Bicarbonate/CO₂-Facilitated Conversion of 1-Aminocyclopropane-1-carboxylic Acid to Ethylene in Model Systems and Intact Tissues

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ABSTRACT

Bicarbonate markedly enhances ethylene production from 1-aminocyclopropane-1-carboxylic acid (ACC) in model chemical systems where the conversion is free radical-mediated, in thylakoid membrane suspensions of Phaseolus vulgaris L. cv Kinghorn where the reaction is light-dependent, and in microsomal membrane suspensions and intact tissues where the reaction is enzymatically mediated. In two model systems generating free radicals—the Fenton reaction and a reaction mixture containing xanthine/xanthine oxidase, NaHCO₃ (200 millimolar) increased the formation of ethylene from ACC by 84-fold and 54-fold, respectively. Isolated thylakoid membranes also proved capable of ACC-dependent ethylene production, but only upon illumination, and this too was enhanced by added NaHCO₃. As well, light-induced inhibition of ACC-dependent ethylene production by leaf discs was relieved by adding 200 millimolar NaHCO₃. Finally, NaHCO₃ (200 millimolar) augmented ACC-dependent ethylene production from young carnation flowers by about 4-fold, and the conversions of ACC to ethylene by mesosomes isolated from carnation flowers and etiolated pea epicotyls were higher by 1900 and 62%, respectively, in the presence of 200 millimolar NaHCO₃.

This increased production of ethylene appears not to be due to bicarbonate or CO₂-induced release of the gas from putative receptor sites, since the addition of NaHCO₃ to sealed reaction mixtures after the ACC to ethylene conversion had been terminated had no effect. Spin-trapping studies have confirmed that bicarbonate does not facilitate the formation of free radicals thought to be involved in the conversion of ACC to ethylene. Nor did bicarbonate alter the physical properties of the membrane bilayer, which might indirectly modulate the activity of the membrane-associated enzyme capable of converting ACC to ethylene. Rather, bicarbonate appears to directly facilitate the conversion of ACC to ethylene, and the data are consistent with the view that CO₂ derived from bicarbonate is the active molecular species.

Increased production of ethylene in response to enhanced levels of CO₂ has been demonstrated for detached leaves and leaf discs of cocklebur (15), intact sunflower plants (6, 12), tobacco leaf discs (1, 2, 18), and detached senescing oat leaves (14). The effect is obtained when CO₂ is supplied either directly as a gas or indirectly as bicarbonate, and no increase in ethylene production

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Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; cis-PNA, cis-parinaric acid; DMPO, 5,5-dimethyl-1-pyrroline-1-oxide; DMSO, dimethyl sulfoxide; DPH, 1,6-diphenyl-1,3,5-hexatriene; ESR, electron spin resonance; THF, tetrahydrofuran; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene; Tiron, 1,2-dihydroxybenzene-3,5-disulfonic acid; trans-PNA, trans-parinaric acid.
were cut with scissors into 70 ml of extraction buffer containing 0.3 M sorbitol, 1 mM MgCl₂, 1% BSA, and 50 mM Tricine, pH 8.0, and homogenized with a Sorvall Omnimixer for 5 s. The homogenate was filtered through eight layers of cheesecloth and centrifuged for 2 min at 2000g. The chloroplast pellet was resuspended in wash buffer (0.3 M sorbitol and 50 mM Tricine, pH 8.0) and centrifuged at 2000g for 2 min. Thylakoid membranes were obtained by resuspending the chloroplast pellet in 50 mM Tricine, pH 8.0, for 10 min to osmotically disrupt the envelope membranes (16). The released thylakoids were then pelleted by centrifugation at 4000g for 5 min and washed twice by resuspension in 50 mM Tricine, pH 8.0, and centrifugation at 4000g for 5 min. Thylakoid membranes were then resuspended in wash buffer at a concentration of 2 mg of Chl/ml. Chl was determined as described by Arnon (3).

