THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1992 by The American Society for Biochemistry and Molecular Biology, Inc.

Vol. 267, No. 9, Issue of March 25, pp. 5964-5967, 1992
Printed in U.S.A.

Characterization and Kinetic Parameters of Ethylene-forming Enzyme from Avocado Fruit*

(Received for publication, August 19, 1991)

Douglas J. McGarvey and Rolf E. Christoffersen‡

From the Division of Molecular and Cellular Biology, Department of Biological Sciences, University of California, Santa Barbara, California 93106

Biosynthesis of the phytohormone ethylene in higher plants proceeds via the following pathway: *S*-adenosylmethionine → 1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene. Ethylene-forming enzyme (EFE), the enzyme responsible for the oxidation of ACC to ethylene, has been only partially characterized *in vitro*. We have obtained authentic EFE activity *in vitro* from extracts of avocado fruit (*Persea americana* Mill. cv Hass). Ammonium sulfate fractionation revealed the presence of two EFE activities, which we designate as EFE1 and EFE2. EFE1 activity utilizes ACC and O_2 as substrates and requires Fe(II) and ascorbate as cofactors. The enzyme has a relatively low K_m (32 μM) for ACC, discriminates diastereomers of 1-amino-2-ethylcyclopropane-1-carboxylic acid, and is inhibited competitively by 2-aminoisobutyric acid, thus confirming its identity with authentic EFE. Activity is retained in a 100,000 × *g* supernatant and has a pH optimum of 7.5–8.0, suggesting a cytosolic localization.

Ethylene (C_2H_4) is a phytohormone with diverse and manifold roles in the development of higher plants. Its essential role in fruit ripening is well documented (1). Ethylene is produced biologically from *S*-adenosylmethionine via the pathway: *S*-adenosylmethionine → ACC[1] → ethylene. Ethylene-forming enzyme (EFE), the enzyme responsible for oxidation of ACC to ethylene, has not been extensively characterized *in vitro* (2).

Earlier reports have shown that EFE activity is dependent on membrane integrity but not membrane potential (3, 4). These results have led to the assumption that EFE interacts with cellular membranes, but the mechanism of this proposed interaction is far from clear (3, 4). Furthermore, results have shown that most of the EFE activity retained by protoplasts is localized in the vacuole; these results have led to the assumption that EFE is associated with the vacuole *in vivo* (5, 6). The EFE activity associated with the vacuole, however, accounts for less than 4% of the EFE activity of intact tissue (7).

* This research was funded by Grant 90-37261-5658 from the United States Department of Agriculture Competitive Research Grant Program (to R. E. C.) The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 805-893-3599; Fax: 805-893-4724.

[1] The abbreviations used are: ACC, 1-aminocyclopropane-1-carboxylic acid; AEC, 1-amino-2-ethylcyclopropane-1-carboxylic acid; AIB, 2-aminoisobutyric acid; DTT, dithiothreitol; EFE, ethylene-forming enzyme; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Mops, 3-(*N*-morpholino)propanesulfonic acid.

In contrast to these earlier conclusions, Ververidis and John (8) have recently shown that EFE activity can be fully recovered in soluble extracts of melon fruit. In confirmation and extension of this report, we have recovered and partially purified two soluble EFE activities from ripening avocado fruit. We have also determined the cofactor and substrate requirements of EFE1, one of these two EFE activities, as well as inhibition and kinetic parameters *in vitro*. The low K_m for ACC, as well as the ability to discriminate between diastereomers of AEC, identify this activity as authentic EFE activity.

EXPERIMENTAL PROCEDURES

Materials—Avocado fruit (*Persea americana* Mill. cv Hass) were harvested from a local orchard (Cavalletto Ranches, Goleta, CA) and induced to ripen under a constant stream of 500 μl/liter propylene, an ethylene analog. ACC was obtained from Calbiochem, AIB was from Aldrich, AEC diastereomers were a kind gift from Dr. S. F. Yang (University of California, Davis), and all other chemicals were obtained from Sigma.

