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Effects of sodium dodecyl sulfate and methyl viologen on the differential extinction coefficient of P-700 – a band shift of chlorophyll *a* associated with oxidation of P-700

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The magnitude of flash-induced bleaching at 700 nm in the thylakoid membranes isolated from the thermophilic cyanobacterium *Synechococcus* sp. was affected neither by addition of 4 mM methyl viologen, nor by treatment of the membranes with 0.1% sodium dodecyl sulfate (SDS) for 1 h, but appreciably increased on addition of methyl viologen to the SDS-treated membranes. Detailed studies on the light-minus-dark difference spectrum of P-700 revealed that methyl viologen induces a shift-type change consisting of a bleaching at 695 nm and a positive band at 685 nm, and consequently increases the magnitude of the 700 nm bleaching without affecting P-700 photooxidation in the SDS-treated membranes. Other bipyridinium dyes, 1,1′-trimethylene-2,2′-bipyridinium dibromide and 1,1′-trimethylene-5,5′-dimethyl-2,2′-bipyridinium dibromide were equally effective as methyl viologen, and a prolonged treatment of the membranes with SDS caused a similar band shift in the difference spectrum of P-700. The band shift is closely associated with P-700 oxidation because, on redox titration, the magnitude of the band shift varied in parallel to the amount of P-700 oxidized by light. Methyl viologen also induced the band shift in the chemically oxidized-minus-reduced difference spectrum of P-700 in the SDS-treated membranes. Thus, the band shift is not related to reduction of a bound electron acceptor. As a consequence of the band shift, the oxidized-minus-reduced differential extinction coefficient of P-700 in the 700 nm region was increased by 40%. The extinction coefficient was 64 $mM^{-1} \cdot cm^{-1}$ at 701 nm in the thylakoid membranes, whereas the 1-h treated membranes with added methyl viologen, or a Photosystem I reaction center complex prepared by SDS-gel electrophoresis, showed the extinction value of 84–86 $mM^{-1} \cdot cm^{-1}$ at 698 nm. Two different models for the band shift are discussed.

Introduction

The primary photochemistry of PS I is an electron transfer from the special chlorophyll *a* dimer P-700 to the electron acceptor chlorophyll *a* A_O (for reviews, see Refs. 1 and 2). Because the reduced A_O is short-lived due to a rapid reoxidation by the secondary electron acceptor A_1, the main features of the light-minus-dark difference spectrum of PS I preparations are ascribed to oxidation of P-700. In the visible region, the difference spectrum of P-700 consists of two major bleaching bands at 700 nm and 430 nm. The bleaching band at 700 nm is asymmetric, the magnitude of absorption changes decreasing more steeply on the shorter- than the longer-wavelength side of the

Abbreviations: SDS, sodium dodecyl sulfate; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, Photosystem.

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maximum [3]. The 700 nm band is always accompanied by a small negative band at 680 nm. In contrast to the 700 nm band, the magnitude of the 680 nm band is highly variable with preparations [4,5].

Different models have been proposed to explain these spectral features of P-700 in the red region. The similarity between the 700 nm and the 680 nm bands in terms of relaxation kinetics [6] and dependence on the ambient redox potential [7] led to a conclusion that the two bands arise from one and the same pigment P-700. P-700 was suggested to have two absorption bands in the red region due to its dimeric or aggregated structure [8]. Based on the light-induced absorption and circular dichroic spectra of spinach PS I particles, an excitation interaction between two chlorophyll *a* molecules within the reaction center was proposed [9]. Two negative absorption changes at 700 nm and 680 nm are due to light-induced disappearance of the two exciton components, whereas the asymmetry of the 700 nm band is ascribed to appearance of a new positive band at 688 nm owing to a remaining unoxidized chlorophyll *a* molecule of the P-700 dimer [9]. These models provide, however, no explanation for the variability of the 680 nm band. An interpretation is that the 680 nm bleaching results from a long-wavelength shift of chlorophyll *a* molecules associated with P-700 [4]. Flash-induced absorption changes and linear dichroic spectra of several PS I preparations were analyzed by means of a Gaussian deconvolution [5,10]. The best fit was obtained by assuming three components; a bleaching at 695.5 nm, an absorption increase at 690 nm and a long-wavelength shift of a band at 688 nm. The band shift is attributed to the electrochromic response of antenna chlorophyll molecules close to P-700 [10].

P-700 is usually quantified by measuring light- or oxidant-induced bleaching at 700 nm. The spectrophotometric assay of P-700 requires an accurate value of the oxidized-minus-reduced differential extinction coefficient of P-700 in this wavelength region. Currently, the extinction coefficients of P-700 in spinach and *Anabaena variabilis* PS I preparations, which have been determined by measuring TMPD oxidation coupled with P-700 reduction [11] and confirmed by EPR studies of Signal I [12] and polarographic studies on oxygen yield per flash [13], are widely in use.

