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Stimulation of Ammonia and 2-Oxoglutarate-Dependent O_2 Evolution in Isolated Chloroplasts by Dicarboxylates and the Role of the Chloroplast in Photorespiratory Nitrogen Recycling

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ABSTRACT

Intact chloroplasts isolated from spinach (*Spinacia oleracea* L.) leaves showed a light-dependent O_2 evolution (5.5 ± 0.75 micromoles per milligram chlorophyll per hour) when supplied with ammonia and 2-oxoglutarate. This (ammonia, 2-oxoglutarate)-dependent O_2 evolution was stimulated 2- to 4-fold by the dicarboxylates, malate, succinate, fumarate, glutarate, and L-tartarate. Evolution of O_2 in the presence of malate was dependent on the presence of both 2-oxoglutarate and NH_4Cl; malate with only either 2-oxoglutarate and NH_4Cl alone did not support O_2 evolution. Furthermore, in the presence of malate, the amount of O_2 evolved was solely dependent on the amount of NH_4Cl or 2-oxoglutarate added and malate did not affect the ratio of O_2 evolved to NH_4Cl or 2-oxoglutarate consumed. Studies with inhibitors (2-(3,4-dichlorophenyl)-1,1-dimethyl urea, methionine sulfoximine, and azaserine) indicated that the above activity was directly linked to glutamine synthetase and glutamate synthase activity in the chloroplast and was not caused by the metabolism of malate. The $V_{max}/2$ of (ammonia, 2-oxoglutarate)-dependent O_2 evolution was reached at 32 micromolar NH_4Cl and 6 millimolar (approximately) 2-oxoglutarate in the absence of malate, and at 22 micromolar NH_4Cl and 73 micromolar 2-oxoglutarate when malate (3 millimolar) was present.

Intact chloroplasts isolated from pea (*Pisum sativum*) leaves also showed a stimulation of (ammonia, 2-oxoglutarate)-dependent O_2 evolution by malate. However glutamine was required for this activity even though glutamine with only either NH_4Cl or 2-oxoglutarate did not respond to malate stimulation.

The measured rates of (ammonia, 2-oxoglutarate)-dependent O_2 evolution in isolated spinach chloroplasts in the presence of malate were about 19.5 ± 4.5 micromoles O_2 evolved per milligram chlorophyll per hour. This is adequate to sustain photorespiratory NH_3 recycling and the refixation of NH_3 arising from NO_3 under ambient conditions in the light. The role of the chloroplast in photorespiratory NH_3 recycling and the nature of the associated transport of 2-oxoglutarate into the chloroplast is discussed.

The GS[1]/GOGAT pathway is the primary route of NH_3 assimilation in leaves of higher plants (13, 16). In the chloroplast, photosynthetic electron transport provides the ATP and reduced ferredoxin required for GS/GOGAT activity (13). During photosynthesis in C_3 plants, the refixation and recycling of photorespiratory NH_3 released during mitochondrial glycine decarboxylation (25) involve this pathway (12, 26). However, studies with a reconstituted system have shown that NH_3 released during glycine decarboxylation in isolated mitochondria could be reassimilated into glutamine in the presence of glutamine synthetase (12). In protoplasts from *Pisum sativum*, 40% of the total GS activity was found to be associated with the cytosolic fraction (23). For this reason, it has been proposed that the initial photorespiratory NH_3 refixation involves the cytosolic GS, while the subsequent reduction of glutamine by GOGAT takes place in the chloroplast (12, 22). If this is the case, the rate of glutamine transport into the chloroplast must, at least, be equivalent to the rate of photorespiratory NH_3 release. Recent studies indicate that the rate of glutamine transport into chloroplasts as mediated by the dicarboxylate carrier could be limiting (3), and might be inadequate to support photorespiratory NH_3 refixation under physiological conditions. On the other hand, NH_3 could presumably move freely from mitochondria to chloroplasts under conditions of high NH_3 flux in the leaves during photorespiration. If this were so, part of the photorespiratory NH_3 would be reassimilated in the chloroplast.

The transport and supply of 2-OG is an integral part of the GS/GOGAT pathway, irrespective of whether the chloroplast or cytosolic GS is involved in the initial NH_3 assimilation. The chloroplast inner envelope contains a dicarboxylate carrier with wide substrate specificity (7, 10), and, presumably, this carrier is involved in the transport of 2-OG into the chloroplast. But the nature of this 2-OG transport that is linked to NH_3 assimilation via the GS/GOGAT pathway remains to be determined.

The chloroplast also contains a $NADP^+$-specific glutamate dehydrogenase (14), but evidence shows that, in pea chloroplasts, NH_3 assimilation in the presence of glutamate (17) and 2-OG occurred via the GS/GOGAT pathway (1, 2). Furthermore, this activity could be directly linked to photosynthetic O_2 evolution, with 0.5 mol O_2 evolved/mol NH_3 and 2-OG fixed (1).