Enzyme Extraction and Partial Purification—The initial extraction procedure was modified from the method of Britsch and Grisebach (9) for extraction of flavanone 3-hydroxylase, an iron ascorbate-dependent nonheme oxidase, from *Petunia hybrida*. All extraction procedures were carried out at 4 °C. Avocado mesocarp tissue was frozen in liquid nitrogen and ground in a Waring blender. The frozen powder was then mixed with 2 volumes of extraction buffer (100 mM Tris, pH 7.5, 10% v/v glycerol, 33 mM sodium ascorbate, deaerated and equilibrated with nitrogen three times). After stirring at 4 °C for 1 h, the homogenate was filtered through Miracloth (Calbiochem) and centrifuged twice at 20,000 × *g* for 20 min each. To the supernatant ("crude extract"), solid ammonium sulfate was added. The mixture was stirred slowly at 4 °C under nitrogen. After centrifugation at 20,000 × *g* for 20 min, pellets were resuspended in 10 mM Tris, pH 7.5, 10% (v/v) glycerol (deaerated and equilibrated with nitrogen), aliquotted, frozen in liquid nitrogen, and stored at −70 °C. A 30–50% ammonium sulfate fraction was used for all further experiments. Protein concentrations were determined by a dye-binding assay (Bio-Rad) with comparison to bovine serum albumin standards.

Incubations—The standard assay consisted of the following in a 16 × 100-mm glass culture tube with serum stopper: 50–500 μl of extract (0.5–3 mg of protein), 30 mM sodium ascorbate, 100 μM $FeSO_4$, 100 μM ACC, and Buffer A (100 mM Tris, pH 7.5, 10% v/v glycerol) to 1 ml. After a brief vortexing, incubation was carried out at 30 °C with vigorous shaking (180 oscillations/min) for a total of 15 min. A 3-ml sample of the head space was removed and analyzed by gas chromatography on a Shimadzu GC-6AM machine equipped with a flame ionization detector and a Porapak N (Waters Corp.) or, for butene measurements, a 0.19% picric acid/Graphpak (Alltech Corp.) column. Production of ethylene was linear over the first 60 min of incubation (not shown). For determination of the pH optimum, Buffer A was replaced in the assay with the designated buffer. For determining the O_2 dependence, the reaction mix was as above but with 20 mM sodium ascorbate and 5 mM DTT. Reaction mix minus ACC was sealed in a 16 × 100-mm glass culture tube and then deaerated and equilibrated with nitrogen. The tube was flushed with 1 liter of varying concentrations of O_2 in nitrogen, and the reaction was then initiated by injecting ACC through the serum stopper.

EFE measurements *in vivo* were determined in discs of avocado fruit at 30 °C by the method of Starrett and Laties (10).

Enzyme Kinetics—K_m values and standard errors were calculated by a statistical method assuming proportional errors in velocity (11) and confirmed with direct linear plots (11). The K_i value for competitive inhibition was determined from a replot of the apparent K_m values at differing inhibitor concentrations.

RESULTS

Crude extracts of avocado tissue exhibited soluble EFE activity (Table I) with recovery of approximately 40% of the *in vivo* EFE activity (not shown). This activity was proportional to the amount of added extract and inactivated by boiling (not shown). Upon centrifugation at 100,000 × *g*, less then 2% of the activity pelleted with the microsomal fraction (not shown). These results indicate that, under these conditions, EFE activity is soluble, not membrane-bound. Similar results were obtained by Ververidis and John (8) with extracts from melon fruit.

Upon fractionation with ammonium sulfate, the EFE activity separated into two distinct fractions, which we designate as EFE1 and EFE2 (Table I). All further experiments were conducted on a 30–50% ammonium sulfate fraction containing EFE1. The ammonium sulfate precipitation was necessary and sufficient to separate the enzyme from endogenous ACC (approximately 30 μM in crude extracts of ripe fruit, not shown).

As confirmation that EFE1 is an authentic EFE activity, we examined its ability to distinguish between diastereomers of AEC. The authentic enzyme converts only (1*R*,2*S*)-AEC to 1-butene; common EFE artifacts *in vitro* convert all four AEC stereoisomers to 1-butene with equal efficiency (12–14). Using racemic mixtures of the two diastereomers, only the mix of (1*R*,2*S*)-AEC and its enantiomer resulted in 1-butene production; the mix of (1*R*,2*R*)- and (1*S*,2*S*)-AEC resulted in no detectable butene production (Table II). Similar results were obtained *in vitro* with EFE from melon (8). Without further concentration of the enzyme, determination of the stereochemical selectivity of EFE2 has proven impractical (not shown).

