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Evidence for Cyclic Photophosphorylation during $^{14}CO_2$ Fixation in Intact Chloroplasts

STUDIES WITH ANTIMYCIN A, NITRITE, AND OXALOACETATE

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ABSTRACT

This study examines the effect of antimycin A and nitrite on $^{14}CO_2$ fixation in intact chloroplasts isolated from spinach (*Spinacia oleracea* L.) leaves. Antimycin A (2 micromolar) strongly inhibited CO_2 fixation but did not appear to inhibit or uncouple linear electron transport in intact chloroplasts. The addition of small quantities (40–100 micromolar) of nitrite or oxaloacetate, but not NH_4Cl, in the presence of antimycin A restored photosynthesis. Antimycin A inhibition, and the subsequent restoration of photosynthetic activities by nitrite or oxaloacetate, was observed over a wide range of CO_2 concentration, light intensity, and temperature. High O_2 concentration (up to 240 micromolar) did not appear to influence the extent of the inhibition by antimycin A, nor the subsequent restoration of photosynthetic activity by nitrite or oxaloacetate. Studies of O_2 exchanges during photosynthesis in cells and chloroplasts indicated that 2 micromolar antimycin A stimulated O_2 uptake by about 25% while net O_2 evolution was inhibited by 76%. O_2 uptake in chloroplasts in the presence of 2 micromolar antimycin A was 67% of total O_2 evolution. These results suggest that only a small proportion of the O_2 uptake measured was directly linked to ATP generation. The above evidence indicates that cyclic photophosphorylation is the predominant energy-balancing reaction during photosynthesis in intact chloroplasts. On the other hand, pseudocyclic O_2 uptake appears to play only a minimal role.

A stoichiometric balance of ATP/NADPH in the chloroplast is essential for optimum photosynthesis. It is now widely accepted that such a stoichiometric balance of ATP/NADPH could not be achieved by linear electron transport from H_2O to $NADP^+$ alone (11). Assuming that the ATP/NADPH ratio generated via linear electron transport is 1.33, the extra ATP needed would presumably be derived from cyclic and/or pseudocyclic electron transport.

High rates of O_2 uptake have been measured in thylakoid preparations from spinach leaves in the presence of high concentrations of Fd (1; R. T. Furbank and M. R. Badger, unpublished), during photosynthesis in intact chloroplasts and cells (8, 9), and in leaves of C_3 plants (6, 10). The values of O_2 uptake measured in these studies are comparable to and, in several cases, exceeded the value (11.3% of total O_2 evolution) required for the generation of all the extra ATP needed for CO_2 fixation. This evidence suggests that pseudocyclic electron transport plays a significant (dominant) role in the ATP/NADPH balance during photosynthesis.

Marsho *et al.* (18) found that, although there was a significant rate of O_2 uptake in intact chloroplasts during the lag phase of photosynthesis, this diminished to a relatively insignificant level during steady-state photosynthesis. These low rates of O_2 uptake were relatively insensitive to increases in light intensity which greatly stimulated photosynthesis (14). These and a number of other workers (3, 4, 20, 23, 32) have provided evidence for cyclic flow during photosynthesis in thylakoids, chloroplasts, and intact leaves. Quantitative measurements of Cyt turnover in intact chloroplasts (26) have also indicated substantial cyclic flow. But the corresponding rates of O_2 evolution during these measurements were low. Thus, the relative contribution of cyclic *versus* pseudocyclic flow to the ATP/NADPH balance during photosynthesis remains unresolved. Furthermore, the reasons for the discrepancies between high (8, 9) and low (14, 18) rates of O_2 uptake measured during photosynthesis in chloroplasts and cells remain to be resolved.

In thylakoids, antimycin A is a reported potent inhibitor of Fd-dependent cyclic flow (2) but may function as an uncoupler at higher concentrations (7). In intact chloroplasts, antimycin A stimulated CO_2 fixation under anaerobic conditions (19, 22, 25) and in aerobic conditions which were suboptimal (*e.g.* high Pi concentration) for CO_2 fixation (28). But under aerobic conditions at optimal Pi, antimycin A strongly inhibited photosynthetic CO_2 fixation in intact chloroplasts with concomitant decreases in energy-dependent processes such as Chl *a* fluorescence, 9-aminoacridine fluorescence quenching, light scattering changes at 535 nm, and the slow electrochromic absorbance changes at 518 nm (14, 20, 23). This evidence suggests that the inhibition of photosynthesis in intact chloroplasts by antimycin A is directly linked to the inhibition of cyclic electron flow.

This study examines the extent and the role of endogenous cyclic photophosphorylation in photosynthesis in intact spinach chloroplasts. Antimycin A (2 μM) was found to inhibit photosynthesis strongly (60–80%) over a wide range of experimental conditions without apparently inhibiting or uncoupling linear electron flow. Photosynthetic activities inhibited by antimycin A were restored by the additions of small quantities of NO_2^- or oxaloacetate.

MATERIALS AND METHODS

Chloroplasts were isolated with a Polytron at 70% line voltage from spinach (*Spinacia oleracea* L.) plants grown under natural daylight in glass-house as described previously (30). O_2 evolution was measured with a Clark O_2 electrode (YSI 4004, Yellow Springs Instruments) in a standard 2.8-ml assay medium containing 0.33 M sorbitol, 50 mM Hepes-NaOH (pH 7.6), 2 mM EDTA, 0.5 mM KH_2PO_4/K_2HPO_4, 1 mM $MgCl_2$, catalase (200 units/ml), 2 mM $NaHCO_3$, and chloroplasts (30–80 μg Chl) at 25°C and preincubated in the dark for 1 to 2 min prior to illumination. Unless otherwise stated, additions of antimycin A, nitrite, and

oxaloacetate were usually done during this preillumination period. Light intensity (1,300 $\mu E\ m^{-2}\ s^{-1}$) was provided by a slide projector. O_2 concentration at start of assay was usually 30 to 40 μM. Experiments with $NaH^{14}CO_3$ (0.4–1.0 Ci/mol) were usually terminated after 4 to 6 min illumination and 2 × 100-μl aliquots of assay medium were transferred to scintillation vials containing 2 ml 8 N HCOOH/60% ethanol. These samples were air-dried and the radioactivity determined by liquid scintillation counting. The O_2 evolved and the rate of steady-state O_2 evolution in these experiments were also determined. Photosynthetic O_2 exchanges in isolated chloroplasts and cells were determined by a Varian MAT GD150 mass-ratio spectrometer as described (9).

