Stabilization of the Structure and Functions of a Photosystem I Submembrane Fraction by Immobilization in an Albumin-Glutaraldehyde Matrix

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ABSTRACT

The effect of immobilization in an albumin-glutaraldehyde cross-linked matrix on the structure and activity of a photosystem I submembrane fraction has been studied. The photosynthetic activity recovered after immobilization was between 35 and 45% of the oxygen-uptake rates of the native material. Resulting oxygen uptake activities found in immobilized photosystem I preparations with methylviologen as acceptor were as high as 270 μmol O_2 (mg Chl h)$^{-1}$, An enhancement of photosystem I electron transfer, which is produced by incubation of thylakoid membranes at temperatures above 30°C, was detected in native submembrane fractions, but not in the immobilized preparations. It is suggested that the increased activity at high temperature results from conformational modifications not allowed in the immobilization matrix. The insensitivity of immobilized photosystem I particles to prolonged storage at 4°C and to strong light exposure, as well as their high electron-transfer rates, demonstrates that the immobilization procedure used can be successfully applied to submembrane fractions.

Index Entries: Photosystem I; submembrane fraction; immobilization; electron transfer; biotechnology.

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INTRODUCTION

Photosynthetic membranes can be used for the phototransduction of light energy into chemical or electrical energy. This property confers great potential to thylakoid membranes for several types of biotechnological applications. However, any significant progress in this area is limited by the lack of functional stability of the isolated biological material. Several immobilization procedures have been developed in order to improve the stability of the photosynthetic reactions. The electron-transfer activity of the native material is usually reduced by up to 60% after immobilization; however it is still able to perform this function, since the remaining activity is affected to a lesser degree by prolonged storage, by high pH or high temperature treatment, or by saturating light conditions. Some promising results have been obtained using these types of preparations. For example, immobilization greatly improved the operational lifetime of bioreactors or solar cells used for photoproduction of hydrogen (*1*), hydrogen peroxide (*2*), formic acid (*3*), or photocurrent (*4*). A clear advantage of the immobilized material over the native preparations is the fact that the former constitutes a physically insoluble structure, and therefore it can be used in continuous-flow systems (*5*).

Photosystem I is able to catalyze all the reactions that have been studied to date in photosynthetic bioreactors. For example, a photosystem I submembrane fraction has been successfully used for photocurrent generation (*6,7*). The latter authors demonstrated that photosystem I could be used alone, providing that photosystem II is replaced by another electron-donation system. Exhaustion of added artificial donors can often be avoided by using a system that functions in a cyclic manner (*6,7*). An important parameter to consider is the known higher stability of photosystem I compared to photosystem II. In fact, most of the relevant reports emphasize that photosystem I is less sensitive to photoinhibition than photosystem II upon exposure to saturating light intensities (*8,9*). Furthermore, photosystem I is much more heat stable (*10,11*), and its activity, monitored as oxygen uptake with dichlorophenolindophenol (DPIP) as an electron donor, is enhanced following incubation of thylakoid membranes at elevated temperatures (30°C), whereas oxygen evolution by photosystem II is inhibited (*12–14*).

Given the intrinsic stability of photosystem I, it is of interest to determine whether immobilization can increase its stability. In this study, we report immobilization of a photosystem I submembrane fraction in a crosslinked albumin-glutaraldehyde matrix, because this type of matrix maintains better functional stability than several other techniques (*15,16*). Oxygen-uptake rates in native and immobilized photosystem I preparations are compared under several conditions. The effects of immobilization on the structure and activity of the submembrane fraction is discussed.

MATERIALS AND METHODS

Photosystem I submembrane fractions were isolated from spinach using digitonin, according to the method of Peters et al. (*17*). The resulting preparation was kept at a chlorophyll (Chl) concentration of 3.3 mg/mL in 20 m*M* Tricine-NaOH, pH 7.8, containing 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$, unless otherwise specified in the text. Chlorophyll was determined according to Arnon's method (*18*).

Immobilization was performed with freshly isolated material using the procedure described by Thomasset et al. (*19*), although the buffer medium was different. Photosystem I (0.6 mL) was mixed with 1.65 mL of 20 m*M* Tricine-NaOH, pH 7.8, (which contained 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$, unless otherwise specified), 1.25 mL of 20% bovine serum albumin, and 1 mL of 1.5% glutaraldehyde. The final glutaraldehyde concentration was 0.33%. After mixing, the solution was distributed into 75 μl samples and frozen at −20°C for 2 h. Before use, the samples were thawed for 2 h at 4°C and homogenized in a mortar with the appropriate buffer.

