Effect of Viscous Solvents and Monomer on Conservation of Intact and Immobilized Chloroplasts

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O_2 evolution activity of PS II in chloroplast stored in buffer including various supercooling solvents was studied. Viscous solvents such as polyethylene glycol (PEG) was remarkably effective for the increase of lifetime of intact chloroplast. Lifetime of immobilized chloroplast by means of radiation polymerization was prolonged more than 40 days in buffer including PEG 70% (v/v). It was found that viscous solvents having PEG units in molecular chain were effective and methoxypolyethyleneglycol methacrylate monomer (M-23G) was most suitable as a immobilization carrier. The immobilized chloroplast with M-23G retained the high activity yield more than 30 days in buffer including PEG.

Introduction

The chloroplast isolated from plant is very labile and in particular, the O_2 evolution in photosynthesis II(PS II) is most unstable. It disappeared in about two days at 0–4 °C and in 16–20 h at 20 °C. However, it was reported that the chloroplast activity was stabilized by entrapping in polymer gels crosslinked with glutaraldehyde [1] or polymerized with acrylamide [2].

In previous paper [3, 4], the authours clearified that chloroplast could be immobilized stably at lower temperature and the O_2 evolution activity in the immobilized material by authors method had a high activity yield and remarkably prolonged lifetime of 2–3 days even at 20–30 °C as well as increased thermo-stability.

Moreover, the authors found that the addition of some kinds of solvents having large viscosity and supercooling property was very effective for stabilization against inactivations by monomer addition and freezing.

In this report, the effects of various viscous solvents on the stability of PS II activity in the intact and immobilized chloroplasts were investigated in details.

Materials and Methods

The isolation of chloroplasts, immobilization by radiation polymerization and assay of activity in PS II (O_2 evolution) were carried out according to the same methods as described in the previous paper [3].

The intact and immobilized chloroplasts were stored in buffer including viscous solvents at 4 °C. The viscous solvents used without purification the commercial reagents.

Results and Discussion

Effect of viscous solvents on stability of chloroplasts at low temperature

Irradiated and unirradiated chloroplasts were stored for 3 h at various temperatures. The effects of temperature irradiation and storage medium on the O_2 evolution activity of PS II process were studied. The result was shown in Fig. 1. There was an optimum temperature range for activity retention between −20 °C and −80 °C. At higher temperatures above 0 °C, activity of irradiated chloroplasts decreased perhaps due to radiation damage. At extremely low temperatures, activity of both irradiated and unirradiated chloroplasts were reduced perhaps mainly due to the freezing of cell. However, as shown in Fig. 1, it is noticed that in the case of chloroplast stored in buffer including polyethyleneglycol (PEG) as a viscous solvent, activity decreases at higher temperature and also at extremely lower temperatures were very slight. This fact suggested that PEG acts as an effective protectant for the damages of chloroplasts by freezing and radiation. The protection of PEG for freezing can be attributed to supercooling property of this viscous solvent to prevent the crystallization of water arround the cell.

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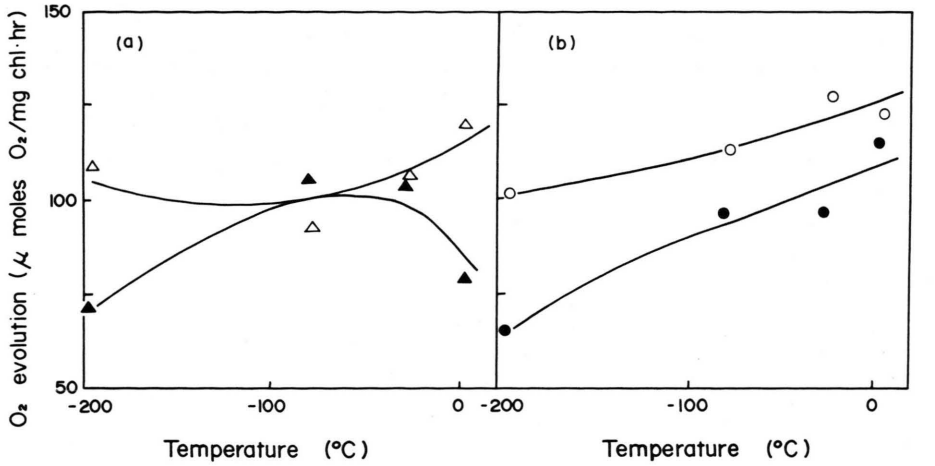

