Note

Effects of *N*-Substituted Glycine Analogues on Photosynthesis in Wheat Protoplasts

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In a previous paper, we reported a simple bioassay method for photosynthesis accelerators using wheat protoplasts and an oxygen electrode. With this bioassay method, choline chloride and its analogues were revealed to be photosynthesis accelerators.¹⁾ In this paper, we report the photosynthesis acceleration due to *N*-substituted glycine analogues in wheat protoplasts.

Wheat (*Triticum aestivum*: Nourin No. 61) seedlings were grown in Vermiculite for 9 to 12 days in a natural-light Phytotron, which was kept at 25/20°C (day/night). Mesophyll protoplasts were isolated enzymatically by the method of Edwards *et al.*²⁾ using a combination of cellulase (Onozuka R-10) and pectinase (Macerozyme R-10) purchased from Yakult Biochemical Co., Ltd. The *N*-substituted glycine analogues were synthesized by the method of Kirino *et al.*³⁾ at the Biochemical Research Division, Mitsubishi Gas Chemical Company Inc.

Each chemical was dissolved in 0.4 M sorbitol, 50 mM Hepes and 1 mM EDTA, and the pH was adjusted to 7.6 with KOH. The photosynthetic O₂ evolution in protoplasts was measured with an oxygen electrode (Hansatech Ltd., Kings Lynn, U.K.) at 25°C in a reaction mixture (1 ml) containing 0.4 M sorbitol, 50 mM Hepes-KOH (pH 7.6), 1 mM EDTA and 10 mM NaHCO₃. After the protoplasts (chlorophyll, 30~50 µg) had been incubated with each chemical for 2 min, photosynthesis was initiated by illumination at 1400 µE m⁻² s⁻¹. The activity of each chemical was expressed as the percentage of O₂ evolution compared to the control rate.¹⁾ Photosynthetic CO₂ fixation was measured at 25°C in a reaction mixture (1.1 ml) containing 0.4 M sorbitol, 50 mM Hepes-KOH (pH 7.6) and 1 mM EDTA. Protoplasts (chlorophyll, 30~50 µg) were mixed with the reaction medium and then

incubated with each chemical for 2 min in the dark. After 10 mM NaH¹⁴C₃ (0.1 µCi/µmol) had been added to the protoplasts, photosynthesis was initiated by illumination at 800 µE m⁻² s⁻¹. After 8 min, the reaction was terminated by the addition of 1 ml 6 N formic acid.

In order to estimate the effects of foliar treatment with *N*-substituted glycine analogues on wheat seedlings, the chemicals were dissolved in water and then each chemical solution was sprayed on wheat seedlings (30 ml/250 cm²), about eight days after sowing. The plants were grown under the same conditions as mentioned above. Protoplasts were prepared from the leaves on the second and third days after the treatment, and their photosynthetic O₂ evolution was measured.

Among a large number of *N*-substituted glycine analogues (Table I), *N*-allyl glycine (compound 3) had a greater accelerative effect on the photosynthetic O₂ evolution. However, *N*-methyl glycine (compound 2) slightly accelerated the photosynthetic O₂ evolution and glycine (compound 1) had no accelerative effect on the photosynthetic O₂ evolution in wheat protoplasts. In the di- and tri-substituted glycine analogues, *N*-allyl-*N*-methyl glycine (compound 5), *N,N*-dimethyl glycine (compound 9) and *N*-allyl-*N,N*-dimethyl glycine ethylester (compound 14) showed comparatively marked accelerative effect on the photosynthetic O₂ evolution. The chemicals showing an accelerative effect had no effect on the mitochondrial O₂ consumption rate in the presence of NADH⁴⁾ (Table II).

Among the chemicals showing an accelerative effect on photosynthetic O₂ evolution, we examined the effects of *N*-allyl glycine, *N*-allyl-*N*-methyl glycine and *N*-allyl-*N,N*-dimethyl glycine on CO₂ fixation. All of them accelerated the rate of CO₂ fixation in protoplasts (Table III).

In order to estimate their effects on intact plants, aqueous solutions of these *N*-substituted glycines were sprayed onto wheat seedlings. *N*-Allyl glycine and *N*-allyl-*N*-methyl glycine increased the photosynthetic O₂ evolution by more than 10% in the isolated protoplasts from treated wheat leaves (Table IV).

The results obtained in these experiments indicated that *N*-substituted glycine analogues are a photosynthesis accelerator. We have previously reported that choline analogues are photosynthesis accelerators.¹⁾ Among these analogues, mono- and di-substituted compounds (ethanolamine derivatives) were not accelerative or rather showed an inhibitory effect. However, *N*-substituted glycine analogues, the hydroxyl group of ethanolamine of which was replaced by a carboxyl group, showed photosynthesis accelerative activity. On the other hand, in the quaternary ammonium salt (choline analogues and betain), the photosynthesis accelerative activity was not related to whether the terminal group was a hydroxyl group or a carboxyl group.

Elucidation of the mechanism of *N*-substituted glycine induced-photosynthesis acceleration and the effects of such analogues plant growth and crop productivity will be the subjects of a future study.