Microsomal membranes capable of converting ACC to ethylene were isolated from epicotyl sections of dark-grown pea seedlings and from cut carnation flowers (Dianthus caryophyllus L. cv White-Sim; Yonder Atkin, Leamington, Ontario, Canada) as described previously (24, 25), except that the isolated membranes were dialyzed at 2°C overnight against 2 mM Epps buffer, pH 8.5, in order to eliminate an endogenous cytosolic inhibitor of ethylene production from ACC (24). The flowers were cut at the commercial stage and maintained for 4 d in deionized H₂O under continuous illumination at 22°C before being used for membrane isolation. By this time, the petals were fully expanded and showing no symptoms of petal-inrolling.

**Assays for Ethylene Production and ACC Levels.** Ethylene production from ACC in the presence of the Fenton reagent or xanthine/xanthine oxidase was carried out essentially as described by Legge et al. (22). For the Fenton reaction, the assay mixture contained 25 mM FeSO₄, 0.03% H₃BO₃, 1 mM ACC, and specified concentrations of NaHCO₃ in 1 of 0.3 M sorbitol-50 mM Tricine, pH 8.0. For the xanthine/xanthine oxidase reaction, the assay contained 0.2 mM xanthine, 1 mM ACC, 0.05 units xanthine oxidase, and specified concentrations of NaHCO₃ in 1 of 0.3 M sorbitol-50 mM Tricine, pH 8.0. In some experiments, catalase at a concentration of 5 units/ml was also added. The reaction mixtures were sealed in 12 × 75-mm test tubes and incubated for 1 h at room temperature. At the end of this period, a 1.0-ml gas sample was removed from the head space and analyzed isothermally (65°C) in a Perkin-Elmer Series 900 gas chromatograph fitted with an Al₂O₃ column and a flame ionization detector.

For measurements of ethylene production from ACC in the presence of thylakoid membranes, the reaction mixture contained 1 mM ACC, thylakoid membranes (0.2 mg Chl), and specified concentrations of NaHCO₃ in 1 of 0.3 M sorbitol-50 mM Tricine, pH 8.0. The reaction mixtures were again sealed in 12 × 75-mm test tubes and placed 10 cm in front of a 150-W light bulb (10 w/m²) for 1 h at room temperature. A 3-cm water filter was placed between the test tubes and the light bulb to protect the reaction mixtures from heat generated by the light bulb. At the end of this period, a 1-ml gas sample was removed from the head space for ethylene measurements. For measurements of ethylene production from ACC in the presence of pea and carnation microsomes, the assay contained 200 μg of membrane protein, 1 or 2 mM ACC, and specified concentrations of NaHCO₃ in 1 of 2 mM Epps buffer, pH 8.5. The reaction mixtures were again sealed in 12 × 75-mm test tubes and incubated for 1 h at 31°C, and a 1-ml gas sample from the head space was analyzed for ethylene. Membrane protein was measured as described by Bradford (9).

Levels of ethylene produced by discs from 2-week-old primary bean leaves were also determined. Eight discs (0.7-mm diameter) were placed adaxial side up in a 25-ml Erlenmeyer flask containing 2 ml of 0.3 M sorbitol-50 mM Tricine, pH 8.0, and specified concentrations of NaHCO₃. The flasks were sealed with rubber septa and incubated in the light (10 w/m² from fluorescent bulbs) for 1 h. For measurements of ethylene production from segments of pea epicotyl grown under etiolating conditions as described previously (25), 10 segments 3 cm in length were placed upright in test tubes (12 × 75 ml) containing 1 ml of 2 mM Epps buffer, pH 8.5, and specified concentrations of NaHCO₃, and incubated for 1 h at room temperature in the light (10 w/m² from fluorescent bulbs). Ethylene production from cut carnation flowers was measured by placing single flowers in 100-ml glass jars fitted with serum stoppers for 1 h at room temperature in the light (10 w/m² from fluorescent bulbs). The glass jars contained 10 ml of 2 mM Epps buffer, pH 8.5, and specified concentrations of NaHCO₃. In each case, 1-ml gas samples from the head space were analyzed for ethylene. ACC levels in carnation petal tissue were measured as described by Lizada and Yang (21).