Initial rates of oxygen uptake were monitored using a water jacketed, Clark type of electrode at 22°C, with white light illumination (160 W/m^2) from a 150 W quartz halogen projector lamp, as described elsewhere (*20*). The light intensity used was saturating for both native and immobilized photosystem I preparations. The reaction medium contained 11 μ g Chl/mL, 0.2 m*M* DPIP, 1 m*M* sodium ascorbate, 0.5 m*M* methylviologen, 1 m*M* NaN_3, 20 m*M* Tricine-NaOH (pH 7.8), 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$, unless otherwise specified.

When oxygen uptake was determined as a function of pH, 20 m*M* Mes-NaOH, 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$ were used as the medium for pHs between 5 and 6.5. For higher pHs, the medium contained 20 m*M* Tricine-NaOH, 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$. The samples were equilibrated for 5 min in the proper medium before measurements were taken. In the temperature studies, samples that were preincubated at various temperatures were transferred to the oxygen electrode cell and equilibrated for 2 min at 22°C before measurements were taken.

The photoinhibition of electron transfer from DPIP to methylviologen was performed at room temperature under aerobic conditions. The photosystem I preparation was diluted to 11 μ g Chl/mL in 20 m*M* Tricine-NaOH (pH 7.8), 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$, and was illuminated (120 W/m^2) with the white light from a 150-W tungsten halogen lamp filtered through a 4-cm water path. At given periods, a 3-mL aliquot from the gently stirred suspension was introduced into the oxygen electrode cell, and the donor/acceptor system was added for oxygen uptake measurements. The percentage of photoinhibition was obtained by comparing

the rates of oxygen uptake obtained from the preilluminated samples with the rates from samples not illuminated but otherwise treated the same way.

RESULTS

Effect of Salt Concentration

In native preparations, the oxygen uptake yields at 22.5°C (with DPIP and methylviologen as electron donor and acceptor, respectively) ranged from 360 to 700 μmol 0_2 (mg Chl-h)$^{-1}$, depending on the ionic content of the final resuspension medium. We therefore expected the activity recovered after immobilization to depend on the salt composition of the immobilization mixture. Immobilization of the photosystem I submembrane fraction in an albumin-glutaraldehyde matrix resulted in a spongy material, as described in the immobilization of whole thylakoid membranes (*19*). When no salt was present, 30% of the activity monitored in the native samples remained after immobilization (Table I). This yield could be improved only when NaCl (10 m*M*), or KCl (1 or 10 m*M*) was present. In these cases, about 35% of the activity was recovered (Table I). When 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$ were used together (this condition corresponds to the ionic composition of the final resuspending medium described by Peters et al. [*17*]), about 36% of the initial activity was also recovered in the immobilized samples in the experiment reported in Table I. The maximal yield of recovery varied with the preparation used and ranged between 35 and 45%, coinciding with typical activities in immobilized photosystem I from 160 to 270 μmol O_2 (mg Chl-h)$^{-1}$.

The salt composition of the immobilization medium also influenced the long-term storage stability at 4°C (Fig. 1). Under most conditions tested, native samples retained between 10 and 35% of their initial activity after a storage period of nearly 2 mo. Even though the native material was quite stable to start with, immobilization still resulted in a significant improvement. As shown in Fig. 1, the treated material preserved from 40 to 70% of its initial oxygen uptake rates after a similar storage period of 2 mo at 4°C.

Optimal storage stabilities of native or immobilized photosystems were obtained with either 10 m*M* KCl or 10 m*M* NaCl (Fig. 1). Although $MgCl_2$ did not improve the percentage of activity recovered after immobilization (Table I), 5m*M* $MgCl_2$ did increase the storage stability of native and immobilized samples (Fig. 1C). The simultaneous presence of 5 m*M* $MgCl_2$, 10 m*M* KCl, and 10 m*M* NaCl in the immobilization medium also provided great stability (Fig. 2): after a storage period of 2 mo at 4°C, the native material retained 30% of its initial electron-transfer rate, but the immobilized material preserved 75%.

