethylene enhanced the development of PAL activity in the apical region of pea epicotyls. Since there is a close relationship between the increase in PAL activity and the synthesis of phenylpropanoids in plants, ethylene may play an important role in enhancing phenylpropanoid synthesis through its regulation of PAL synthesis. However, it should be noted that the concentration of ethylene required to stimulate the PAL activity (Fig. 5) was much higher than that to cause the morphological changes (triple response) in pea seedlings (3, 28). These data suggest that the stimulation of PAL activity by ethylene cannot be the causal factor for the triple response observed in pea seedlings.

LITERATURE CITED

1. ABELES, F. B. 1968. Role of RNA and protein synthesis in abscission. Plant Physiol. 43: 1577-1586.
2. ATTRIDGE, T. H. AND H. SMITH. 1967. A phytochrome-mediated increase in the level of phenylalanine ammonia-lyase activity in the terminal buds of *Pisum sativum*. Biochim. Biophys. Acta 148: 805-807.
3. BURG, S. P. 1962. The physiology of ethylene formation. Annu. Rev. Plant Physiol. 13: 265-302.
4. BURG, S. P. AND E. A. BURG. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42: 144-152.
5. CHALUTZ, E., J. E. DEVAY, AND E. C. MAXIE. 1969. Ethylene-induced isocoumarine formation in carrot root tissue. Plant Physiol. 44: 631-635.
6. CHRISPEELS, M. J. AND J. E. VARNER. 1967. Hormonal control of enzyme synthesis: On the mode of action of gibberellic acid and abscisin in aleurone layers of barley. Plant Physiol. 43: 1008-1016.
7. CONN, E. E. 1964. Enzymology of phenolic biosynthesis. *In:* J. B. Harborne, ed., Biochemistry of Phenolic Compounds. Academic Press, New York. pp. 399-435.
8. CREASY, L. L. 1968. The increase in phenylalanine ammonia-lyase activity in strawberry leaf disks and its correlation with flavonoid synthesis. Phytochemistry 7: 441-446.
9. DURST, F. AND H. MOHR. 1966. Phytochrome-mediated induction of enzyme synthesis in mustard seedlings (*Sinapsis alba* L.). Naturwissenschaften 53: 531-532.
10. ENGELSMA, G. 1967. Effect of cycloheximide on the inactivation of phenylalanine deaminase in gherkin seedlings. Naturwissenschaften 54: 319-320.
11. ENGELSMA, G. 1968. Photoinduction of phenylalanine deaminase in gherkin seedlings. Planta 82: 355-368.
12. FARKAS, G. L. AND J. SZIRMAI. 1969. Increase in phenylalanine ammonia-lyase activity in bean leaves infected with tobacco necrosis virus. Neth. J. Plant Pathol. 75: 82-85.
13. GAYLER, K. R. AND K. T. GLASZIOU. 1968. Plant enzyme synthesis: Decay of messenger RNA for peroxidase in sugar-cane stem tissue. Phytochemistry 7: 1247-1251.
14. HADWIGER, L. A. 1968. Changes in plant metabolism associated with phytoalexin production. Neth. J. Plant Pathol. 74: 163-169.
15. HADWIGER, L. A. AND M. E. SCHWOCHAU. 1970. Induction of phenylalanine ammonia lyase and pisatin in pea pods by polylysine, spermidine or histone fractions. Biochem. Biophys. Res. Commun. 38: 683-691.
16. HERRERO, F. A. AND W. C. HALL. 1960. General effects of ethylene on enzyme systems in the cotton leaf. Physiol. Plant. 13: 736-750.
17. HIGUCHI, T. 1966. Role of phenylalanine deaminase and tyrosinase in the lignification of bamboo. Agr. Biol. Chem. 30: 667-673.
18. HOLM, R. G., T. J. O'BRIEN, J. L. KEY, AND J. H. CHERRY. 1970. The influence of auxin and ethylene on chromatin-directed ribonucleic acid synthesis in soybean hypocotyl. Plant Physiol. 45: 41-45.
19. HORTON, R. F. AND D. J. OSBORNE. 1967. Senescence, abscission and cellulase activity in *Phaseolus vulgaris*. Nature 214: 1086-1088.
20. IMASEKI, H., M. UCHIYAMA, AND I. URITANI. 1968. Effect of ethylene on the inductive increase in metabolic activities in sliced sweet potato roots. Agr. Biol. Chem. 32: 387-389.
21. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.
22. MAIER, V. P. AND S. HASEGAWA. 1970. L-Phenylalanine ammonia-lyase activity and naringenin glycoside accumulation in developing grapefruit. Phytochemistry 9: 139-144.
23. MINAMIKAWA, T. AND I. URITANI. 1964. Phenylalanine deaminase and tyrosine deaminase in sliced or black rot-infected sweet potato roots. Arch. Biochem. Biophys. 108: 573-574.