Fig. 1. Change of activity in irradiated and unirradiated chloroplasts stored for 3 h at various temperatures. (a) Irradiated chloroplast: (▲) in buffer, (△) in PEG 400 90/buffer 10 (v/v%), (b) unirradiated chloroplast: (●) in buffer, (○) in PEG 400 90/buffer 10 (v/v%). Irradiation dose, 1×10^6 r.

So, the irreversible change of cell structure such as destroy of cell membrane might be avoided. The protection for radiation damage might be due to the decrease of water content in buffer. Because it is known that radiolysis species (radicals) of water highly accelerate the decomposition of solute molecules.

Effect of viscous solvents on stability of stored chloroplasts

Various viscous solvents such as ethyleneglycol, glycerine, trimethyrolpropyleneglycol, carbitol, polyethyleneglycol 300, 400, 600, 1000 and methyl cellosolve were added to the suspension of intact chloroplasts in buffer. The decays of chloroplasts activity in buffer including carbitol, PEG and glycerine were less than that in buffer including no additive solvent. The activity in viscous solvents remained slightly even after storage for 100 h. The activity decays of chloroplast in buffer including solvents other than the viscous ones was similar to that in buffer only. Fig. 2 showed the change of PS II (O_2 evolution) of intact chloroplasts with the storage time in buffer-various viscous solvents mixture. According to this result, certain kinds of viscous solvents such as methyl carbitol and PEG showed remarkable effect on stabilization almost comparable to the case of protectant addition such as bovin serum albumin. Fig. 3 showed the effect of composition of some viscous solvents such as PEG and glycerine in buffer. With the increase of PEG and glycerine concentration, the lifetime of chloroplast was prolonged and chloroplast suspended in buffer 10/PEG 90 (v/v%) composition retained the

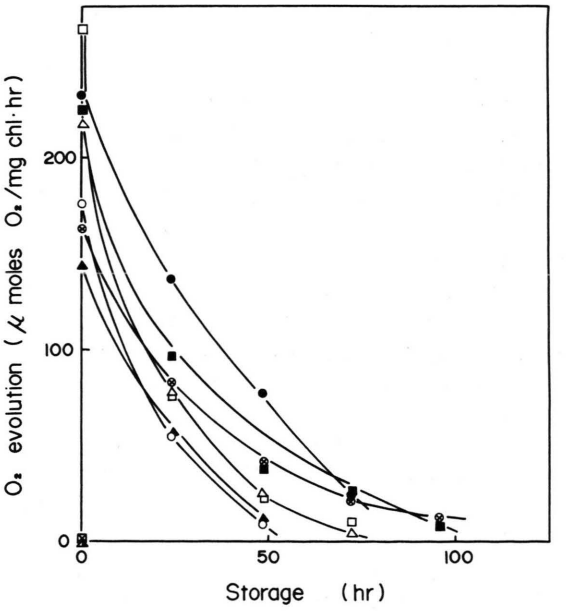

Fig. 2. Effect of various viscous solvents on PS II (O_2 evolution) activity in the stored intact chloroplasts. (○) in buffer only, (●) in buffer including bovin serum albumin, (△) in ethyleneglycol 70/buffer 30 (v/v%), (▲) in glycerine 70/buffer 30, (□) in trimethyl propyleneglycol 70/buffer 30, (■) in methylcarbitol 70/buffer 30, (⊗) in PEG 70/buffer 30, (⊠) in buthylcellosorp 70/buffer 30, (◭) in DMSO 70/buffer 30.

