Note

Aminomethanesulfonic Acid as an Inhibitor of Photorespiration

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In the search for a potent inhibitor of photorespiration, we have been screening various chemicals by measuring the so-called oxygen compensation point in isolated spinach cells with an oxygen electrode, and have found that aminoacetonitrile (AAN) and aminomethanesulfonic acid (AMS) are effective inhibitors.¹⁾ The subsequent experiment characterized AAN as a specific and competitive inhibitor of photorespiration at the site of mitochondrial glycine oxidation in the glycolate pathway.²⁾ Since then, AAN has been used for a study of the photorespiratory carbon metabolism in higher plants.^{3,4)} AMS, however, has not yet been characterized as a photorespiration inhibitor with respect to its specificity and inhibition site.

This study aims, firstly, at the characterization of AMS as an inhibitor of photorespiration; and, secondly, the photosynthesis under photorespiratory conditions is examined to check whether it is influenced by a photorespiration inhibitor.

Rice plants (*Oryza sativa* L. cv. Nipponbare) were grown in soil for about 2 months, and pea (*Pisum sativum* L.) and wheat (*Triticum aestivum* L.) were grown in vermiculite for 9 to 11 days in a natural-light phytotron at 25/20°C (day/night).

Four excised rice leaves were set in a leaf chamber with their bases submerged in distilled water. Photorespiratory and respiratory CO₂ exchanges were measured as CO₂ evolution into CO₂-free air in the light and dark, using the system previously reported.⁵⁾ Photosynthetic CO₂ uptakes under non-photorespiratory and photorespiratory conditions were measured in air containing 2% O₂, and in normal atmospheric air. AMS, adjusted to pH 7.0 with KOH, was applied with a syringe to the distilled water immediately after the rate of CO₂ exchange reached the steady state.

The photosynthetic O₂ evolution by wheat protoplasts in the presence of 10 mM NaHCO₃ and the respiratory O₂ uptake by pea mitochondria were measured polarographically with an oxygen electrode (Hansatech, King's Lynn, UK) according to Edwards *et al.*⁶⁾ and Usuda *et al.*²⁾ respectively. The photosynthetic rate thus measured in the presence of 10 mM NaHCO₃ represents the rate under non-photorespiratory conditions, because the CO₂ concentration was sufficient to suppress the RuBP oxygenase reaction.

The inhibition site of AMS was identified by investigating the effect of AMS on several enzymes in the glycolate pathway under suitable conditions. The activity of glycolate oxidase was assayed at 30°C with an oxygen electrode according to Jewess *et al.*⁷⁾ The activities of hydroxypyruvate reductase and glutamate:glyoxylate aminotransferase, and of serine:glyoxylate aminotransferase were assayed spectrophotometrically at 25°C according to Brock *et al.*⁸⁾ and Rehfeld and Tolbert,⁹⁾ respectively. Mitochondrial glycine oxidation was measured at 25°C with an oxygen electrode according to Usuda *et al.*²⁾

Glycolate oxidase and hydroxypyruvate reductase were purchased from Sigma, the other enzymes being prepared according to Jenkins *et al.*¹⁰⁾

TABLE I. INHIBITORY EFFECT OF AMS ON PHOTORESPIRATION, RESPIRATION AND PHOTOSYNTHESIS

AMS	Inhibition (%)				
	Photorespiration	Respiration		Photosynthesis*	
	(Leaf) ^a	(Leaf) ^b	(Mito.) ^c	(Leaf) ^d	(Proto.) ^e
5 mM	15	0	0	0	0
10 mM	28	0	0	7	5

Light intensity during the measurement was 800 $\mu\text{E m}^{-2}\text{s}^{-1}$ in *a*, *d* and *e*. Temperature was 25°C throughout the measurement. The control rates for *a*, *b* and *d* were 4.5~5.0, 2.5~3.5 and 31.3~34.8 mg CO₂/dm²/hr, respectively; *c* and *e* were 105~120 nmol O₂/mg protein/min and 115~125 $\mu\text{mol O}_2/\text{mg chr/hr}$, respectively.

* Photosynthesis under the non-photorespiratory condition.

FIG. 1. Concentration Effect of AMS on Apparent Photosynthesis in Excised Rice Leaves.

The rate of apparent photosynthesis in the absence of AMS was $25 \sim 27 \text{ mg CO}_2/\text{dm}^2/\text{hr}$. The light intensity and temperature were the same as these in Table I.

Table I shows the effect of AMS on photorespiration, respiration and photosynthesis under the non-photorespiratory condition. AMS inhibited photorespiration as strongly as aminooacetoneitrile,¹¹⁾ but did not affect either respiration or photosynthesis under this condition. These results could indicate that AMS has a high specificity for the inhibition of photorespiration.

An assay of the inhibitory effect of AMS on the enzymes in the glycolate pathway and of the mitochondrial glycine oxidation revealed that AMS inhibited the photorespiration by affecting glycolate oxidase and hydroxypyruvate reductase. AMS showed a non-specific inhibition ($K_i = 1.1 \times 10^{-5} \text{ M}$) in glycolate oxidase and a noncompetitive inhibitor ($K_i = 2.7 \times 10^{-3} \text{ M}$) in hydroxypyruvate reductase.

We further investigated the effect of AMS on photosynthesis under the photorespiratory condition; this is, the apparent photosynthesis under normal air conditions. As shown in Fig. 1, AMS clearly inhibited the apparent photosynthesis, and the inhibition extent increased with the increase of AMS that was applied. The inhibition of photosynthesis under the photorespiratory condition was not due to direct inhibition of the photosynthetic mech-

anism, but to the inhibition of photorespiration, because AMS did not affect at all photosynthesis under the non-photorespiratory condition (Table I). Mulligan *et al.*¹²⁾ have shown, with spinach chloroplasts, that glycolate inhibited CO_2 fixation. Therefore, it is postulated that the inhibition of photosynthesis under the photorespiratory condition by AMS is due to the accumulation of glycolate caused by the inhibition of glycolate oxidase.

It has been reported by us⁵⁾ and some workers^{2,13)} that AAN, a specific inhibitor of mitochondrial glycine oxidation, decreased photosynthetic CO_2 fixation under the photorespiratory condition. AMS also decreased photosynthesis under photorespiratory condition, although AMS had an inhibition site different from AAN in the photorespiratory glycolate pathway. From these results, it seems difficult to accelerate the net photosynthetic CO_2 uptake by means of photorespiration inhibitors.

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