RESULTS

The effect of antimycin A on photosynthetic activities in thylakoids and intact chloroplasts is complex and apparently depends on the experimental conditions involved (7, 14, 19, 20, 22, 23, 25, 28). Figure 1 shows that the photoreduction of methyl viologen in intact chloroplasts is insensitive to antimycin A up to 4 μM in the presence and absence of 10 mM NH_4Cl. This evidence indicates that low concentrations of antimycin A did not inhibit or uncouple linear electron transport in intact chloroplasts used in this study. In contrast, 2 μM antimycin A strongly inhibited photosynthetic O_2 evolution (Fig. 2). The addition of 20 and 50 μM NO_2^- restored O_2 evolution to about 60 and 80% of the control, respectively (Fig. 2b). In the absence of antimycin A, 20 μM NO_2^- stimulated O_2 evolution by about 11%, but 50 μM NO_2^- was highly inhibitory (Fig. 2a). Indeed, the activity measured at 50 μM NO_2^- in the presence of antimycin A was 63% higher than that measured in the absence of antimycin A. The effects of antimycin A and NO_2^- on photosynthetic O_2 evolution were not greatly affected by the order of addition of these compounds.

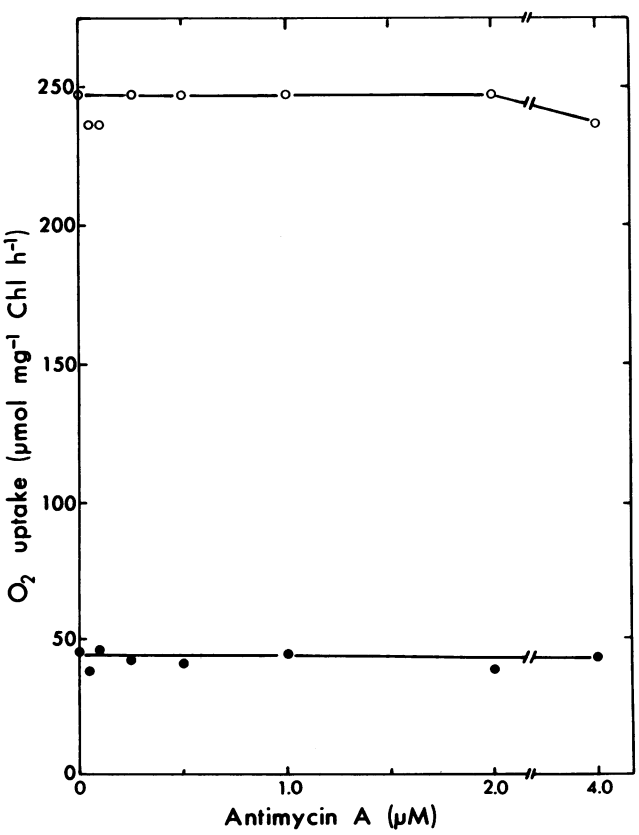

FIG. 1. Effect of antimycin A on photoreduction of methyl viologen in intact spinach chloroplasts (47 μg Chl) in the presence (○) and absence (●) of NH_4Cl. Concentrations of methyl viologen and NH_4Cl used were 0.1 and 10 mM, respectively.

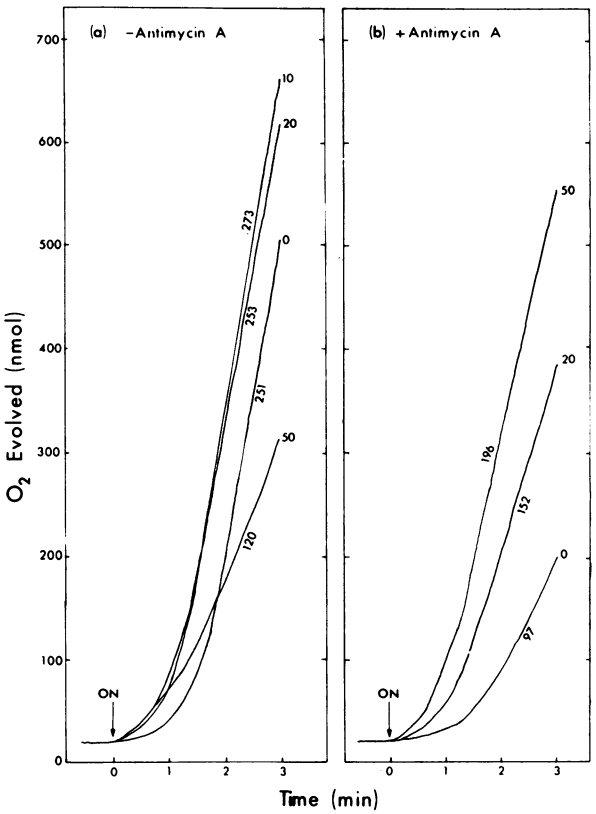

FIG. 2. Time course of O_2 evolution in illuminated spinach chloroplasts (73 μg Chl) in 2 mM $NaHCO_3$ in the absence (a) and presence (b) of antimycin A (2 μM) at different concentrations of NO_2^-. The values beside traces are rates of O_2 evolution in $\mu mol\ mg^{-1}$ Chl h^{-1} and the values at end of traces are μM NO_2^- concentration added.

Figure 3 shows that $^{14}CO_2$ fixation inhibited by antimycin A was also restored by the addition of NO_2^-. Increasing NO_2^- concentrations up to 50 μM increased the rate of $^{14}CO_2$ fixation from 68 to 125 $\mu mol\ mg^{-1}$ Chl h^{-1}. But further increase in NO_2^- concentration (80 μM) caused a decline in activity. The optimum NO_2^- concentration required was found to vary slightly (50–100 μM) between different chloroplast preparations, but in all cases, NO_2^- was found to decrease the lag phase of photosynthesis. Figure 4 shows that maximum inhibition of photosynthetic activities (*viz.* the amount of $^{14}CO_2$ fixed and O_2 evolved, and the rate of steady state O_2 evolution) was observed at antimycin A concentration $\geq$ 0.5 μM. NO_2^- was found to restore these photosynthetic activities at concentrations of antimycin A ($\geq$ 0.2 μM) where substantial inhibition of photosynthesis by antimycin A occurred.

