Biochimica et Biophysica Acta, 000 (1981) 175–184
Elsevier/North-Holland Biomedical Press

BBA 48084

MEMBRANE-SURFACE ELECTRIC PROPERTIES OF TRITON-FRACTIONATED SPINACH SUBCHLOROPLAST FRAGMENTS *

YASUSI YAMAMOTO ** and BACON KE

Charles F. Kettering Research Laboratory, Yellow Springs, OH 45387 (U.S.A.)

(Received February 24th, 1981)

Key words: Photosystem I; Photosystem II; Chlorophyll fluorescence; Surface charge density; Thylakoid membrane; (Spinach subchloroplast fragment)

Surface charge density of subchloroplast fragments fractionated from spinach by Triton X-100 treatment was estimated from cation-induced quenching of chlorophyll fluorescence, with the premise that the fluorescence yield is dependent on the surface electric potential of the preparations. Application of the Gouy-Chapman theory of diffuse double layer to the subchloroplast preparations, or treating the surface of the preparations under electric charge regulation conditions yielded a result suggesting the Photosystem II reaction-center preparation (TSF-IIa) to be more negatively charged than the Photosystem I reaction-center preparation (TSF-I). Isoelectric points of the subchloroplast fragments were determined by measuring 90° light scattering and more directly by gel isoelectric focusing. Isoelectric points of TSF-I and -IIa were estimated to be 4.8 and 4.0 from light-scattering experiments, and 4.5 and 4.1 from gel electrophoresis, respectively. The TSF-II preparation that contains both a light-harvesting complex and the reaction-center (core) complex showed a small cation-induced quenching of chlorophyll fluorescence. This fluorescence quenching may be ascribed mostly to the regulation of energy transfer in the preparation (Yamamoto, Y. and Ke, B. (1980) Biochim. Biophys. Acta 592, 296–302). Furthermore, the TSF-II preparation showed a broad and indefinite peak in light scattering in the pH range 3–8, suggesting that the complex probably carries a small amount of charge in this pH range. The physiological role of the membrane surface charge of the subchloroplast preparations in membrane structure and cation regulated processes in chloroplast is discussed.

Electric surface properties of photosynthetic membrane play an important role in various aspects of photosynthesis, including the primary photochemical and physiological processes [1]. In chloroplasts, cations have a significant regulatory action on the structure of thylakoid membranes, i.e., cation-induced membrane stacking and unstacking [2,3]. Regulation of excitation-energy distribution between Photosystems I and II in the presence of cations might be the most prominent phenomenon related to the structural change of the thylakoid membrane [4–6]. The importance of the surface electric properties of the membrane in these processes have been suggested theoretically and demonstrated experimentally [1–7].

The thylakoid membranes have a net negative charge at neutral pH, and its isoelectric point was reported to be about 4.3 [8–10]. However, the fixed

* Contribution No. 687 from the Charles F. Kettering Research Laboratory.

** Permanent address: Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan.

Abbreviations: TSF-I and -II (TSF-IIa), Triton-fractionated Photosystem I and Photosystem II subchloroplast fragments (subfragments by further fractionation of TSF-II); DCIP, 2,6-dichlorophenolindophenol; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; Q, primary electron acceptor of Photosystem II; Chl, chlorophyll; Mes, 4-morpholineethanesulfonic acid, Tes, *N*-Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid; SDS, sodium dodecyl sulfate.

negative charges do not seem to be distributed uniformly over the membrane, because small particles of various sizes, which probably correspond to the chlorophyll-protein complexes and carry net negative charges at neutral pH, have been shown to move relatively freely on the fluid membrane and change their distribution depending on the ionic condition of chloroplasts [11–13]. Thus the differentiation and estimation of distribution of the complexes on the membrane seem to be quite necessary for understanding the related cation-regulation processes.

In the present work, we examined cation-induced quenching of chlorophyll fluorescence in Triton-fractionated spinach subchloroplast fragments, and estimated their surface charge densities, with the premise that fluorescence yield is dependent on the membrane surface potential. These results were correlated with the isoelectric points separately determined for the subchloroplast fragments by 90° light-scattering measurements and by gel isoelectric focusing.

Materials and Methods

Subchloroplast fragments, TSF-I, -II and -IIa were prepared as described previously [14–16]. The TSF-I particle had one P-700 per 26 chlorophyll molecules. The TSF-IIa particle had one cytochrome *b*-559 per 34 chlorophyll molecules, no detectable P-700, and a DCIP-reduction activity of 1000 μmol/mg Chl per h. The TSF-II preparation showed very little or no contamination of P-700 and had a DCIP-reduction activity of 200 μmol/mg Chl per h. TSF-I and TSF-IIa were shown to be Photosystem I and Photosystem II reaction-center particles, and TSF-II contains both a light-harvesting chlorophyll *a*/*b*-protein complex and the Photosystem II reaction-center complex. Detailed characteristics of the preparations including absorbance and fluorescence spectra, lipid composition, etc. were described elsewhere [15–18]. The preparations were stored frozen at −80°C until needed.

