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PROPERTIES OF THYLAKOIDS AND THYLAKOID PARTICLES DERIVED FROM STRUCTURALLY DIFFERENT CHLOROPLASTS

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Structurally and functionally different tobacco chloroplasts were subjected to digitonin treatment and subsequent fractional centrifugation. The light-harvesting chlorophyll *a*/chlorophyll *b*-protein complex was found to be enriched in the most dense fraction regardless of the presence of grana in the original preparation. It is suggested that isolated thylakoid membranes and fragments thereof which contain sufficient light-harvesting protein may, under appropriate ionic conditions, form aggregates even when they originate from unstacked thylakoid systems. Comparative studies of fluorescence properties and polypeptide composition of the thylakoids suggest that the light-harvesting protein does not contribute significantly to the fluorescence spectrum of isolated chloroplasts as long as this protein is intimately associated with the Photosystem II (PS II) pigment-protein complex responsible for the 685 nm emission. While the PS II-deficient mutant chloroplasts of the variegated tobacco variety *NC 95* lacked both the 685 nm fluorescence component and two or three PS II proteins, one of these proteins was found to be very prominent in our chlorophyll *b*-deficient mutant thylakoids which also displayed an intense 685 nm fluorescence peak. This correlation supports the contention that a 45 kdalton polypeptide is an apoprotein of pigments associated with the PS II reaction center.

Introduction

Light absorbed by the chlorophyll proteins of photosynthetic membranes is partially reemitted as fluorescence. Since the yield of the fluorescence from a given pigment aggregate depends on the rate constants of the competing radiationless deexcitation events, fluorescence analyses give valuable insight into the fate of light absorbed by photosynthetic systems.

With chloroplast thylakoids, fluorescence measurements at room temperature monitor only the light emission from pigments associated with PS II because the chlorophyll proteins of PS I fluorescence to any significant extent only at temperatures well below 0°C. Usually measurements are made at 77 K. The observed emission peaks have been correlated with the presence of specific pigment proteins on the basis of measurements with chloroplast particles enriched in either PS I or PS II, and with chloroplasts lacking certain components due to genetic alterations, or incomplete development [1,2]. While many assignments are still not unequivocal, emissions below 700 nm are generally attributed to PS II, and those above that wavelength to PS I.

Recently, Rijgersberg et al. [3] have analyzed the changes of chloroplast fluorescence during cooling to, and below, 77 K. These changes were discussed in relation to the pigment composition of the chloro-

Abbreviations: Chl, chlorophyll; *F*, fluorescence peak, used in conjunction with number indicating wavelength; JWB, tobacco variety John Williams Broadleaf; LHCP, light-harvesting Chl *a*/*b*-protein complex; NC yellow, yellow leaf areas of the variegated tobacco mutant derived from variety *NC 95*; PS, photosystem; SDS, sodium dodecyl sulfate; Tricine, *N*-tris(hydroxymethyl)methylglycine; DCIP, 2,6-dichlorophenolindophenol.

plast material, and the pathways of energy transfer between them. Gasanov et al. [4] have studied excitation transfer in chloroplasts and thylakoid particles through measurements of the wavelength dependence of fluorescence quantum yields. Both research groups paid particular attention to the contribution of the different PS II-associated pigment proteins to the short-wavelength fluorescence. In another investigation, Menke and Schmid [5] have addressed this problem by showing that a constitutional deficiency in PS II, as found in certain naturally occurring plastids, is reflected by a corresponding attenuation of the fluorescence below 700 nm which, in the extreme case, may be altogether lacking.

Independently, we have extended earlier work [6] on the pattern of detergent-induced disruptions of thylakoids differing in structure, composition and photosynthetic properties. These studies provided us with an opportunity to analyze the fluorescence properties and the composition of a great variety of thylakoid preparations and thylakoid-derived membranous particles. We have reconfirmed that the presence of LHCP, but not a native granal structure, is essential for an enrichment of PS I in small membrane fragments. We also suggest that the contribution of LHCP to the fluorescence emission between 680 and 685 nm is usually small and is regulated by the presence of the PS II antenna.