In intact chloroplasts, NO_2^- is reduced to NH_3 in the light by Fd-dependent NO_2^- reductase (EC 1.7.7.1). NH_3 has been shown to stimulate $^{14}CO_2$ fixation in intact chloroplasts (12) and cells (29). Table I shows that, in the presence of antimycin A, NH_4Cl, at concentrations similar to those of NO_2^- used in the present study, has no significant effect on photosynthetic activities. This suggests that the effect of NO_2^- in restoring photosynthetic activities (inhibited by antimycin A) is primarily linked to its Fd-dependent reduction by NO_2^- reductase and the consequent generation of ATP via linear electron transport with no NADPH formation. This suggestion is supported by the evidence that oxaloacetate was equally effective in restoring photosynthetic activities inhibited by antimycin A (Fig. 5).

The inhibition of photosynthesis by antimycin A was observed over a wide range of $NaHCO_3$ concentrations (Fig. 6), light intensities (Fig. 7), and temperatures (Fig. 8). In all cases, the

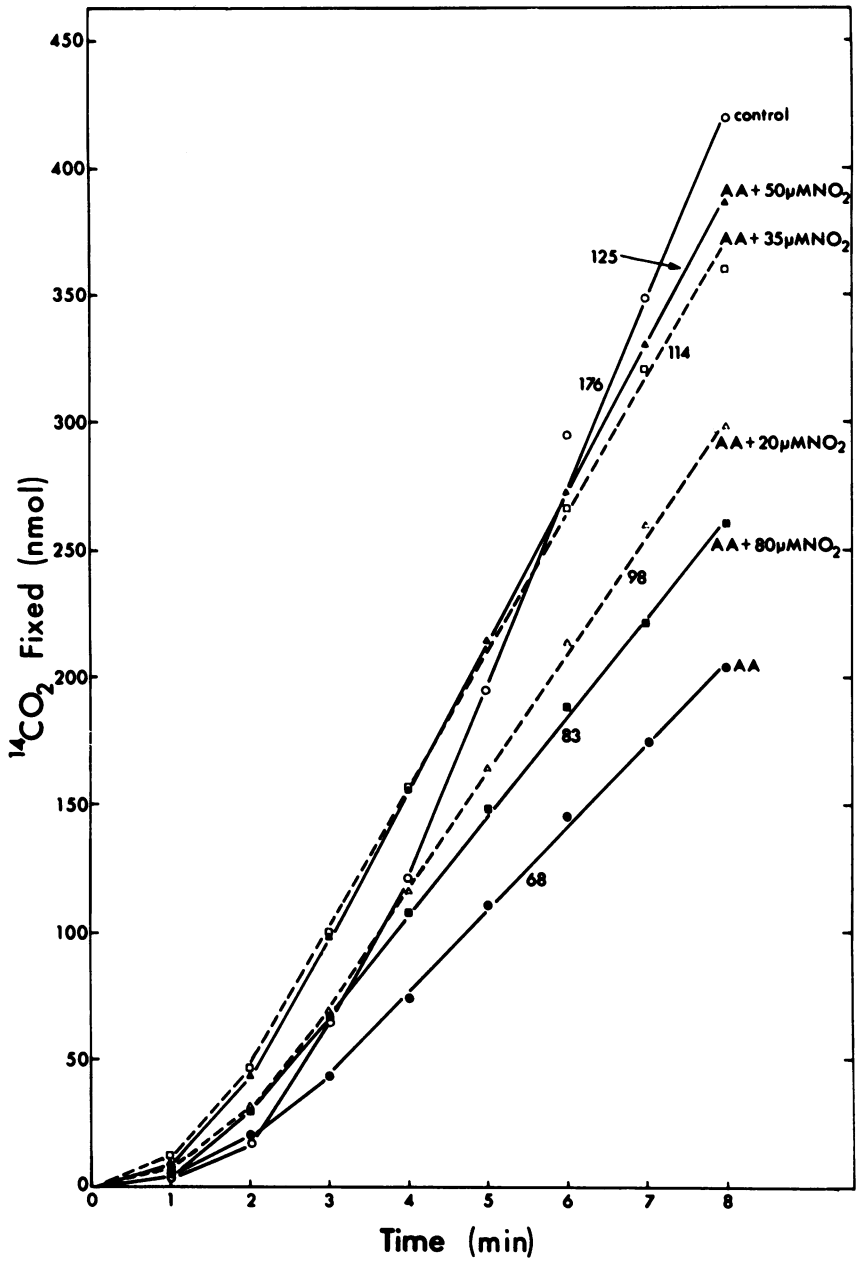

FIG. 3. Time course of $^{14}CO_2$ fixation in illuminated spinach chloroplasts (28 μg Chl) at different NO_2^- concentrations. The concentration of antimycin A (AA) used was 2 μM. The values beside traces are rates of $^{14}CO_2$ fixation in μmol mg^{-1} Chl h^{-1}.

inhibition by antimycin A was relieved by NO_2^- (data for temperature not shown). The extent of the inhibition by antimycin A was relatively similar over the range of $NaHCO_3$ concentrations examined (Fig. 6). The relative inhibition at low light was substantially greater than that observed at saturating light intensity (Fig. 7). In contrast, Heber *et al.* (14) have shown that the inhibition of photosynthesis in intact chloroplasts by antimycin A at concentrations up to 5 μM was substantially lower at 12 than at 245 w m^{-2}. The reasons for these differences are not known.