**ESR Spin Trapping.** Free radicals formed during the conversion of ACC to ethylene in the presence of the Fenton reagent and specified concentrations of NaHCO₃ were detected as spin adducts of DMPO added to the reaction mixture at a concentration of 100 mM. ESR spectra were recorded on a Varian E-12 ESR spectrometer at room temperature as described (22).

**Fluorescence Depolarization.** Fluorescence depolarization of isolated membranes labeled with fluorescent probes was carried out essentially as described previously (30). Stock solutions of cis-PNA and trans-PNA (4 mM in absolute ethanol) as well as DPH (2 mM in THF) and TMA-DPH (2 mM in DMSO) were flushed with N₂ and stored at −20°C. For membrane labeling, aliquots of the stock solutions were diluted 1000-fold in 0.3 M sorbitol-50 mM Tricine, pH 8.0, or 2 mM Epps buffer, pH 8.5, by stirring vigorously for 15 min, and equal volumes of fluorescent probe solution and membrane suspension were mixed to give a final probe concentration of 2 μM for cis-PNA and trans-PNA and 1 μM for DPH and TMA-DPH. Upon addition of probe to the membrane suspension, the mixture was quickly vortexed for 10 to 15 s. The final concentration of membrane was 20 μg Chl/ml for thylakoid membranes and 25 μg protein/ml for microsomal membranes.

Steady state fluorescence depolarization (P) was measured at room temperature using an SLM 8000 spectrophotometer. For DPH and TMA-DPH, an excitation wavelength of 360 nm and an emission cutoff filter of 418 nm were used. For cis-PNA and trans-PNA, excitation wavelengths of 325 and 320 nm, respectively, were used, and emitted fluorescence was passed through a 370-nm cutoff filter. When measurements for thylakoid membranes were being recorded, the emitted fluorescence was passed through an additional band filter (transmitting from 400-450 nm) to eliminate Chl fluorescence.

**RESULTS**

Ethylene can be generated chemically from ACC in the presence of OH⁻ formed either by the Fenton reaction (Fe²⁺ and H₂O₂) or in the presence of xanthine and xanthine oxidase (22). In the xanthine/xanthine oxidase reaction, OH⁻ is apparently formed through the Haber-Weiss reaction (22, 26). When NaHCO₃ (200 mM) was added to these chemical systems, ethylene production from ACC increased by 84-fold in the Fenton reaction and by more than 54-fold in the presence of xanthine and xanthine oxidase (Table I). ACC plus NaHCO₃ alone produced no ethylene, and no ethylene was produced by the Fenton reaction with bicarbonate unless ACC was also present (Table I). As well, both Fe²⁺ and H₂O₂ had to be present to obtain the bicarbonate enhancement of ethylene production by the Fenton reaction, indicating that bicarbonate facilitates the OH⁻-mediated conversion of ACC to ethylene (Table I). Catalase is known to scavenge H₂O₂ generated through spontaneous dismutation of O₂⁻, thereby minimizing the conversion of O₂⁻ to OH⁻ through
the Haber-Weiss reaction (22). Accordingly, the sensitivity to catalase of the bicarbonate effect in the xanthine/xanthine oxidase system again suggests that bicarbonate is facilitating the OH\textsuperscript{-}mediated conversion of ACC to ethylene (Table 1). The bicarbonate-induced enhancement of ethylene production in the Fenton reaction proved to be dependent upon the concentration of NaHCO\textsubscript{3}. Maximum enhancement was obtained at 200 mM NaHCO\textsubscript{3} (Fig. 1), the same concentration of bicarbonate that caused maximum stimulation of ethylene production in situ in detached leaves and leaf disks of cocklebur (15). The enhancement effect of NaHCO\textsubscript{3} in the Fenton reaction also appeared to be highly specific in that the sodium salts of chloride, acetate, and formate at a concentration of 200 mM caused only a slight elevation of ethylene levels, and 200 mM NaHSO\textsubscript{3} actually inhibited ethylene formation (Table 1). Indeed, further experiments demonstrated that NaHSO\textsubscript{3} when present with NaHCO\textsubscript{3} (200 mM) in a molar ratio of 1:20 inhibited ethylene formation in the Fenton reaction by as much as 80%.

