Short Communication

Search for Photorespiration Inhibitors; Glycine and Serine Derivatives

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The acceleration of the apparant photo-synthesis of C_3 plants by inhibiting photorespiration with chemicals would be an attractive means towards an improvement in crop productivity.

The strategy which we have adopted so far in the search for a photorespiration inhibitor was to synthesize various derivatives of glycine and serine, expecting their effects as competitive or feedback inhibitors of glycine conversion to serine in the mitochondria, which is presumably the main site for CO_2 release in the photorespiratory glycolate pathway. One success in this strategy has been to discover that aminoacetonitrile is an effective and specific inhibitor of photorespiration.^{1~3)} In this paper, the effects of other derivatives of glycine and serine synthesized in our laboratory are investigated, and the specificity of the effects on the photorespiratory reaction are discussed.

The chemicals tested, except for aminoacetonitrile hydrochloride which was purchased from Sigma, were synthesized according to Kirino *et al.*⁴⁾ and Baldwin *et al.*⁵⁾

The photorespiration rate was measured with an infrared CO_2 analyzer (Fuji Electric Co., Ltd., ZAL) as the CO_2 evolution into

CO_2 -free air in excised rice (*Oryza sativa* L. cv. Nihonbare) leaves according to Yun *et al.*,²⁾ and with a polarographic O_2 electrode (Rank Brothers, Cambridge, UK) as the glycine oxidation rate in pea (*Pisum sativum* L.) mitochondria according to Usuda *et al.*³⁾ Respiration was measured as the O_2 consumption rate due to malate oxidation in pea mitochondria according to Usuda *et al.*,³⁾ and as the O_2 consumption rate in spinach (*Spinacia oleracea* L.) leaf segments according to Hyeon *et al.*⁶⁾ Carbon dioxide fixation rates in the presence of 10 mM $KHCO_3$ were measured polarographically as the O_2 evolution rate in wheat (*Triticum aestivum* L.) chloroplasts isolated according to Edwards *et al.*,⁷⁾ and in spinach leaf segments according to Hyeon *et al.*⁶⁾

Rice was cultured for two months with soil in the natural light phytotron where the temperature was controlled between 25/20°C (day/night). The youngest and fully expanded leaf blades were used for the measurement of CO_2 evolution rate. Pea and wheat were grown for 9 to 11 days with vermiculite, in the artificial light phytotron where the temperature and the day length were kept at 23/17°C (day/night) and for 12 hr with an intensity of 10 klux. Spinach was purchased from the market.

We commenced the synthesis of glycine derivatives by substituting the site of R^1 in the formula for glycine by ethyl, *n*-propyl, 2-butenyl, benzyl, and allyl groups, fixing R^2 and X as H and CO_2H respectively. Preliminary experiments showed that the derivatives with an allyl group had a marked inhibitory effect on photorespiration, although benzyl and *n*-propyl had no effect. Based on this result, we fixed R^1 as allyl and 2-butenyl, R^2 as H, and introduced CO_2Et , $CONH_2$, CO_2K and CN at the site of X (Compound 1~4 and 10~11 in Table I). Among these, *N*-allylglycine (3) and *N*-2-butenylaminoacetonitrile (11) showed comparatively strong inhibition of photorespiration, but the latter inhibited both the respiration and CO_2 fixation in the same manner as all other cases of the compounds having a nitrile group (1, 5, 9). The introduction of CH_3