Various detergents affect the difference spectrum of P-700. The 700 nm band is shifted to a shorter wavelength in detergent-treated preparations. Variations in the magnitude of the 680 nm change relative to that of the 700 nm change are attributed to a detergent effect [4]. In particular, several detergents were reported to increase the 700 nm bleaching in the chemically oxidized-minus-reduced difference spectrum of Tobacco PS I particles [14]. A 30% increase in the bleaching was also observed after addition of SDS to PS I membrane fractions from the thermophilic cyanobacterium *Phormidium laminosum* [15]. In spite of these observations, effects of detergents on the differential extinction coefficient of P-700 has not yet been studied in detail.

In the present work, we have examined effects of SDS on light-induced absorption changes of PS I in the thylakoid membranes isolated from the thermophilic cyanobacterium *Synechococcus* sp. A prolonged treatment of the thylakoid membranes with the detergent caused an apparent band-shift of chlorophyll *a* molecules at 690 nm in the light-minus-dark difference spectrum of P-700. A similar effect of methyl viologen on the difference spectrum of P-700 was discovered in SDS-treated membranes. As a result of the band shift, the oxidized-minus-reduced differential extinction coefficient of P-700 was increased by 40%.

Materials and Methods

Synechococcus sp. was grown for two days at 55 °C in the light (20000 lx) with continuous bubbling with air containing 5% CO_2 [16]. The thylakoid membranes were prepared as described previously [17]. Cells were incubated in a medium containing 50 mM Tris-HCl (pH 7.5), 10 mM NaCl and egg-white lysozyme (1 mg/ml) at 37 °C for 2 h. The protoplasts thus prepared were passed through a French pressure cell at 400 kg/cm^2 and then centrifuged at 20000 × *g* for 40 min. The supernatant was supplemented with 1 mM $MgCl_2$ and DNase (20 μg/ml) and left at 25 °C for 30 min. The thylakoid membranes were collected by centrifugation at 140000 × *g* for 1 h, suspended in 0.5 M sucrose, 50 mM Tris-HCl (pH 7.5) and 10

mM NaCl and stored at −30 °C. A PS I reaction center complex was prepared by the procedures of Takahashi et al. [17,18].

All spectrophotometric measurements were carried out at room temperature. Flash-induced absorbance changes were measured with a Union Giken single-beam spectrophotometer [19,20]. The basal reaction mixture contained 50 mM Tris-HCl (pH 7.5), 10 mM NaCl, 1 mM TMPD and the thylakoid membranes equivalent to 10 μg chlorophyll *a* per ml. For measurement of absorption changes in the red region, xenon flashes with a half height duration of 5 μs were passed through a Corning 4-96 filter and the photomultiplier was guarded with a Toshiba VR-65 filter. Absorbance changes in the blue region were measured with flashes passed through two Toshiba VR-65 filters. The photomultiplier was blocked with two Corning 4-96 filters. Flashes were fired at 1 Hz and signals were stored and averaged with a microcomputer (Sord M223 Mark II) attached to the spectrophotometer, then analyzed by another microcomputer (NEC PC -9801 VM2).

The reduced-minus-oxidized differential extinction coefficient of P-700 was determined by measuring oxidation of TMPD coupled with reduction of flash-oxidized P-700 as described by Hiyama and Ke [11] except that experiments were carried out under aerobic conditions. Reaction mixture was the same as described above, except TMPD concentration was varied in the range of 125 μM to 2 mM. When the thylakoid membranes were used, 1 mM KCN and 10 μM DCMU were added to inhibit a respiratory oxidation of TMPD and PS II-dependent reduction of oxidized TMPD, respectively. TMPD oxidation was determined at 575 nm, an isosbestic point of P-700 oxidation, and the extinction coefficient of oxidized TMPD at this wavelength was assumed as 10.7 $mM^{-1} \cdot cm^{-1}$ [11]. Routinely, absorption changes of P-700 and TMPD were determined in separate but identical reaction media, both several times, and mean values were presented. Variation of the data was less than 5%.

The chemically oxidized-minus-reduced difference spectrum of P-700 was determined with a Hitachi 320 spectrophotometer. Membranes (10 μg chlorophyll *a* per ml) were suspended in 50 mM Tris-HCl (pH 7.5), 10 mM NaCl and 0.3 mM ferricyanide, and 2 ml each of the suspension was placed in the sample and reference cuvettes. The difference spectrum was determined immediately after addition of 20 μl of 1 M ascorbate and 2 μl of 1 M TMPD to the sample and 22 μl of water to the reference cuvettes. The back-ground correction was made with a microprocesser attached to the spectrophotometer.

Chlorophyll *a* was determined by the method of Mackinney [21].