To further examine the mechanism by which bicarbonate might be enhancing the conversion of ACC to ethylene in the Fenton system, the ESR spectra of DMPO spin adducts were examined in the presence and absence of 200 mM NaHCO\textsubscript{3} as described by Legge et al. (22). When DMPO was added to the Fenton reaction in the absence of ACC, a spectrum representing the OH\textsuperscript{-} spin adduct of DMPO was formed (Fig. 2A). NaHCO\textsubscript{3} (200 mM) reduced the amplitude of the spectrum by about 80% (Fig. 2B). Hydroxyl radicals are known to react readily with NaHCO\textsubscript{3} to produce the CO\textsubscript{3}\textsuperscript{2-} radical (13, 19). However, no spin adduct of DMPO other than that attributable to OH\textsuperscript{-} could be detected. The CO\textsubscript{3}\textsuperscript{2-} and CO\textsubscript{2}\textsuperscript{-} radical species have strong transient absorption peaks at 600 (with a molar extinction coefficient of 2900 M\textsuperscript{-1} cm\textsuperscript{-1}) and 260 nm (with a molar extinction coefficient of 2250 M\textsuperscript{-1} cm\textsuperscript{-1}), respectively (19), and hence the Fenton reaction system containing 200 mM NaHCO\textsubscript{3} was scanned from 200 to 700 nm. However, no evidence was obtained for the presence of either of these radical species. When ACC was added to the Fenton reaction mixture, an additional spectrum tentatively identified as the DMPO adduct of a carbon-centered radical derived from ACC (22) was superimposed on the spectrum for the OH\textsuperscript{-} adduct of DMPO (Fig. 2C); bicarbonate (200 mM) reduced the amplitude of the hydroxyl radical spin adduct, but had no effect on the adduct for the ACC-derived radical (Fig. 2D).

The effect of bicarbonate on ethylene production by discs from primary bean leaves was also tested. Low levels of ethylene were obtained from the discs under both light and dark regimes (Fig. 3A). ACC enhanced ethylene production in both the light and dark regimes but, in accordance with earlier observations (11, 14, 15, 18), light inhibited the formation of ethylene from ACC relative to levels obtained in darkness (Fig. 3A). Moreover, this inhibition was largely overcome by the addition of 200 mM NaHCO\textsubscript{3} (Fig. 3A). Treatment of leaf discs with 200 mM NaHCO\textsubscript{3} alone did not result in any significant increase in ethylene production (Fig. 3A).

In view of the light sensitivity of ACC-dependent ethylene production from leaf discs, the ability of isolated thylakoid membranes to convert ACC to ethylene in the presence and absence of NaHCO\textsubscript{3} was examined. Thylakoid membranes maintained in darkness produced only trace levels of ethylene even in the presence of ACC and 200 mM NaHCO\textsubscript{3} (Fig. 3B). In the light, addition of ACC to the thylakoid membranes resulted in significant levels of ethylene being produced, and 200 mM bicarbonate caused a further 5-fold enhancement in ethylene production (Fig. 3B). Again, the enhancement effect appeared to be a
specific response to bicarbonate in that various other sodium salts (chloride, acetate, and formate) had little effect on ethylene levels, and NaHSO₃ almost completely inhibited its formation (Table II). The effect of bicarbonate on the thylakoid-mediated conversion of ACC to ethylene was also concentration-dependent, with optimum levels of ethylene being obtained at 200 mM NaHCO₃ (Fig. 1).

