Effect of Irradiation and Immobilization on Spinach Chloroplast Activities

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The effect of γ-ray irradiation and immobilization by means of radiation polymerization on PS II activity (O_2 evolution) of isolated chloroplasts from spinach was investigated. Reduction of O_2 evolution activity by irradiation was small at lower temperatures below −24 °C, but the activity decreased slightly by freezing at extremely low temperature below −78 °C. So the optimum low temperature range for the treatment was observed.

The immobilized chloroplast in a hydrophilic polymer matrix showed the stable duration of O_2 evolution activity more than 700 h at 4 °C. Thermo-stability of chloroplast was also improved greatly by immobilization. The active center of PS II in immobilized chloroplasts was retained even after 60 min standing at 50 °C.

Introduction

Recently, chloroplasts have attracted the large interest from the viewpoint of solar energy utilization [1–6]. The authors studied the immobilization of various biofunctional bodies and substances such as enzymes [7], microbial cell [8] and drugs [9], by means of radiation polymerization at low temperatures using the supercooled monomers for the purpose of artificial utilization of those biofunctional materials. Therefore, it is expected that the stability of chloroplast activity in its electron transfer system might increase by entrapping immobilization with the polymer matrices especially at low temperatures.

However, it is also necessary to study the radiation effect on the intact chloroplast activity before immobilization. Although many studies have been done on the radiation effect on organisms [10, 11], no work has been published on the influence of irradiation on the chloroplast activity excepting a few works for radiation effect on the chlorophyll activity [12]. On the other hand, there has been a few reports on the immobilization of chloroplast [3, 4, 13, 14] by the entrapping with polyacrylamide and polyvinylalcohol at temperatures above 0 °C. However, these studies have been limitted within the PSI (O_2 uptake) activity and PSII (2,6-dichlorophenol indophenol photoreduction activity) and used the relatively higher temperatures for the immobilization.

The present work concerns the effects of irradiation and the immobilization on the stability of PSII (O_2 evolution) activity of chloroplasts mainly at low temperatures.

Materials and Methods

Chloroplasts were prepared by homogenizing 50 g of commercially available spinach leaves at near 0 °C for 10 sec in 200 ml of STN buffer (50 mM Tris-HCl, pH 7.4, 0.4 M Sucrose and 10 mM NaCl). The homogenate was squeezed through ten layers of gauze. The sap obtained was centrifuged at $300 \times g$ for 2 min. The supernatant was then centrifuged at $2100 \times g$ for 10 min and the pellet was resuspended in 2 ml of the same buffer solution or the buffer including 90 vol% of polyethylene glycol 600 (PEG) to give a chlorophyll content of 250–300 µg/100 µl buffer. The mixture was kept at 4 °C.

The immobilization was carried out as follows. One ml of chloroplast suspension was mixed with 0.4 ml of 10% (v/v) bovin serum albumin, 0.4 ml of

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0.1% (v/v) D-mannitol, 0.4 ml of 0.6% (v/v) sodium ascorbate and 0.4 ml of 2-hydroxyethyl acrylate (HEA) which had been purified by distillation under a reduced pressure and ascertained to have no impurities. The resultant mixture was cooled to a determined low temperature and irradiated with γ-ray from 100000 Ci Co^{60} source. Also, 0.9 ml of chloroplast suspension including PEG was mixed with 0.1 ml of HEA monomer, then the mixture was irradiated by γ-ray at -24 °C.

After irradiation, the immobilized product was immersed in cold STN buffer, cut down and washed with cold STN buffer. The immobilized chloroplast was stored in STN buffer or in the mixture of STN buffer 30% and PEG 70% (v/v%).

Chloroplast activity was estimated by 2,6-dichlorophenol indophenol photoreduction (DCIP) and O_2 evolution in $K_3Fe(CN)_6$ reduction. The activity measurement was carried out as follows. The immobilized chloroplast was suspended in buffer by homogenizing before measurement of activity. The chloroplast suspension was introduced into the cell of a spectrophotometer with 10µl of 0.2M $K_3Fe(CN)_6$. The rate of O_2 evolution in the sample was measured with a clerk type oxygen electrode at 20 °C under an illumination of white light from a 300 W projector, which was transmitled through an aqueous solution of 1% (v/v%) coupper (II) sulfate. For DCIP photoreduction measurement, the mixture (3.0 ml) containing 30 µg of chlorophylles and 0.5 µM of DCIP was introduced to a cuvette in Shimazu Recording Spectrophotometer Model 500. A red light from a 300 W Nikon projector through a Toshiba VR-67 filter was illuminated for 1 min and the change of absorbance at 593 nm was measured during illumination. Activity of DCIP photoreduction with s-diphenylcarbazide (DCP) of 1.5 µM asan electron donor was also measured at 593 nm in the same manner as described above. The chlorophyll content was determined according to the Mackinney method [15].