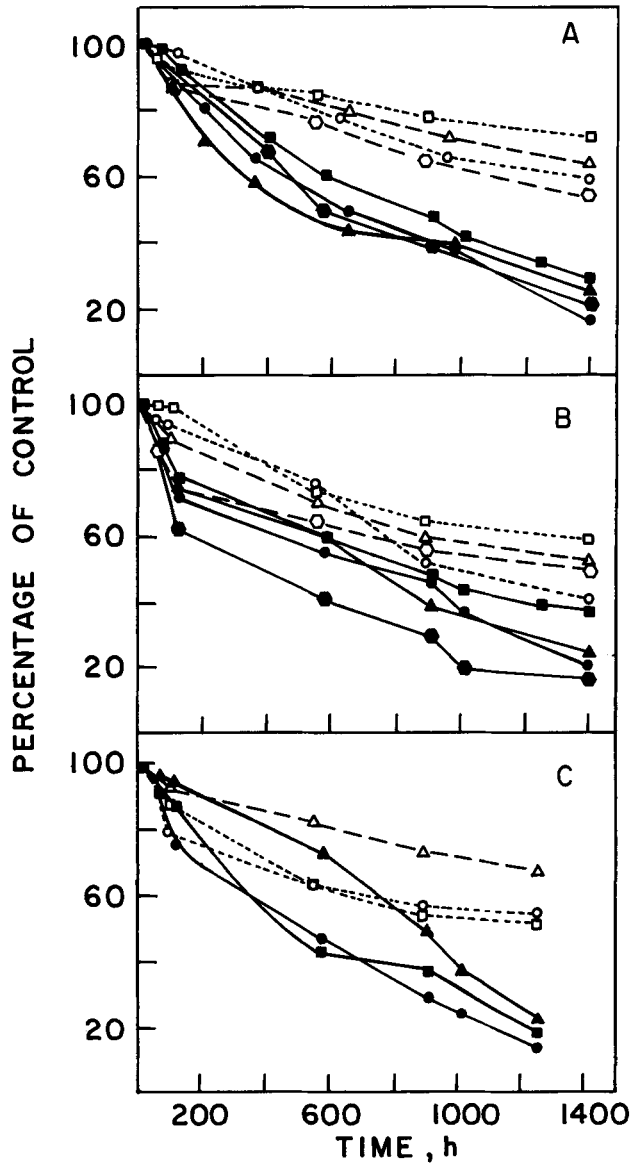

Fig. 1. Stability of the oxygen uptake activity of the photosystem I submembrane fractions during storage at 4°C in the dark. Closed symbols, native preparations; open symbols, immobilized. Control rates (μmol O_2[mg Chl-h]$^{-1}$) are given in brackets. (A) Effect of NaCl; 0.1 m*M*, ●, ○ [453,129]; 1 m*M*, ●, △ [507,150]; 10 m*M*, ■, □, [507,174]; 100 m*M*, ⬢, ⬡ [575,142]. (B) Effect of KCl: 0.1 m*M*, ●, ○ [361,95]; 1 m*M*, ▲, △ [531,183]; 10 m*M*, ■, □ [503,186]; 100 m*M*, ⬢, ⬡ [709,199]. (C) Effect of $MgCl_2$; 0.5 m*M*, ●, ○ [504,131]; 5 m*M*, ▲, △ [572,174]; 50 m*M*, ■, □ [518,97]. Ions were present at the above concentrations in the final resuspension buffer as well as in the immobilization and oxygen uptake assay medium. Other details are given in Materials and Methods.

Table 1
Effect of the Salt Composition
of the Final Resuspension and Immobilization Medium
on the Oxygen Uptake Activity Recovered After Immobilization

Salt (s)	Concentration, m*M*	Activity recovered,[a] %
None	-	30±4
NaCl	1	30±3
	10	34±3
	100	25±3
KCl	1	35±3
	10	37±3
	100	28±3
$MgCl_2$	5	30±3
	50	19±4
NaCl	10	
+KCl	10	
+$MgCl_2$	5	36±3

[a] Activity monitored as μmol O_2(mg Chl-h)$^{-1}$ in the assay medium described in Materials and Methods, with the exception that the salt composition was the same as that in the final resuspension and immobilization buffer. The values presented are the average of four experiments.

The results obtained in the absence of salts are also described in Fig. 2. The much faster decrease of electron-transfer rates observed during storage at 4°C under these conditions demonstrates an important stabilization effect of salts. For example, after a storage period of 75 d in the presence of salts, the native photosystem I preparation studied in Fig. 2 retained electron-transfer activity of 46 μ mol O_2 (mg Chl-h)$^{-1}$. However, after the same storage period, the immobilized preparation retained an activity of 118 μ mol O_2 (mg Chl-h)$^{-1}$.