Results

P-700 photooxidation in the thermophilic cyanobacterium *Synechococcus* sp. is extremely resistant against detergents such as SDS and five PS I reaction center complexes containing photoactive P-700 have been isolated by means of SDS-polyacrylamide gel electrophoresis from SDS-solubilized thylakoid membranes [17,18]. During experiments to determine the recovery of P-700 in the SDS preparations, we have occasionally encountered an unexpected situation that SDS-treatment of the thylakoid membranes increased the content of P-700 which was determined by measuring photobleaching at 700 nm. Systematic investigations such as shown in Fig. 1 revealed that treatment of the membranes with 0.1% SDS for 1 h had no significant effect on light-induced absorption decrease at 700 nm (trace b) but addition of methyl viologen, a good electron acceptor of PS I, to the SDS-treated membranes induced an appreciable increase in the magnitude of the 700 nm bleaching (trace c). The effect of methyl viologen was not seen in untreated membranes but appeared slowly during incubation of the membranes with SDS to reach the maximum level after 1 h of the treatment. The SDS-treatment also resulted in a retardation of the decay kinetics which was largely reversed by methyl viologen. The effect of methyl viologen on the decay kinetics was not further studied.

To examine the unexpected effect of methyl viologen on the absorption changes, the light-minus-dark difference spectrum of P-700 was determined under various conditions. The difference spectrum of untreated thylakoid membranes shows a bleaching at 701 nm and a minor negative band at 682 nm, both of which are ascribed to P-700

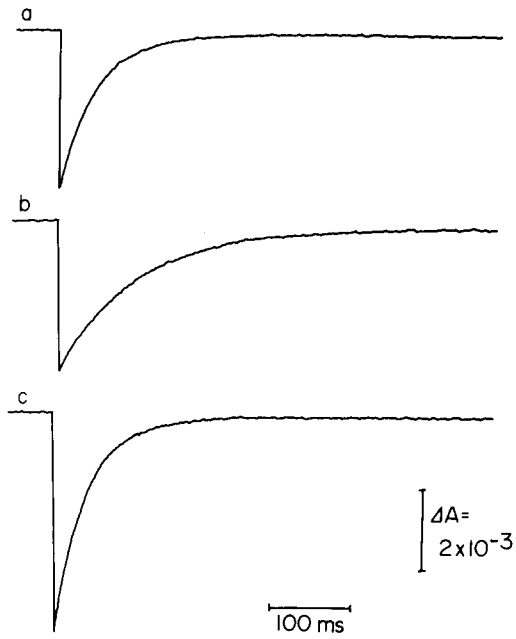

Fig. 1. Effects of SDS and methyl viologen on flash-induced absorption changes in the thylakoid membranes. (a) Untreated membranes in the presence of 4 mM methyl viologen; (b) membranes treated with 0.1% SDS for 1 h; (c) b plus 4 mM methyl viologen. Signals obtained with 16 flashes fired at 1 Hz were averaged.

oxidation (Fig. 2 curve a). Photoresponse of the PS II reaction center chlorophyll could not be resolved in the time range used here. Treatment of the membranes with 0.1% SDS for 1 h slightly affected the difference spectrum (curve b). The difference between the two spectra (curve c) shows that the detergent treatment induced an upward shift of the entire spectrum and a blue-shift of the main bleaching band. Thus, SDS appears to alter the membrane structure and the microenvironment surrounding P-700, leaving the photochemical activity of PS I unaffected.

Addition of methyl viologen to the SDS-treated membranes caused a large change in the difference spectrum: the magnitude of the main bleaching band was increased while the 682 nm band was diminished (Fig. 3 curve b). The maxima of the two bands were shifted to shorter wavelengths. The effect of methyl viologen is more clearly seen when the difference spectrum determined in the absence of methyl viologen is subtracted from that determined in the presence of methyl viologen (curve c). The resulting spectrum, which consists of a negative band at 695 nm and a positive band at 685 nm, suggests a band shift of chlorophyll *a*

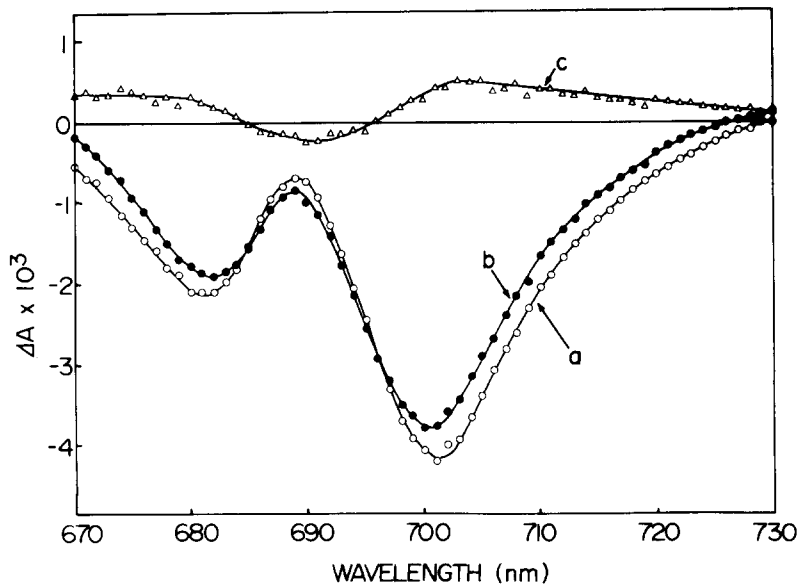

Fig. 2. Effects of SDS on the difference spectrum for flash-induced absorption changes. (a) Untreated membranes; (b) membranes treated with 0.1% SDS for 1 h; (c) b minus a.