Results and Discussion

Effect of irradiation temperature on PS II activity (O_2 evolution) of chloroplast

Effect of γ-ray irradiation on PSII activity of chloroplast was investigated before immobilization study. Chloroplast in buffer was irradiated at various temperatures and subjected to activity measurement at 20 °C. The result was shown in Fig. 1. Curves B, D, F and H of solid circles in Fig. 1 show the change of O_2 evolution activity of irradiated chloroplasts. Curves G, E, C and A with open circles show the activity change of unirradiated chloroplasts during storage for the same times and at the same temperatures as in irradiation. At -24 °C (curves C and H), almost no activity change occurred during the low temperature storage, but a higher (at 20 °C, curve G) or a lower temperature (at -78 °C, curve A), the activity decreased considerably after the low temperature storage. Therefore, the loss of activity caused by irradiation can be estimated by the difference of activities between irradiated and unirradiated samples. In the irradiation at 20 °C, the O_2 evolution activity decreased markedly with increasing dose and the original activity was almost completely lost on 3.5 Mrad irradiation. With lowering the irradiation temperature, however, the activity loss by irradiation was reduced. At temperatures below -24 °C, the difference in activity between the irradiated chloroplast and the stored one without irradiation became slight and negligible. The activities of irradiated and of stored samples decreased gradually with time at -78 °C. This might be attributed to an irradiation with freezing at extremely low temperatures. Therefore, there is an optimum temperature range for activity retention between -24 °C and -78 °C.

Effect of immobilization by radiation polymerization on duration of PS II activity (O_2 evolution) of chloroplast for a long period

The isolated chloroplasts from spinach were immobilized by means of radiation polymerization using supercooled vinyl monomer at low temperatures. The changes of PSII activity (O_2 evolution) in intact and immobilized chloroplasts were shown in Fig. 2. Both intact and immobilized chloroplasts were immersed in buffer or in buffer including 70% PEG, and stored for a long period at 4 °C. The activity of intact chloroplast disappeared completely within 150 h. On the other hand, the activity of immobilized chloroplast was retained far longer, though the activity yield from original spinach activity was lower than that of intact one. Especially, in the case of immobilized chloroplast suspended in buffer including PEG, high activity continued more

