THE WARBURG EFFECT IN MAIZE BUNDLE SHEATH PHOTOSYNTHESIS

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SUMMARY

Bundle sheath strands isolated from maize are capable of photosynthesizing glycolate in the presence of an inhibitor of glycolate oxidase. Glycolate synthesis is stimulated by high oxygen concentrations and suppressed by high levels of CO_2. This data, together with the reversible oxygen inhibition of bundle sheath photosynthesis reported earlier [Ref. 6], demonstrates the occurrence of the Warburg effect in maize bundle sheath photosynthesis. These results also suggest a mechanism to account for the inhibition of photorespiration in intact maize leaves.

Oxygen inhibition of photosynthetic CO_2 fixation with concomitant glycolate production is termed the Warburg effect: glycolate formation and oxygen inhibition of photosynthesis are favored by either high oxygen or low CO_2, and are suppressed by low oxygen or high CO_2 [1]. Previous work in this laboratory indicates that the Warburg effect is mediated by ribulose-1,5-diphosphate (RuDP) carboxylase. Oxygen competitively inhibits RuDP carboxylase with respect to CO_2 [2,3] and substitutes for CO_2 in the carboxylase reaction to yield 2-phosphoglycolate [4], a glycolate precursor. In many algae the glycolate produced is excreted into the suspending medium, while in photorespiratory higher plants (C_3 species), glycolate is excreted from the chloroplast and subsequently oxidized to CO_2 by the photorespiratory pathway in the peroxisomes and mitochondria [5].

In contrast to photorespiratory higher plant species, leaves of a second class of plants, the C_4 species, do not exhibit the Warburg effect or the external manifestations of photorespiration [5]. The data presented here show that the Warburg effect does occur in the bundle sheath cells of maize, a C_4

species, and suggest that the absence of this effect in intact maize leaves may be the result of a CO_2 concentrating mechanism.

MATERIALS AND METHODS

Bundle sheath strands from primary and secondary leaves of 12-14-day old Zea mays L. were isolated and suspended as previously described [6], except that 50 mM tris-HCl (pH 8.0) was substituted for Tricine in all media. Vessels containing assay medium, 8.0 mM α-hydroxy-2-pyridinemethanesulfonic acid (α-HPMS), and strands (6-9 μg chl) were preilluminated (2,000 ft-c, 24°) and gassed with vigorous shaking for 4 min and sealed. The reactions were initiated by injecting $NaH^{14}CO_3$ (3-10 μCi/μmole) and terminated after 10 min by acidification. Contents of the reaction vessels were pooled, centrifuged, and the supernatant fractionated on columns of Dowex 1-X8-acetate. Glycolate and glycerate were eluted with 4 N acetic acid, concentrated, and separated by one-dimensional descending paper chromatography in n-pentanol-5 N formic acid (1:1, upper phase) [7]. The radioactive areas were located with a radiochromatogram scanner, eluted from the paper, and dpm determined by scintillation spectroscopy. Recovery of glycolate-1-^{14}C internal standard was 90%. Total $^{14}CO_2$ incorporated ranged from 6-11 X 10^4 dpm for the various experiments. Control rates of $^{14}CO_2$ incorporation at 20.0 mM $NaH^{14}CO_3$ and 8.0 mM α-HPMS in 2% oxygen were 3-8 μmoles $^{14}CO_2$ fixed mg chl^{-1} hr^{-1}.

RESULTS AND DISCUSSION

We have previously shown that oxygen inhibits photosynthetic CO_2 fixation by maize bundle sheath strands, and that this inhibition is rapidly and completely reversible and relieved by increased levels of CO_2 [6]. Maize bundle sheath strands are also capable of synthesizing large amounts of glycolate in the presence of 1.0 mM $NaH^{14}CO_3$ and 100% oxygen, and glycolate synthesis is suppressed either by reducing the oxygen concentration or by increasing the bicarbonate concentration (Table 1). Thus the Warburg effect occurs in maize bundle sheath photosynthesis.

Glycolate accumulation during maize bundle sheath photosynthesis is depen-

Table 1. Glycolate production during maize bundle sheath photosynthesis.

Expt. No.	mM $NaH^{14}CO_3$	Gas phase	^{14}C-Glycolate Percent of total ^{14}C fixed
I	1.0	2% O_2	2.1
	1.0	100% O_2	20.4
II	1.0	100% O_2	21.2
	20.0	100% O_2	3.3

Table 2. Dependence of glycolate accumulation on the inhibition of glycolate oxidase.

mM α-HPMS	mM $NaH^{14}CO_3$	Gas phase	^{14}C-Glycolate Percent of total ^{14}C fixed
0	1.0	100% O_2	2.6
8.0	1.0	100% O_2	14.3

dent on the addition of a competitive inhibitor of glycolate oxidase, α-HPMS [8] (Table 2). In the absence of inhibitor, glycolate is likely metabolized through the photorespiratory pathway. Maize bundle sheath cells contain photorespiratory enzymes [9] and numerous peroxisomes and mitochondria [10], and maize leaf discs convert added glycolate to CO_2 [11]. From mass spectrometer analysis of gas exchange in atmospheres of $^{13}CO_2$ and $^{18}O_2$, Volk and Jackson [12] estimate the rate of photorespiration in maize to be 0.35-0.70 mg CO_2 dm^{-2} hr^{-1} in 8% oxygen. This rate is considerably less than that in soybean [13], a C_3 plant.

In C_4 panicoid grasses such as maize, sugarcane, and crabgrass, atmospheric CO_2 is initially fixed by phosphopyruvate (PEP) carboxylase in the mesophyll, and the resultant oxaloacetate is predominantly reduced to malate. The malate is transported to the bundle sheath chloroplasts and decarboxylated to pyruvate and CO_2 by "malic" enzyme. The CO_2 is refixed by RuDP carboxylase and further metabolized through the Calvin cycle [14,15]. The PEP carboxylase-"malic"

enzyme system presumably functions as a CO_2 pump, increasing the CO_2 concentration in the bundle sheath two orders of magnitude higher than the dark equilibrium level [16]. The reduced photorespiration rate in maize is likely caused by the increased level of CO_2 in the bundle sheath which results from the PEP carboxylase-"malic" enzyme CO_2 concentrating mechanism [3,6]. CO_2 and oxygen compete for RuDP through RuDP carboxylase, yielding two moles of 3-phosphoglycerate or one mole each of 3-phosphoglycerate and 2-phosphoglycolate, respectively [2-4]. An increased level of CO_2 in the bundle sheath, the site of RuDP carboxylase [6,17], would lessen 2-phosphoglycolate production and thereby decrease the amount of glycolate available for photorespiratory oxidation to CO_2. Any CO_2 produced in the bundle sheath during photorespiration would be refixed by PEP carboxylase in the surrounding mesophyll before it escapes from the leaf [3,12, 18,19].

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