The standard reaction mixture contained subchloroplast fragments equivalent to 5.0 μg Chl/ml in 0.2 mM sodium-phosphate buffer (pH 7.0). When measuring the effects of cations on Photosystem II preparations, DCMU was added to avoid changes in fluorescence yield due to change in the redox level of Q.

Chlorophyll fluorescence was measured in a double-beam fluorometer chamber (Cary accessory 56-231). For detecting low-level fluorescence emitted from TSF-I particles, the emission monochromator was removed and the photomultiplier (Hamamatsu R928) was placed close behind the cuvette. The photomultiplier was protected against the actinic light by two Corning 2-58 filters and a 680-nm interference filter. The actinic light was isolated by a combination of two Corning 4-96, two 1-69 heat-absorbing filters and a Baird-Atomic broadband (60 nm wide) 430 nm interference filter. Other conditions for fluorescence measurements were the same as previously described [19].

Light scattering at 90° was measured in the same fluorometer chamber. The actinic light was isolated by a 540-nm interference filter, a Corning 4-96 filter and two 1-69 heat-absorbing filters. A 540-nm interference filter and a Corning 4-96 filter were placed on the detecting side of the cuvette. A Jobin Yvon model H10 monochromator (bandwidth 4 nm; wavelength set at 540 nm) was placed before the photomultiplier. Intensity of the 540-nm actinic light was 20 erg/cm^2 per s.

Isoelectric focusing of the subchloroplast preparations was carried out in a slab-electrophoresis apparatus (Medical Research Apparatus, model 150). Usually, 4% polyacrylamide gel containing 2% LKB carrier ampholyte (pH range either 3–10 or 4–6) was electrophoresed at 0.9 W for 8–10 h at 4°C. After electrophoresis, the gel was cut into vertical strips approx. 1 cm wide. Each strip was cut into 2.5-mm sections and the latter placed in narrow test tubes containing 1.0 ml water and dispersed by a Voltex mixer, and then let to stand for 30 min. If necessary, the gel was frozen before being cut into strips. pH of the solution in each test tube was measured with a combination electrode connected to a Radiometer pH-meter. Each gel strip after pH measurement was extracted with 80% acetone and chlorophyll concentration was determined by measuring the absorbance at 663 nm in a Cary spectrophotometer.

Results

When TSF-I and -IIa preparations were suspended in a buffer solution of low concentration (0.2 mM sodium-phosphate buffer at pH 7.0), addition of cations induced a significant quenching of chlorophyll

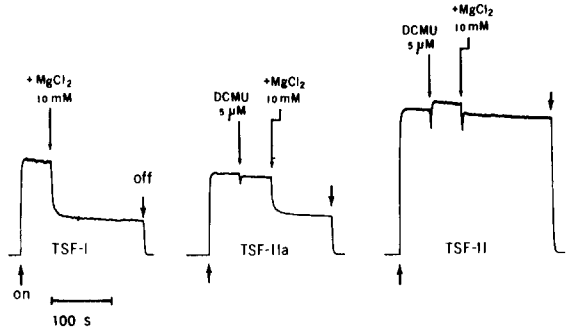

Fig. 1. Time-course of Mg^{2+}-induced quenching of chlorophyll fluorescence in subchloroplast preparations. Upward and downward arrows indicate actinic light on and off. Instrument time constant was 1 s. Fluorescence intensity was not normalized on a chlorophyll basis.

fluorescence (Fig. 1). In each preparation, $MgCl_2$ was found to be more effective than KCl in quenching fluorescence (Fig. 2). The extent of fluorescence quenching was dependent on the valence of the cation used (Table I). Among the cations examined, trivalent La^{3+} was most effective in quenching fluorescence. Quenching of fluorescence was only slightly dependent on the anionic species. In contrast to the TSF-I and -IIa particles, cations did not induce any significant quenching of fluorescence in TSF-II fragments.