Materials and Methods

Plant material. Four previously used and described [7,8] tobacco (*Nicotiana tabacum* L.) varieties were the sources of the structurally different chloroplasts used in this study: JWB, our control with normal grana-containing chloroplasts; its aurea mutant *Su/su* with rudimentary grana and variable LHCP content; the *Su/su*-derived mutant *Su/su* var *area* with very low LHCP content and essentially no grana; and the variegated mutant of *NC 95* which contains granaless, PS II-deficient chloroplasts in its yellow-green leaf areas (NC yellow). From our *Su/su* mutants, and from NC yellow, chloroplasts with varying Chl *a/b* ratios could be selected. Young *Su/su* mutant leaves (less than half expanded) had ratios above 10, thus yielding chloroplasts quite similar to those of leaves from *Su/su* var. *aurea*. The older leaves could reach Chl *a/b* ratios close to 3.5. The Chl *a/b* ratio of chloroplasts from NC yellow changes from approx. 4 to 8 as exposure of the leaves to the sun led to a conspicuous bleaching. The general properties of the chloroplast material are described in previous articles [7,8]. Analyses of the main four carotenoids [9] revealed as one noteworthy difference a higher relative violaxanthin content in *Su/su* var. *aurea*. Our plants were grown as previously except that some of our *Su/su* mutant was occasionally maintained in growth chambers illuminated with 'cool-white' fluorescent lights. Mn^{2+}-deficient plants were grown in water culture [10] and kept in the same growth cabinets.

Chloroplast preparation and measurements. Methods of chloroplast preparation and treatment have been described earlier [6,11] but the media were varied according to the desired ionic conditions. The osmoticum was sucrose or sorbitol at 0.4 M and the buffer usually Tricine-NaOH at 25 mM. In 'low-salt' media we included 5 mM NaCl and a further addition of 10 mM $MgSO_4$ provided 'high-salt' conditions. Salt-free media contained no added buffer and salt except for 0.2–1 mM Tris for adjustment of the pH to about 7.2. 5 mM ascorbate was frequently added to the medium used for grinding in order to prevent the formation of oxidation products from phenolic cell constituents. For parallel preparations of low- and high-salt chloroplasts, we often macerated the leaves in low-salt media and then added 10 mM $MgSO_4$ to half of the suspension prior to further processing.

Digitonin-treated fractions are designated according to the value (in thousands) used for their sedimentation with the duration of centrifugation being 15 min at or below $12\,000 \times g$ and 45 min above. The supernatant from centrifugations at $145\,000 \times g$ (digitonin-145S) was centrifuged for 5–8 h at $160\,000 \times g$ so that essentially all green material accumulated as a concentrated suspension at the bottom of the centrifuge tube. It was freed from the clear medium above by aspiration.

Photosynthetic activies were determined as described elsewhere [11,12]. Acrylamide gel electrophoresis of SDS-solubilized membrane fractions was performed in slabs cast from 10% acrylamide plus 0.27% *N,N'*-methylenebisacrylamide and 5 M urea according to the method of Hoyer-Hanson et al. [13] and Chua and Bennoun [14]. For chlorophyll ana-

lyses we sometimes extracted chlorophyll-containing bands from our gels according to Ref. 14.

Total chlorophyll and Chl *a/b* ratios were estimated from the formula derived by McKinney [15]. When the Chl *b* content was low, we used the fluorescence method of Boardman and Thorne [16]. A comparison of the measured relative Chl *b* content with visual or actual estimates of the LHCP bands on the developed polyacrylamide gel electrophoresis lanes revealed a close correlation. We shall, therefore, refer to the relative LHCP content of our fractions even when only their Chl *a/b* ratios were determined.

Fluorescence spectra at 77 K were measured in 60% glycerol according to the method of Boardman et al. [17] with a Varian model SF 330 fluorimeter, and excitation light at 436 nm. The slid widths for excitation and emission light were 3 and 5 nm, respectively.

Results

Fractionation patterns of digitonin-treated thylakoids

With digitonin-treated, grana-deficient mutant chloroplasts of NC yellow, Salin and Homann [6] have shown that grana are not essential for an enrichment of Chl *b* in the heaviest, and of PS I activity in the light fractions. These results were confirmed in the present study as shown in Fig. 1. The influence of cations on the fractionation pattern was the same as is known from investigations on normal, granal thylakoid systems [18], i.e., a significant degree of separation of pigment proteins and of photochemical activities occurred in Mg^{2+}-supplemented media, or in the absence of any salts (not shown; see Ref. 10) but not in low-salt media. Yet, with all LHCP-containing chloroplasts some separation of chlorophylls and photosynthetic activity occurred even in low-salt media (Fig. 1). Therefore, we used as criteria for an ineffectual fractionation the essentially unattenuated PS I activity in the heavy, and the retention of PS II activity in the lightest fractions.

