# Behavior of the Early Products of Photosynthesis in $C_3$- and $C_4$-Plants

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Since it is said that the population of the world may double at the end of this century and the hunger problem should become more severe in the world, we hold ourselves responsible for the increase of the agricultural productivity to prevent this crisis.

Reclamation, irrigation and improvement of soil are the most important methods to get more production, especially in developing countries. Genetic methods and using fertilizers and pesticides are also very effective to increase production.

However, the yields may have already reached a zenith in the developed districts by those methods.

In these districts, increasing the efficiency of the photosynthesis in the leaves of the crop is one of the possible methods to raise agricultural productivity[1].

Higher plants can be classified for the rate of net photosynthesis per unit leaf area at high light intensities in normal air into two groups such as efficient species ($C_4$-plants) and inefficient species ($C_3$-plants). During the last decade, there was great progress of the comparative studies on the photosynthesis between $C_4$ and $C_3$-plants. Table 1 shows the various photosynthetic characteristics of these species, which were published by many workers[2].

It is a prerequisite to understand the intrinsic nature of the mechanism of photosynthesis and photorespiration to control them and to increase the net photosynthesis rate. The present paper is a synopsis which has been under study regarding the photosynthesis and photorespiration in $C_3$- and $C_4$-plants in our laboratory.

## Metabolism of glycolate in $C_3$-and $C_4$-plants

The glycolate pathway proposed by Kisaki and Tolbert is believed to be a main mechanism of photorespiration[3], but another pathway of glycolate metabolism is claimed by Zelitch[4].

The influence of light and oxygen on the evolution of $^{14}CO_2$ from glycine-1-$^{14}$C and glycine-2-$^{14}$C were examined in the tomato (*Lycopersicon esculentum Mill.*, $C_3$-plant) and

**Table 1. Photosynthetic characteristics of the $C_3$- and $C_4$-plants**

(adapted from Black)

| Characteristic | $C_3$-plants | $C_4$-plants |
|---|---|---|
| Kranz type of leaf anatomy | No | Yes |
| Compensation point (ppm $CO_2$) | 30—70 | 0—10 |
| Saturation of net photosynthesis to the light intensity | 1/2—1/3 of full sunlight | Tending to saturate at full sunlight |
| Maximum growth rate gr/dm$^2$/hr | 0.5— 2 | 4— 5 |
| Net photosynthesis rate mg $CO_2$/dm$^2$/hr | 15—40 | 40—80 |
| Photorespiration | Present | Difficult to detect |
| Major carboxylation reaction | RuDP carboxylation | PEP carboxylation and RuDP carboxylation |

**Table 2. $^{14}CO_2$ Evolution from glycine-1-$^{14}$C or glycine-2-$^{14}$C supplied to tomato and maize leaves and the influence of light and oxygen on the $CO_2$ evolution**

| Leaves | $^{14}CO_2$ evolution from glycine-1-$^{14}$C | $^{14}CO_2$ evolution from glycine-2-$^{14}$C |
|---|---|---|
| | % of total $^{14}$C incorporated | |
| Light, $O_2$ 21% : Tomato | 12.23 | 6.05 |
| Maize | 4.08 | 1.52 |
| Light. $O_2$ 100% : Tomato | 29.24 | 24.57 |
| Maize | 5.40 | 1.09 |
| Dark, $O_2$ 21% : Tomato | 35.48 | 1.45 |
| Maize | 59.10 | 4.81 |

Substrates were fed to tomato and maize for 60 min at 30°C

maize (*Zea mays L.*, $C_4$-plant) leaves to study whether the photorespiration occurred only by the glycolate pathway.

As shown in Table 2, a considerable evolution of $^{14}CO_2$ was detected from glycine-2-$^{14}$C and it was accelerated by light and oxygen. And the influence of the addition of glycine on the evolution of $^{14}CO_2$ from glycolate-1-$^{14}$C was examined in the tomato and maize leaves in the light. The evolution of $^{14}CO_2$ was strongly inhibited in the maize leaves but unexpectedly there was no effect in the tomato leaves. As proposed by Zelitch these results show that the mechanism of glycolate metabolism may exist in $C_3$-plants besides the glycolate pathway, which is accelerated by light and oxygen[5].