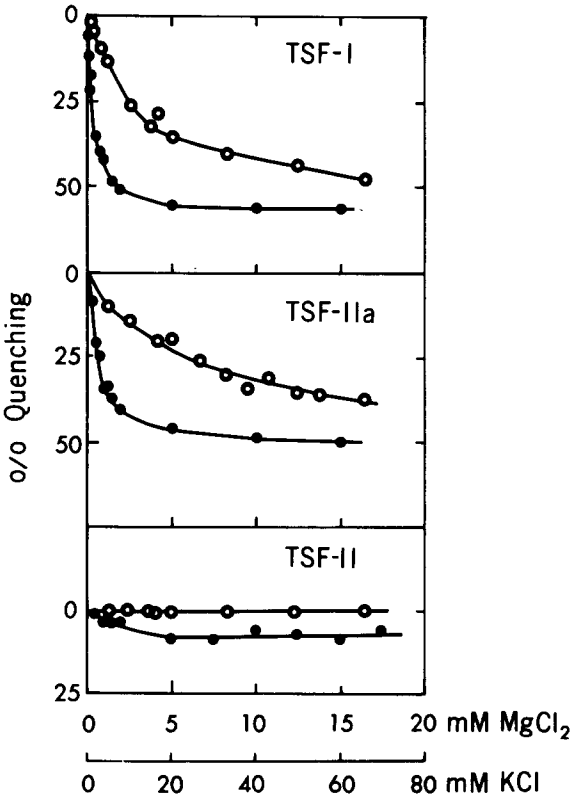

Fig. 2. K^+(○)- and Mg^{2+}(●)-induced quenching of chlorophyll fluorescence in subchloroplast preparations.

TABLE I

EFFECT OF THE VALENCE STATE ON CATION-INDUCED QUENCHING OF CHLOROPHYLL FLUORESCENCE IN SUBCHLOROPLAST FRAGMENTS AT pH 7.0

Salt added	% Quenching	
	TSF-IIa	TSF-I
0.5 mM		
NaCl	3	4
LiCl	3	3
$MgCl_2$	37	36
$CaCl_2$	32	36
$MgSO_4$	31	36
$LaCl_3$	45	saturating
0.1 mM		
$MgCl_2$	–	14
$CaCl_2$	–	13
$LaCl_3$	–	25

Effects on the fluorescence yield of TSF-I and -IIa were examined over a low concentration range of Mg^{2+}. For convenience of comparing calculated results of surface potential and surface charge density, a symmetrical electrolyte ($MgSO_4$) was also used. The half-effective Mg^{2+} concentration in quenching fluorescence was 0.3 mM in TSF-I and 0.5 mM in TSF-IIa particles, respectively.

Fluorescence yield of the subchloroplast preparations was dependent on pH of the suspending medium (Fig. 3). In the alkaline region, the particles showed a high fluorescence yield. Minimum fluorescence yield was observed at pH ranging from 3 to 5 depending on the preparation.

At each given pH, $MgCl_2$ quenched fluorescence to different extents in different preparations (Fig. 4). In the TSF-IIa preparation, 5 mM $MgCl_2$ induced a greater quenching of fluorescence at alkaline pH and it became less toward acidic pH values. Conversely, Mg^{2+}-induced quenching in the TSF-II preparation

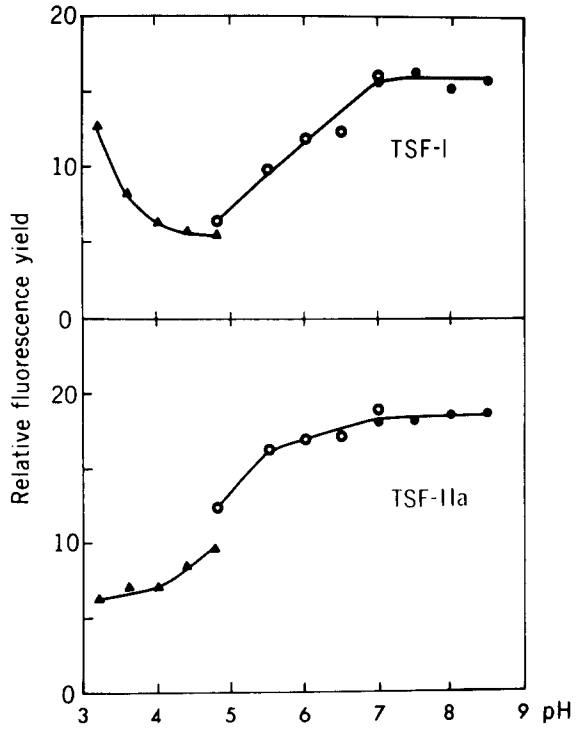

Fig. 3. pH-dependence of fluorescence yields in TSF-I and TSF-IIa. Buffers (4 mM) were: citric acid/sodium citrate for pH 3.2–4.8; Mes/NaOH for pH 4.8–7.0; Tes/NaOH for pH 7.0–8.5.

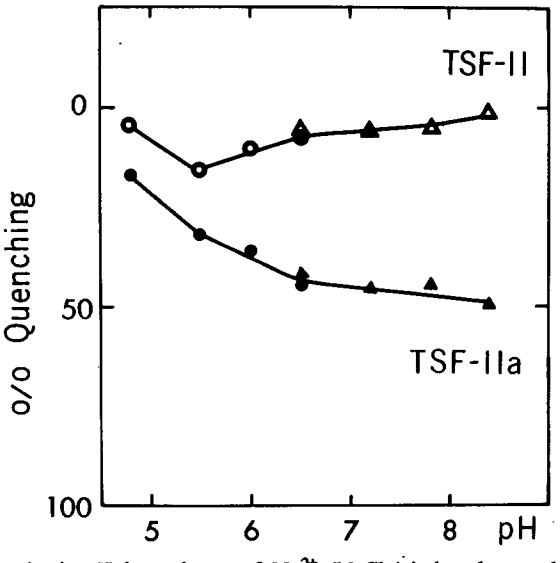

Fig. 4. pH-dependence of Mg^{2+} ($MgCl_2$)-induced quenching of chlorophyll fluorescence in TSF-II and IIa preparations. Mes/NaOH and Tes/NaOH (both at 4 mM) were used for pH ranges 4.8–6.5 and 6.5–8.4, respectively.