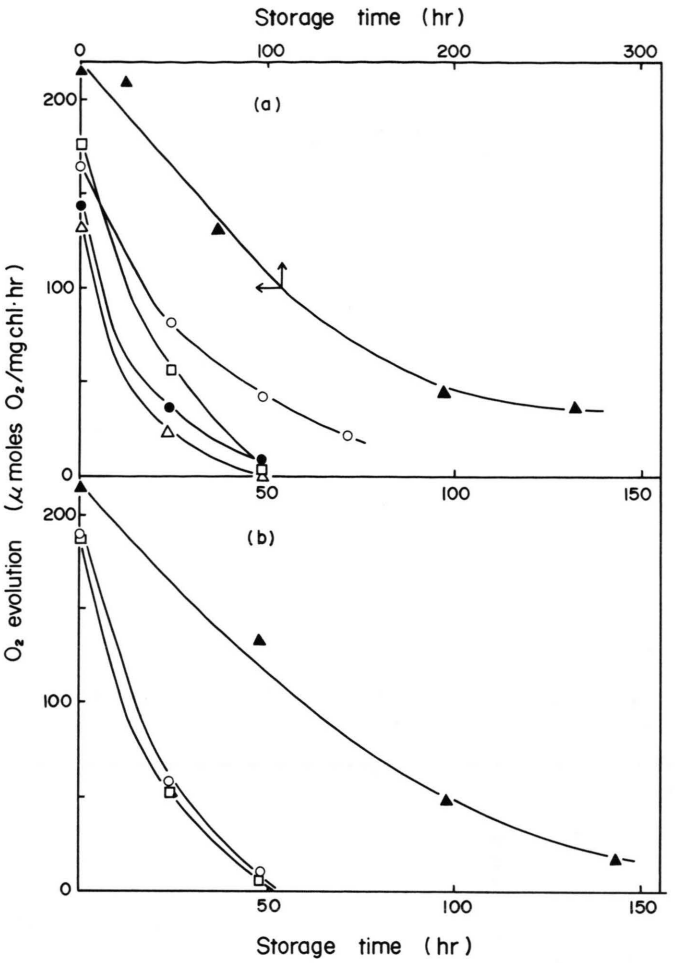

Fig. 3. Effect of composition of the buffer-PEG or glycerine-buffer on PS II (O_2 evolution) activity in the stored intact chloroplasts. (a) buffer-PEG system (v/v%): (▲) buffer 10-PEG 400 90, (○) 30–70, (●) 50–50, (△) 70–30, (□) buffer only. (b) buffer-glycerine (v/v%): (▲) buffer 10-glycerine 90, (○) 30–70, (□) buffer only.

25% of original activity after storage for 200 h. However, in low concentration of PEG in buffer, activity decreased quicker than that of pure buffer and chloroplast suspended in pure PEG or glycerine lost the activity immediately in storage. Consequently, it is necessary to store the chloroplast in buffer including PEG or glycerine of relatively high concentration more than 70% (v/v) in order to prolong the lifetime sufficiently. Asada *et al.* [5] reported that the activity of Hill reaction at −20 °C was retained for several months with a small loss, when spinach chloroplasts were suspended in 50% glycerine. The result in Fig. 3 showed that the lifetime of chloroplast activity was prolonged by addition of other solvents such as PEG at higher temperature such as 4 °C.

Effect of viscous solvents on stability of immobilized chloroplasts

In the previous paper [3], it was found that when the immobilized chloroplast was stored in buffer including bovin serum albumin as a protectant, it was retained PS II activity for very long time at 4 °C. It was expected that PS II activity of immobilized chloroplast was further prolonged in storage in buffer including various solvents. The immobilized sample was stored in buffer including 70% (v/v) viscous solvents such as glycerine, polyethyleneglycol (300, 400, 600 and 1000), methylcarbitol, ethyleneglycol, triethyleneglycol, tripropleneglycol, methoxypolyethyleneglycol methacrylate (M-23G) and polyethyleneglycol dimethacrylate (14G). The PS II activities in these systems were lost in earlier stage than in

intact chloroplast stored in buffer 10/PEG 90 (v/v%), except some effective systems including PEG, 14G and glyceline. The immobilized chloroplasts in buffer including 70% (v/v) of PEG, glycerine and 14G retained the activity after storage for 300 h.