The often discussed importance of LHCP for a successful separation of the photosystems in detergent-liberated thylakoid particles was confirmed by our results with strongly LHCP-deficient *Su/su* chloroplasts. As can be seen from Fig. 1, the presence of Mg^{2+} during fractionation of such material favored an enrichment of the residual LHCP in the heavy fractions, but the properties of the particles were otherwise very similar under all conditions. The observed curtailment of PS II and PS I reactions in particles isolated from Mg^{2+}-containing media was attributed to Mg^{2+}-induced inhibitions of photoreactions which we noted with our chloroplast particles and which have also been reported by others [19].

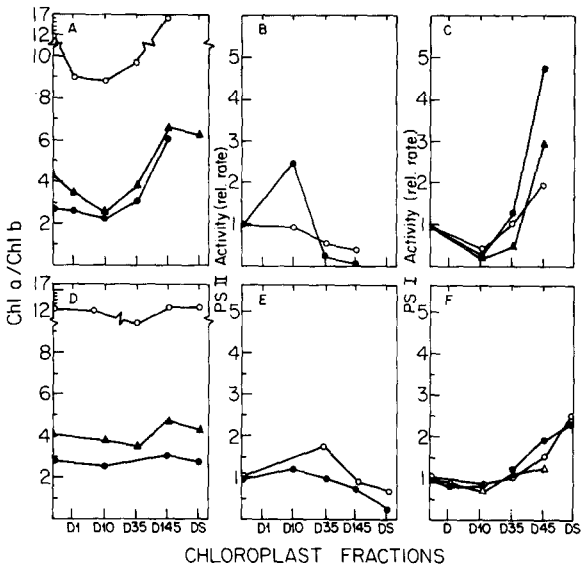

Fig. 1. Distribution of properties among thylakoids and thylakoid particles derived from various tobacco chloroplasts. ●——●, JWB (control); ○——○, *Su/su* var. *aurea*; ▲——▲, NC yellow. PS II activity determined as photoreduction of DCIP by diphenylcarbazide; PS I activity measured as methyl viologen-mediated Mehler reaction with reduced DCIP as electron donor to 3-(3,4-dichlorophenyl)-1,1-dimethylurea-poisoned preparations. A–C, digitonin treatment in Mg^{2+}-containing media; D–F, treatment in Mg^{2+}-free low-salt media.

The distribution of particles generally followed the pattern predicted by the work of Argyroudi-Akoyunoglou [20], i.e., the abundance of the heavy pellet depended on the extent of grana formation of the thylakoids. It is noteworthy, nevertheless, that our *Su/su* chloroplasts yielded a relatively much larger digitonin-145S fraction (approx. 45% of the total chlorophyll) than the green control under the unstacking conditions of a low-salt medium (approx. 20%).

Fluorescence spectra at 77 K

Since fluorescence properties of thylakoids and

thylakoid particles reflect the abundance and interactions of their pigment proteins [1,4], the great similarity of the spectra of untreated control and LHCP-deficient *Su/su* var. *aurea* chloroplasts was remarkable. Fig. 2 documents this apparent lack of correlation between F_{685} and LHCP content which had been seen previously with developing plastids by Davis et al. [21]. Like these authors, we made the surprising observation that the relative intensity of the short-wavelength emission increased with decreasing LHCP content, probably as a result of a higher PS II/PS I ratio [22]. Relevant data are summarized in Table I and contrasted with corresponding measurements on the PS II-deficient NC yellow chloroplasts. In this material the relative intensity of the short-wavelength fluorescence was directly dependent on the abundance of LHCP. However, as shown in Fig. 2, the fluorescence peak of NC yellow was located at 680 instead of 685 nm. This is the wavelength where purified, detergent-solubilized LHCP fluoresces (Refs. 1 and 23, and our own observations). In spite of the fundamentally different relationship between LHCP content and fluorescence emission in *Su/su* and NC yellow thylakoids, the data of Table I show that the relative extent of Mg^{2+}-induced changes of the short-wavelength emission was strictly dependent on LCHP in both.