showed a maximum at pH 5.5 and became smaller on both sides of the optimum pH. Mg^{2+} could quench fluorescence even in the presence of a high concentration of buffer, suggesting that quenching may not be solely explained by a change in surface potential of the membrane. As reported by us recently [20], this quenching can readily be interpreted as a process related to the control of energy distribution in TSF-II preparations, and the peak observed here in the pH profile of cation-induced chlorophyll fluorescence quenching may be related to the optimum pH for energy transfer in this preparation.

Relationship between cationic concentration, pH, and the extent of fluorescence quenching in TSF-IIa fragments suggests that fluorescence yield of subchloroplast preparations is dependent on surface electric properties of the membrane, probably the surface electric potential.

When the net negative surface charge of the subchloroplasts was shielded by the addition of cations, the thylakoid membranes coalesce and a change in light-scattering properties would be expected in these subchloroplast suspensions. We measured light scattering at 90° to obtain an indication of aggregation in the various subchloroplast preparations (Fig. 5). When the $MgCl_2$ concentration in the reaction mixture (pH 7.0) was increased, light scattering reached saturation level in the sequence of TSF-II, TSF-I and

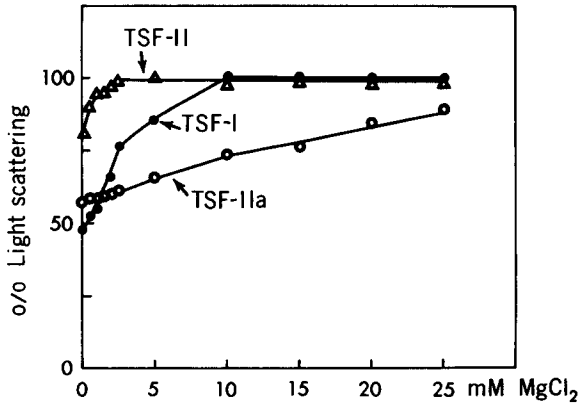

Fig. 5. Effecr os Mg^{2+} on the 90° light scattering in subchloroplast preparations. The scattering level in each preparation was normalized to its maximum attainable scattering level (≡100%) in the presence of saturating amount of $MgCl_2$.

TSF-IIa preparations. These results suggest that at neutral pH, the amount of net charge that each preparation carried is in the order of TSF-IIa > TSF-I > TSF-II.

The isoelectric point of each preparation may be determined from light-scattering measurements, as shown in Fig. 6. TSF-I fragments showed a light-scattering maximum at pH 4.8, a pH corresponding to its isoelectric point. The TSF-IIa fragments showed a light-scattering maximum at pH 4.0, which is more acidic than that of TSF-I. The TSF-II preparation had a structureless pH profile (a small peak was observed near neutral pH, but it could not be located precisely). Judging both from cation-induced fluorescence quenching and the 90° light-scattering properties, the TSF-II preparation seems to carry the least amount of charges in this pH region.

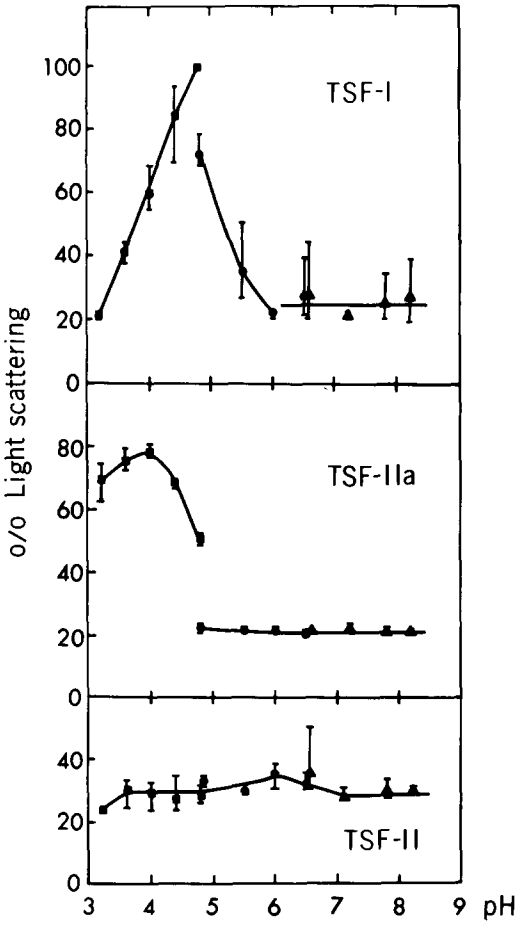

Fig. 6. Dependence of the 90° light scattering in subchloroplast preparations on the pH of the reaction mixture. Buffers (4 mM) were: citric acid/sodium citrate for pH 3.2–4.8; Mes/NaOH for pH 4.8–6.5 and Tes/NaOH for 6.5–8.4. The data are average of three independent measurements.