Ethylene production by epicotyl segments cut from etiolated pea seedlings was not differentially sensitive to light and dark regimes. Moreover, whereas ACC caused a 6- to 7-fold increase in ethylene production under both light and dark conditions, there was very little further enhancement upon addition of 200 mM NaHCO₃ (Fig. 4A). However, microsomal membranes isolated from the pea epicotyl segments, which have been previously characterized as a model system capable of converting ACC to ethylene (25), were responsive to bicarbonate. NaHCO₃ (200 mM) raised the level of ethylene production from ACC in the presence of the microsomal membranes by about 62% (Fig. 4B). The enhancement effect appeared to be a specific response to bicarbonate in that sodium chloride had no effect and NaHSO₃ inhibited ethylene formation (Fig. 4B). Heat-denatured micro-

| Reaction Mixtures | Ethylene* |
|-------------------|-----------|
| Control           | 0.55 ± 0.01 |
| Control plus 200 mM NaHCO₃ | 2.22 ± 0.28 |
| Control plus 200 mM NaCl | 0.61 ± 0.05 |
| Control plus 200 mM Na acetate | 0.35 ± 0.01 |
| Control plus 200 mM Na formate | 0.52 ± 0.01 |
| Control plus 200 mM NaHSO₃ | 0.03 |

* Standard errors of the means for three separate experiments are indicated.

** Contained thylakoid membranes (0.2 mg Chl) and 1 mM ACC in 1 ml of wash buffer.

somes in the absence of NaHCO₃ were incapable of converting ACC to ethylene.

The conversion of ACC to ethylene by young carnation flowers was not affected by light and dark regimes, but was enhanced by NaHCO₃. Exogenous ACC raised ethylene production from less than 0.1 nl/h-flower to 2.5 nl/h-flower in the light and to 1.6 nl/h-flower in darkness, and 200 mM NaHCO₃ induced a further 7- to 8-fold increase (Fig. 5A). NaHCO₃ had no effect in the
The absence of ACC (Fig. 5A). Analysis of the carnation petal tissue for ACC content after the 1-h incubation in the presence of either ACC alone or ACC plus NaHCO_3 demonstrated that the bicarbonate-induced enhancement of ethylene production was not attributable to an increased uptake of ACC into the petals in the presence of NaHCO_3. Microsomal membranes from carnation flowers are also capable of converting ACC to ethylene (24), and also responded to bicarbonate. Indeed, 200 mM NaHCO_3 increased microsome-mediated ethylene production from ACC by ~20-fold, and again the enhancement appeared to be a specific response to ACC and was heat-denaturable (Fig. 5B).

To assess the prospect that NaHCO_3 might be enhancing ethylene production from ACC by perturbing membrane lipids and thus altering the activity of the membrane-associated enzyme that converts ACC to ethylene, various fluorescent probes were used to monitor the lipids in different regions of thylakoid and carnation microsomal membranes following treatment with bicarbonate. Measurements of P (the degree of polarization) for membranes labeled with DPH, which probes the interior hydrocarbon region of the membrane, and with TMA-DPH, a probe which is anchored at the lipid-water interface, indicated that bicarbonate had no apparent effect on the physical organization of the lipid bilayer. Similarly, there were no significant changes in P for membranes labeled with cis-PNA, which partitions equally between liquid crystalline and gel phase lipid, or with trans-PNA, which partitions preferentially into gel phase lipid, following treatment of either thylakoid membranes or carnation microsomal membranes with NaHCO_3.