In this study, we have reexamined (NH_3, 2-OG)-dependent O_2 evolution in isolated spinach and pea chloroplasts, to evaluate and determine the role of the chloroplast for photorespiratory NH_3 assimilation and the role of the dicarboxylate carrier for 2-OG transport directly linked to photorespiratory NH_3 assimilation. This study reports the stimulation of (NH_3, 2-OG)-dependent O_2 evolution by dicarboxylic acids. The nature and significance of this stimulation by dicarboxylates in relation to 2-OG transport and the photorespiratory NH_3 pathway is discussed.

MATERIALS AND METHODS

Plants were grown in a glasshouse under natural daylight. Only young leaves were used for chloroplast isolation. Spinach and pea chloroplasts were isolated according to the procedures of Heber (8) and Anderson and Done (2), respectively. O_2 evolution was

[1] Abbreviations: GS, glutamine synthetase; GOGAT, glutamate synthase; 2-OG, 2-oxoglutarate; MSO, methionine sulfoximine; OAA, oxaloacetate; FCCP, *p*-trifluoromethoxy (carbonylcyanide) phenyl hydrazone.

measured at 25°C with an O_2 electrode (Rank Bros., Cambridge, England). The standard assay medium contained 0.33 M sorbitol, 50 mM Hepes-NaOH (pH 7.6), 0.5 mM K_2HPO_4/KH_2PO_4, 1 mM $MnCl_2$, 2 mM EDTA, and catalase (600 U) in a total volume of 3 ml.

For CO_2-dependent O_2 evolution, the assay also contained 10 mM $NaHCO_3$, 5 mM NaP_2O_7, and 1 mM $MgCl_2$ and measurements were made at 1,300 $\mu E\ m^{-2}s^{-1}$. And for measurements of (NH_3, 2-OG)-dependent O_2 evolution, the assay medium also contained 10 mM malate plus 10 mM DL-glyceraldehyde to inhibit endogenous O_2 evolution (19). Measurements were made at 600 $\mu E\ m^{-2}s^{-1}$. Chloroplast intactness was determined by the ferricyanide reduction test (9). All acid compounds were adjusted to pH 7.0 with NaOH before use.

RESULTS

Chloroplasts isolated from spinach leaves showed a light-dependent O_2 evolution when supplied with NH_4Cl and 2-OG (trace A, Fig. 1). There was an initial fast rate which lasted 1 to 2 min followed by a slower steady rate. In contrast, Anderson and Done (1) observed a long lag period of about 10 min for (NH_3, 2-OG)-dependent O_2 evolution (8.3 μmol mg^{-1} Chl h^{-1}) in isolated pea chloroplasts. These authors also reported a high rate of O_2 evolution (10.6 μmol mg^{-1} Chl h^{-1}) with glutamine and 2-OG (2). But, in spinach chloroplasts (Gln, 2-OG)-dependent O_2 evolution is low (2–3 μmol mg^{-1} Chl h^{-1}) and glutamine did not affect (NH_3, 2-OG)-dependent O_2 evolution significantly (trace B and C, Fig. 1). On the other hand, malate increased (NH_3, 2-OG)-dependent O_2 evolution dramatically, both in the absence and in the presence of glutamine (traces D and E, Fig. 1). The above activity was sensitive to DCMU and FCCP (trace C and D, Fig. 1). The inhibition by DCMU indicates the involvement of photosynthetic electron transport for the observed (NH_3, 2-OG)-dependent O_2 evolution. The inhibition by FCCP was apparently not related to its uncoupling effect on photosynthetic electron transport since the presence of glutamine and 2-OG would have supported O_2 evolution under these conditions. Rather, the inhibitory effect of FCCP suggests the requirement of a membrane potential or proton transport across the chloroplast inner envelope presumably for the transport of the metabolites involved. Such a requirement could be associated with the ATPase activity reported to be present in chloroplast envelope preparations from spinach leaves (6).

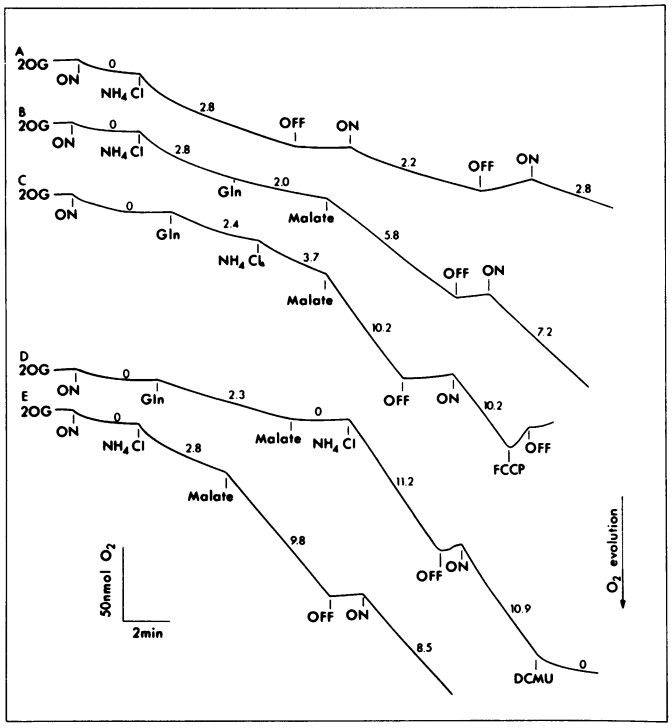

FIG. 1. Effect of glutamine, malate, DCMU, and FCCP on (NH_3, 2-OG)-dependent O_2 evolution in intact spinach chloroplasts. Standard assays containing 2 mM 2-OG were used. Additions: NH_4Cl, 1 mM; glutamine, 5 mM; malate, 3 mM; DCMU, 10 μM; FCCP, 10 μM. Values beside curves are rates of O_2 evolution in μmol mg^{-1} Chl h^{-1}. Chloroplasts containing 124 μg Chl were used in each assay; intactness, 65%. Rate of CO_2-dependent O_2 evolution of chloroplast preparation was 129 μmol mg^{-1} Chl h^{-1}.