## Fate of $C_4$-dicarboxylic acid in $C_3$-and $C_4$-plants

The transfer of the C-4 of malate-4-$^{14}$C to PGA in the maize and tomato leaves was studied. This transfer occurred only in the maize leaves in the light[6]. To ascertain the light dependence of the transformation from aspartate-$^{14}$C(U) via PGA was examined in the maize leaves. The activity in sugar increased with the increase of light intensity, and more than 45% of the activity supplied was recovered in sugar fraction at 30 k lux as shown in Table 3[7].

The transformation of the $C_4$ compounds to sugar was apparently light dependent reactions, but the large value cannot be explained by the $C_4$-pathway theory proposed by Hatch et al., and suggests the possibility of the incorporation of the three carbons except C-4 of $C_4$-compounds into sugar.

The effect of the oxygen on the sugar formation from $C_4$-dicarboxylic acid was studied in the maize leaves at 50 k lux. The sugar formation from malate-$^{14}$C(U) under aerobic condition was 3 times larger than that under

**Table 3. The effect of the light intensity on the sugar formation from exogenous Asp-$^{14}$C(U) fed to the maize leaves**
**Data for each fraction were given as % of the total metabolized. 0.5$\mu$ Ci Asp-$^{14}$C(U) was fed for 30 min to maize leaves in an atmosphere of $CO_2$-free air under several light intensities. Illumination was provided by a bank of 100-watt incandescent lamps**

| Light intensity k lux | $CO_2$ | Soluble fraction | | | Residue | | Total sugar |
|---|---|---|---|---|---|---|---|
| | | a. a. | org. a. | sugar | a. a. | sugar | |
| | | (% of total $^{14}$C incorporated) | | | | | |
| 5.0 | 1.57 | 48.0 | 26.5 | 15.6 | 4.4 | 4.0 | 19.6 |
| 12.5 | 0.98 | 53.9 | 11.1 | 21.0 | 5.6 | 8.6 | 29.6 |
| 25.0 | 0.20 | 37.0 | 16.9 | 31.0 | 7.3 | 7.2 | 38.2 |
| 30.0 | 0.70 | 47.9 | 8.8 | 43.3 | 3.1 | 1.8 | 45.1 |

**Table 4. Metabolism of malate-$^{14}$C(U) by maize leaves under aerobic and anaerobic conditions. The feedings were carried out for 30 min in the light. Illumination was provided by a 1,000-watt projection lamp. Light intensity was 50 k lux at the leaf surface**

| Metabolic intermediates | $CO_2$-free air | $N_2$ gas |
|---|---|---|
| $CO_2$ | 0.2 | 1.2 |
| Sugar | 43.8 | 13.2 |
| Malate | 28.6 | 46.5 |
| Aspartate | 3.6 | 5.7 |
| Phosphate ester | 0.2 | — |
| Alanine | 16.2 | 30.9 |
| Glutamate | 1.7 | — |
| Citrate | 0.8 | — |
| Fumarate+succinate | 2.0 | 1.5 |
| Glycine+Serine | 1.5 | — |
| Glycolate | 1.0 | — |
| Others | 0.3 | 0.4 |

anaerobic condition as shown in Table 4. However in the case of malate-4-$^{14}$C, there was no detectable difference of the sugar formation between two conditions. These results show that the three carbons except C-4 of $C_4$-compounds were also utilized for the sugar formation in the normal air[8].

## Fate of $C_3$-compound in $C_3$-and $C_4$-plants

The sugar formation from alanine-1-$^{14}$C under anaerobic condition was examined in the maize and rice (*Oryza sativa*, $C_3$-plant) leaves. Sugar was formed from alanine-1-$^{14}$C in maize leaves but not in rice leaves under anaerobic condition.

As the TCA cycle does not work under this condition, $^{14}CO_2$ may not be evolved from TCA cycle. Accordingly the sugar formation from alanine-1-$^{14}$C might occur via pyruvate→PEP→PGA by the backward glycolysis reaction, as the mesophyll cell in maize leaves has a characteristic enzyme to form PEP from pyruvate (Pyruvate Pi-dikinase)[8].

The influence of oxygen on the evolution of $^{14}CO_2$ and the sugar formation from alanine-1-$^{14}$C was was examined in $C_3$-plants (*Oryza sativa, Phalaris arundinacea, Panicum disculcatum*) and $C_4$-plants (*Eragrostis ferrunginea, Zoysia japonica*). The evolution of $^{14}CO_2$ increased with the augmentation of the ambient oxygen concentration, whereas the radioactivity was quite low in sugar fraction in $C_3$-plants.