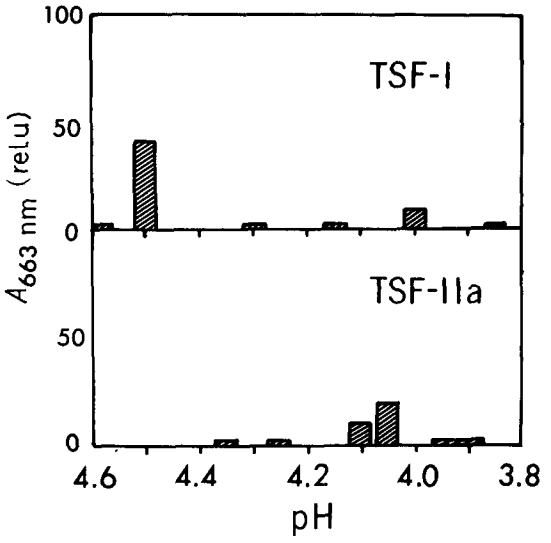

Fig. 7. Profile of gel isoelectric focusing of TSF-I and -IIa preparations. A carrier ampholyte (LKB) of 4–6 pH range was used. The absorbance represented by the low-level bars was practically nil.

The isoelectric points of the subchloroplast preparations were also determined more directly by gel isoelectric focusing using a slab electrophoresis apparatus (Fig. 7). The isoelectric points obtained for TSF-I and -IIa were 4.5 and 4.1, respectively, which are in excellent agreement with values obtained from light-scattering measurements. We could not obtain the isoelectric point of the TSF-II preparation, because these fragments were too large to enter into the electrophoresis gel, even one prepared with a low concentration of acrylamide.

Discussion

Characteristics of cation-induced chlorophyll fluorescence change in the subchloroplast fragments. When the subchloroplast preparations were suspended in a low-salt medium, addition of cations induced a very large quenching of fluorescence, except for the TSF-II preparation. Similar quenching was reported

previously by Gross and coworkers [21–23], who interpreted the results as regulation of energy transfer by cations within the subchloroplast particles.

Our results, however, suggest that the change in the fluorescence yield may be related to a change in the surface electric properties of the preparations, probably the surface electrical potential. This suggestion is based on the following observations: (i) the fluorescence quenching was caused by adding cations of various valencies and was independent on the anions used; (ii) cations were effective in the order of $C^{3+} > C^{2+} > C^{+}$; (iii) the fluorescence yield of the subchloroplast showed a characteristic pH dependence; that of TSF-I and -IIa became greater with increasing pH from 4 to 9. In TSF-II and -IIa, electron transport was inhibited by the addition of DCMU, so there was no fluorescence change due to the change in the redox state of Q. Contribution of aggregation of the preparation to the fluorescence change might not be so significant because the cation-induced membrane aggregation monitored by 90° light scattering had a different characteristic in its dependence on pH and on concentration of cations added. As seen from Figs. 2 and 5, the half-effective concentration of $MgCl_2$ in inducing the 90° light scattering was 10–30 times higher than that necessary for fluorescence quenching. Furthermore, parallel measurements of surface potential of the preparation using an artificial fluorescence probe, 9-aminoacridine, yielded results consistent with that obtained from chlorophyll fluorescence measurements (data not shown).

Our previous results on Mg^{2+}-induced chlorophyll fluorescence change in the Photosystem II subchloroplast preparations show the effect of regulation of electron transport and excitation-energy distribution by Mg^{2+}, depending on the structural properties of the preparations [19,20,24]. Regulation of energy transfer can be observed in Photosystem II preparation containing the light-harvesting chlorophyll-protein complexes, but not in the core photosystem II preparation [20]. These results were obtained in the presence of a high concentration of buffer (20 mM sodium phosphate, pH 7.0) in the reaction medium, where the fluorescence yield was kept low and change in surface potential might also be negligible.

Estimation of the surface charge density on the subchloroplast preparations; application of the Gouy-Chapman theory. Initially we simply applied the Gouy-Chapman theory that has been described for explaining the nature of the diffuse electrical layers associated with charged surface [25,26]. As thylakoid membranes are negatively charged at neutral pH, cations tend to be drawn close to the membrane for the sake of neutrality and form a diffuse layer. The Gouy-Chapman theory describes the relationship between the surface charge density and the surface potential on a membrane surface in a solution of diffusable ions as follows:

$$q = \pm\left|\frac{RT\epsilon}{2\pi}\sum C_b[\exp(-FZ\Psi_0/RT) - 1]\right|^{1/2} \quad (1)$$

where q is the surface charge density ($\mu C/cm^2$), C_b the bulk concentration of salt (mol/l), ψ_0 the membrane surface potential (mV), ϵ the permittivity of water and is equal to 80 ϵ_0 at 20°C (where ϵ_0 is the permittivity of a vacuum), and Z the valence of ions. R, T, and F have their usual meanings.