Figure 2 shows the response of (NH_3, 2-OG)-dependent O_2 evolution in spinach chloroplasts to increasing malate concentration. Maximum stimulation was achieved by about 2 mM malate and the concentration required for half-maximal velocity was about 0.3 M. The stimulation by malate required the presence of both NH_4Cl and 2-OG (Fig. 3). Malate, with either NH_4Cl or 2-OG alone, did not support O_2 evolution (traces C and D, Fig. 3). This observation suggests that the stimulation of O_2 evolution by malate was associated directly with the metabolism of NH_3 and 2-OG in the chloroplast *per se* and was not caused by the metabolism of malate in the chloroplast.

The above suggestion is supported by the evidence that, in the presence of malate and NH_4Cl, the amount of O_2 evolved was determined by the amount of 2-OG present (trace A, Fig. 4). Conversely, in the presence of 2-OG, with or without malate, the amount of O_2 evolved was determined by the amount of NH_4Cl added (traces B and C, Fig. 4). It is assumed that the limiting substrate added was totally consumed when O_2 evolution ceases. Table I shows that there was little difference in the ratio of O_2 evolved to NH_4Cl and 2-OG added either in the presence or absence of malate. These values are comparable to the expected ratio of 0.5 and are similar to those obtained by Anderson and Done (1) in pea chloroplasts.

Figure 5 shows that (NH_3, 2-OG)-dependent O_2 evolution was abolished by MSO and azaserine, which are specific inhibitors of GS and GOGAT, respectively. Malate did not restore O_2 evolution in these chloroplasts. In contrast, O_2 evolution resumed in the presence of OAA, which was presumably reduced to malate by

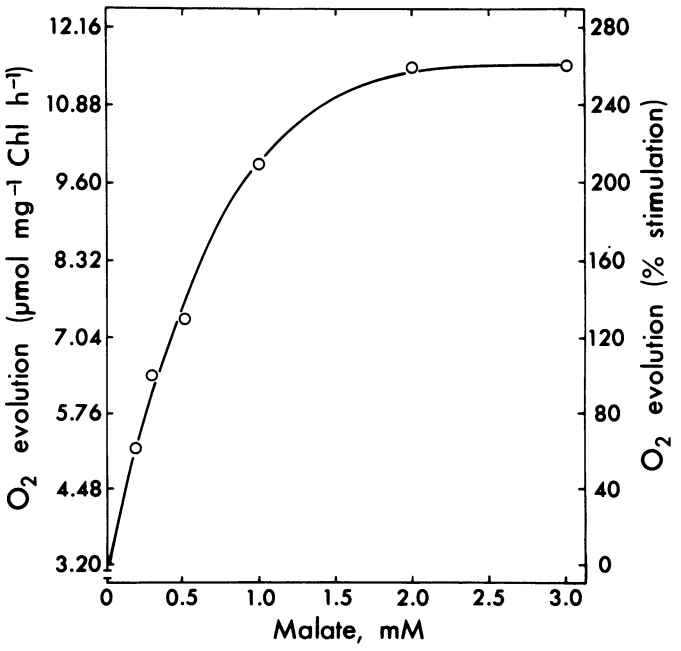

FIG. 2. The response of (NH_3, 2-OG)-dependent O_2 evolution to malate in intact spinach chloroplasts (176 μg Chl; 56% intactness). The activity of control treatments was 2.9 μmol mg^{-1} Chl h^{-1}.