On the contrary, the evolution of $^{14}CO_2$ was very slow and it was slightly affected by the ambient oxygen concentration but the sugar formation was stimulated with the increased concentration of oxygen as shown in Fig. 1.

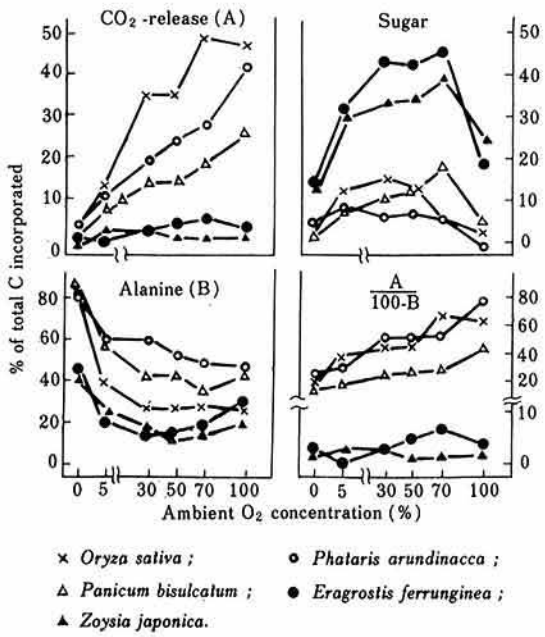


Fig. 1. Metabolism of alanine-1-$^{14}$C by excised leaves of *Gramineaes* under various $O_2$ concentrations

This indicates that $CO_2$ fixation is inhibited in the high concentration of ambient oxygen; hence, the internal $^{14}CO_2$ due to dark respiration is not refixed and released from the leaves in $C_3$-plants.

However, internal $^{14}CO_2$ can be efficiently refixed by a PEP carboxylase (with low Km)

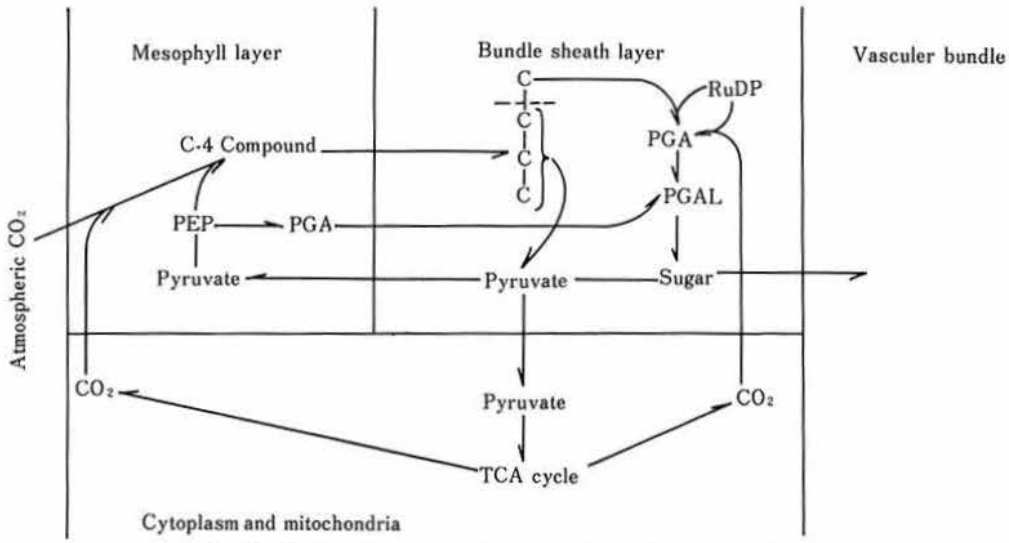


Fig. 2. Proposed pathway for sugar formation in $C_4$-plants

in the mesophyll cell, and then metabolized to sugar in the bundle sheath cell of $C_4$-plants[9].

Inhibition of TCA cycle by the light has been reported by many workers[10], however, the feeding experiments of labelled compounds showed that TVA cycle activity in detached leaves was not inhibited to any extent, dark respiration was sufficiently preserved during photosynthesis and they were rather closely connected with each other.