TABLE I. EFFECTS OF *N*-SUBSTITUTED GLYCINE ANALOGUES (10 mM) ON PHOTOSYNTHETIC O₂ EVOLUTION IN WHEAT PROTOPLASTS^a

$\begin{array}{c} \text{R}_1 \\ \\ \text{R}_2-\text{N}-\text{CH}_2-\overset{\text{O}}{\parallel}\text{C}-\text{O}-\text{R}_3 \end{array}$				
Compound				O ₂ Evolution ^b (%)
No.	R ₁	R ₂	R ₃	
1	H	H	H	101
2	H	CH ₃	H	103
3	H	CH ₂ CH=CH ₂	H	110
4	CH ₃	CH ₃	H	108
5	CH ₃	CH ₂ CH=CH ₂	H	114
6	C ₂ H ₅	CH ₂ CH=CH ₂	H	107
7	C ₂ H ₅	CH ₂ C(CH ₃)=CH ₂	H	110
8	CH ₂ CH=CH ₂	CH ₂ CH=CH ₂	H	110
9	CH ₂ C(CH ₃)=CH ₂	CH ₂ C(CH ₃)=CH ₂	H	115
10	CH ₃	CH ₂ CH=CH ₂	C ₂ H ₅	108
11	C ₂ H ₅	CH ₂ CH=CH ₂	C ₂ H ₅	110

$\begin{array}{c} \text{R}_1 \\ \\ \text{R}_2-\text{N}^+-\text{CH}_2-\overset{\text{O}}{\parallel}\text{C}-\text{O}-\text{R}_4 \\ \\ \text{R}_3 \end{array}$				
Compound				O ₂ Evolution (%)
No.	R ₁	R ₂	R ₃	R ₄
12	CH ₃	CH ₃	CH ₃	H
13	CH ₃	CH ₃	CH ₂ CH=CH ₂	H
14	CH ₃	CH ₃	CH ₂ CH=CH ₂	C ₂ H ₅

^a The control photosynthetic O₂ evolution rate was 90 ~ 130 μmol O₂ · mg Chl⁻¹ · hr⁻¹. The rate was uncorrected on dark respiration in mitochondria.

^b The values are the averages of three measurements.

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TABLE II. EFFECTS OF *N*-SUBSTITUTED GLYCINE ANALOGUES (10 mM) ON RESPIRATION IN MITOCHONDRIA^a

Compound	O ₂ Consumption rate ^b
CH ₂ =CHCH ₂ -N-CH ₂ COOH H	100
CH ₂ =CHCH ₂ -N-CH ₂ COOH CH ₃	99
CH ₂ =CHCH ₂ -N ⁺ -CH ₂ COOH CH ₃ Br ⁻	100
Control	100

^a Mitochondria were isolated from wheat leaves by homogenization of leaf segments in an ice-cold grinding medium (10 g tissue in 500 ml medium) containing 0.3 M mannitol, 30 mM MOPS-KOH (pH 7.6), 4 mM cysteine, 1 mM EDTA, 0.2% bovine serum albumin (BSA) and 0.6% insoluble PVP. The leaf segments were ground for 3~4 sec with a Polytron. The homogenate was squeezed through four layers of cheesecloth and then centrifuged at 1200 × *g* for 4 min to remove chloroplasts and cell debris. The supernatant was then centrifuged at 10,000 × *g* for 10 min and the resulting pellet resuspended with a Teflon/glass homogenizer in 40 ml wash medium containing 0.4 M mannitol, 25 mM TES (pH 7.6) and 0.1% BSA, then after centrifugation at 10,000 × *g* for 10 min, the pellet was resuspended in 2 ml wash medium. Mitochondrial respiration was measured with an oxygen electrode at 25°C in an assay medium containing 0.4 M mannitol, 25 mM TES-KOH (pH 7.2), 2 mM MgSO₄, 5 mM KH₂PO₄, 0.1% BSA and 1.5 mM NADH. When investigating the effects of *N*-substituted glycine analogues on the mitochondrial O₂ consumption rate, the mitochondria were incubated with the test chemical for 2 min prior to addition of the 1 mM ADP.

^b The values are the averages of three measurements.

TABLE III. EFFECTS OF *N*-SUBSTITUTED GLYCINE ANALOGUES ON CO₂ FIXATION IN WHEAT PROTOPLASTS^a

Compound	Concentration (μmol CO ₂ · mg Chl ⁻¹ · hr ⁻¹) ^b
CH ₂ =CHCH ₂ -N-CH ₂ COOH H	0 mM 135 ± 2.5 10 mM 145 ± 3.4 (108%)
CH ₂ =CHCH ₂ -N-CH ₂ COOH CH ₃	0 mM 145 ± 3.2 10 mM 155 ± 1.5 (107%)
CH ₂ =CHCH ₂ -N ⁺ -CH ₂ COOH CH ₃ Br ⁻	10 mM 142 ± 2.8 150 ± 1.2 (106%)

^a The values in parenthesis are the relative rates as to the isolated protoplasts from control and treated leaves.

^b The values are the averages of three measurements.

TABLE IV. EFFECTS OF FOLIAR APPLICATION OF *N*-SUBSTITUTED GLYCINE ANALOGUES ON PHOTOSYNTHESIS IN WHEAT PROTOPLASTS^a

Compound	Concentration	(μmol O ₂ · mg Chl ⁻¹ · hr ⁻¹) ^b	
		2 days	3 days
<i>N</i> -Allylglycine	0 ppm	108 ± 1.2	90 ± 2.1
	300 ppm	122 ± 3.7 (114%)	101 ± 2.9 (110%)
<i>N</i> -Allyl- <i>N</i> -methylglycine	0 ppm	101 ± 2.3	91 ± 2.1
	300 ppm	115 ± 1.9 (113%)	100 ± 1.7 (110%)

^a The values in parenthesis are the relative rates as to the isolated protoplasts from control and treated leaves.

^b The values are the averages of three measurements.