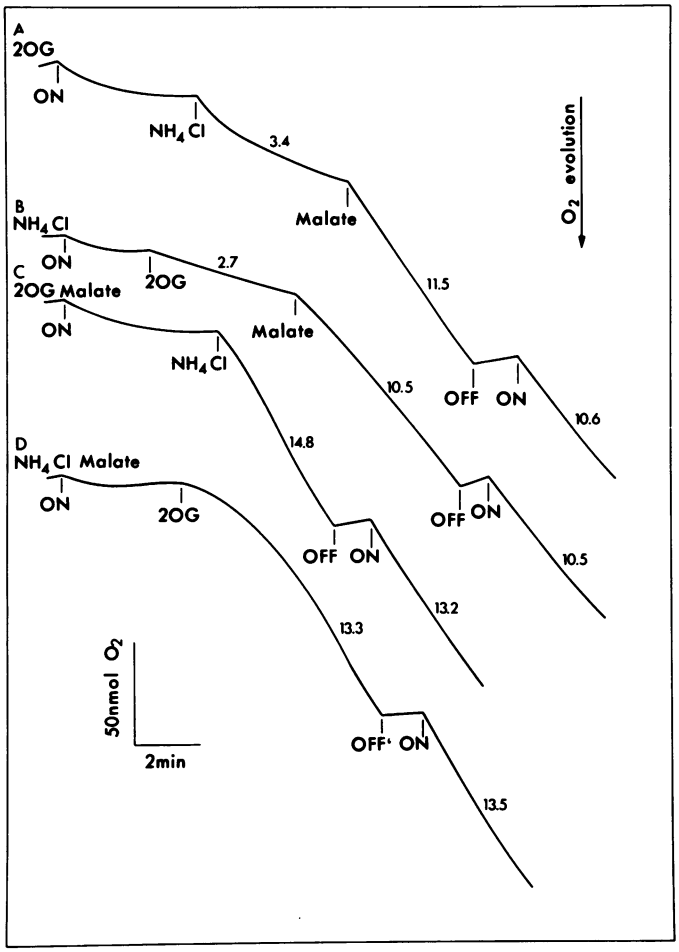

FIG. 3. Requirements for 2-OG and NH_4Cl in the stimulation of (NH_3, 2-OG)-dependent O_2 evolution by malate in intact spinach chloroplasts (116 μg Chl; 75% intactness). Additions: 2-OG, 2 mM; NH_4Cl, 1 mM; malate, 3 mM. Values beside curves are rates of O_2 evolution in μmol mg^{-1} Chl h^{-1}. Rate of CO_2-dependent O_2 evolution of chloroplast preparations was 104 μmol mg^{-1} Chl h^{-1}.

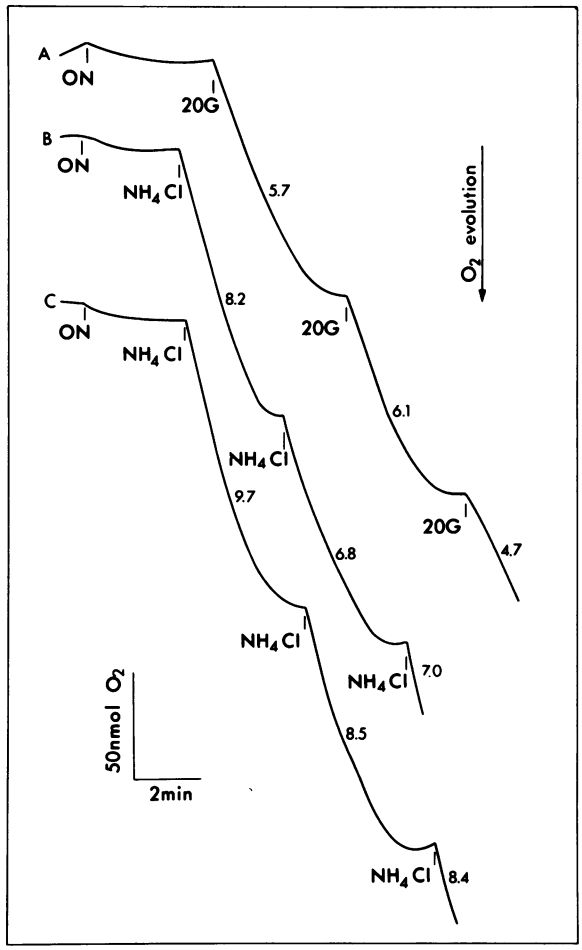

FIG. 4. Effect of limiting (300 nmol) 2-OG and NH_4Cl supply on (NH_3, 2-OG)-dependent O_2 evolution in intact spinach chloroplasts (148 μg Chl; 77% intactness). Trace A contained 1 mM NH_4Cl and 3 mM malate; trace B, 10 mM 2-OG; and trace C, 2 mM 2-OG and 3 mM malate. Values beside curves are rates of O_2 evolution in μmol mg^{-1} Chl h^{-1}. Rate of CO_2-dependent O_2 evolution of chloroplast preparation was 103 μmol mg^{-1} Chl h^{-1}.

$NADP^+$-malate dehydrogenase. These data suggest that MSO and azaserine did not significantly affect either photosynthetic electron transport or malate dehydrogenase activity.

Figure 6 shows the response of (NH_3, 2-OG)-dependent O_2 evolution to NH_4Cl concentration in the presence and absence of malate; the $K_{1/2}$ (NH_4^+) was 32 and 22 μM, respectively. In contrast, the apparent affinity for 2-OG in (NH_3, 2-OG)-dependent O_2 evolution was increased by about two orders of magnitude in the presence of malate compared to treatments in which malate was absent (Fig. 7). This increase in the apparent affinity of (NH_3, 2-OG)-dependent O_2 evolution for 2-OG in the presence of malate could account for the observed stimulation of O_2 evolution by malate. The double reciprocal plot gave a linear slope for the activity determined in the presence of malate and a $K_{1/2}$ (2-OG) of 73 μM. This $K_{1/2}$ (2-OG) value obtained is comparable to a value of 150 μM obtained for partially purified GOGAT from leaves of *Vicia faba* (24). In contrast, a hyperbola was obtained in the set of data for 2-OG determined in the absence of malate. Evidently, the kinetics of the reaction for 2-OG during (NH_3, 2-OG)-dependent O_2 evolution in the absence of malate are very complex. Under these conditions, 2-OG could act both as a substrate and an activator since it could potentially substitute for malate in the dicarboxylate carrier.