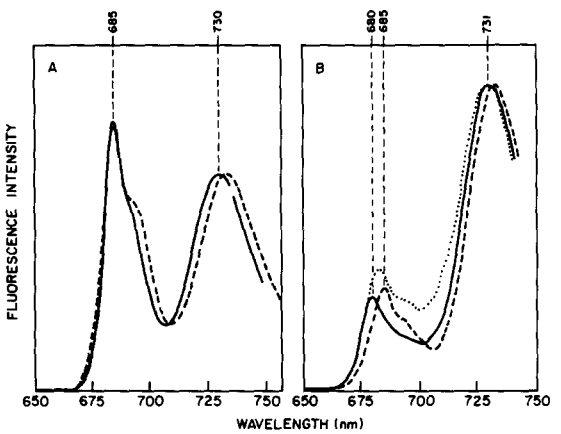

Fig. 2. Fluorescence spectra of chloroplasts at 77 K normalized for the long-wavelength peak. (A) Mg^{2+}-containing suspension of chloroplasts from JWB (------) and *Su/su* (———); (B) Mg^{2+}-free suspension of chloroplasts from JWB (– – –); Mg^{2+}-containing suspensions from NC yellow (———) and Mn^{2+}-deficient JWB (·····). The Chl *a/b* ratios were: JWB, 2.8; *Su/su*, 6.2; NC yellow, 7.5; Mn^{2+} deficient, 2.6.

TABLE I

Mg^{2+} EFFECT ON THE FLUORESCENCE AT 77 K OF VARIOUS TYPES OF CHLOROPLASTS

Suspension media either contained, or were devoid of 10 mM Mg^{2+}; other constituents were as described in Materials and Methods. The Mg^{2+} effect is expressed as $\frac{F_{II}/F_I\ (+Mg^{2+})}{F_{II}/F_I\ (-Mg^{2+})}$ where F_{II} and F_I refer to the short- and long-wavelength peak, respectively.

	Chl *a/b*	F_{II}/F_I		Mg^{2+} effect
		$+Mg^{2+}$	$-Mg^{2+}$	
JWB	2.8	1.00	0.43	2.30
Su/su	3.5	1.35	0.75	1.80
	7.5	1.90	1.32	1.45
	9.8	2.0	1.80	1.11
Mn^{2+}-deficient *Su/su*	2.3	0.48	0.38	1.23
NC yellow	5.5	0.50	0.22	2.27
	6.8	0.42	0.31	1.35
	7.5	0.30	0.30	1.00
	8.0	0.28	0.24	1.17

According to Burke et al. [23], thylakoid-bound LHCP fluoresces at 680 nm only when it is detached from the antenna of PS II. This contention would appear to explain the fluorescence properties of the PS II-deficient mutant chloroplasts of Burke et al. [23], Bennoun [25] and ourselves and also the blue shift of the short-wavelength emission in granal chloroplasts disorganized due to Mn^{2+} deficiency (Fig. 2). In the latter, however, the emission intensity remained low in all media in spite of a very high LHCP content (Table I).

The fluorescence properties of thylakoid particles obtained in Mg^{2+}-containing media after detergent treatment of normal chloroplasts are well known. They reflect the relatively high concentration of PS II in the heavy pellet, and the enrichment of PS I in the small particles. Accordingly, we found in the digitonin-145 fraction of our control chloroplasts a very low F_{685} value, and a high one in the digitonin-10 pellet. This is shown in Fig. 3 which also provides data on the relative fluorescence intensities of particles isolated from NC yellow and *Su/su* mutants. As expected, there was essentially no difference between the fluorescence properties of particles from *Su/su*

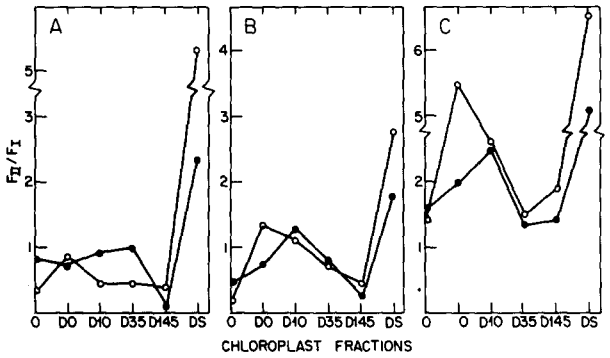

Fig. 3. Ratio of short-wavelength (F_{II}) to long-wavelength (F_I) height of fluorescence peak at 77 K for various thylakoid preparations. •———•, digitonin treatment in the presence of 10 mM Mg^{2+}; ○———○, digitonin treatment in Mg^{2+}-free low-salt medium; (A) JWB, (B) NC yellow, (C) *Su/su*. Chl *a/b* ratios were: JWB, 2.7; NC yellow, 4.2; *Su/su*, 12.5.