TABLE I. THE EFFECTS OF SOME CHEMICALS ON PHOTORESPIRATION, RESPIRATION AND CO₂ FIXATION

Compound					Photorespiration		Respiration		CO ₂ Fixation	
					Leaf ^a	Mitochon. ^{b*}	Mitochon. ^c	Segment ^d	Segment ^e	Chloropl. ^f
No. Control					100	100	100	100	100	100
R ¹ -NH-CH-X R ²	1	CH ₂ =CHCH ₂	H	CN	88	86	—	48	62	—
	2	CH ₂ =CHCH ₂	H	CO ₂ Et	78	88	—	100	103	100
	3	CH ₂ =CHCH ₂	H	CO ₂ K	51	86	95	100	100	105
	4	CH ₂ =CHCH ₂	H	CONH ₂	90	—	—	100	100	—
	5	CH ₂ =CHCH ₂	CH ₃	CN	43	36	35	46	14	0
	6	CH ₂ =CHCH ₂	CH ₃	CO ₂ Et	98	104	—	100	100	—
	7	CH ₂ =CHCH ₂	CH ₃	CO ₂ K	100	—	—	100	100	—
	8	CH ₂ =CHCH ₂	CH ₃	CONH ₂	91	99	—	100	94	—
	9	C ₂ H ₅	H	CN	84	80	—	41	54	—
	10	CH ₃ CH=CHCH ₂	H	CO ₂ Et	91	75	88	100	80	0
	11	CH ₃ CH=CHCH ₂	H	CN	86	55	32	74	69	—
	12	H	CH ₂ =CH	CO ₂ H	54	52	95	100	100	95
	13	H	CH ₃ CH=CH	CO ₂ H	69	95	—	100	100	—
R-CH-CH-CO ₂ H OH NH ₂	14	R: Thienyl			83	106	104	100	100	94
	15	R: Phenyl			86	109	98	100	100	97
	16	R: 2,4-Dichlorophenyl			61	82	91	100	100	79
	17	R: 3,4-Methylenedioxyphenyl			46	55	60	100	100	31
Aminoacetonitrile (H ₂ N-CH ₂ -CN)					70	20	95	100	100	100

Light intensity during the measurement was 45, 40 and 100 klux in *a*, *e* and *f* respectively. Temperature was 25°C throughout all measurements. The control rate of each measurement was 5~6 mg CO₂/dm²/hr in *a*, 200~300 μmol O₂/dm²/hr in *e* and 112 μmol O₂/mg Chl/hr in *f*. ADP/O ratio in mitochondrial glycine (*b*) and malate (*c*) oxidation was 2.5~2.7. Treating the concentration of chemicals gave 10 mM in *a* and 5 mM in *b* to *f*.

* Glycine oxidation in the mitochondria.

to the site of R^2 (5~8), in the next step, resulted in a reduction of the inhibitory effects of photorespiration. In the last trial, we fixed H and CO_2H at the sites of R^1 and X respectively, and substituted vinyl and 1-propenyl groups at R^2 (12, 13), finding that the vinylglycine (12) showed a specific inhibitory effect on photorespiration.

As already mentioned, *N*-allylglycine and vinylglycine could be possible inhibitors of photorespiration. Table I shows that *N*-allylglycine exhibited a weaker inhibitory effect on mitochondrial glycine oxidation than vinylglycine, whereas both of them inhibited photorespiratory CO_2 evolution to a similar extent, suggesting that the inhibition mechanism could be different between them. This means that *N*-allylglycine might have a stronger inhibitory effect on other reactions in the glycolate pathway than mitochondrial glycine oxidation, although there is no denying the possibility that the chemicals are selective to the plant species.

Through the glycine derivatives shown in Table I, we were greatly interested that all of the nitrile compounds inhibited both the respiration and CO_2 fixation, probably through the electron transport chain. Nevertheless, aminoacetonitrile showed no effect in either reaction. In this respect, aminoacetonitrile is exceptionally the specific inhibitor of mitochondrial glycine conversion to serine.

Among the serine derivatives shown in Table I, thienylserine (14) exhibited a weak inhibitory effect on CO_2 evolution without the effects on respiration and CO_2 fixation. 2,4-Dichlorophenylserine (16) and 3,4-methylenedioxyphenylserine (17) showed comparatively strong inhibition of photorespiration, but these two compounds inhibited both respiration and CO_2 fixation.

It was clarified in this paper that vinylglycine, *N*-allylglycine, and thienylserine were specific inhibitors of photorespiration. The inhibition mechanism and the effects on apparent photosynthesis of these chemicals are under investigation.

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