REACTION BETWEEN SUCCINATE AND GLYOXYLATE AS A POSSIBLE SOURCE OF CO_2 DURING PHOTORESPIRATION IN WHEAT LEAVES

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1. Introduction

Illuminated leaves of C_3 plants release recently fixed CO_2 during photorespiration; also known as glycolate oxidation pathway [1]. The light reaction involves the formation of phosphoglycolate in the chloroplasts by the action of ribulose 1,5-biphosphate oxygenase. Glycolate is then produced by a phosphatase. Subsequent oxidative decarboxylation of glycolate is known to be the source of photorespiratory CO_2. Thus it was shown that glycolate is transported to peroxisomes, where it is converted into glyoxylate and glycine by oxidation and aminotransferase reactions [2]. Glycine is then transported to the mitochondria, where 2 mol glycine give rise to 1 each of serine and CO_2. This mechanism was confirmed in [3]. However, alternative mechanisms of CO_2 evolution from glycolate and glyoxylate have also been suggested. A direct non-enzymic decarboxylation of glyoxylate by H_2O_2 was demonstrated in the peroxisomes [4]. We now suggest the possible formation of isocitrate by the condensation of succinate with glyoxylate via the reversal of the isocitrate lyase reaction. Further metabolism of isocitrate would generate CO_2. The possibility of photorespiration as a source of carbon for citric acid cycle during active photosynthesis is discussed.

2. Materials and methods

Leaf discs (4 × 4 mm) were obtained from 10–15 day wheat seedlings (CV-Sonalika) grown in pots. Leaf tissue (200 mg) was incubated in Warburb flasks containing in a total reaction vol. 3 ml; phosphate buffer (pH 5.0), 100 mM; succinate, 5 mM (containing 0.25 μCi [1,4-^{14}C]succinate) and malonate, 25 mM. Unlabelled glycolate or glyoxylate were added at 10 mM as indicated in table 1. KOH (0.2 ml, 20%) was placed in the central well and flasks were stoppered. After 30 min incubation at 30°C, 0.1 ml alkali was removed into a counting vial containing 5 ml scintillation fluid (Napthalene PPO in 1,4-Dioxone). $^{14}CO_2$ evolved from 1,4-succinate was counted in a Packard tri-carb liquid scintillation counter.

To study the incorporation of ^{14}C from [1-^{14}C]-glycolate into citric acid cycle intermediates leaf discs (1 g) were placed in 50 ml Erlenmyer flasks, containing in 10 ml, phosphate buffer (pH 5.0) 100 mM, glycolate, 5 mM (containing 2 μCi [1-^{14}C]glycolate) and succinate, 5mM. KOH (0.2 ml 20%) was placed in a small tube which was suspended in the flask with the help of a pin in the stopper. The flasks were exposed to bright sunlight (70–108 kLux) in a water bath at 30°C, for 2 h. For the dark experiments the flasks were covered with dark paper. At the end of the experiment, leaf discs were separated from the reaction mixture, extracted with hot 80% ethanol and the extract concentrated under reduced pressure [5]. The extract was passed through an Amberlite IRA 400 anion exchange column to purify organic acids as in [6]. The eluate was concentrated and organic acids were separated by descending paper chromatography using ethanol:ammonia:water (80:10:10) solvent [7]. The spots were identified, eluted and radioactivity counted. Since distinct separation of citrate from isocitrate and of succinate from malate and 2-oxogluterate could not be obtained in the chromatograms, the total radioactivity in these positions is indicated in table 2.

3. Results

Leaf discs obtained from 10–15 day wheat seedlings were incubated in the presence of [1,4-^{14}C]-succinate. Normal oxidation of succinate was inhibited by malonate. It was observed that 25 mM malonate inhibited $^{14}CO_2$ release by ~70%. As shown in table 1, in malonate-treated tissues, addition of glyoxylate or glycolate stimulated $^{14}CO_2$ evolution from [1,4-^{14}C]-succinate by 40–75% and 35–56%, respectively, in different experiments. In separate experiments it was observed that malonate inhibition of a purified succinate dehydrogenase was not affected by glyoxylate. Thus since succinate dehydrogenase step is inhibited by treatment with malonate, the stimulation of succinate oxidation by glyoxylate appears to involve an alternate pathway. Although the equilibrium of the enzyme reaction of isocitrate lyase is towards the formation of glyoxylate and succinate, the reaction is known to be reversible in the presence of higher concentrations of the products [8]. This enzyme is present in leaf mitochondria [9]. While the function of the enzyme in mitochondria is not clear, the enzyme may operate in the reverse direction, towards the synthesis of isocitrate, which could release CO_2 in the citric acid cycle.