(NH_3,2-OG)-dependent O_2 evolution was also stimulated by other dicarboxylates (succinate, fumarate, glutarate, L-tartarate)

Table I. *Relationship of O_2 Evolution to NH_4Cl and 2-Oxoglutarate Consumed in (NH_3,2-OG)-Dependent O_2 Evolution in Isolated Spinach Chloroplasts*

Assays contained 2 mM 2-OG (10 mM 2-OG when malate is not present), 3 mM malate, and 1 mM NH_4Cl when these are present as nonlimiting substrates.

Nonlimiting Substrates	Limiting Substrate	Amount Added (A)	O_2 Evolved (B)	B:A
		nmol		*ratio*
2-OG	1. NH_4Cl	150	58	0.38
	2. NH_4Cl	150	63	0.42
	3. NH_4Cl	300	123	0.41
2-OG + malate	1. NH_4Cl	150	69	0.46
	2. NH_4Cl	150	63	0.42
	3. NH_4Cl	300	131	0.44
NH_4Cl + malate	1. 2-OG	300	107	0.36
	2. 2-OG	300	100	0.33
	3. 2-OG	600	207	0.35

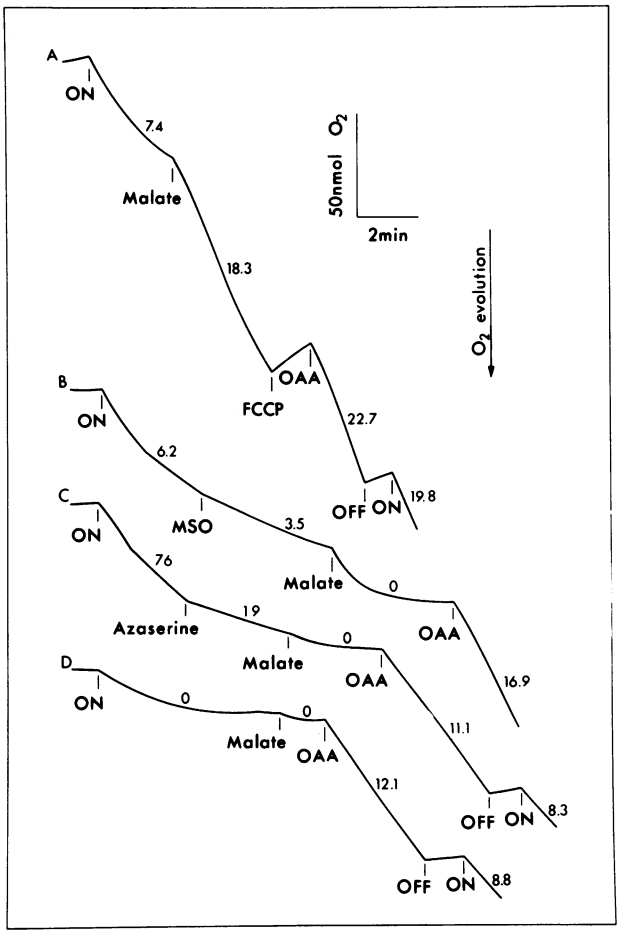

FIG. 5. Effect of MSO and azaserine on (NH_3, 2-OG)-dependent O_2 evolution in intact spinach chloroplasts (116 μg Chl; 75% intactness). All assays contained 2 mM 2-OG and 1 mM NH_4Cl. Additions: malate, 3 mM; OAA, 0.2 mM; FCCP, 10 μM; MSO, 2.5 mM; azaserine, 2.5 mM. For trace D, chloroplasts were preincubated with 0.5 mM azaserine for 10 min in the dark. Values beside curves are rates of O_2 evolution in μmol mg^{-1} Chl h^{-1}. Rate of CO_2-dependent O_2 evolution of chloroplast preparation was 104 μmol mg^{-1} Chl h^{-1}.

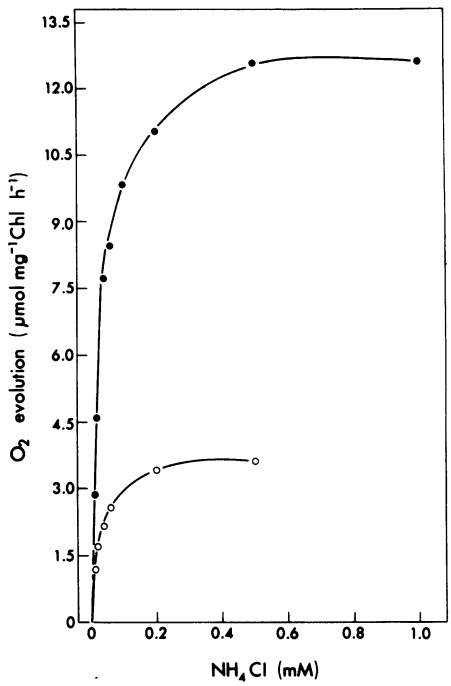

FIG. 6. Response of (NH_3, 2-OG)-dependent O_2 evolution to NH_4Cl in the absence (○) and in the presence (●) of malate in intact spinach chloroplasts (140 μg Chl; 60% intactness). Assays contained 2 mM 2-OG in the absence and presence of malate (3 mM).