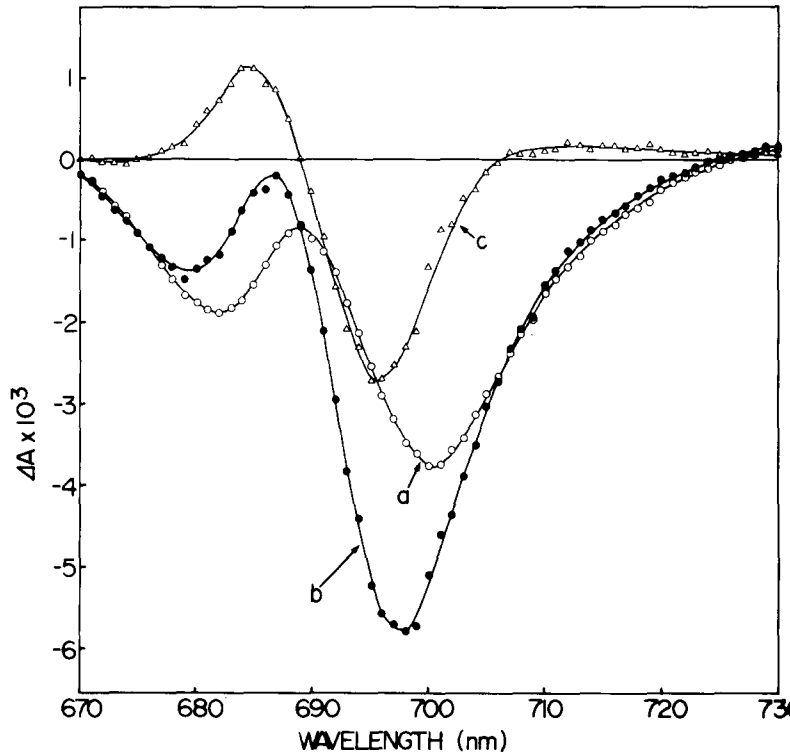

Fig. 3. Effects of methyl viologen on the difference spectrum for flash-induced absorption changes in the thylakoid membranes treated with 0.1% SDS for 1 h. (a) No addition; (b) 4 mM methyl viologen was added; (c) b minus a.

molecules at about 690 nm to a shorter wavelength. The P-700 photoresponse was little affected, or occasionally somewhat diminished by the addition of methyl viologen. Thus, methyl viologen increased the magnitude of photobleaching at 700 nm by inducing an additional spectral change in the P-700 difference spectrum.

In the blue region, the difference spectrum of the SDS-treated membranes was also affected by methyl viologen but to a lesser extent. The main negative band at 433 nm and a positive band at 450 nm were both reduced in magnitude (Fig. 4). The difference between the two spectra suggests that methyl viologen also induces a band shift in this wavelength region (curve c).

A prolonged treatment of the membranes with SDS also caused a shift-type change in the difference spectrum of P-700. Fig. 5 shows that, as compared with the 1h-treated membranes (curve a), the main bleaching band was blue-shifted and the 682 nm band was considerably reduced in magnitude and shifted to a shorter wavelength in the membranes which had been treated with 0.1% SDS for 13 h (curve b). However, the prolonged SDS-treatment did not increase the magnitude of photobleaching around 700 nm. Subtraction of the difference spectrum of the 1h-treated membranes from that of the 13-h treated membranes resulted in a spectrum with a positive band at 684 nm and a negative band at 694 nm (curve c). Clearly, the long-term SDS-treatment is also effective in inducing the band-shift. Further addition of methyl viologen had no effect on the difference spectrum of the 13-h treated membranes (not shown). Curve c additionally shows a band at 704 nm, indicating a partial inactivation of P-700 photooxidation. The extent of the inactivation was estimated as 33%, by comparing amplitudes of the photobleaching at 707 nm, an isosbestic point of the band-shift (see curve c of Fig. 3), between the 1-h and 13-h treated membranes. When curve c is corrected for the inactivation, curve d is obtained, which is similar in shape to, but approx. 30% smaller in magnitude than, the methyl viologen-in-