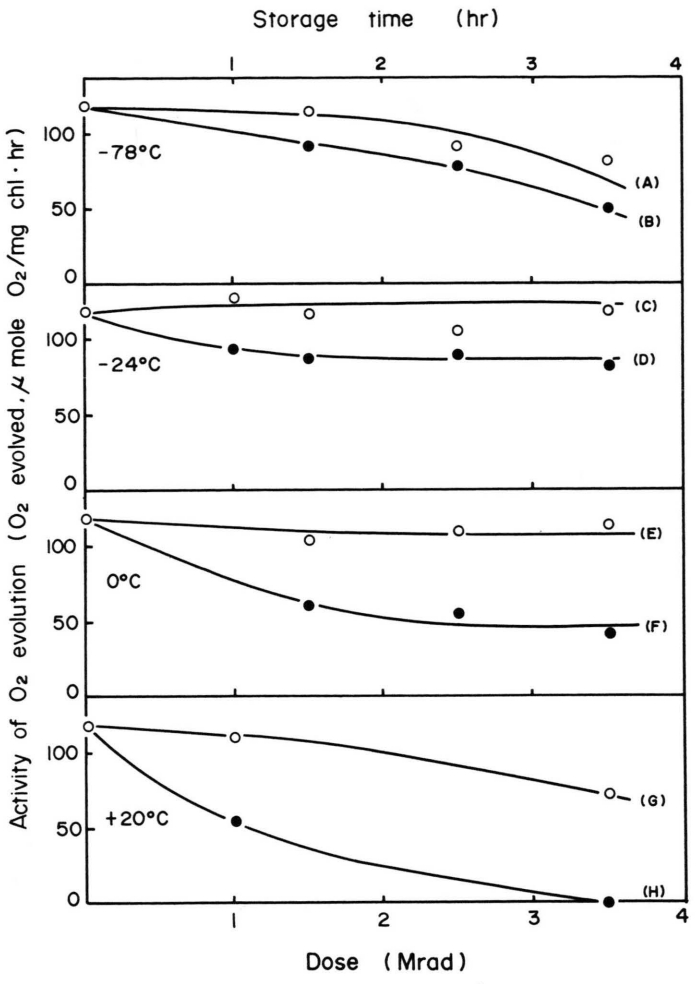

Fig. 1. Change of O_2 evolution activity (PS II) in the irradiated and unirradiated chloroplasts. Irradiated sample: (B) at −78 °C, (D) at −24 °C, (F) at 0 °C, (H) at 20 °C. Unirradiated sample: (A) stored at −78 °C, (C) stored at −24 °C, (E) stored at 0 °C, (G) stored at 20 °C. Dose rate for irradiation: 1×10^6 r/h.

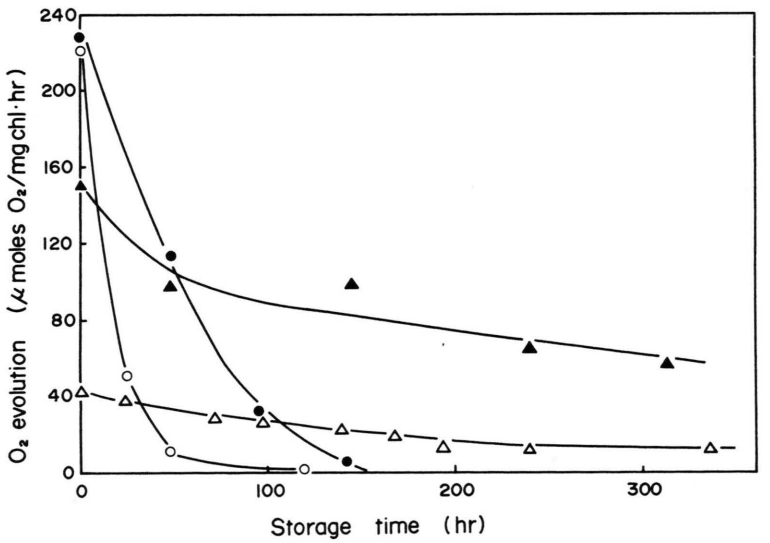

Fig. 2. Change of the O_2 evolution activity (PS II) of intact and immobilized chloroplasts for a long period at 4 °C. Intact chloroplast: (○) in buffer, (●) in PEG 90/buffer 10 (v/v%). Immobilized chloroplast: (△) chloroplast was isolated in buffer and after immobilization, immobilized chloroplast was stored in buffer, (▲) chloroplast was isolated in PEG 90/buffer 10 (v/v%) and after immobilization, immobilized chloroplast was stored in PEG 90/buffer 10 (v/v%).

Table I. Photoreduction activity of DCIP with DPC as electron donor in immobilized chloroplast.

Storage time after immobilization [h]	DCIP photoreduction (mol DCIP/mg Chl; h)	
	without DPC	DPC
24	27.3	48.6
120	30.4	38.0

than 300 h. The activity yield of chloroplast immobilized in pure buffer is very low perhaps due to inactivation by the monomer. So, bovine serum albumin and D-mannitol as protectant were added in buffer including chloroplast on immobilization. Those additives were used by Ochiai *et al.* [13] for the same purpose on catalytic immobilization.

On the other hand, the activity of chloroplast in buffer including PEG is hardly lost by the monomer addition as well as by irradiation and freezing, so the immobilization scarecely decreased the high activity.

The increase in DCIP photoreduction activity by addition of DPC as an electron donor was investigated and the result is shown in Table I. For 24 h after immobilization, increase of photoreduction by addition of is slight and difference in activity between systems with DPC and without DPC is not clearly observed. From these results, it was suggested that not only O_2 evolution activity with storage time but also active center in whole PSII process was destroyed by inactivation.