Both the activity recovered after immobilization and the storage stability at 4°C were maximal when 5 m*M* $MgCl_2$, 10 m*M* NaCl, and 10 m*M* KCl were included in the immobilization medium. Therefore, this condition was used throughout the following experiments.

Effect of pH

The effect of pH on the activity of the photosystem I submembrane fraction is presented in Fig. 3. The optimal pH for native membranes ranges from pH 7.0 to 8.5. The activity decreases abruptly when increasing or decreasing pH outside of these limits. On the other hand, immobilized membranes were less affected by low pH, and their maximal oxygen uptake rates were obtained between pH 8 and 9. The immobilized material thus required higher pH for optimal activity.

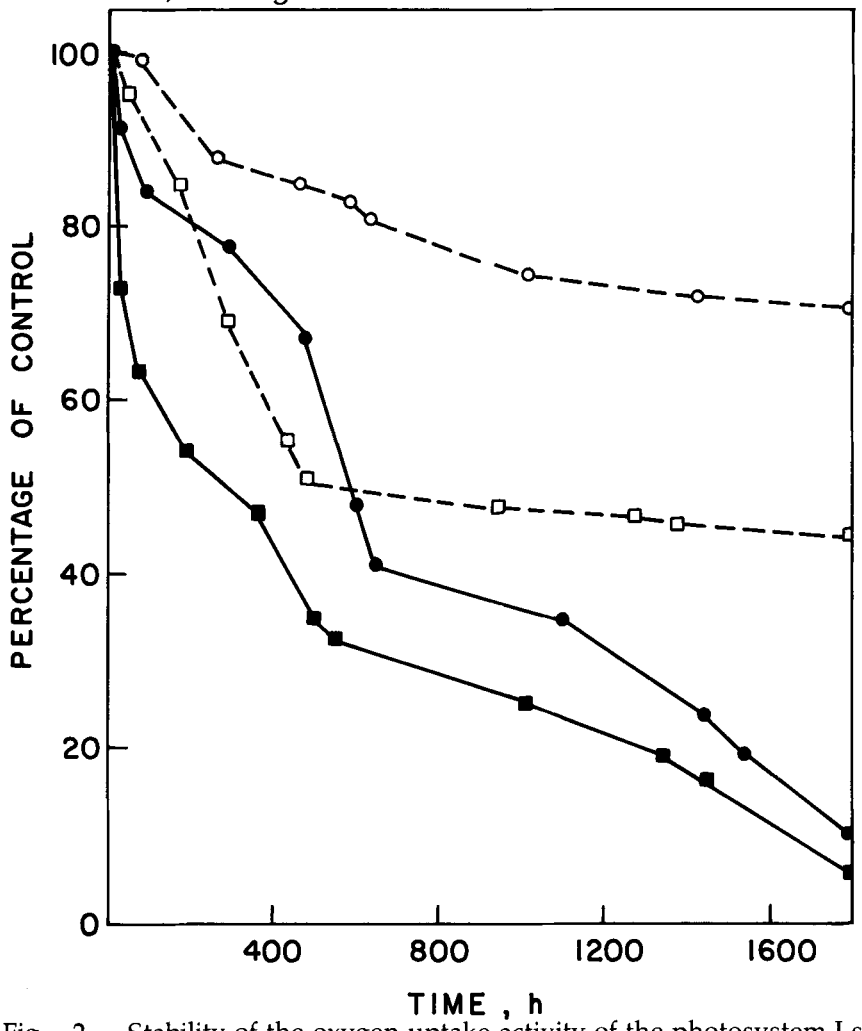

Fig. 2. Stability of the oxygen uptake activity of the photosystem I submembrane fractions during storage at 4°C for native (closed symbols) and immobilized preparations (open symbols). (Control rates (μmol O_2[mg Chl-h]$^{-1}$) are given in brackets.) With 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$, ●, ○ [461,164]; without salts, ■, □ [572,172]. Other details as in Fig. 1.

Effect of Temperature

Figure 4 shows the effect of a 5-min incubation at various temperatures on the rate of oxygen uptake monitored at 22.5°C. In native membranes, electron transfer from DPIP to methylviologen was activated by high-temperature incubation with a maximum of 50°C. At this temperature, the activity was enhanced by a factor of about 300% after incubation. At higher temperatures, the electron-transfer rate started to decline, owing to increasing concurrent degradation of membrane constituents.