The possibility of incorporation of radioactive glycolate into citric acid cycle intermediates was studied under conditions of active photorespiration, namely high light intensity and high temperature. KOH solution was placed in the flask to decrease the ambient CO_2 concentration. In table 2 the results are compared with a dark control. Incorporation of radioactivity from [1-^{14}C]glycolate in citrate (isocitrate) and succinate (malate + 2-oxoglutarate) was ~2-fold higher in light than in the dark. These results thus confirm the incorporation of ^{14}C from glycolate in citric acid cycle intermediates. [2-^{14}C]Glyoxylate was incorporated into malate, succinate and citrate in a variety of plant tissues including pea leaves and maize coleoptile [10]. Thus both the carbons of glyoxylate are incorporated into organic acids.

Table 2
Incorporation of [^{14}C]glycolate into citric acid cycle intermediates in wheat leaf discs in light and dark (cpm/flask)

Radioactivity	Light	Dark
Glycolate	55 620	57 300
Citrate (Isocitrate)	6840	3050
Succinate (malate, 2-oxoglutarate)	51 120	23 900

As described in section 2, leaf discs were incubated with [1-^{14}C]glycolate under photorespiratory conditions of high light intensity and high temperature. Organic acids were separated by chromatography and incorporation of ^{14}C was counted

Decarboxylation of [1-^{14}C]glyoxylate, glycolate and glycine was then studied. Results in table 3 show that malonate and succinate inhibited $^{14}CO_2$ evolution from [1-^{14}C]glyoxylate quite significantly. The inhibitory effect was proportional to the concentrations added. Succinate similarly inhibited $^{14}CO_2$ release from [1-^{14}C]glycolate. It was also observed that 25 mM malonate inhibited $^{14}CO_2$ evolution from [1-^{14}C]glycine by 73%. At 100 mM malonate almost completely inhibited decarboxylation of glycine in carrot tissues [11]. Results in table 3 also show that 5 mM succinate inhibited $^{14}CO_2$ evolution from glycine by 35%. However, further increase in the succinate concentration did not enhance the inhibitory effect.

Table 1
Stimulation of $^{14}CO_2$ evolution from [1,4-^{14}C]succinate in malonate-treated wheat leaf discs (cpm/flask)

Expt. no.	Succinate + malonate	Succinate + malonate + glyoxylate	Succinate + malonate	Succinate + malonate + glycolate
1	324	520	388	524
2	240	416	346	488
3	234	384	368	492
4	568	800	396	612

Effect of unlabelled glyoxylate and glycolate on $^{14}CO_2$ evolution from [1,4-^{14}C]succinate in the presence of malonate was as in section 2

Table 3
Inhibition of $^{14}CO_2$ evolution from [1-^{14}C]glyoxylate, glycolate and glycine by succinate in wheat leaf discs (cpm/flask)

Additions (mM)	Carboxyl-labelled substrate			
	Glyoxylate		Glycolate	Glycine
	Malonate	Succinate	Succinate	Succinate
Nil (control)	676	848	3236	1932
2.5	576	872	2776	1724
5	420	600	2364	1260
10	352	500	2100	1212
20	304	416	1828	1112
30	272	324	1708	1158

Experimental details as in table 1. Glyoxylate, glycolate or glycine were added at 5 mM containing 0.25 units of 1-^{14}C-labelled substrates as indicated. Different concentrations of malonate and unlabelled succinate were added in different treatments. After 2 h incubation at 30°C, the $^{14}CO_2$ evolved from photorespiratory substrates was counted as in table 1