We presumed from the above results that the following pathways function in the photosynthetic sugar formation in $C_4$-plants as shown in Fig. 2[8]. 1) C-4 of $C_4$-dicarboxylic acid is transferred to RuDP, resulting in the formation of PGA and is metabolized to sugar. 2) After the transfer of C-4 of $C_4$-dicarboxylic acid, the remaining three carbons are introduced into TCA cycle and completely degraded there, and so the produced $CO_2$ is refixed by PEP carboxylase in the mesophyll cell and metabolized to sugar via the same pathway as in atmospheric $CO_2$ fixation. 3) The remaining $C_3$-compound is changed to PEP by pyruvate Pi-dikinase, and a part of PEP directly converts to sugar via PGA by the backward glycolysis and the rest acts as an acceptor of $CO_2$. 4) The shortage of $C_3$-compounds may be supplemented from sugar by glycolysis.

## Internal evolution of $CO_2$ and leaf anatomy

The evolution of $^{14}CO_2$ from asparate-$^{14}$C(U) and alanine-1-$^{14}$C was examined under various light intensities in three $C_4$-plants (*Paspalum urvillei*, *Eragrostis ferrunginea* and *Zoysia japonica*).

At high light intensities, the evolution of $^{14}CO_2$ correlated with the thickness of mesophyll layers surrounding the vascular bundle sheath. The thicker the mesophyll layers, the smaller the evolution of $^{14}CO_2$[8].

This evidence supported the theory that the mesophyll layers played a vital role for refixation of the internal $CO_2$ in the light in $C_4$-plants as mentioned above.

Accordingly, the apparent lack of photorespiration in $C_4$-plants results at least in part from refixation of the $CO_2$ by PEP carboxylase in the mesophyll layers.

## Conclusion

1) The metabolic pathway of glycolate, which evolves $CO_2$ from C-2 of glycolate, may also exist in $C_3$-plants besides the glycolate pathway. And this pathway may also play a role of the photorespiration.

2) TCA cycle is sufficiently preserved even in the light; accordingly the respiration in the light should be considered both of the dark respiration and photorespiration (as a narrow meaning).

3) Actually, each of the photosynthesis and respiration doesn't work independently in the leaves in the light and they are rather closely connected with each other.

4) It is assumed that the actual form of $C_4$-pathway in an intact leaf is a combination of the complicated reactions which include glycolysis and TCA cycle besides the pathway proposed by Hatch et al.

5) The $C_4$-plants release $CO_2$ with very slow rates and show a greater productivity, as the PEP carboxylase in the mesophyll cell of those plants refix the internal $CO_2$ evolved by the respiration in the light.

## References

1) Yamada, Y.: Behavior of the early products of photosynthesis, Kali Symposium. 1971, Tokyo, Kali Kenkyukai, 155–175 (1972).

2) Black, C. C. Jr.: Photosynthetic carbon fixation in relation to net $CO_2$ uptake. *Ann. Rev. Plant Physiol.*, 24, 253–286 (1973).

3) Kisaki, T. & Tolbert, N. E.: Glycine as a substrate for photorespiration. *Plant Cell Physiol.*, 11, 247–258 (1970).

4) Zelitch, I.: Comparison of the effectiveness of glycolic acid and glycine as substrates for photorespiration. *Plant Physiol.*, **50**, 109–113 (1972).

5) Ono, S. & Yamada, Y.: Unpublished.

6) Imai, H., Yamada, Y. & Harada, T.: Comparative studies on the photosynthesis of higher plants. *Soil Sci. Plant Nutr.*, 17, 110–114 (1971).

7) Imai, H., Yamada, Y. & Harada, T.: Comparative studies on the photosynthesis of higher plants. *Soil Sci. Plant Nutr.*, 18, 133–141 (1972).

8) Imai, H., Iwai, S. & Yamada, Y.: Unpublished.

9) Imai, H. et al.: *Soil Sci. Plant Nutr.*, **19**, 61–71 (1973).

10) Santarius, K. A. & Heber, U.: Changes in the intracellular level of ATP, ADP, AMP and Pi and regulatory function of the adenylate system in leaf cells during photosynthesis. *Biochim. Biophy. Acta*, **102**, 39–54 (1965).