24. NITSCH, C. AND J. P. NITSCH. 1966. Effect de la lumière sur l'induction de la phénylalanine déaminase dans les tissus de tubercule d'*Helianthus tuberosus* L. C. R. Acad. Sci. (Paris). 262: 1102-1105.
25. OGAWA, M. AND I. URITANI. 1969. Metabolic changes in sweet potato roots induced by gamma radiation in response to cutting. Radiat. Res. 39: 117-125.
26. OSBORNE, D. J. 1968. Ethylene as a plant hormone. S.C.I. Monograph No. 31. Society of Chemical Industry, London. pp. 236-249.
27. POWELL, R. D. AND P. W. MORGAN. 1970. Factors involved in the opening of the hypocotyl hook of cotton and beans. Plant Physiol. 45: 548-552.
28. PRATT, H. K. AND J. D. GOESCHL. 1969. Physiological roles of ethylene in plants. Annu. Rev. Plant Physiol. 20: 541-584.
29. RAHE, J. E., J. KUC, C.-M. CHUANG, AND E. B. WILLIAMS. 1969. Correlation of phenolic metabolism with histological changes in *Phaseolus vulgaris* inoculated with fungi. Neth. J. Plant Pathol. 75: 58-71.
30. RIOV, J., S. P. MONSELISE, AND R. S. KAHN. 1968. Effect of gamma radiation on phenylalanine ammonia-lyase activity and accumulation of phenolic compounds in citrus fruit peel. Radiat. Bot. 8: 463-466.
31. RIOV, J., S. P. MONSELISE, AND R. S. KAHN. 1969. Ethylene-controlled induction of phenylalanine ammonia-lyase in citrus fruit peel. Plant Physiol. 44: 631-635.
32. RUBERY, P. H. AND D. E. FOSKET. 1969. Changes in phenylalanine ammonia-lyase activity during xylem differentiation in *Coleus* and soybean. Planta 87: 54-62.
33. RUSSELL, D. W. AND A. W. GALSTON. 1969. Blockage by gibberellic acid of phytochrome effect on growth, auxin responses, and flavonoid synthesis in etiolated pea internodes. Plant Physiol. 44: 1211-1216.
34. SACHER, J. A. AND S. O. SALMINEN. 1969. Comparative studies of effect of auxin and ethylene on permeability and synthesis of RNA and protein. Plant Physiol. 44: 1371-1377.
35. SCHERF, H. AND M. H. ZENK. 1967. Induction of anthocyanin and phenylalanine ammonia-lyase formation by a high energy light reaction and its control through the phytochrome system. Z. Pflanzenphysiol. 56: 203-206.
36. SIEGEL, M. R. AND H. D. SISLER. 1965. Site of action of cycloheximide in cells of *Saccharomyces pastorianus*. III. Further studies on the mechanism of action and the mechanism of resistance in *Saccharomyces* species. Biochim. Biophys. Acta 103: 558-567.
37. STAHMANN, M. A., B. G. CLARE, AND W. WOODBURY. 1966. Increased disease resistance and enzyme activity induced by ethylene and ethylene production by black rot infected sweet potato tissue. Plant Physiol. 41: 1505-1512.
38. WALTON, D. C. AND E. SONDHEIMIER. 1968. Effects of abscisin II on phenylalanine ammonia-lyase activity in excised bean axes. Plant Physiol. 43: 467-469.
39. WEBSTER, B. D. 1968. Anatomical aspects of abscission. Plant Physiol. 43: 1512-1544.
40. WEIDNER, M., I. RISSLAND, L. LOHMANN, C. HUAULT, AND H. MOHR. 1969. Die Regulation der PAL-Aktivität durch Phytochrom in Senfkeimlinger (*Sinapis alba* L.). Planta 86: 33-41.
41. YOSHIDA, S. 1969. Biosynthesis and conversion of aromatic amino acids in plants. Annu. Rev. Plant. Physiol. 20: 41-62.
42. YOSHIDA, S. AND M. SHIMOKORIYAMA. 1965. Studies on phenylalanine deaminase in buckwheat plant. Bot. Mag. Tokyo 78: 14-19.
43. YOUNG, R. E., H. K. PRATT, AND J. B. BIALE. 1952. Manometric determination of low concentration of ethylene. Anal. Chem. 24: 551-555.
44. ZUCKER, M. 1965. Induction of phenylalanine deaminase by light and its relation to chlorogenic acid synthesis in potato tuber tissue. Plant Physiol. 40: 779-785.
45. ZUCKER, M. 1968. Sequential induction of phenylalanine ammonia-lyase and a lyase-inactivating system in potato tuber disks. Plant Physiol. 43: 365-374.
46. ZUCKER, M. 1969. Induction of phenylalanine ammonia-lyase in *Xanthium* leaf disks. Photosynthetic requirement and effect of daylength. Plant Physiol. 44: 912-922.