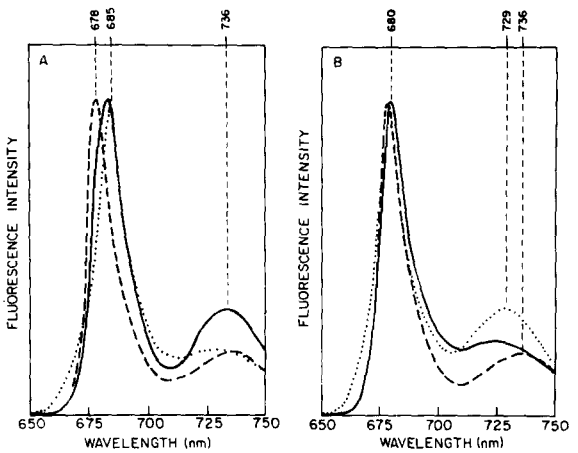

Fig. 4. Fluorescence spectra (77 K) of digitonin-145S fractions from various chloroplasts (normalized for short-wavelength emission). Curves represent the following starting materials (Chl *a/b* ratios in parentheses): (A) ------, JWB (2.8); ———, *Su/su* (6.0); · · · · · ·, *Su/su* (12). (B) ------, JWB (2.8); ———, NC yellow (3.8); · · · · · ·, NC yellow (4.5).

thylakoids isolated in media containing, or devoid of, Mg^{2+}. Surprisingly, an influence of the ionic conditions also was not evident in the fluorescence spectra of NC yellow particles even though the separation of LHCP from PS I activity had been found to be dependent on the presence of Mg^{2+} (see above). Especially noteworthy was the nearly complete lack of PS II fluorescence in both digitonin-145S fractions.

In Mg^{2+}-free low-salt media, all types of thylakoids displayed a significantly elevated short-wavelength fluorescence in the unfractionated digitonin-0 suspension, and the supernatant fraction digitonin-145S. This reflected an apparently more extensive disruption of the pigment organization during digitonin treatment under such conditions. Since the intense short-wavelength emission from the digitonin-145S fraction of LHCP-rich control chloroplasts peaked between 678 and 680 nm, we assume that the detergent had converted part of the LHCP into a strongly fluorescent form. In support of this interpretation we observed that the principal emission of digitonin-145S particles from *Su/su* var. *aurea* chloroplasts difering in LHCP content was gradually shifted towards 685 nm as the original preparation became lower in LHCP (Fig. 4). An emergence of an otherwise obscure emission from LHCP could be seen even in unfractionated digitonin-0 particles when moderately LHCP-deficient *Su/su* chloroplasts were subjected to detergent treatment [25].

An intermediate position of the digitonin-generated fluorescence peak as shown in Fig. 4A for particles from moderately LHCP-deficient *Su/su* thylakoids was not observed with NC yellow preparations of identical LHCP content. Their digitonin-145S fractions fluoresced maximally at 680 nm or less regardless of the original Chl *a/b* ratio (Fig. 4B). In fact, the chloroplasts with the lowest LHCP content yielded particles fluorescing at a somewhat shorter wavelength than particles from LHCP-richer chloroplasts, quite the opposite of what we expected.