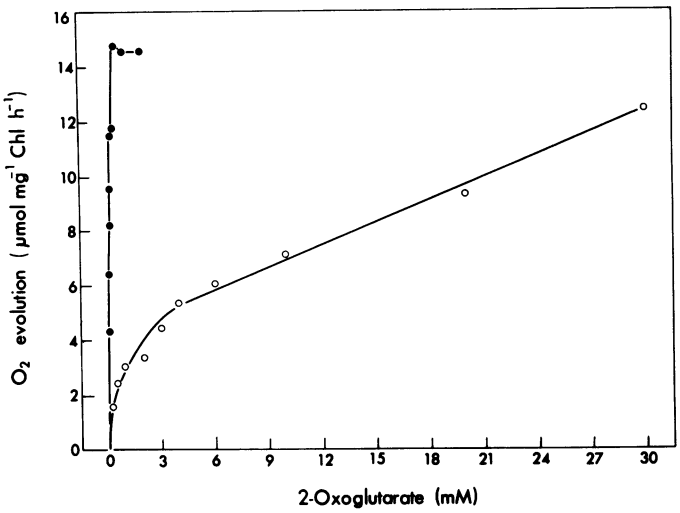

FIG. 7. Response of (NH_3, 2-OG)-dependent O_2 evolution to 2-OG in the absence (○) and in the presence (●) of malate in intact spinach chloroplasts (150 μg Chl; 78% intactness). Assays contained 1 mM NH_4Cl and, when present, 3 mM malate.

to about the same extent as malate (Fig. 8). All these dicarboxylates are transported by the dicarboxylate carrier (10). In contrast, maleate and malonate, which are not transported by the dicarboxylate carrier, had only a very small effect on O_2 evolution (data not shown).

Malate also stimulated (NH_3, 2-OG)-dependent O_2 evolution in isolated pea chloroplasts (Fig. 9). But, in contrast to spinach chloroplasts (Fig. 1), glutamine was required for malate stimulation in these pea chloroplast preparations (trace A, Fig. 9). This requirement for glutamine suggests that these pea chloroplast preparations probably contained a very low level of glutamine and glutamate compared to isolated spinach chloroplasts. There was no increase in (Gln, 2-OG)-dependent O_2 evolution in the presence of malate unless NH_4Cl was also present (trace B, Fig. 9). Presumably, ADP was limiting in the absence of NH_4Cl. As in spinach chloroplasts, the above activity was sensitive to DCMU and FCCP (traces A and B, Fig. 9).

DISCUSSION

Chloroplasts isolated from spinach and pea leaves showed a light-dependent O_2 evolution in the presence of NH_4Cl and 2-OG (and glutamine). This O_2 evolution was sensitive to MSO and azaserine inhibition, indicating that it was directly linked to GS/GOGAT activity in the chloroplast. The dicarboxylates, malate, succinate, fumarate, glutarate, and L-tartarate, stimulated (NH_3, 2-OG)-dependent O_2 evolution 2- to 4-fold. This stimulation was associated with a dramatic increase in the affinity for 2-OG in (NH_3, 2-OG)-dependent O_2 evolution. The apparent $K_{1/2}$ (2-OG) decreased from about 6 mM to 73 μM in the presence of malate. This decrease could in part be due to a stimulation of the transport of 2-OG into the chloroplast. In addition, there is evidence that GS from rat liver was activated by 2-OG (20), but to our knowledge, there is no evidence of such an activation of the GS isolated from plant tissue.

The transport of 2-OG into the chloroplast involves a carrier in the chloroplast inner envelope. In contrast to plant and animal

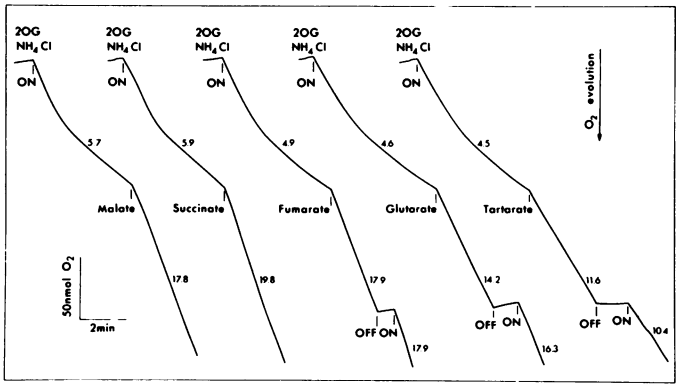

FIG. 8. Stimulation of (NH_3, 2-OG)-dependent O_2 evolution by dicarboxylates in intact spinach chloroplasts (136 μg Chl; 83% intactness). Assays contained 2 mM 2-OG and 1 mM NH_4Cl. Additions: malate, succinate and fumarate, 5 mM; glutarate and L-tartarate, 10 mM. Values beside curves are rates of O_2 evolution in μmol mg^{-1} Chl h^{-1}. Rate of CO_2-dependent O_2 evolution of chloroplast preparation was 148 μmol mg^{-1} Chl h^{-1}.