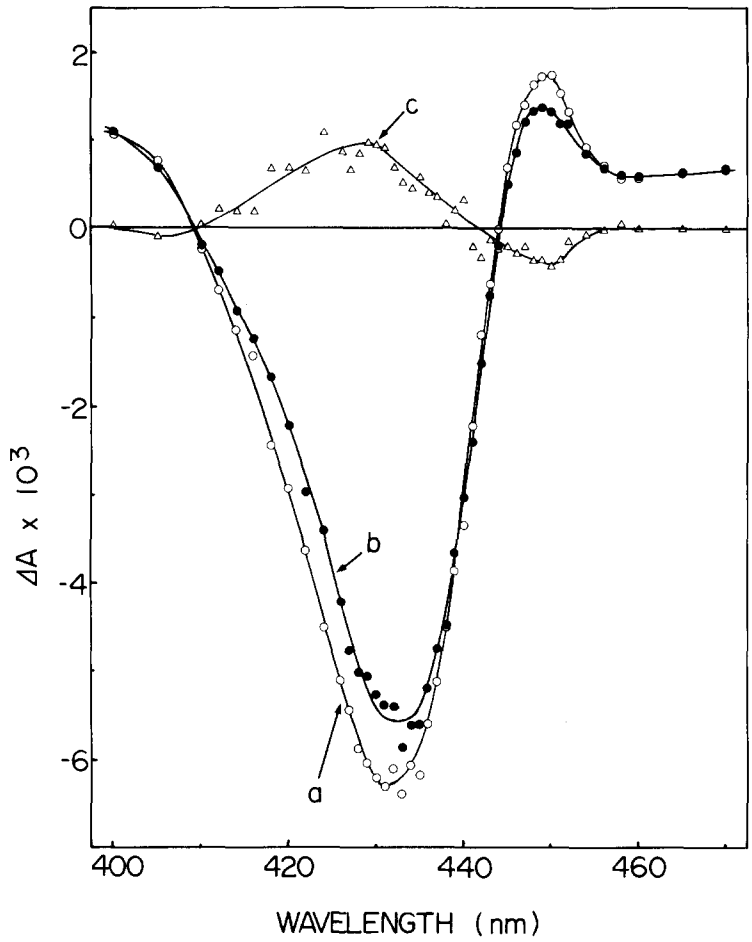

Fig. 4. Effect of methyl viologen on the difference spectrum for flash-induced absorption changes in the blue region in the thylakoid membranes which were treated with 0.1% SDS for 1 h. (a) No addition; (b) 4 mM methyl viologen was added; (c) b minus a.

duced band shift (Fig. 1B, curve c). This indicates that a partial suppression of P-700 photooxidation is accompanied by a comparable diminution of the band-shift.

Redox titration of the band-shift and P-700 oxidation in the SDS-treated membranes shows that the band shift is indeed closely related to photooxidation of P-700 (Fig. 6). Magnitudes of the photobleaching at 696 nm decreased as the ambient redox potential was raised. Deviations from the theoretical curve for a one-electron transfer process in the low-potential region may be ascribed to a partial oxidation of ferrocyanide added. A midpoint potential of P-700 was determined as 0.38 V, which is lower than that of P-700 in untreated membranes (0.42 V). A drop in the midpoint potential of P-700 by SDS has been reported [22]. Note that the magnitude of the methyl viologen-induced spectral change, i.e., the difference between the photobleachings with and without methyl viologen, varied in parallel to the extent of P-700 photooxidation. The linear relationship between the two photoresponses strongly suggests that the band shift is caused by photooxidation of P-700.

The effect of methyl viologen on the magnitude of photobleaching at 696 nm was saturated at 4 mM (Fig. 7). Two other low potential electron acceptors of PS I, 1,1′-trimethylene-2,2′-bipyridinium dibromide (−521 mV) and 1,1′-trimethylene-5,5′-dimethyl-2,2′′-bipyridinium dibromide (−670 mV) were also effective in enhancing the

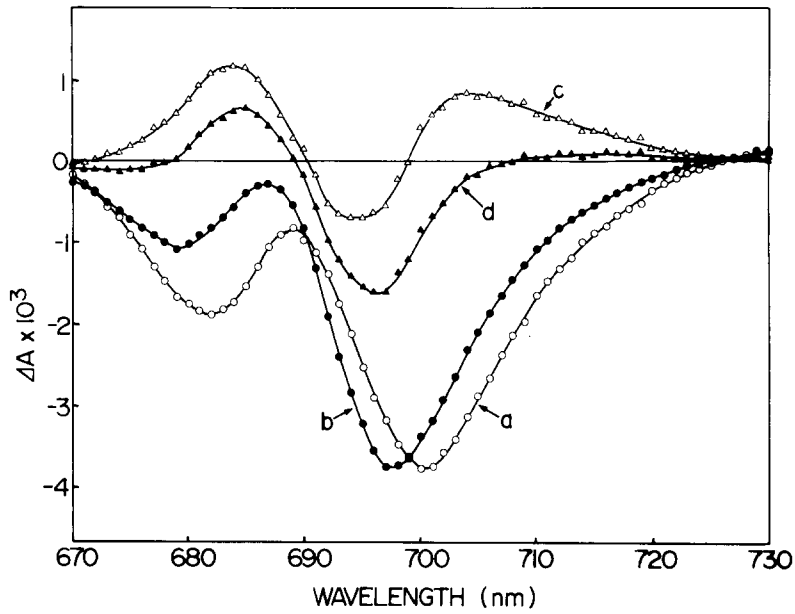

Fig. 5. The difference spectra for flash-induced absorption changes in the thylakoid membranes treated with 0.1% SDS for 1 h and 13 h. (a) 1-h treated membranes; (b) 13-h treated membranes; (c) b minus a; (d) c corrected for an inactivation of P-700 photooxidation. The correction was made by adding curve a, which had been reduced in magnitude by a factor of 0.33, to curve c.