The effect of PEG-buffer composition on PS II activity of immobilized chloroplast was shown in Fig. 4. The immobilized chloroplast in buffer including PEG of 50, 70 and 90% (v/v) retained the activity for a long period. The decay of activity was the smallest the composition of PEG 70/buffer 30 (v/v). In intact chloroplast, the activity decay was the minimum in PEG 90/buffer 10 (v/v%) composition. Thus, the most suitable composition of PEG and buffer to keep the activity exists. PEG was most suitable as protectant among various viscous solvents. Then, the effect of molecular structure, ethyleneglycol chain length on activity protection was studied. The result was shown in Fig. 5. According to this result, ethyleneglycol was not effective. In case of triethyleneglycol, activity was kept up to 20 days. In buffer including PEG 300, 400 and 600, activity was remained constantly for long period more than 40 days. But the activity decay was small in the order to PEG 600, 400 and 300. This fact shows that the protection effect in viscous solvents having ethylene glycol

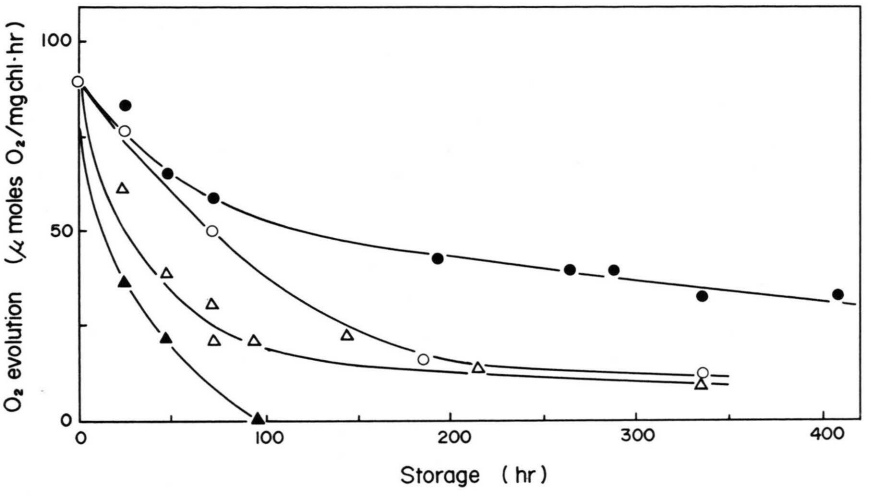

Fig. 4. Effect of composition of buffer-PEG on PS II (O_2 evolution) activity in the immobilized chloroplasts storage. Composition during storage: (○) buffer 10-PEG 400 90 (v/v%), (●) 30–70, (△) 50–50, (▲) buffer only. Immobilization: carrier, M-23G; irradiation, 1×10^6 r at −24 °C.