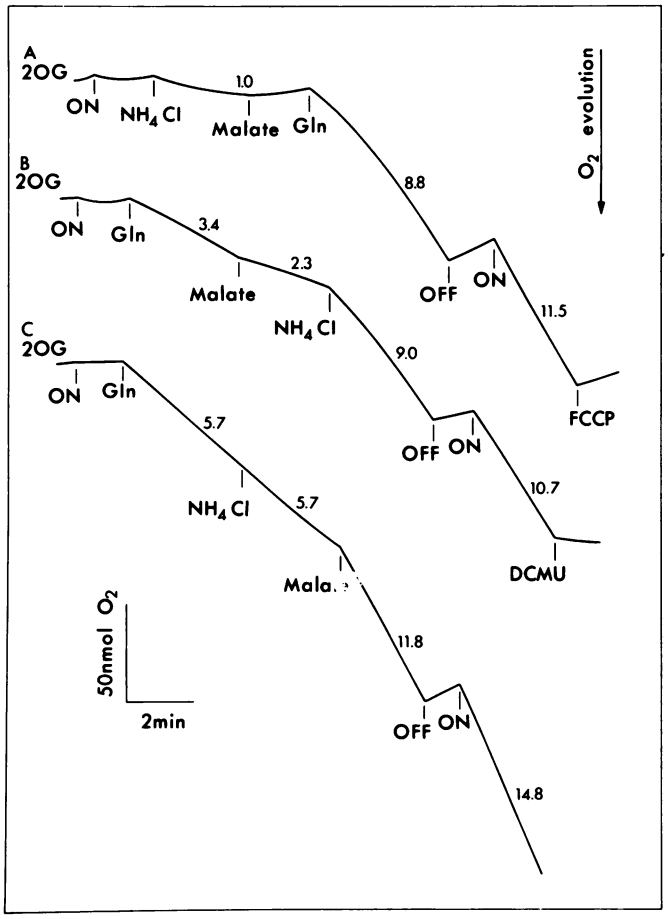

FIG. 9. Effect of malate, glutamine, FCCP, and DCMU on (NH_3, 2-OG)-dependent O_2 evolution in intact pea chloroplasts (156 μg Chl; 65% intactness). All assays contained 2 mM 2-OG and 1 mM ADP. Additions: NH_4Cl, 1 mM; malate, 3 mM; glutamine, 5 mM; DCMU, 10 μM; FCCP, 5 μM. Values beside curves are rates of O_2 evolution in μmol mg^{-1} Chl h^{-1}. Rate of CO_2-dependent O_2 evolution of chloroplast preparation was 66 μmol mg^{-1} Chl h^{-1}.

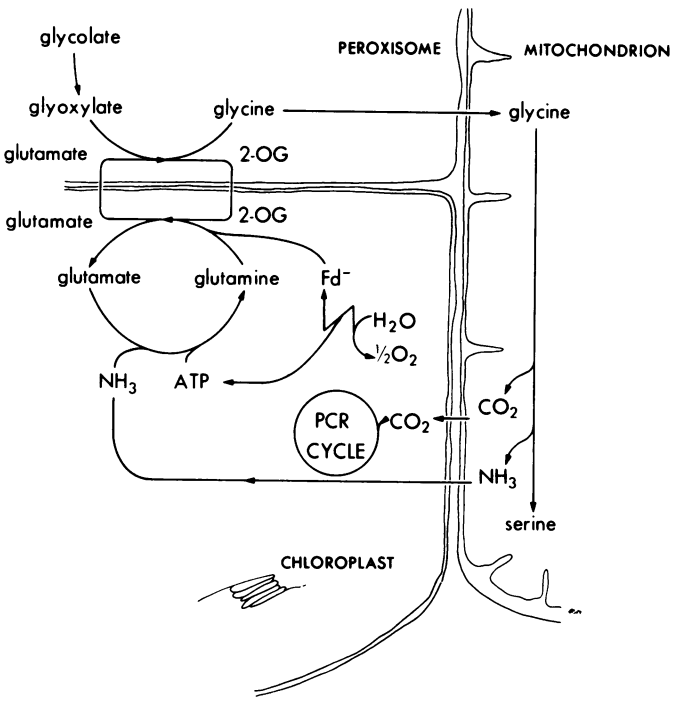

FIG. 10. Proposed pathway for the recovery of photorespiratory NH_3 via the chloroplast glutamine synthetase and glutamate synthase pathway. This recovery is shown coupled, via a (glutamate, 2-OG) shuttle, to the (NH_2) requirements of the peroxisome. Note that only one of 2 mol of glycine are shown in this scheme; the (NH_2) requirement for the synthesis of the 2nd mol of glycine is satisfied by serine-glyoxylate transaminase in the peroxisome (21).