EFE1 activity was determined over a temperature range of 10–50 °C (not shown). Activity was maximal at 25–30 °C, dropping to half-maximal activity at 15 and 40 °C. Similarly, EFE activity *in vivo* is sensitive to high and low temperatures, with optimal activity at 30 °C (15, 16).

Fig. 1 shows the pH-dependent activity of EFE1. Over a range of pH from 4 to 9, EFE1 activity was optimal at pH 7.5–8.0; the enzyme was inactive below pH 6.0.

Following precipitation with ammonium sulfate, EFE1 activity demonstrated a requirement for Fe(II) (Fig. 2). Fe(II) is also essential for EFE activity *in vivo* (17). Of several divalent cations tested, none were capable of replacing Fe(II) (not shown). In the complete assay mix, addition of Co(II), Cu(II), and Zn(II) strongly inhibited EFE1 activity; Mn(II) and Ni(II) were partially inhibitory, whereas Mg(II) and

TABLE I

Ammonium sulfate fractionation of EFE activities

Ammonium sulfate cut	Specific activity[a]	Fraction of total activity[a,b]
	pmol/min/mg protein	*%*
Crude extract	0.98 ± 0.12	
0–20%	0.33 ± 0.12	0.2 ± 0.1
20–30%	0.16 ± 0.04	1.5 ± 0.3
30–40%	0.12 ± 0.09	1.3 ± 1.0
40–50%	1.29 ± 0.09	31.8 ± 2.2[c]
50–60%	0.11 ± 0.11	0.7 ± 0.7
60–70%	0.25 ± 0.13	2.4 ± 1.3
70–80%	0.62 ± 0.16	3.2 ± 0.8
Supernatant	1.56 ± 0.08	58.9 ± 3.0[d]

[a] Mean of three assays ± standard error.
[b] Based on total extractable EFE activity. (Approximately 40% of the EFE activity *in vivo*.)
[c] EFE1.
[d] EFE2.

TABLE II

Conversion of AEC diastereomers to 1-butene

Racemates of AEC diastereomers	1-Butene[a]
	pmol/min/mg protein
(1*R*,2*S*)- and (1*S*,2*R*)-AEC (200 μM)	0.49 ± 0.04
(1*R*,2*R*)- and (1*S*,2*S*)-AEC (200 μM)	<0.04

[a] Mean of four assays ± standard error.

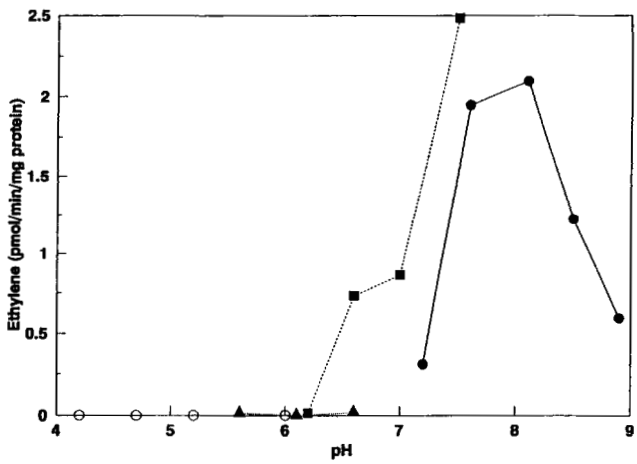

FIG. 1. **pH dependence of EFE1 activity.** EFE1 activity was assayed at differing pH values with the following buffers: sodium acetate (○), Na-Mes (▲), Na-Mops (■), and Tris-Cl (●). Each *point* represents the mean of three or more assays.

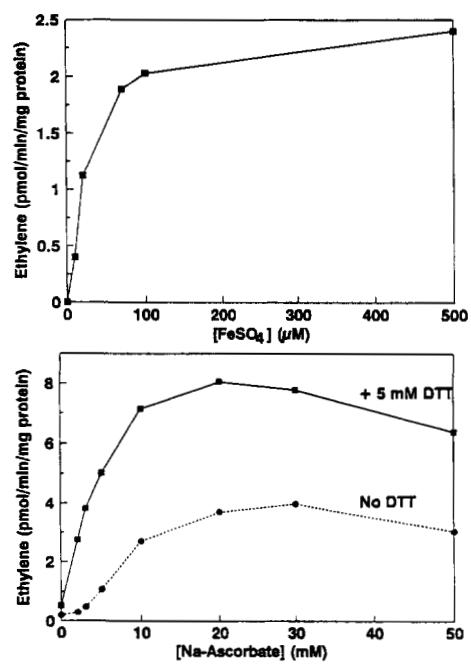

FIG. 2. **Cofactor dependence of EFE1 activity.** *Upper panel*, Fe(II) dependence. *Lower panel*, ascorbate dependence, with and without addition of 5 mM DTT. Each *point* represents the mean of three or more assays.