photobleaching. Note that the three acceptors gave identical concentration curves. Benzyl viologen seems similarly effective, although only a low concentration of the acceptor was examined due to its poor water solubility.

Fig. 8 shows the ferricyanide-oxidized minus TMPD-reduced difference spectra of P-700 in the

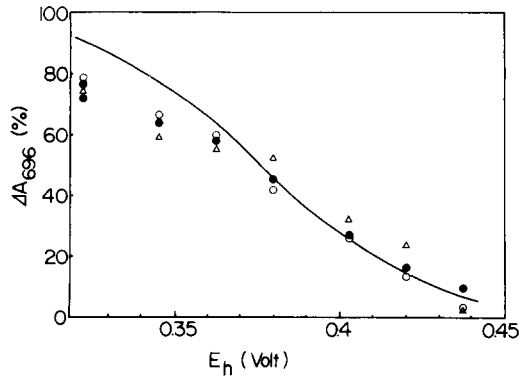

Fig. 6. Oxidation-reduction titrations of light-induced absorption changes at 696 nm in the thylakoid membranes treated with 0.1% SDS for 1 h. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 10 mM NaCl, 10 μg chlorophyll *a* per ml and varied ratios of ferricyanide and ferrocyanide. The midpoint potential of the ferricyanide-ferrocyanide couple was assumed as 0.42 V [23]. ● and ○, magnitudes of absorption changes determined in the presence and absence of 1 mM methyl viologen, respectively. △, ● minus ○. Magnitudes of the absorption changes determined in the presence of 10 mM ascorbate were taken as 100%. The solid curve is a theoretical curve for one electron transfer.

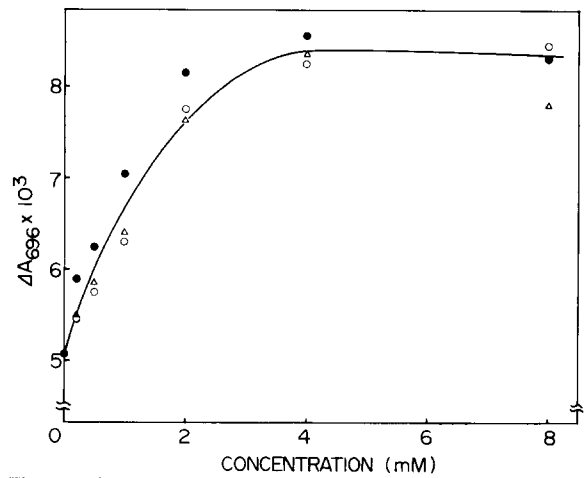

Fig. 7. Effects of various concentrations of methyl viologen and other bipyridinium dyes on flash-induced absorption change at 696 nm in the membranes treated with 0.1% SDS for 1 h. ●; methyl viologen. ○, 1,1′-trimethylene-2,2′-bipyridinium dibromide. △, 1,1′-trimethylene-5,5′-dimethyl-2,2′-bipyridinium dibromide. ▲, benzyl viologen.

SDS-treated membranes measured in the absence and presence of methyl viologen. Although noise level of the spectra were considerably larger as compared with the light-minus-dark difference spectrum, bleachings in the 700 and 680 nm regions are evident in the spectrum determined in the absence of methyl viologen (Fig. 9a). Addition of methyl viologen caused notable changes in the difference spectrum, which may be explained by assuming an increase in the magnitude of the bleaching around 700 nm and a diminution of the 680 nm bleaching band. In contrast to photooxidation of P-700, chemical oxidation of P-700 with ferricyanide is not accompanied by reduction of any bound electron acceptors of PS I. It is concluded therefore that the methyl viologen-induced band shift is not related to reduction of a bound electron acceptor.