The substantial increase in photosynthetic O_2 uptake observed at saturating CO_2 with increasing O_2 concentrations in intact chloroplasts (9, 13) suggests an increasingly greater role for pseudocyclic electron transport at higher (>80 μM) O_2 concentrations. But no corresponding measurements of $^{14}Co_2$ fixation were made in these studies. In experiments where low Chl concentrations were used (<3 μg Chl/ml) to minimize changes in O_2 concentration during photosynthesis, we found that the rate of $^{14}CO_2$ fixation in intact chloroplasts at saturating CO_2 remained relatively unchanged between 35 and 100 μM O_2, but this activity decreased by about 20% between 100 and 240 μM O_2 (data not shown). Under conditions where there is no increase in photosynthesis (and ATP requirements), to maintain the proper ATP/NADPH balance any increase in pseudocyclic electron transport would presumably be balanced by an equivalent decrease in cyclic flow. Figure 9 shows that there was relatively little change in the extent of the inhibition of photosynthetic $^{14}CO_2$ fixation and the steady-state rate of O_2 evolution by antimycin A over a wide range of O_2 concentrations in the assay medium. Furthermore, even at 240 μM O_2 where pseudocyclic O_2 uptake is saturated (9, 13), the addition of oxaloacetate restored more than 90% of the control activities (Fig. 10). The above evidence suggests that cyclic flow predominates even at O_2 concentrations which would normally support maximum rates of pseudocyclic O_2 uptake.

Table II shows the effect of antimycin A on light-dependent photosynthetic O_2 exchanges in intact chloroplasts. O_2 uptake was stimulated by about 25% at 2 μM antimycin A when total and net

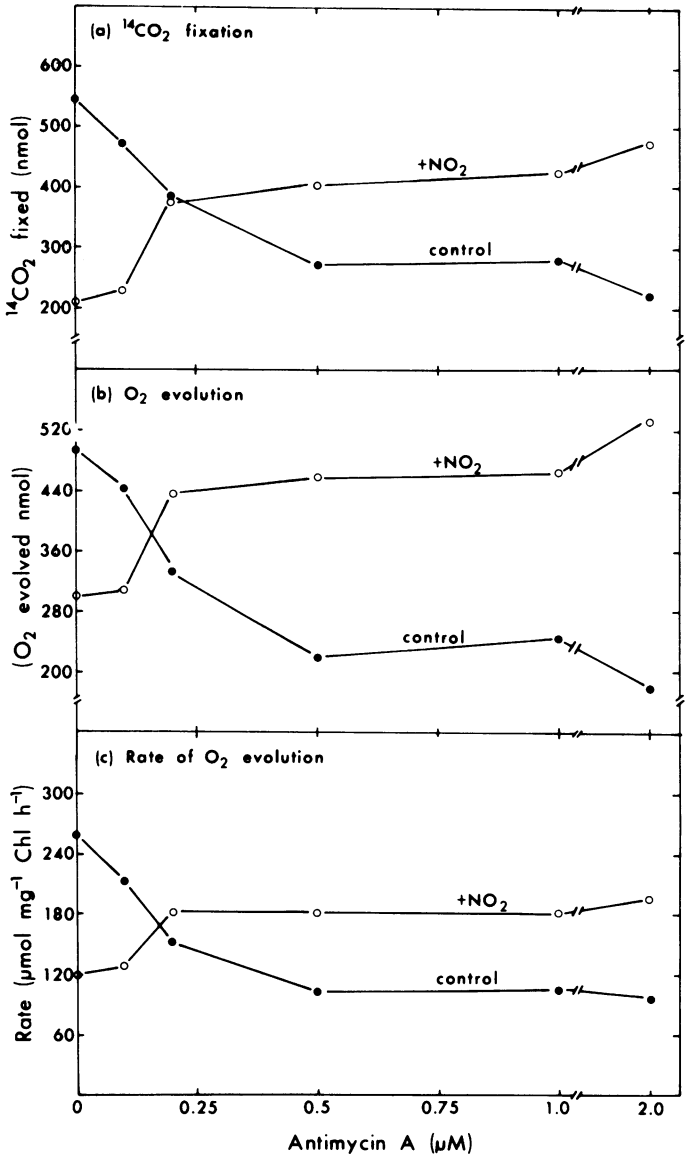

FIG. 4. Effect of antimycin A on (a) $^{14}CO_2$ fixation, (b) O_2 evolution, and (c) steady-state rate of O_2 evolution in the absence (●) and presence (○) of NO_2^- (50 μM) in spinach chloroplasts (73 μg Chl) after 3 min of photosynthesis.

Table I. *Effect of NH_4Cl on Photosynthetic O_2 Evolution in the Presence of Antimycin A (2 μM) in Spinach Chloroplasts (55 μg Chl) after 4 Minutes in the Light*

NH_4Cl	O_2 Evolved	Rate of O_2 Evolution
μM	nmol	μmol/mg Chl·h
0	161	64
10	166	60
40	169	60
100	152	52

O_2 evolution were inhibited by 48 and 76%, respectively. At 2 μM antimycin A, O_2 uptake was 67% of total O_2 evolution. Similar results were obtained with isolated cells although a higher (5- to 10-fold) concentration of antimycin A was required for equivalent inhibition of photosynthesis (data not shown). Evidently, most of this O_2 uptake is not linked to pseudocyclic electron transport and ATP generation because a rate of pseudocyclic O_2 uptake of only about 12% of total O_2 evolution would have been sufficient to generate all the extra ATP needed to maintain an ATP/NADPH