In the experiments reported in Fig. 4, the incubation was performed for 5 min at various temperatures. We also studied the influence of pro-

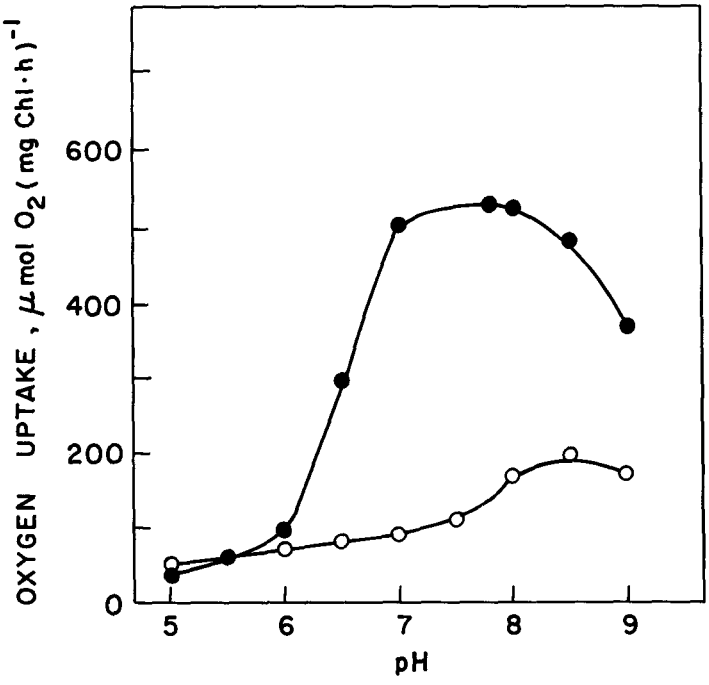

Fig. 3. Effect of the pH of the oxygen uptake assay medium on the activity of native (●) and immobilized (○) photosystem I preparations. Samples were immobilized and assayed in the presence of 10 m*M* NaCl, 10 m*M* KCl, and 5 m*M* $MgCl_2$. Other details are given in Materials and Methods.

longed periods of incubation at 22.5 and 50°C (Fig. 5). In this case, the activation took about 20 min to reach its maximal value and was then followed by a progressive decrease in activity at both temperatures studied. The immobilized samples were very stable for 30 min incubation, but lost about 40–50% of their activity after a 60-min incubation at 22.5 or 50°C. Therefore, the activity enhancement observed with native samples was not detected in immobilized preparations.

It is also relevant at this point to mention that the saturating concentration of the donor (DPIP) was similar in native and immobilized preparations at the two temperatures studied in Fig. 5. However, the I_{50} value for DPIP saturation in native samples treated at 50°C was slightly lower than that for 22.5°C ($\cong$20 μM vs 40 μM, respectively). The maximal oxygen uptake activity was obtained with 100 μM DPIP (not shown).

Effect of Strong Illumination

In Fig. 6, we show the photoinhibitory effect of strong illumination on native and immobilized preparations. The initial rate of photoinactivation was 50% faster in native than in immobilized samples. Furthermore,