The prospect that ethylene formed from ACC remains bound, either specifically or nonspecifically, to microsomal and thylakoid membranes and is released by NaHCO_3 was also examined. Carnation microsomes, pea epicotyl microsomes, and thylakoid membranes were incubated for 2 h in standard reaction mixtures, and 200 mM NaHCO_3 was added either at the beginning of the reaction or after 1 h. As expected, bicarbonate enhanced ethylene production in all three systems over the 2-h incubation period when added at time 0, and to a lesser extent when added after 1 h (Fig. 6, A-C). Ethylene production from ACC was almost totally inhibited when thylakoid membranes were placed in darkness, and when 20 mM Tiron (a scavenger of O_2^-; 25) was added to either microsomal system (Fig. 6D). To determine whether bicarbonate was releasing bound ethylene, production by each membrane system was terminated after 1 h, and the terminated reaction mixture was incubated in the presence or absence of 200 mM NaHCO_3 for an additional hour (Fig. 6, E and F). Ethylene levels measured after the 2-h period in these inhibited systems were not significantly different in the presence
or absence of 200 mM NaHCO₃ (Fig. 6, E and F), indicating that NaHCO₃ is not releasing ethylene bound to the membranes.

**DISCUSSION**

Bicarbonate markedly enhances ethylene production from ACC in chemical systems where the conversion is driven by free radicals (Fenton reaction, xanthine/xanthine oxidase); in thylakoid membrane suspension where the reaction is dependent upon light; and in microsomal membrane suspensions and intact tissues where the reaction is enzymically mediated. It has been proposed that bicarbonate could achieve this effect by promoting release of bound ethylene (15) or facilitating the actual conversion of ACC to ethylene (18). The former prospect is consistent with previous observations that CO₂ can displace bound [¹⁴C] ethylene from putative receptor sites of both intact tissue and cell-free preparations (7, 28). However, Sisler (28) has calculated that the level of ethylene released from these specific receptor sites is too low to permit ordinary chemical detection. Thus, even if CO₂ does release bound ethylene, the contribution of such ethylene to the increased production measured in the presence of exogenous bicarbonate would be insignificant. Sisler (29) has also demonstrated that 69% of the ethylene binding sites are located in a 12,000 to 100,000g fraction, which is essentially a microsomal fraction. Accordingly, the experiments reported in the present study, in which the ability of bicarbonate to release ethylene from microsomal membranes was tested, also indicate that the increased levels of ethylene detected in the presence of NaHCO₃ cannot be attributed to CO₂-mediated release of bound ethylene.

Thus, the bicarbonate enhancement of ACC-dependent ethylene evolution appears likely to be effected through a more efficient conversion of ACC to ethylene. The prospect that bicarbonate facilitates this conversion indirectly by altering the physical properties of the membrane lipid bilayer so as to modulate the activity of the enzyme converting ACC to ethylene appears to be ruled out by the finding that treatment of carnation microsomal membranes with 200 mM NaHCO₃ has no significant effect on DPH, TMA-DPH, cis-PNA, or trans-PNA polarization values. Several lines of evidence from both intact tissues and model systems suggest that ethylene formation from ACC may be mediated by free radicals. For example, the reaction can be inhibited *in situ* by radical scavengers (4, 5), is driven by OH− in a strictly chemical system consisting of ACC and the components of the Fenton reaction (22), and appears to be facilitated by O₂•− when catalyzed by isolated microsomal membranes (25).

Thus, it is conceivable that bicarbonate might enhance ACC-dependent ethylene production by facilitating the formation of these reactive species of oxygen. However, spin-trapping experiments with the chemical system, in which the conversion of ACC to ethylene is driven by OH− formed through the Fenton reaction, indicated that bicarbonate actually reduces the pool size of OH−. Moreover, bicarbonate had no effect on levels of the ACC-derived radical thought to be an intermediate in the chemical conversion of ACC to ethylene (Fig. 2, C and D; 22). The reduced pool size of OH− presumably reflects quenching by NaHCO₃, although increased levels of either CO₂− or CO₃²−, which are known to be products of reactions involving OH− and NaHCO₃ (13, 19), were not detected. Moreover, if CO₂− were responsible for the enhanced conversion of ACC to ethylene in the Fenton reaction system, the addition of sodium formate, which reacts with OH− to produce CO₂− (8, 27) should have increased ethylene production rather than having no effect.