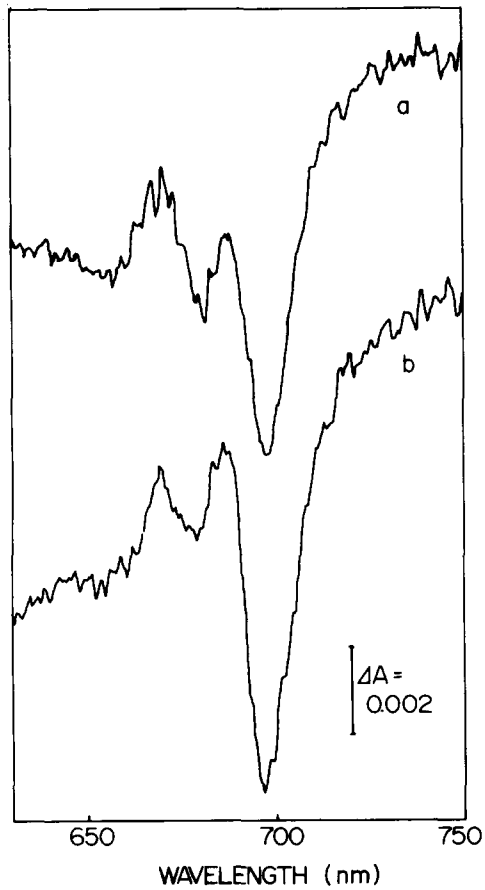

Fig. 8. Effects of methyl viologen on the chemically oxidized-minus-reduced difference spectrum of the thylakoid membranes treated with 0.1% SDS for 1 h. (a) No addition; (b) 4 mM methyl viologen was added.

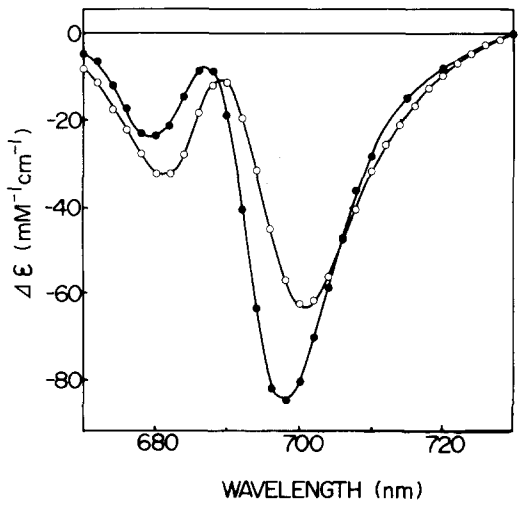

Fig. 9. The light-minus-dark differential extinction coefficients of P-700 in CP1-a and the thylakoid membranes in the red region. ●, CP1-a; ○, thylakoid membranes.

For the quantification of P-700 in cyanobacterial preparations, the oxidized-minus-reduced differential extinction coefficient of P-700 in the PS I particles prepared from *Anabaena variabilis* with Triton X-100 has been used widely [11]. However, the differential extinction coefficient of P-700 can no longer be regarded as a constant under conditions where the spectral change occurs because the band shift increases the amplitude of photobleaching around 700 nm without affecting the P-700 photooxidation.

The effects of SDS and methyl viologen on the differential extinction coefficient of P-700 in the thylakoid membranes were examined by measuring oxidation of TMPD coupled with reduction of flash-oxidized P-700 [11]. Measurements were carried out under aerobic conditions to prevent accumulation of reduced methyl viologen, and a slow autooxidation of TMPD was corrected by a computer subtraction. The details of the experimental procedures will be described elsewhere. The post-illumination reduction of P-700 is well coupled with oxidation of TMPD, both proceeding monophasically with an identical half time. The differential extinction coefficient of P-700 in the thylakoid membranes was determined as 64 $mM^{-1} \cdot cm^{-1}$ at 701 nm (Table I). The value decreased

TABLE I

DIFFERENTIAL EXTINCTION COEFFICIENTS OF P-700 IN THE THYLAKOID MEMBRANES AND CP1-a

MV, methyl viologen.

Preparations	Additions	Extinction coefficient ($mM^{-1} \cdot cm^{-1}$)
Thylakoid membranes	4 mM MV	64
SDS-treated membranes	–	61
	4 mM MV	85
CP1-a	–	84
	4 mM MV	86

slightly on treatment of the thylakoid membranes with 0.1% SDS for 1 h, but increased 85 $mM^{-1} \cdot cm^{-1}$ on addition of 4 mM methyl viologen to the SDS-treated membranes. As stated above, the P-700 photooxidation was occasionally inhibited to some extent on addition of methyl viologen to the SDS-treated membranes. The differential extinction coefficient of P-700 was, however, 40% larger than that determined in the absence of methyl viologen, irrespective of a diminished P-700 response.

The membranes treated with 0.1% SDS for 13 h yielded erratic results, possibly due to an interaction of solubilized chlorophyll with TMPD under illumination (not shown). Instead, we examined a PS I reaction center complex, CP1-a which had been isolated by SDS-polyacrylamide gel electrophoresis from SDS-solubilized *Synechococcus* thylakoid membranes [17,18]. The extinction value of CP1-a was similar to that of the SDS-treated membranes determined in the presence of methyl viologen (Table I). Fig. 9 compares the extinction values of P-700 in CP1-a with those of the thylakoid membranes at different wavelengths. Evidently, the harsh SDS-treatment employed for preparation of CP1-a have caused the band shift. The height ratio of the 680 nm to the 700 nm band was 0.51 in the difference spectrum of the untreated membranes, whereas CP1-a had the ratio of 0.27, which is compared with the ratio of 0.25 in the SDS-treated membranes with methyl viologen added.