V(IV) had little effect (Table III). Co(II) also inhibits EFE activity *in vivo* (18, 19).

EFE uses ACC and O_2 as substrates, producing ethylene and cyanoformic acid, which decomposes to HCN and CO_2 (1). EFE1 also demonstrated a requirement for ascorbate (Fig. 2). Glutathione, DTT, and NADH were incapable of replacing ascorbate as a reducing agent, although trace levels of activity were obtained with 5–10 mM DTT (not shown).

Although DTT was incapable of replacing ascorbate, maximal EFE1 activity increased 2–3-fold in the presence of ascorbate plus 5 mM DTT (Fig. 2). These results indicate that although DTT can only partially replace the specific function of ascorbate, it can enhance EFE1 activity, presumably by maintaining a reducing environment.

Fig. 3 shows the dependence of EFE1 activity on its two known substrates, ACC and O_2. The K_m for ACC was 32 ± 6 μM, which is consistent with values estimated for EFE activity *in vivo* (20) and somewhat lower than the K_m reported for melon EFE *in vitro* (8). A low K_m for ACC is diagnostic of authentic EFE activity; common artifactual activities exhibit K_m values in the millimolar range (12).

The K_m for O_2 was 4.6 ± 0.8 kPa. Cellular O_2 concentrations estimated for ripening avocado fruit indicate that O_2 is unlikely to be a limiting factor for EFE activity *in vivo* (21). EFE1 activity in the presence of 21 kPa of O_2 in nitrogen was nearly 2-fold less than the corresponding activity in air (not shown). Since CO_2 stimulates EFE activity *in vivo* (22), we assayed the effect of CO_2 on EFE1 activity. Addition of CO_2 (as $NaHCO_3$) to the reaction mix with 21 kPa of O_2 restored EFE1 activity to the levels observed under air (not shown).

AIB, a structural analog of ACC, has been shown to act as a competitive inhibitor of EFE *in vivo* (23, 24). Fig. 4 shows the effect of AIB on EFE1 activity. The K_i for AIB was 0.3 mM, nearly 10-fold higher than the K_m for ACC. AIB is a relatively poor inhibitor, requiring millimolar amounts for significant inhibition *in vivo* as well (23, 24).

TABLE III

Effect of divalent metals on EFE1 activity

Metal (100 μM each)[a]	Specific activity[b]	Inhibition
	pmol/min/mg protein	%
Fe(II)	1.99 ± 0.09	
Mg(II)/Fe(II)	1.67 ± 0.29	16
V(IV)/Fe(II)	1.71 ± 0.18	14
Mn(II)/Fe(II)	0.97 ± 0.10	51
Ni(II)/Fe(II)	0.75 ± 0.13	62
Co(II)/Fe(II)	0.04 ± 0.02	98
Cu(II)/Fe(II)	0.07 ± 0.06	96
Zn(II)/Fe(II)	0.08 ± 0.04	96

[a] Metals were added as the sulfate salt. V(IV) was added as $VOSO_4$.
[b] Mean of three assays ± standard error.

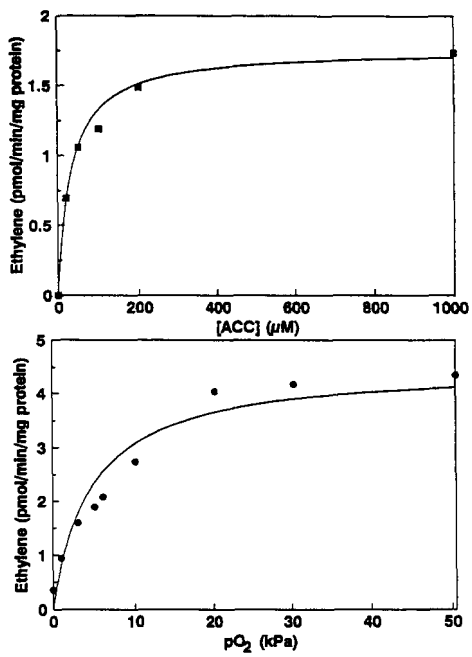

FIG. 3. **Substrate dependence of EFE1 activity.** *Upper panel*, ACC dependence. *Lower panel*, oxygen dependence. Oxygen is reported as the partial pressure of O_2 in the gas phase. Each *point* represents the mean of three or more assays.