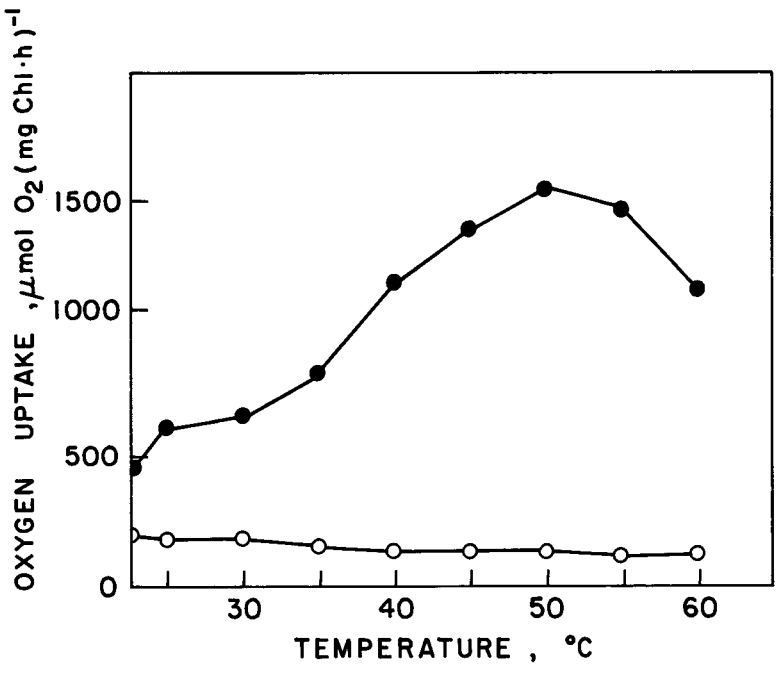

Fig. 4. Effect of the incubation temperature on the oxygen uptake activity of native (●) and immobilized (○) photosystem I preparations. Samples were incubated for 5 min at the given temperature and transferred to the oxygen electrode cell.

when complete loss of activity was produced in the native samples, immobilized submembrane fractions still reduced methylviologen at 50% of their initial rate. After a 6-h illumination, the activity in immobilized membranes used in the experiment reported in Fig. 6 corresponded to 110 μmol O_2 (mg Chl-h)$^{-1}$.

DISCUSSION

We have demonstrated in this study that the photosystem I submembrane fraction used can be immobilized advantageously in an albumin-glutaraldehyde crosslinked matrix. The percentage of activity recovered after immobilization was similar to the percentage of recovery obtained when immobilizing whole thylakoid membranes using the same procedure (*16,19*). The electron-transfer rates measured after immobilization were as high as 270 μmol O_2 (mg Chl-h)$^{-1}$.

The decreased photoactivity after immobilization of whole thylakoid membranes or photosystem II submembrane fractions has been explained tentatively by a reduced mobility of the electron carriers in the membrane,

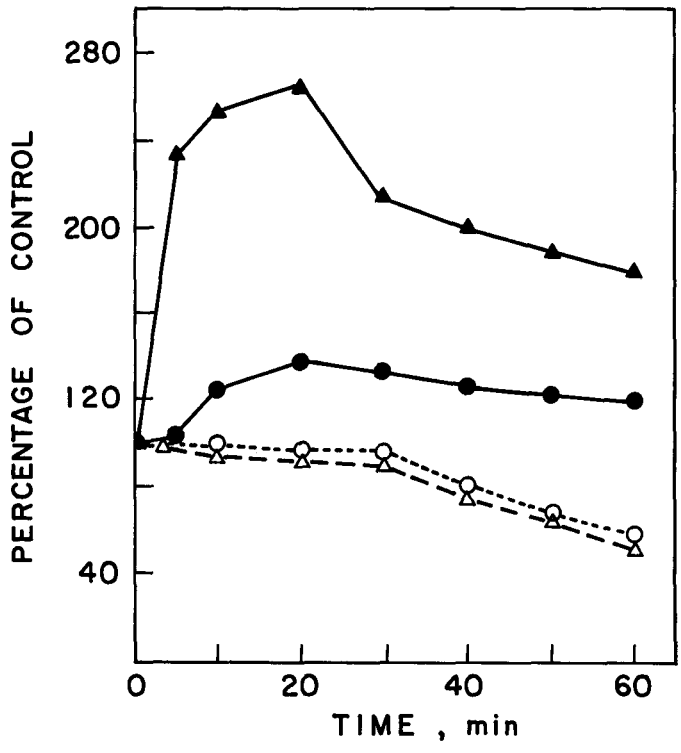

Fig. 5. Effect of the incubation period at 22.5°C (●, ○) and 50°C (▲,△) on the oxygen uptake rates. Control rates were 514 μmol O_2(mg Chl-h)$^{-1}$ for native samples (closed symbols) and 200 μmol O_2(mg Chl-h)$^{-1}$ for immobilized samples (open symbols).

because of crosslinking between glutaraldehyde and the membrane proteins, and/or by reduced access of artificial donors and acceptors at their respective sites (*21,22*). Glutaraldehyde inhibits photosystem I electron transfer at the level of plastocyanin (*23,24*). Therefore, the yields of methylviologen reduction observed in our immobilized preparations could also be affected by partial inhibition by glutaraldehyde.