mitochondria, which have specific carriers for 2-OG transport (4, 5), the transport of 2-OG into chloroplasts is mediated by a dicarboxylate carrier with wide substrate specificity (10). It has been suggested that this dicarboxylate carrier might function, physiologically, as a shuttle for the transport of 2-OG and aspartate into the chloroplast in exchange for malate and glutamate, thereby leading to an effective transfer of reducing equivalents from the chloroplast to the cytosol (7). Recent studies in pea chloroplasts (3) have indicated that, to maintain electroneutrality, exchange transfers by the dicarboxylate carrier might be coupled to proton movements. In the light, it seems likely that the dicarboxylate carrier could function basically as a transporter of 2-OG for (photorespiratory) NH_3 assimilation. Under these conditions, the dicarboxylate shuttle could conceivably have 2-OG/OAA moving into the chloroplast in exchange for malate/glutamate, with protons moving in to maintain electroneutrality. Such a shuttle would be sensitive to uncoupler inhibition (trace D, Fig. 1), but OAA reduction would not be affected (trace A, Fig. 5) since the stoichiometric exchange of OAA and malate alone, via the carrier, would be electroneutral. Alternatively, a different carrier specific for 2-OG transport and stimulated by dicarboxylates could be involved.

In the light, the refixation and recycling of photorespiratory NH_3 involves the GS/GOGAT pathway (12, 26). Alloenzymes of GS are present in the chloroplast and cytosol (22). GS activity was also reported to be present in the mitochondria (11), but subsequent studies by Wallsgrove *et al.* (22) failed to confirm this observation. In barley leaves, very little GS activity was detected in the cytoplasm (15), but this has not been supported by recent studies (22). The refixation of NH_3 released during mitochondrial glycine decarboxylation in a reconstituted system containing mitochondria, glutamine synthetase, and ATP has led Miflin and coworkers (12, 22) to propose that the cytosolic alloenzyme was

involved in the initial refixation of photorespiratory NH_3 and that the product, glutamine, was subsequently transported to the chloroplast for reduction by GOGAT. This suggests that the rate of glutamine transport into the chloroplast must, at least, be equivalent to the rate of photorespiratory NH_3 release.

Glutamine transport into chloroplasts also involves the dicarboxylate carrier, but the measured rate was only about 4.2 μmol mg^{-1} Chl h^{-1} at 1 mM glutamine (3). Furthermore, this transport was competitively inhibited by dicarboxylates. Comparable rates (0.6–4.2 μmol mg^{-1} Chl h^{-1}) of light-dependent glutamate synthesis from 2-OG and glutamine were obtained in intact chloroplasts isolated from different species (24). In contrast, rates of about 10.6 μmol O_2 evolved mg^{-1} Chl h^{-1} were reported by Anderson and Done (2) for (Gln, 2-OG)-dependent O_2 evolution in isolated pea chloroplasts, suggesting that glutamine transport into pea chloroplasts was substantial. However, in these chloroplast preparations, it is uncertain whether a carrier-mediated transport process was involved. Our studies show that the rates of (Gln, 2-OG)-dependent O_2 evolution in the absence of NH_4Cl in both isolated spinach (trace D, Fig. 1) and pea (trace B, Fig. 9) chloroplasts were low and inhibited by malate even though high rates were obtained when NH_4Cl was present.

In contrast, our studies indicate that the GS/GOGAT pathway in the chloroplast can sustain high rates of NH_3 assimilation in the presence of dicarboxylates. When this activity was corrected for chloroplast breakage, in nine separate spinach chloroplast preparations, the rate of (NH_3, 2-OG)-dependent O_2 evolution in the absence and in the presence of malate was 5.5 ± 0.75 and 19.5 ± 4.5 μmol O_2 evolved mg^{-1} Chl h^{-1}, respectively. These values are equivalent to 11 and 39 μmol NH_3 and 2-OG fixed mg^{-1} Chl h^{-1}, respectively. The higher rates obtained are adequate to account for the estimated rate of photorespiratory NH_3 recycling (25, 26). Thus, like the cytosol, the chloroplast is a potential site for the refixation and recycling of photorespiratory NH_3 release during photosynthesis in C_3 plants.

The above evidence also suggests a physiological involvement for malate (and other dicarboxylates) in photorespiratory NH_3 reassimilation. Such a role is apparently consistent with the observed light activation of $NADP^+$-malate dehydrogenase in chloroplasts (18). Furthermore, if photorespiratory NH_3 refixation is linked to the modified dicarboxylate shuttle outlined above, then there would be an extra cost of 1 mol NADPH/mol NH_3 refixed.

In conclusion, our evidence suggests that the chloroplast has the capacity to directly reassimilate the NH_3 released during photorespiration in leaf mitochondria via the GS/GOGAT pathway. In this scheme (Fig. 10), glutamine formed in the chloroplast could be shuttled to the peroxisome for glycine synthesis and the 2-OG returned to the chloroplast for NH_3 refixation. In contrast, a system relying on glutamine transport into the chloroplast (12, 22) does not appear, at least in spinach chloroplasts, to have the capacity of sustaining high rates of photorespiratory NH_3 recycling. It is probable that, *in vivo*, both pathways would be involved and the relative role and contribution of each pathway would depend, to a large extent, on the relative positions of the mitochondria and chloroplasts and the concentrations of the reactants involved.

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