DISCUSSION

The ability to discriminate stereoisomers of AEC, as well as its micromolar K_m for ACC identify EFE1 as an authentic EFE activity. Furthermore, the inhibition by Co(II) and by AIB, as well as the requirement for Fe(II), correlate well with the characteristics of EFE *in vivo* (17–19, 23, 24).

In contrast to *in vivo* experiments implying location of EFE at the plasma membrane or tonoplast (2, 5, 6, 15), our results indicate that at least 40% of the *in vivo* EFE activity of avocado fruit is soluble at 100,000 × *g* and hence not membrane-bound. Similar solubility has been demonstrated for a potential EFE antigen from avocado fruit (25) and for EFE activity *in vitro* from melon (8). Earlier work has shown a correlation of membrane integrity, but not membrane potential (3, 4), with EFE activity *in vivo*. These results may be due to a change in the reducing environment or leakage of Fe(II) and ascorbate upon disruption of the cell membrane.

Work from Kende's (5, 6) laboratory has suggested an association of EFE with the vacuole. It shows that 80% of the EFE activity in protoplasts of *Vicia faba* or *Pisum sativum* L. is associated with the vacuole. However, protoplast ethylene production typically accounts for only a small fraction of the ethylene production by whole tissue (7, 26, 27); the vacuolar EFE activity accounts for less than 4% of the activity of intact tissue (7). Furthermore, Mitchell *et al.* (28) demonstrated a recovery of only 0.5% of the EFE activity of intact tissue in a tonoplast membrane fraction of kiwi fruit. The association of EFE with vacuolar preparations could be due to a small amount of cytosolic EFE trapped within membrane vesicles. Our results and those of Ververidis and John (8) show that at

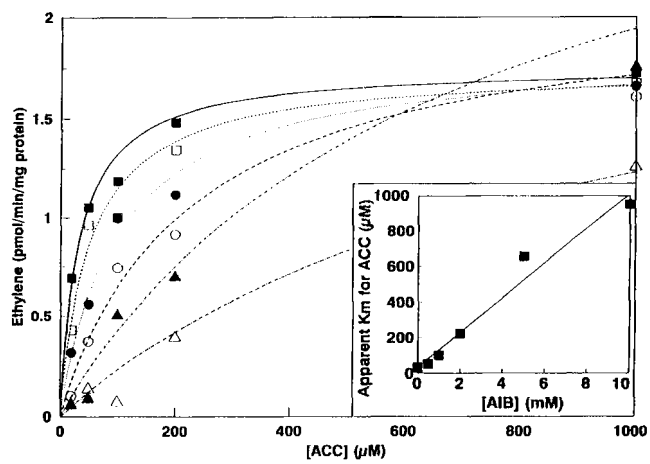

FIG. 4. **Competitive inhibition of EFE1 activity by AIB.** ACC-dependent activity with no AIB (■), 0.5 mM (□), 1 mM (●), 2 mM (○), 5 mM (▲), and 10 mM (△) AIB. Each *point* represents the mean of three or more assays. *Inset*, replot of apparent K_m for ACC at each inhibitor concentration.

least a major fraction of the EFE activity in avocado and melon fruit is not membrane-associated nor does it require membranes for activity. Furthermore, the lack of activity below pH 6 precludes the possibility of EFE1 being localized as a soluble enzyme within the vacuole. The antigen of a potential EFE gene of avocado is retained by protoplasts (25). Furthermore, EFE from tomato fruit has been expressed in Xenopus oocytes (39). Thus EFE does not require the cell wall for activity. Hence, we conclude that EFE1 is a soluble cytosolic enzyme.