4. Discussion

In the absence of accumulated succinate decarboxylation of glycolate and glyoxylate would take place as mentioned in section 1 by pathways suggested in [2,4]. These reactions appear to be inhibited by succinate concentrations as shown in table 3. Thus in the presence of succinate, glyoxylate would accumulate in the leaves. The reversal of isocitrate lyase reaction described in table 1 can occur only in the presence of higher concentrations of both succinate and glyoxylate. It is seen from table 3 that succinate even at the highest concentration used, inhibited $^{14}CO_2$ evolution from glyoxylate and glycolate by $\leqslant$60% and 50%, respectively. Hence it appears that when pathways of $^{14}CO_2$ evolution [2,4] are inhibited by succinate, an alternative pathway of decarboxylation of glycolate comes into operation, via the formation of isocitrate as suggested in tables 1 and 2. Thus succinate appears to have the following effects on the metabolism of glyoxylate. Firstly it inhibits decarboxylation of the latter by pathways proposed in [2,4]. When succinate and glyoxylate concentrations are enhanced, and only then, the second reaction of reversal of isocitrate lyase would come into operation and $^{14}CO_2$ would be evolved by further metabolism of isocitrate.

Inhibition of decarboxylation of substrates involved in photorespiration (table 3) by succinate could be of physiological significance as explained below. In actively photosynthesizing tissues, the light-dependent increase in the adenylate energy charge, which is transmitted to the mitochondria, may inhibit the electron transfer chain to O_2 [12]. Inhibition of mitochondrial respiration in light by ATP levels in bean leaves has also been observed [13]. It was shown [14] that in moss spores the cytochrome oxidase pathway is not functional under high light intensities, since it is under the control of ATP/ADP ratios.

These reports thus show that oxidation of NADH as well as of succinate via the cytochrome oxidase pathway in the mitochondria would be inhibited in light. NADH could be utilised via shuttles such as malate/oxaloacetate or by the cytoplasmic nitrate reductase as reported in [15–17]. Accumulated succinate could however inhibit the oxidation of photorespiratory substrates as shown in table 3. In the presence of high concentrations of succinate and glyoxylate in the mitochondria, the isocitrate lyase reaction could be reversed as shown in table 1. How much of the succinate added to the medium in table 1 is actually absorbed by the leaf discs is not known. Under physiological conditions within the mitochondria at the localised sites succinate could accumulate in sufficient concentrations in actively photosynthesizing tissues, for this reaction to occur. Glyoxylate is known to be transported from the peroxisomes [1]. Thus accumulation of both glyoxylate and succinate in the mitochondria is a distinct possibility.

It is perhaps possible that CO_2 is also derived from decarboxylation of malate, formed by malate synthetase as a result of reaction between glyoxylate and acetyl CoA. However, the presence of malate synthetase in leaf mitochondria is not known. Moreover, in table 1 only succinate was labelled and not glyoxylate. Hence $^{14}CO_2$ was derived from metabolism of succinate, which was stimulated by the addition of unlabelled glyoxylate. Hence formation of isocitrate, and its subsequent decarboxylation appears to be the source of $^{14}CO_2$ when succinate dehydrogenase is inhibited by malonate.

If the above proposition is reasonably correct, $^{14}CO_2$ from [1,4-^{14}C]succinate would be evolved during the oxidative decarboxylation of isocitrate to form 2-oxogluterate. Subsequent oxidation of the latter to regenerate succinate would result in the evolution of $^{14}CO_2$, originating from [1-^{14}C]glyoxylate. This could be a source of photorespiratory CO_2.

This pathway could be significant as a supplement or substitute for the anaplerotic carboxylation reactions, required to replenish the 2-oxo acids, which are rapidly consumed for the synthesis of amino acids in photosynthetic tissues. The triose phosphates exported from the chloroplasts may not at all be a significant source of carbon for the citric acid cycle during active photosynthesis, since they are utilised for sucrose synthesis in the cytoplasm [18]. Serine or some other substance may be acting as a carbon source for citric acid cycle in light [18]. As shown here, it could well be glyoxylate.

The relative contribution of the proposed pathway, towards photorespiratory evolution of CO_2 in the mitochondria, as compared with the well-documented glycine–serine reaction is not known. However, the pathway suggests a physiological function for photorespiration in the sense that glyoxylate could be a source of carbon for the generation of carbon skeletons via the citric acid cycle in light.

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