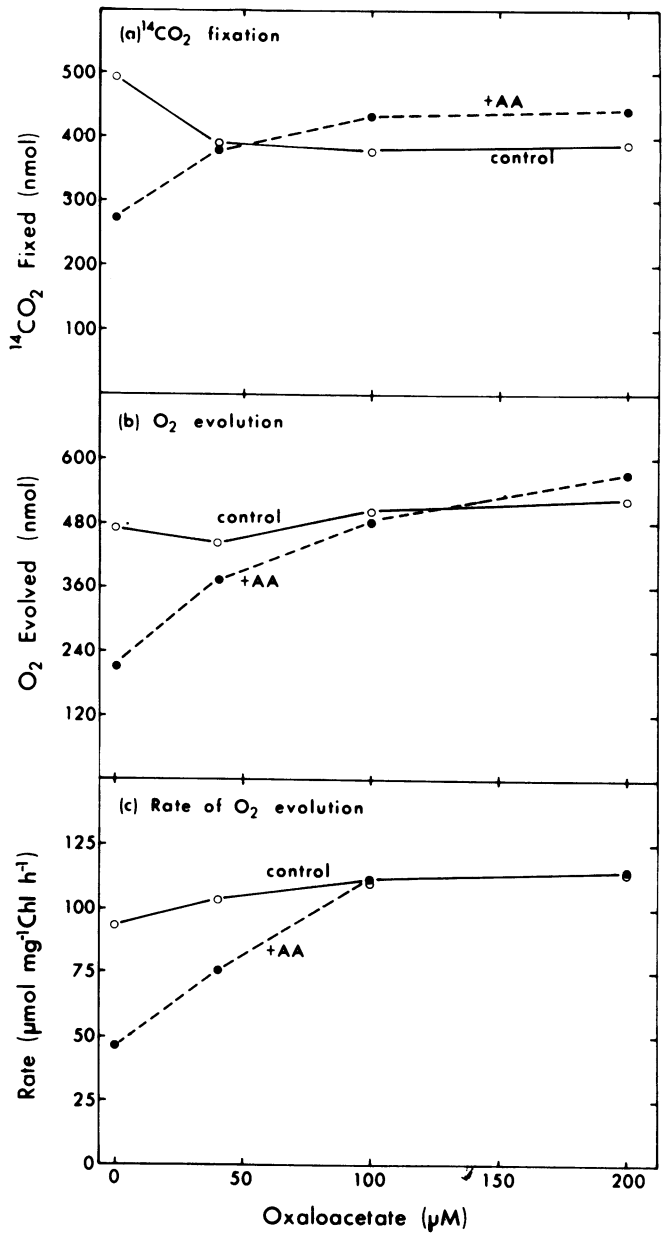

FIG. 5. Effect of oxaloacetate on (a) $^{14}CO_2$ fixation, (b) O_2 evolution, and (c) steady-state rate of O_2 evolution in the absence (○) and presence (●) of antimycin A (AA) in spinach chloroplasts (55 μg Chl) after 6 min of photosynthesis. O_2 concentration at start of experiment was 35 μM.

ratio of 1.5 for optimum photosynthesis in chloroplasts if it is assumed that the ratio of ATP/NADPH generation via linear electron transport is 1.33. Thus, although high rates of O_2 uptake were observed, the rate of pseudocyclic flow appeared to be minimal and ineffective in supporting photosynthesis in intact chloroplasts under the conditions employed in these studies.

DISCUSSION

This study shows that low concentrations (<4 μM) of antimycin A had little effect on coupled or uncoupled linear electron transport (Fig. 1) but strongly inhibited photosynthetic activities in intact chloroplasts (Fig. 4). NO_2^- or oxaloacetate (Figs. 2, 3, and 5), but not NH_4Cl (Table I), restored these activities. The higher rates of O_2 evolved compared to $^{14}CO_2$ fixed in the presence of NO_2^- in all the experiments reported in this study (*e.g.* Figs. 6, a and b, and 7, a and b) indicate the reduction of NO_2^- in these

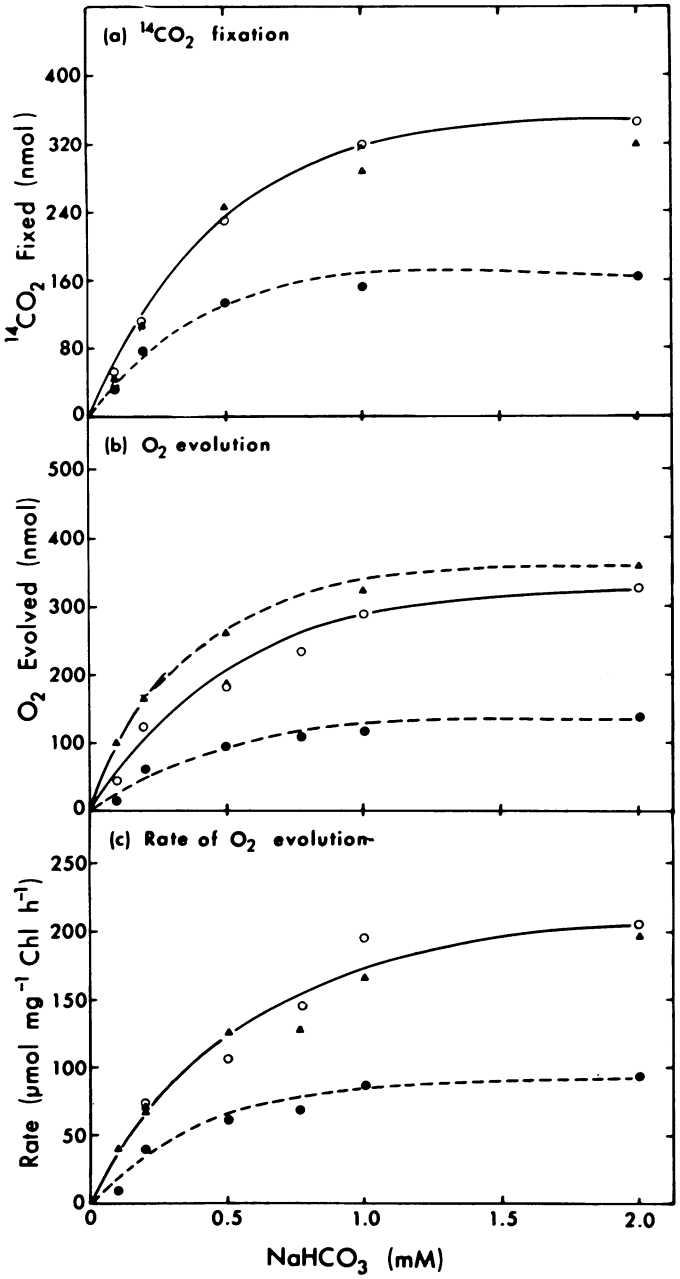

FIG. 6. Effect of NO_2^- (60 μM) and antimycin A (2 μM) on (a) $^{14}CO_2$ fixation, (b) O_2 evolution, and (c) steady-state rate of O_2 evolution in spinach chloroplasts (42 μg Chl) after 4 min photosynthesis at different concentrations of $NaH^{14}CO_3$ (0.1–0.5 mM and 0.8–2 mM were 8.9 and 1.2 Ci/mol, respectively). (○), No addition; (●), antimycin; (▲), antimycin and NO_2^-.