The effect of bicarbonate on ethylene production from ACC is concentration-dependent, and maximum stimulation of ethylene production was achieved with 200 mM bicarbonate for leaf discs (15), with the chemical system using the Fenton reaction, and with microsomal and thylakoid membranes (Fig. 1). Maximum stimulation of ACC-dependent ethylene production in leaf discs can also be achieved by direct application of 1.5 to 3% CO₂ (18). If the CO₂ were converted entirely to bicarbonate in the system used by Kao and Yang (18), the maximum HCO₃− concentration would be 33 mM. Since 200 mM HCO₃− is required for maximal enhancement, it appears that CO₂ is a much more potent stimulus of ACC-dependent ethylene formation than HCO₃−. At physiological pH and over the pH range employed in the *in vitro* reactions examined in the present study, the equilibrium among CO₂, HCO₃−, and CO₃²− favors HCO₃−. Under these conditions, the equilibrium concentration of CO₂ in the head space over 200 mM HCO₃− would be about 3%. It therefore seems likely that the effect observed with HCO₃− is attributable to CO₂. This contention is supported by the fact that introduction of only 0.1% CO₂ into the head space above the Fenton reaction enhanced the conversion of ACC to ethylene by about 2-fold.

In view of the apparent role of CO₂ in facilitating the conversion of ACC to ethylene, it is conceivable that the inhibitory effect of light on this conversion in leaf discs reflects partial depletion of endogenous CO₂ pools by photosynthetic fixation. The fact that light-induced inhibition of ACC-dependent ethylene formation can be overcome by addition of bicarbonate supports the contention that the inhibitory effect does reflect
partial depletion of CO₂ pools. The fact that photosynthetic fixation can influence ethylene formation suggests that chloroplasts may also be capable of forming ethylene from ACC. Konze and Elstner (20) have reported that isolated thylakoid membranes produce ethylene from methionine upon illumination by a mechanism contingent upon the formation of OH through the Haber-Weiss reaction. In the present study, we demonstrate that illuminated thylakoid membranes also convert ACC to ethylene, and that this conversion can be stimulated by added bicarbonate. However, since this reaction only occurs in the light, it is presumably free radical-mediated and nonenzymatic. Bean leaf discs and carnation flowers both responded to exogenous bicarbonate by producing more ACC-dependent ethylene, suggesting that in both instances availability of CO₂ was a rate-limiting factor. By contrast, ACC-dependent ethylene production by etiolated pea epicotyl segments proved to be only marginally responsive to bicarbonate. This may simply reflect a high respiratory activity in this tissue, which would lead to correspondingly high endogenous levels of bicarbonate. Indeed, ACC-dependent ethylene production by microsomal membranes isolated from pea epicotyls, which are in effect removed from endogenous sources of bicarbonate, did prove responsive to exogenous NaHCO₃.

In conclusion, NaHCO₃ clearly promotes the conversion of ACC to ethylene, presumably by serving as a source of CO₂. This proved to be true for intact tissues; for microsomal membranes, which serve as an in vitro model system capable of enzymically converting ACC to ethylene (24, 25); and for chemical model systems able to convert ACC to ethylene through a free radical mechanism (22). Notwithstanding this common response to bicarbonate, the extent to which these model systems are a true simulation of the in situ conversion remains unclear (17). Since the conversion of ACC to ethylene is enhanced by HCO₃⁻ in chemical systems where no enzyme is present, it is conceivable that CO₂ achieves its effect by interacting directly with ACC, perhaps by forming an ACC-carbamate complex (23).

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