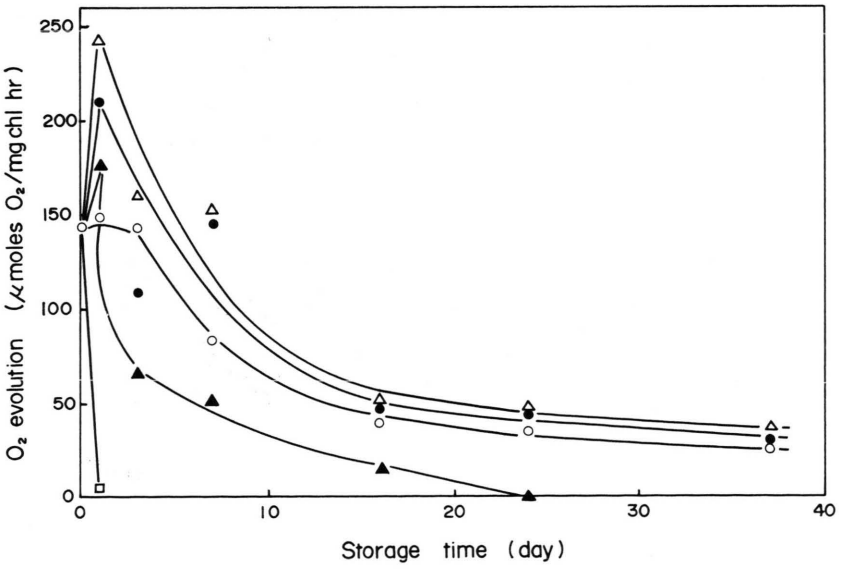

Fig. 5. Change of the activity for storage of immobilized chloroplast in buffer including various solvents having ethyleneglycol chain. Composition of buffer-various solvents during storage, 30–70 (v/v%). (□) ethyleneglycol, (▲) triethyleneglycol, (○) PEG 300, (●) PEG 400, (△) PEG 600. Immobilization: carrier, M-23G; irradiation, 1×10^6 r at −24 °C.

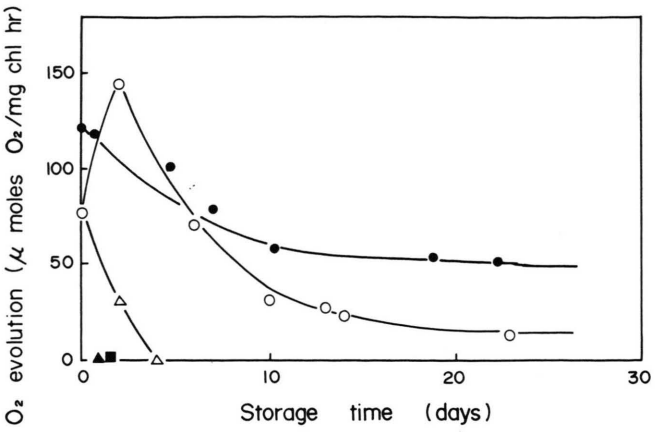

Fig. 6. Change of the activity for storage of immobilized chloroplast with various carriers. Storage of immobilized chloroplast, in PEG 400 70-buffer 30 (v/v%). (▲) HEA, (■) HEMA, (○) 14G, (●) M-23G, (△) A-9G. Immobilization, 1×10^6 r irradiated at −24 °C.

unit increases with the increase of ethylene glycol units length. PEG of average molecular weight above 1000 are solid. Though they are completely dissolved in buffer at 25 °C, they can not be used at low temperatures owing to crystallization.

Effect of viscous long chain monomers on stability of immobilized and stored chloroplasts

Various hydrophilic monomers such as 2-hydroxyethyl acrylate (HEA), 2-hydroxyethyl methacrylate (HEMA), polyethyleneglycol diacrylate (A-9G), 14G and M-23G were used for immobilization. These monomers except for M-23G inactivated the activity of intact chloroplast in buffer by contacting before immobilization. Thus, the protectant such as bovin serum albumin was added to buffer before monomer addition, but activity was reduced immediately by monomer addition even in the presence of bovin serum albumin [3]. Fig. 6 shows the activity of chloroplast immobilized with various monomers in the absence of protectant. In the case of immobilization by HEA and HEMA, activity was completely lost after immobilization. This attributed to inactivation perhaps by the monomer and irradiation. In immobilization by A-9G, 14G and M-23G carriers, activity was remained after immobilization and gradually decayed with the time. In particularly, in the case of immobilization by M-23G, high activity retained for a long time and this decay curve was same as that in the presence of protectant (bovin serum albumin) [3]. It is sure that chloroplast in buffer was hardly inactivated by M-23G monomer addition even in absence of protectant perhaps due to the specific effect of monomer having the long polyethyleneglycol chain structure. Although, the activity yield immediately after immobilization was slightly lower than the original activity immediately after isolation, this monomer might be the most excellent carrier for the chloroplast immobilization.

M-23G polymer including chloroplast swelled easily with buffer containing PEG and rather brittle. It was also recognized that M-23G was effective for stabilization of chloroplast even in buffer including no PEG. That is, PEG can be replaced by M-23G having the similar chemical structure as PEG.

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