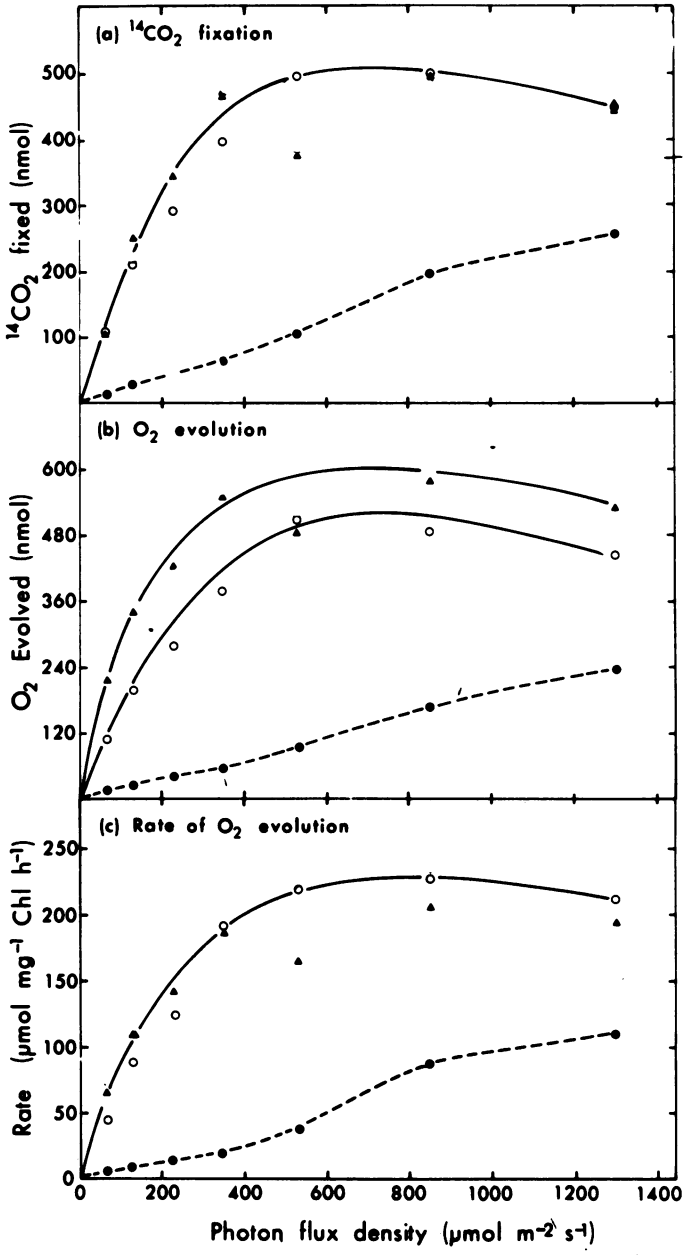

FIG. 7. Effect of NO_2^- (60 μM) and antimycin A (2 μM) on (a) $^{14}Co_2$ fixation, (b) O_2 evolution, and (c) steady-state rate of O_2 evolution in spinach chloroplasts (56 μg Chl) after 4 min photosynthesis at different light intensities. (○), No addition; (●), antimycin; and (▲), antimycin and NO_2^-.

chloroplast preparations in the presence of antimycin A. The simplest explanation for these observations is that antimycin A primarily inhibited cyclic electron flow and caused a decrease in the supply of ATP for photosynthesis (4, 14, 20, 23). However, the reduction of NO_2^- or oxaloacetate via linear electron flow would lead to O_2 evolution and a net generation of ATP without the formation of NADPH and thus would restore the ATP/NADPH balance. Consequently, photosynthesis was restored.

The extensive inhibition (60–80%) of photosynthesis by antimycin A and the subsequent restoration of these activities by NO_2^- and oxaloacetate over a wide range of experimental conditions of $NaHCO_3$ (Fig. 6), light intensity (Fig. 7), and temperature (Fig. 8) indicate that the cyclic electron transport pathway is the predominant energy-balancing reaction during photosynthesis in intact chloroplasts. Inasmuch as substantial activity of cyclic photophosphorylation remains at 2 μM antimycin A (31), the actual contribution of cyclic electron transport may be even higher than that suggested by the above values.

The evidence for the involvement of pseudocyclic electron transport in photosynthesis is based mainly on the observation of high rates of O_2 uptake measured during photosynthesis at saturating CO_2 in chloroplasts, cells, and leaves (6, 8–10). It is generally assumed that most of this O_2 uptake is Fd-dependent and linked to ATP synthesis. However, net O_2 evolution in intact chloroplasts (and cells) was strongly inhibited by antimycin A, whereas O_2 uptake was stimulated by it (Table II). Furthermore, antimycin A had little effect on Fd-dependent O_2 uptake in thylakoids when O_2 was the only subtrate used (R. T. Furbank and M. R. Badger,

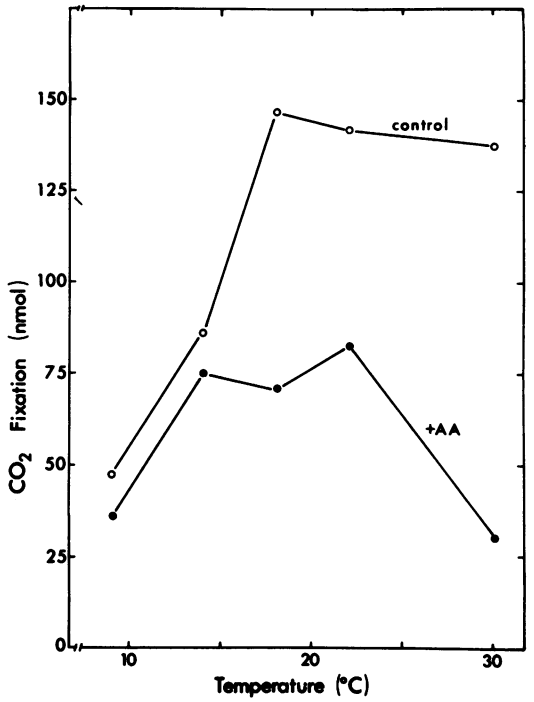

FIG. 8. Effect of temperature on the inhibition of $^{14}Co_2$ fixation by antimycin A (AA) in intact chloroplasts (56 μg Chl) after 6 min of photosynthesis.