EFE1 was inhibited by Co(II), as is EFE activity *in vivo* (18, 19). Cu(II) and Zn(II) were also effective inhibitors (Table III). The inhibitory metals may act by replacing Fe(II) and forming an inactive enzyme-metal complex. Since equimolar amounts of Co(II), Cu(II), or Zn(II) with Fe(II) abolished nearly all EFE1 activity, these metals may associate with the enzyme with greater affinity than does Fe(II). On the other hand, Mn(II) and Ni(II), which only reduced activity by half, presumably associate with about the same affinity as Fe(II).

Nonenzymatic oxidation of dideuterated ACC by transition metal oxidants proceeds with a loss of stereochemistry, resulting in both cis- and trans-dideuterioethylene (29), as does the biosynthetic process (30, 31). The requirement of Fe(II) for EFE1 activity suggests that the enzyme operates through a similar mechanism, utilizing iron as an electron acceptor (29), with consequent transfer of electrons to dioxygen. This hypothetical mechanism proceeds via nitrogen and carbon radical intermediates, thus allowing free rotation and loss of stereochemistry (29).

Ascorbate is thought to be required in iron ascorbate-dependent oxidases to protect the iron from fortuitous oxidation (32). Ascorbate may act in a similar fashion as a reducing agent for Fe in EFE1 *in vivo*, interacting specifically with the enzyme but not taking part in the overall reaction. Alternatively, ascorbate may be a required substrate in the reaction, contributing two electrons to the reduction of dioxygen. Experiments with purified enzyme will help to determine the stoichiometry of this reaction.

Non-heme-iron-containing enzymes typically incorporate one or both atoms of dioxygen into their substrates (33). EFE, however, transfers neither atom of dioxygen into its substrate (1, 34). Isopenicillin N synthase, a nonheme-iron-containing enzyme with structural similarity to EFE (35), utilizes oxygen with a similar stoichiometry (35, 36). Whereas isopenicillin N synthase catalyzes a four-electron reduction of O_2 to H_2O; however, oxidation of ACC only involves two electrons. In the EFE reaction, dioxygen may act as the final acceptor of two electrons/cycle, resulting in production of H_2O_2 by the following scheme.

$$\text{ACC} + \text{O}_2 \xrightarrow[\text{Ascorbate}]{\text{Fe(II)}} \text{C}_2\text{H}_4 + \text{HCN} + \text{CO}_2 + \text{H}_2\text{O}_2 \qquad (1)$$

Alternately, if ascorbate acts as a substrate, it may contribute two more electrons for a four-electron reduction of O_2 to water, with oxidation of both ACC and ascorbate (see following scheme). Experiments with purified enzyme will determine the validity of these schemes.

$$\text{ACC} + \text{O}_2 + \text{ascorbate} \xrightarrow{\text{Fe(II)}} \text{C}_2\text{H}_4 + \text{HCN} + \text{CO}_2 + \text{dehydroascorbate} + 2\text{H}_2\text{O} \qquad (2)$$

We are presently undertaking the purification of EFE1 and EFE2, which would allow for further study of the mechanism of this unusual reaction. We have previously identified a potential EFE gene in avocado fruit (25, 37), which bears homology to EFE genes from tomato fruit (38–40). We are currently investigating the relationship of this gene to EFE1 and EFE2 activity.

Acknowledgments—We are grateful to Dr S. F. Yang for his gift of AEC diastereomers and for critical reading of the manuscript. We thank Dr. N. O. Reich for helpful discussions and critical reading of the manuscript. We thank Cavalleto Ranches for donating avocado fruit.