Discussion

The present work shows the occurrence of a shift-type spectral change in the oxidized-minus-reduced differential spectrum of P-700 under the influence of SDS. The spectral change, which indicates a band shift of chlorophyll *a* with a maximum at about 690 nm to a shorter wavelength, could not be kinetically distinguished from the P-700 photoresponse and its magnitude is diminished by an inactivation or prior oxidation of P-700 proportionally to decreases in the amount of P-700 photooxidized. Thus the band shift is closely associated with P-700 oxidation. On the other hand, the spectral change is not related to the reduction of any endogenous electron acceptor of PS I because the band shift is also associated with chemical oxidation of P-700.

There are two explanations for the band shift. The first explanation assumes that, responding to the electric field generated by P-700 oxidation, the chlorophyll *a* molecules located near P-700 slightly shift their absorption band to a longer wavelength, resulting in the appearance of a band at 695 nm and a bleaching at 685 nm in the light-minus-dark difference spectrum. The electrochromic shift is largely diminished or totally abolished by a harsh SDS-treatment of the membranes, which would strongly affect the binding, location or orientation of the field-sensitive chlorophyll *a* molecules. Thus, the treatment results in an apparent red shift, i.e., appearance of a band at 685 nm and disappearance of a band at 695 nm in the difference spectrum.

The second explanation postulates a band shift of P-700 itself. The asymmetry of the 700 nm band and the occurrence of a small negative band at 680 nm in the difference spectrum of P-700 are explained by an assumption that a bleaching of the P-700 dimer is associated with an appearance of a chlorophyll *a* monomer band, which is slightly blue shifted and has a narrower bandwidth as compared to the dimer [5,8]. Any treatments which affect the absorption spectrum of either reduced or oxidized P-700 will result in a shift-type change in the difference spectrum of P-700. The observed band shift is well explained by assuming that SDS causes a slight blue-shift of the absorption band of the oxidized P-700 with a maximum at 690 nm.

Methyl viologen also induced the band shift. However, methyl viologen was totally ineffective in the absence of SDS or in the membranes, which had been intensively treated with SDS, and a moderate treatment of the membranes with the detergent was a prerequisite for the effect of methyl viologen to appear. The P-700 photooxidation was strongly resistant to the 1-h treatment of the membranes with SDS but occasionally diminished to some extent by further addition of methyl viologen. Preliminary experiments showed that methyl viologen causes a small but significant bleaching in the 673–695 nm region of the absorption spectrum in the 1-h treated but not in the untreated membranes. We suggest therefore that methyl viologen enhances the effect of SDS on the chlorophyll species responsible for the band shift. The effect of methyl viologen may be related to lipid solubility of the molecule because various inorganic cations are ineffective in inducing the band shift (data not shown). The equal effectiveness of other bipyridinium molecules suggests an importance of positive charges the methyl viologen molecules carry.

Whatever the mechanism of the band shift is, the occurrence of the spectral change in the difference spectrum of P-700 has an important consequence on the spectrophotometric quantification of P-700. The oxidized-minus-reduced differential extinction coefficient of P-700 in the *Synechococcus* thylakoid membranes was determined as 64 $mM^{-1} \cdot cm^{-1}$ at 701 nm. The extinction value reported by Hiyama and Ke [11] for P-700 in PS I particles prepared from *Anabaena variabilis* with Trition X-100 is 70 $mM^{-1} \cdot cm^{-1}$. The small difference may be ascribed to an effect of Triton or the difference in the cyanobacteria used. The differential extinction coefficient of P-700 was found to be 84–86 $mM^{-1} \cdot cm^{-1}$ in a PS I reaction center complex, CP1-a, and the SDS-treated membranes with methyl viologen added. Thus the band shift causes a 40% increase in the extinction coefficient.

There are two lines of evidence suggesting that the band shift occurs in the difference spectrum of other organisms and detergents other than SDS are also effective in this respect. First, SDS has been reported to increase the magnitude of ferricyanide-induced bleaching at 700 nm in tobacco PS I particles [14] and in PS I membranes isolated from *Phormidium laminosum* [15]. Several other detergents were also effective in increasing the bleaching in tobacco preparations [14]. Second, difference spectra of P-700 in the literature show a large variation in the magnitude of the 680 nm band relative to the 700 nm band [4,5]. This may be related to the band shift because the present work shows that the 680 nm band/the 700 nm band ratio is 0.51 in the thylakoid membranes but decreases to 0.25–0.27 when the band shift occurs. Thus, the present work strongly urges reexamination of the the oxidized-minus-reduced differential extinction coefficient of P-700 in PS I preparations isolated from various organisms with different detergents.

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