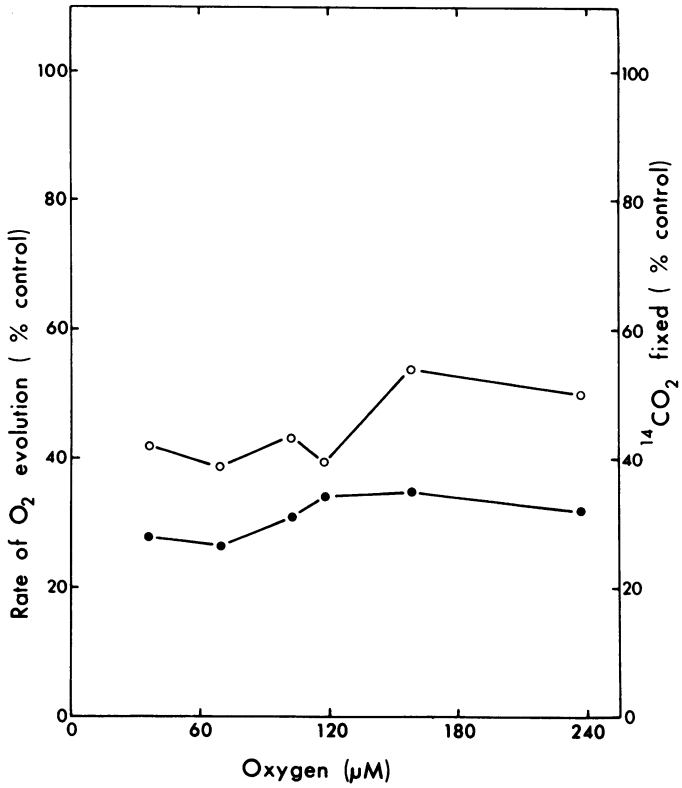

FIG. 9. Effect of antimycin A (2 μM) on $^{14}CO_2$ fixation (●) and the steady-state rate of O_2 evolution (○) in spinach chloroplasts after 5 min photosynthesis at different O_2 concentrations. The results are the average of three different experiments containing 6 to 9 μg Chl in 2.8 ml assay medium, and the O_2 concentrations represent the concentrations present at the start of the experiments.

unpublished). Thus, the O_2 uptake measured in isolated cells and chloroplasts (Table II; Refs. 8 and 9) overestimates true rates of pseudocyclic electron transport. For example, the rate of pseudocyclic electron transport needed to totally support the measured rate of photosynthesis at 2 μM antimycin A (Table II) would only be about 7 μmol mg^{-1} Chl h^{-1}. The value of O_2 uptake measured was 38.2 μmol mg^{-1} Chl h^{-1}. This is more than 5-fold greater (4-fold if corrected for dark rate) than the maximum rate of pseudocyclic flow required. In isolated cells, the measured value was about 4-fold greater than required (data not shown). Evidently, a very large proportion (>80%) of the O_2 uptake measured in cells and chloroplasts reported here and elsewhere (8, 9) is not linked to ATP synthesis. In contrast, Marsho *et al.* (18) have found that, at saturating CO_2, O_2 uptake was only 2 to 3% of O_2 evolution in spinach chloroplasts and cells. Similarly, Heber *et al.* (14) have shown that in spinach chloroplasts there was little change in O_2 uptake at saturating CO_2 at light intensities ranging from 9 to 84 w m^{-2} when net photosynthetic O_2 evolution increased from 16 to 67 μmol mg^{-1} Chl h^{-1}. The nature of the light-dependent but Fd-independent O_2 uptake observed in cells and chloroplasts (Table II) remains to be determined. It is also uncertain whether the high rates of O_2 uptake measured during photosynthesis at saturating CO_2 in leaves (6, 10) are qualitatively different from those measured in chloroplasts and cells.

Studies in thylakoids indicate that high rates of O_2 uptake can occur at high concentrations of Fd and O_2 (1), but this activity was strongly inhibited by low (μM) concentrations of $NADP^+$ (R. T. Furbank and M. R. Badger, unpublished). The K_i ($NADP^+$) determined was about 8 to 15 μM. Antimycin A greatly decreased the $NADP^+$ level in chloroplasts (27) which presumably would favor Fd-dependent O_2 uptake. The observed increase in O_2 uptake in chloroplasts (Table II) and cells in the presence of antimycin A may partly reflect such an increase in this activity. However, the ineffectiveness of high O_2 concentrations to restore photosynthesis inhibited by antimycin A (Fig. 9), compared to the positive effect of oxaloacetate at 240 μM O_2 (Fig. 10), indicates only a minimal contribution of pseudocyclic electron flow to ATP production in intact chloroplasts even under conditions (high Fd and low $NADP^+$ level) which presumably would have been favorable to such pseudocyclic activity. Under conditions of high level of $NADP^+$ (10–25 nmol/mg Chl) observed in illuminated chloroplasts (15, 27), this activity would presumably be further suppressed during photosynthesis in intact chloroplasts. Thus, although significant rates of Fd-dependent O_2 uptake can occur under favorable conditions *in vitro*, the above evidence indicates that the physiological conditions prevailing in intact chloroplasts (and cells) do not appear to support significant activity of pseudocyclic O_2 uptake *in vivo*.

O_2 is involved in the regulation of cyclic electron flow (14, 17, 31, 32) presumably by preventing the overreduction of the electron transport components involved. Electron transport to O_2 would generate ATP (18), but the experiments of Ziem-Hanck and Heber (32) showed that only a minute quantity of O_2 at the start was sufficient to poise cyclic electron flow and enable photosynthesis to proceed. Hence, the generation of ATP in this manner does not appear to contribute significantly to photosynthesis. NO_2^- could presumably also influence cyclic flow in a similar manner. The effects of small quantities of NO_2^- on photosynthesis (Fig. 2) might conceivably reflect the balance between the poising of cyclic flow by NO_2^- and the drainage of electrons from $NADP^+$ for NO_2^- reduction. The observed stimulation of photosynthesis by NO_2^- in isolated spinach cells (29) may also partly reflect the involvement of similar processes. High concentrations (2 mM) of NO_2^- have been shown to inhibit photosynthesis in intact chloroplasts by reducing the stromal pH (21). However, the low concentrations of NO_2^- used in the present study are unlikely to have much effect on the stromal pH.