REFERENCES

1. McKeon, T. A., and Yang, S. F. (1987) in *Plant Hormones and Their Role in Plant Growth and Development* (Davies, P. J., ed), pp. 94–112, Martinus Nijhoff, Dordrecht
2. Kende, H. (1989) *Plant Physiol.* **91,** 1–4
3. Guy, M. (1990) *Physiol. Plant.* **79,** 526–530
4. John, P., Porter, A. J. R., and Miller, A. J. (1985) *J. Plant Physiol.* **121,** 397–406
5. Mayne, R. G., and Kende, H. (1986) *Planta (Heidelberg)* **167,** 159–165
6. Guy, M., and Kende, H. (1984) *Planta (Heidelberg)* **160,** 281–287
7. Porter, A. J. R., Borlakoglu, J. T., and John, P. (1986) *J. Plant Physiol.* **125,** 207–216
8. Ververidis, P., and John, P. (1991) *Phytochemistry (Oxford)* **30,** 725–727
9. Britsch, L., and Grisebach, H. (1986) *Eur. J. Biochem.* **156,** 569–577
10. Starrett, D. A., and Laties, G. G. (1991) *Plant Physiol.* **95,** 921–927
11. Cornish-Bowden, A. (1976) *Principles of Enzyme Kinetics,* Butterworths, London
12. McKeon, T. A., and Yang, S. F. (1984) *Planta (Heidelberg)* **160,** 84–87
13. Venis, M. A. (1984) *Planta (Heidelberg)* **162,** 85–88
14. Hoffman, N. E., Yang, S. F., Ichihara, A., and Sakamura, S. (1982) *Plant Physiol.* **70,** 195–199
15. Apelbaum, A., Burgoon, A. C., Anderson, J. D., Solomos, T., and Lieberman, M. (1981) *Plant Physiol.* **67,** 80–84
16. Yu, Y.-B., Adams, D. O., and Yang, S. F. (1980) *Plant Physiol.* **66,** 286–290
17. Bouzayen, M., Felix, G., Latché, A., Pech, J.-C., and Boller, T. (1991) *Planta (Heidelberg)* **184,** 244–247
18. Yu, Y.-B., and Yang, S. F. (1979) *Plant Physiol.* **64,** 1074–1077
19. Lürssen, K., Naumann, K., and Schröder, R. (1979) *Z. Pflanzenphysiol.* **92,** 285–294
20. Yip, W.-K., Jiao, X.-Z., and Yang, S. F. (1988) *Plant Physiol.* **88,** 553–558
21. Tucker, M. L., and Laties, G. G. (1985) *Plant Cell Environ.* **8,** 117–127
22. Kao, C. H., and Yang, S. F. (1982) *Planta (Heidelberg)* **155,** 261–266
23. Satoh, S., and Esahi, Y. (1980) *Plant Cell Physiol.* **21,** 939–949
24. Apelbaum, A., Wang, S. Y., Burgoon, A. C., Baker, J. E., and Lieberman, M. (1981) *Plant Physiol.* **67,** 74–79
25. McGarvey, D. J., Sirevåg, R., and Christoffersen, R. E. (1992) *Plant Physiol.*, in press
26. Anderson, J. D., Lieberman, M., and Stewart, R. N. (1979) *Plant Physiol.* **63,** 931–935
27. Percival, F. W., Cass, L. G., Bozak, K. R., and Christoffersen, R. E. (1991) *Plant. Cell Rep.* **10,** 512–516
28. Mitchell, T., Porter, A. J. R., and John, P. (1988) *New Phytol.* **109,** 313–319
29. Baldwin, J. E., Jackson, D. A., Adlington, R. M., and Rawlings, B. J. (1985) *J. Chem. Soc. Chem. Commun.* **1985,** 206–207
30. Adlington, R. M., Baldwin, J. E., and Rawlings, B. J. (1983) *J. Chem. Soc. Chem. Commun.* **1983,** 290–292
31. Pirrung, M. C. (1983) *J. Am. Chem. Soc.* **105,** 7207–7209
32. Englard, S., and Seifter, S. (1986) *Annu. Rev. Nutr.* **6,** 365–406
33. Ingraham, L. L., and Meyer, D. L. (1985) *Biochemistry of Dioxygen,* Plenum, New York
34. Peiser, G. D., Wang, T.-T., Hoffman, N. E., Yang, S. F., Liu, H., and Walsh, C. T. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 3059–3063
35. Baldwin, J. E., and Abraham, E. (1988) *Natl. Prod. Rep.* **5,** 129–145
36. White, R. L., John, E.-M. M., Baldwin, J. E., and Abraham, E. P. (1982) *Biochem. J.* **203,** 791–793
37. McGarvey, D. J., Yu, H., and Christoffersen, R. E. (1990) *Plant. Mol. Biol.* **15,** 165–167
38. Hamilton, A. J., Lycett, G. W., and Grierson, D. (1990) *Nature* **346,** 284–287
39. Spanu, P., Reinhardt, D., and Boller, T. (1991) *EMBO J.* **10,** 2007–2013
40. Hamilton, A. J., Bouzayen, M., and Grierson, D. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 7434–7437