When the membrane is suspended in a medium containing a Z-Z type symmetrical electrolyte such as KCl or $MgSO_4$, Eqn. 1 is reduced to

$$q = 2\left(\frac{RT\epsilon}{2\pi}\right)^{1/2}(C_b)^{1/2}\sinh(ZF\Psi_0/2RT) \quad (2)$$

Numerical substitution (for 20°C) gives

$$q = 11.75(C_b)^{1/2}\sinh(Z\Psi_0/50.6) \quad (3)$$

When monovalent cation (concentration C') and divalent cation (concentration C'') induce the same extent of quenching of chlorophyll fluorescence, we can derive q and ψ_0 in terms of C' and C'' from the following relationships:

$$q = -11.75[(C'^2 - 4C'C'')/4C'']^{1/2} \quad (4)$$

$$\Psi_0 = 50.6\sinh^{-1}\{-[(C'/4C'') - 1]^{1/2}\} \quad (5)$$

We adopted a negative value for q and ψ_0, because it is reasonable to assume a net negative surface charge of the preparation at neutral pH, judging from their isoelectric points. Applying the experimental data of Figs. 2 and 3 to Eqns. 4 and 5, we could estimate the surface potential and surface charge density of TSF-I and TSF-IIa preparations. The calculated values are

TABLE II

SURFACE ELECTRIC PROPERTIES OF TRITON-FRACTIONATED SUBCHLOROPLAST PREPARATIONS

The surface potential (Ψ_0) and surface charge density (q) were calculated from fluorescence-quenching data where a linear relationship existed between the concentration of cations and the extent of quenching. Calculated values are the average of duplicate measurements.

Preparation	Surface potential (Ψ_0), (mV)	Surface charge density (q), ($\mu C/cm^2$)	Isoelectric point	
			By light scattering	By gel isoelectric focusing
TSF-I	−58	1.09	4.8	4.5
TSF-IIa	−90	2.9	4.0	4.1

shown in Table II, along with the isoelectric points of the preparations.

However, some remarks should be made on the application of the Gouy-Chapman theory to our results. The first problem is that the value of q is sometimes sensitive to the choice of C'' and C'. The preparations were always suspended in a buffer of low concentration and they also seem to have tightly associated cations on their surface, providing a certain background level of cations that could not be determined experimentally in our study. Therefore, for more valid estimation of q, the following equations should be used instead of Eqn. 2:

$$q = 2(RT\epsilon/2\pi)^{1/2}(C_d + C_e + C')^{1/2}\sinh(F\Psi_0/2RT) \tag{2a}$$

and

$$q = 2(RT\epsilon/2\pi)^{1/2}\{\sinh^2(F\Psi_0/RT) + 0.5(C_d + C_e)\cosh(F\Psi_0/RT) - 1\}^{1/2} \tag{2b}$$

where C_d is the concentration of added cation and C_e is the concentration of the endogenous cations.

The second and perhaps more notable shortcoming is that the basic assumption in the Gouy-Chapman theory of an infinitely flat membrane is not satisfied due to the size of the subchloroplast fragments. TSF-I and -IIa are 100–200 Å particles. TSF-II is a planar and sometimes vesicular membrane fragment with an average size of several thousand angstroms. In practice, the membrane can be considered infinitely flat if the radius of curvature of the surface is 30-times the perpendicular distance of the ion from the center of the membrane, and that might not be met in the case of the subchloroplast preparations. Thus, a three-dimensional treatment rather than the two-dimensional planar Gouy-Chapman theory should be applied to the subchloroplast preparations for a more quantitative analysis.

Estimation of the surface charge density on the subchloroplast preparation: treatment of the surface of the preparation under charge regulation conditions. When the subchloroplast preparations are treated under electric charge regulation conditions [27], the surface charge density is given by

$$q_i = er_i/(1 + [H_s^+]/k_i) \tag{6}$$

where r_i is the number of ionizable surface groups per unit area, k_i the dissociation constant of the ith group species, $[H_s^+]$ the concentration of aqueous H^+ at the surface of the particle and q_i is the effective surface charge density of the ith group species under specified conditions. On the thylakoid membrane, carboxyl groups of the proteins seem to be the major group determining the surface electric properties under the present conditions [10]. Then the net surface charge density of the preparation can be expressed as follows in relation to their isoelectric points:

$$q = er/(1 + [H_I^+]/k) - er/(1 + [H_s^+]/k) \tag{7}$$

where r is the number of carboxyl groups per unit area, k is the dissociation constant of carboxyl group (10^{-4}–$10^{-4.5}$), and $[H_I^+]$ and $[H_s^+]$ are the concentration of H^+ at the isoelectric point of the preparation and that at the surface of the preparation, respectively. According to Boltzmann's law, $[H_s^+]$ at surface potential ψ_0 is related to the bulk H^+ concentration $[H_b^+]$ by

$$[H_s^+] = [H_b^+]\exp(-e\psi_0/kT) \tag{8}$$

in which e is the unit electron charge, k is the Boltzmann constant, and T the absolute temperature. At

pH 7.0 and assuming ψ_0 of −50 mV, which is sometimes met in thylakoid membrane suspended in salt-free medium, the concentration of dissociated carboxyl groups at the surface of the membrane will be nearly 100-times larger than that of the protonated form, then the contribution of $[H_s^+]$ in Eqn. 7 becomes small enough to be neglected. In that case, q is expressed as

$$q = er/(1 + [H_I^+]/k) - er \quad (9)$$

Assuming er = 2.5 μC/cm^2, and substituting the measured value of $[H_I^+]$ for subchloroplast into Eqn. 9, surface charge density of each particle was obtained, as shown in Table III.

The calculations shown here, however, include some unknown key values such as er, k (the dissociation constant) and the actual value of surface potential under the present condition. We also should point out the possibility that the surface electric properties in the preparations might be changed during the preparation steps. Especially exposure of the preparations to a high concentration of surface-active agents such as Triton X-100 might induce some changes in the membrane properties that produces preparations having different characteristics from the integral membrane pigment-protein complexes in situ. In our experiments, however, the final concentration of Triton was kept low and photochemical activities of the preparations which are quite sensitive to the concentration of Triton were checked routinely to minimize the effects of the surfactants on the preparations.

TABLE III

SURFACE CHARGE DENSITY OF SUBCHLOROPLAST PREPARATIONS

Preparation	Surface charge density (q) (μC/cm^2)	
	A *	B **
TSF-I	−0.83	−1.25
TSF-IIa	−1.89	−1.78

* Light scattering was used for pI determination.

** Gel isoelectric focusing was used for pI determination. Calculation was carried out by treating the particle surface under electrical charge regulation conditions.

Surface electric properties of the subchloroplast preparations and their relevance to the structure of thylakoid membrane. Through examination by freeze-fracture electron microscopy, thylakoid membrane has been shown to consist of small particles of various sizes, embedded in what is assumed to be a fluid lipid matrix of the membrane. The details of the identity of the small particles as well as the functional relationship between the particles and the subchloroplast preparations derived from the thylakoid membrane were discussed in recent reviews [13,18–30]. TSF-I and -IIa are enriched with Photosystem I and Photosystem II reaction centers, respectively, and judging from the size (100–200 Å), they seem to be small membrane fragments containing small reaction-center particles. TSF-II contains the light-harvesting chlorophyll-protein complexes in addition to the reaction centers of Photosystem II and is a much larger membrane fragment.

The results of cation-induced fluorescence change and measurement of isoelectric points of the subchloroplast suggest the TSF-II to have a smaller amount of charge on the surface than TSF-I or -IIa. However, according to Thornber et al. [31], the amino-acid composition of the light-harvesting chlorophyll-*a*/*b*-protein complex is not significantly different from that of the total SDS-solubilized chloroplast membrane or the P-700-protein complex. The light-harvesting complex isolated by Satoh and Butler [32] showed an isoelectric point of 4.1, which is smaller than those of the Photosystem-I and -II fractions. These results suggest that the light-harvesting complex in the isolated form should be negatively charged nearly the same or more than the reaction-center complexes at neutral pH.

Although no exact information is yet available from our experiments on the electric properties of the light-harvesting complex, the variance between our results and those obtained with the isolated light-harvesting complex might be explained partly by the structural interaction of the light-harvesting complex and the Photosystem II reaction-center complex in TSF-II. The current model of thylakoid-membrane structure derived from the freeze-fracture electron microscopic evidence shows that the light-harvesting complex tends to associate with the Photosystem II core complex and has a characteristic conformation in the thylakoid membrane [29] that might result in

reducing negative charges on its surface. This highly negatively charged surface of the Photosystem II complex is accordingly shielded by the light-harvesting complex. Consistent with the above idea are the results of mild trypsin treatment of thylakoid membrane which digests the light-harvesting complex [33, 34] and exposes the more negatively charged surface [35].