NO_2^- was found to reduce the lag phase of photosynthesis in the absence or presence of antimycin A (Figs. 2 and 3). This

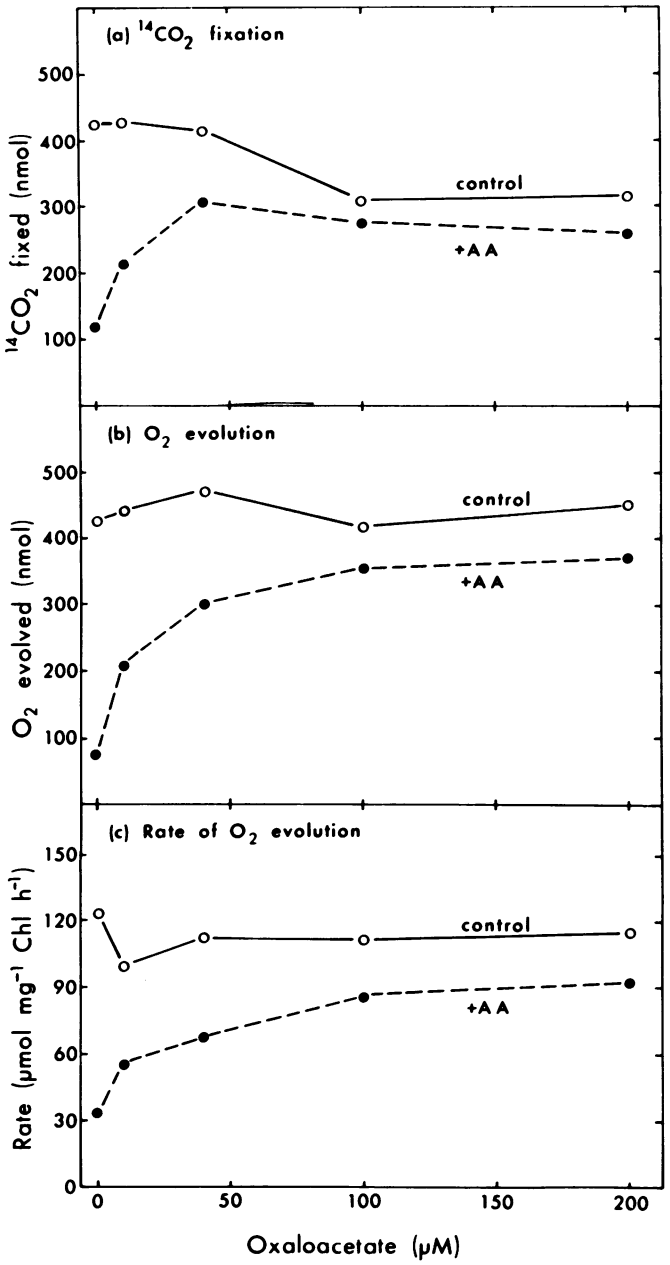

FIG. 10. Effect of oxaloacetate on (a) $^{14}CO_2$ fixation, (b) O_2 evolution, and (c) the steady-state rate of O_2 evolution in spinach chloroplasts (55 μg Chl) in the absence (○) and presence (●) of antimycin A (AA) after 6 min photosynthesis in 240 μM O_2. The concentrations of antimycin A and $NaH^{14}CO_3$ used were 2 μM and 5 mM, respectively.

suggests that ATP is indeed a limiting factor during this period. A high flash yield of Cyt *f* turnover and a pronounced rise in the slow 518 nm *A* changes during this period had been used to suggest that cyclic electron transport was involved to provide the extra ATP needed (26). However, recent studies have shown that, during the lag phase, $NADP^+$ reduction (and electron transport) was restricted by the availability of $NADP^+$ and resulted in the build-up of a large proton gradient (27). Under these conditions, the reduction of NO_2^- would, presumably, alleviate this proton back pressure, increase ATP turnover and electron transport, and thereby shorten the lag phase.

Figure 7 shows that, at saturating CO_2, cyclic photophosphorylation contributes ATP to photosynthesis from very low to saturating light intensities. On the other hand, Heber *et al.* (14) concluded that cyclic photophosphorylation was involved mainly

Table II. *Effect of Antimycin A on Photosynthetic O_2 Uptake and Evolution in Isolated Spinach Chloroplasts (5 μg Chl/ml) in the Presence of $NaHCO_3$ (10 mM)*

O_2 concentration at start of experiment was 80 to 95 μM.

Antimycin A	O_2 Exchanges		
	Uptake	Total Evolution	Net Evolution
μM		*μmol/mg Chl·h*	
Light			
0	30.6	109.1	78.5
0.05	32.2	90.7	58.5
0.1	35.0	80.7	45.7
0.2	36.3	71.3	35.0
2.0	38.2	56.9	18.7
Dark	8.9	−0.3	−9.2

at moderate and high light intensities, whereas the evidence in the reviews of Simonis and Urbach (24) and Gimmler (11) indicated that cyclic photophosphorylation was saturated at low light intensities. The reasons for these discrepancies are not known, but might be partly related to the different experimental conditions used. For example, red light was used in the experiments of Heber *et al.* (14) while the uptake of phosphate had been used as an indicator reaction in the evidence discussed by Simonis and Urbach (24) and Gimmler (11).

The reported stimulation of photosynthesis in chloroplasts by antimycin A under anaerobic and aerobic conditions (19, 22, 25, 28) indicates the complex nature of the direct and indirect effect of antimycin A on photosynthetic carbon metabolism. However, like Heber *et al.* (14), we have never observed stimulation of photosynthesis by antimycin A in intact chloroplasts. Thus, the inhibitory effect of antimycin A reported in this study and elsewhere (14, 20) could be attributed primarily to the inhibition of cyclic electron flow. In mitochondria, antimycin A inhibition is associated with binding of the inhibitor to *b*-type Cyt (5). The inhibition of cyclic electron flow by antimycin A in thylakoids (2), the demonstration of a high binding site in chloroplasts for antimycin A (20), and the partial inhibition of oxidoreductase activity in the isolated Cyt f/b_6 complex from spinach leaves by antimycin A (16) suggest a similar interaction between antimycin A and Cyt b_6.

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