Activation of photosystem I electron transfer by incubation at a relatively high temperature was demonstrated in whole thylakoid membranes when DPIP was used as electron donor (*14*). It has been inferred that new DPIP donation sites are exposed at the level of the cytochrone b-f complex after a temperature-induced conformational change that would increase the apparent activity of the photosystem (*14*). We have shown in the experiments reported in Figs. 4 and 5 that this property is retained in isolated photosystem I submembrane fractions. However, the absence of any increase in oxygen uptake activity in the immobilized preparations supports the hypothesis that the conformational modification is not allowed in the immobilized matrix. The absence of temperature-increased activity

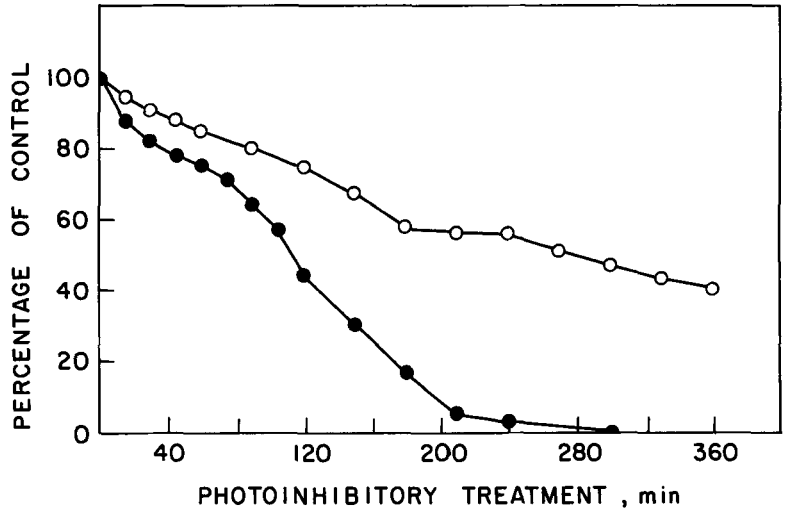

Fig. 6. Photoinhibition of oxygen uptake activity following strong illumination of native (●) and immobilized (○) preparations; the control rates were 694 and 270 μmol O_2(mg Chl-h)$^{-1}$, respectively, and the initial rates of photoinhibition were 0.83 and 0.38% per min, respectively.

in the immobilized membranes was not caused by limited access of DPIP to its electron donation site(s), because the saturating concentration was similar in both native and immobilized membranes studied either at 22.5 or 50°C. However, our results are in disagreement with the interpretation of Thomas et al. (*14*) that activation of photosystem I activity results from the opening of new DPIP oxidation sites. If that were the case, the saturation concentration of DPIP should increase simultaneously. Instead, we report a slightly lower I_{50} for DPIP saturation of native photosystem I preparations treated at 50°C than for preparations treated at 22.5°C. Therefore, additional experiments will be necessary to clarify the true nature of the thermal activation of photosystem I.

The results in Figs. 4 and 5 provide evidence that temperature treatments had a much smaller influence on the immobilized preparations than on the native ones. However, the native samples always supported higher activities than immobilized photosystem I. This is because of both the higher initial activity in the native material and the activation effect produced during incubation on the dark. On this basis, native submembrane fractions would be more useful than immobilized ones, if they could be used in the absence of illumination. However, it is important to consider the effects of photoinhibition, which are induced by the strong illumination required during photobiotechnological experiments. Exposure to strong light has been shown to damage the acceptor side of photosystem I at the level of the iron-sulfur centers (*25*). The results in Fig. 6 clearly demonstrate that the immobilized preparation is more resistant to photo-

inactivation and will retain more activity than the native photosystem I after prolonged exposure to working conditions. However, some of the reduced photosensitivity obtained in the immobilized preparations could be caused by an increased light scattering in this type of preparation in comparison to the native material.

On the other hand, the relative insensitivity of the immobilized preparation to temperature changes, as compared to the large variations of electron-transfer rates produced by temperature changes in native samples, is a great advantage for applications in which stable photoactivity is required but temperature is difficult to control

Finally, in comparison to immobilized whole thylakoid membranes or photosystem II submembrane fractions, the immobilized photosystem I preparation was far more resistant to prolonged storage at 4°C (ref. *19,22;* Figs. 1 and 2) and to strong light exposure (ref. *26,27;* Fig. 6). Provided that an electron-donor system is either included in a sacrificial manner or regenerated in a cyclic system, the relatively high electron-transfer rate of the preparation described here demonstrates that the immobilization procedure used can be applied successfully to this type of material.

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