Role of the lowly-charged light-harvesting complex in cation-regulated processes. The light-harvesting complex is considered to play a significant role in membrane stacking and regulation of energy transfer from Photosystems II to I ('spill-over') in the presence of cations [29]. According to the recent model of Barber [36], the relationship of excitation spill-over and membrane stacking can be explained by screening of surface charge of the thylakoid membrane by the addition of cations and redistribution of the chlorophyll-protein complexes over the thylakoid membrane. Highly-charged Photosystem I complexes migrate away from the stacked regions, while lowly charged light-harvesting/Photosystem II complexes are predominantly located in the stacked region. The resulting reduction of electrostatic repulsive force among the complexes and the reorganization of the complexes in the presence of cations are responsible for the change in the efficiency of spill-over of excitation energy between the photosystems. However, one of the basic assumption of the model that the light-harvesting complex is neutral in its charge on the surface has not been directly demonstrated experimentally. Our present study, although being rather qualitative, suggests a smaller surface charge density on the light-harvesting/Photosystem II complex compared to the Photosystem I complex. For a more quantitative treatment of surface charge density of small subchloroplast particles, either a spherical or ellipsoidal non-linear Poisson-Boltzmann analysis should be applied.

Acknowledgement

The authors thank E.R. Shaw for preparing the subchloroplast fragments and Dr. R.A. Darrow for the electrophoresis facilities. This work was supported in part by a National Science Foundation grant PCM 77-08455.

References

1 Barber, J. (1981) Biochim. Biophys. Acta, in the press
2 Izawa, S. and Good, N. (1969) Plant Physiol. 41, 533–543, 544–552
3 Murakami, S. and Packer, L. (1971) Arch. Biochem. Biophys. 146, 337–347
4 Murata, N. (1969) Biochim. Biophys. Acta 189, 171–181
5 Homann, P. (1969) Plant Physiol. 44, 932–936
6 Murata, N. (1971) Biochim. Biophys. Acta 226, 422–432
7 Barber, J. (1978) in Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Foundation Symposium 61, pp. 283–304, Elsevier, Amsterdam
8 Mercer, F.V., Hodge, A.J., Hope, A.B. and McLean, J.D. (1955) Aust. J. Biol. Sci. 8, 1–18
9 Dilley, R.A. and Rothstein, A. (1967) Biochim. Biophys. Acta 135, 427–443
10 Nakatani, H.Y., Barber, J. and Forrester, J.A. (1978) Biochim. Biophys. Acta 504, 215–225
11 Wang, A.Y.I. and Packer, L. (1973) Biochim. Biophys. Acta 305, 488–492
12 Ojakian, G.K. and Satir, P. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 2052–2056
13 Anderson, J.M. (1975) Biochim. Biophys. Acta 416, 191–235
14 Vernon, L.P. and Shaw, E.R. (1971) Methods Enzymol. 23, 277–289
15 Ke, B., Sahu, S., Shaw, E.R. and Beinert, H. (1974) Biochim. Biophys. Acta 347, 36–48
16 Klein, S.M. and Vernon, L.P. (1974) Photochem. Photobiol. 19, 43–49
17 Vernon, L.P., Shaw, E.R., Ogawa, T. and Raveed, D. (1971) Photochem. Photobiol. 14, 343–357
18 Ke, B., Vernon, L.P. and Chaney, T.H. (1972) Biochim. Biophys. Acta 256, 345–357
19 Yamamoto, Y. and Ke, B. (1980) Biochim. Biophys. Acta 592, 285–295
20 Yamamoto, Y. and Ke, B. (1980) Biochim. Biophys. Acta 592, 296–302
21 Prochaska, L.J. and Gross, E.L. (1977) Arch. Biochem. Biophys. 181, 147–154
22 Davis, D.J., Janovitz, E.B. and Gross, E.L. (1977) Arch. Biochem. Biophys. 184, 197–203
23 Gross, E.L. and Grenier, J. (1978) Arch. Biochem. Biophys. 187, 387–398
24 Yamamoto, Y. and Ke, B. (1979) Febs Lett. 107, 137–140
25 Delahay, P. (1965) Double Layer and Electrode Kinetics, John Wiley, New York
26 Shaw, D.J. (1969) Electrophoresis, Academic Press, New York
27 Duniec, J.T., Sculley, M.J. and Thorne, S.W. (1979) J. Theor. Biol. 79, 473–484
28 Park, R.B. and Sane, P.V. (1971) Annu. Rev. Plant Physiol. 22, 395–430
29 Arntzen, C.J. (1978) Curr. Top. Bioenerg. 7, 111–160

30 Boardman, N.K., Anderson, J.M. and Goodchild, D.J. (1978) Curr. Top. Bioenerg. 7, 35–109
31 Thornber, J.P., Stewart, J.C., Hatton, M.W.C. and Bailey, J.L. (1967) Biochemistry 6, 2006–2014
32 Satoh, K. and Butler, W.L. (1978) Plant Physiol. 61, 373–379
33 Steinback, K.E., Burke, J.J. and Arntzen, C.J. (1979) Arch. Biochem. Biophys. 195, 546–557
34 Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117
35 Nakatani, H.Y. and Barber, J. (1980) Biochim. Biophys. Acta 591, 82–91
36 Barber, J. (1980) FEBS Lett. 118, 1–10