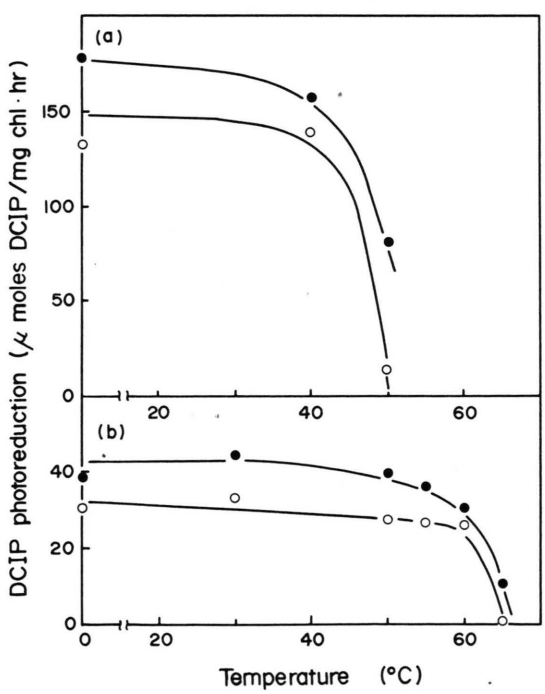

Fig. 3. Thermo-stability in photoreduction activity for DCIP with DPC as electron donor in intact and immobilized chloroplast. (a) intact chloroplast, (b) immobilized chloroplast. (○) without DPC, (●) DPC addition. Treatment time at various temperatures, 5 min.

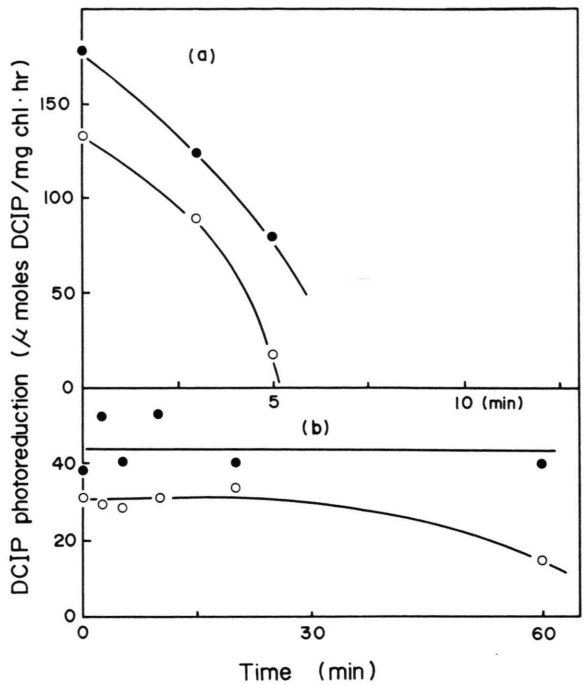

Fig. 4. Thermo-stability of photoreduction activity for DCIP with DPC as electron donor in intact and immobilized chloroplast at 50 °C. (a) intact chloroplast, (b) immobilized chloroplast. (○) without DPC, (●) DPC addition.

Thermo-stability of immobilized and intact chloroplast

It was expected that thermo-stability of chloroplast at higher temperatures is increased by immobilization. Fig. 3 shows the change in DCIP photoreduction activity by heating for 5 min at various temperatures. In the case of intact chloroplast, activity decreased quickly above 40 °C and almost completely lost at 50 °C in the system without DPC. On the other hand, in the immobilized chloroplast, activity was retained even at higher temperature than in intact chloroplast. At a temperature above 60 °C, activity in DPC including system was lost. This fact suggests that active center in PSII is lost thermally at this temperature.

Fig. 4 shows the change of DCIP photoreduction activity when intact and immobilized chloroplast were stored for a long time at 50 °C. In intact chloroplast, PS II activity disappeared within 10 min and in case of immobilized chloroplast, 30% of original activity remained even after 1 h. Moreover, the activity in DPC including system was same as that of original (at zeri time) even after 1 h. This fact shows that active center in PS II remains more stable on heating than O_2 evolution activity. From these results, it is obvious that thermo-stability for both O_2 evolution and DCIP photoreduction activity of chloroplast was increased greatly by immobilization.

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