Taken together, these observations suggest that detergent treatment caused a drastic increase in the fluorescence emission from two PS II-associated pigment-protein complexes, one fluorescing at 685 nm, and the other, presumably LHCP, at about 680 nm. Most of these highly fluorescent forms of the chlorophyll-pigment complexes were associated with the lightest (digitonin-145S) fraction, perhaps mainly solubilized in detergent micelles. However, some complexes made detergent-labile remained associated with the digitonin-35 fraction while the digitonin-145 particles were essentially devoid of them (Figs. 3 and 5). Unfortunately, these data do not allow any conclusions to be made about cause and effect in the ob-

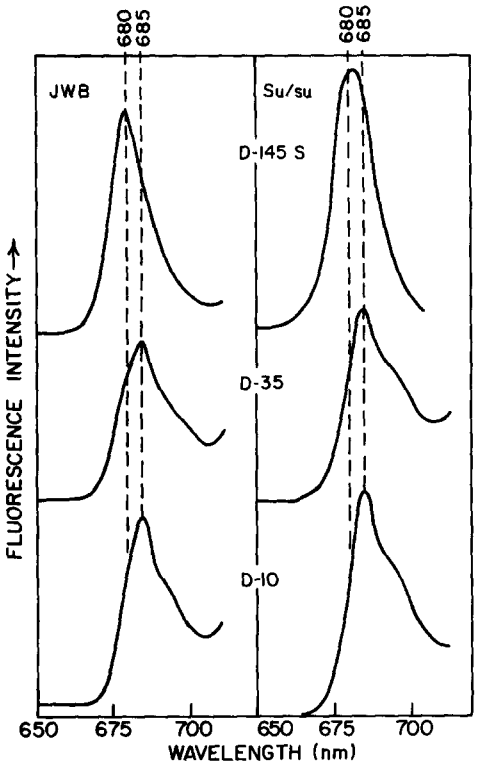

Fig. 5. Short-wavelength fluorescence emission of digitonin-liberated particles from *Su/su* and JWB chloroplasts at 77 K. Treatment in Mg^{2+}-free low-salt media. left, JWB; right, *Su/su*.

served relationship between particle mass or density and pigment composition.

Much controversy surrounds the origin of F_{695} in chloroplast fluorescence spectra [1], but recent work of Satoh [26] confirms its origin in an antenna pigment of PS II. F_{695} was present in the spectra of all our chloroplasts but became obscured by detergent treatment especially in our *Su/su* var. *aurea* chloroplasts and in Mg^{2+}-free media. We have shown elsewhere [25] that a shoulder at 695 nm became recognizable in digitonin-145S particles when the intense short-wavelength emission was attenuated by an addition of Mg^{2+} to the low-salt medium. Such an Mg^{2+}-induced decrease in the short-wavelength fluorescence is known from PS I particles [19], and was observable not only in the light fractions of all our chloroplasts, but also in the heavier pellets of detergent-treated *Su/su* thylakoids.

Mullet et al. [27] have shown that the antenna pigments of PS I are laid down successively during plastid development with the innermost antenna pigments having the lowest excitation energy level. Hence, the synthesis of the PS I assembly results in a red shift of the PS I fluorescence towards 736 nm. Since our *Su/su* var. *aurea* chloroplasts resemble, in many respects, LHCP-deficient developing chloroplasts from intermittent-light-grown peas, it was not surprising to find a long-wavelength fluorescence peak below 735 nm in those preparations with a particularly low LHCP content (Fig. 2). However, in confirmation of data published by Menke and Schmid [5], the shift was never more than to 728 nm even with a Chl *a/b* ratio as high as 15. A comparably low LHCP content in developing cucumber chloroplasts was coupled with a long-wavelength emission at 725 nm [27]. We noted a fluorescence peak at lower than 735 nm also with LHCP-deficient NC yellow preparations, and with all our small thylakoid particles. The latter observation agrees with the finding of Mullet et al. [27] that the external F_{736} antenna is most readily detached by detergent action.

SDS-polyacrylamide electrophoresis of thylakoid proteins

In the course of our studies we wished to correlate the properties of our thylakoid preparations directly with their protein composition. Analyses of the relative abundance and composition of the main three pigment bands are shown in Table II. One note-

TABLE II

DISTRIBUTION OF Chl *a* + *b* AMONG THE THREE MAIN PIGMENTED BANDS SEPARABLE BY SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

Values are averages of at least four determinations and are subject to error by approx. ± 10%. CP I, slowly moving pigment-protein complex representing PS I pigment-protein complex; FP, free solubilized pigments; n.d., not determinable.

Chloroplast source	CP I		LHCP		FP	
	%	Chl *a/b*	%	Chl *a/b*	%	Chl *a/b*
JWB	15	10	41	1.3	44	1.8
Su/su	23	13	28	1.4	49	3.5
Su/su var. *aurea*	31	16	n.d.	–	68	6
NC yellow	31	10	20	1.3	48	1.7

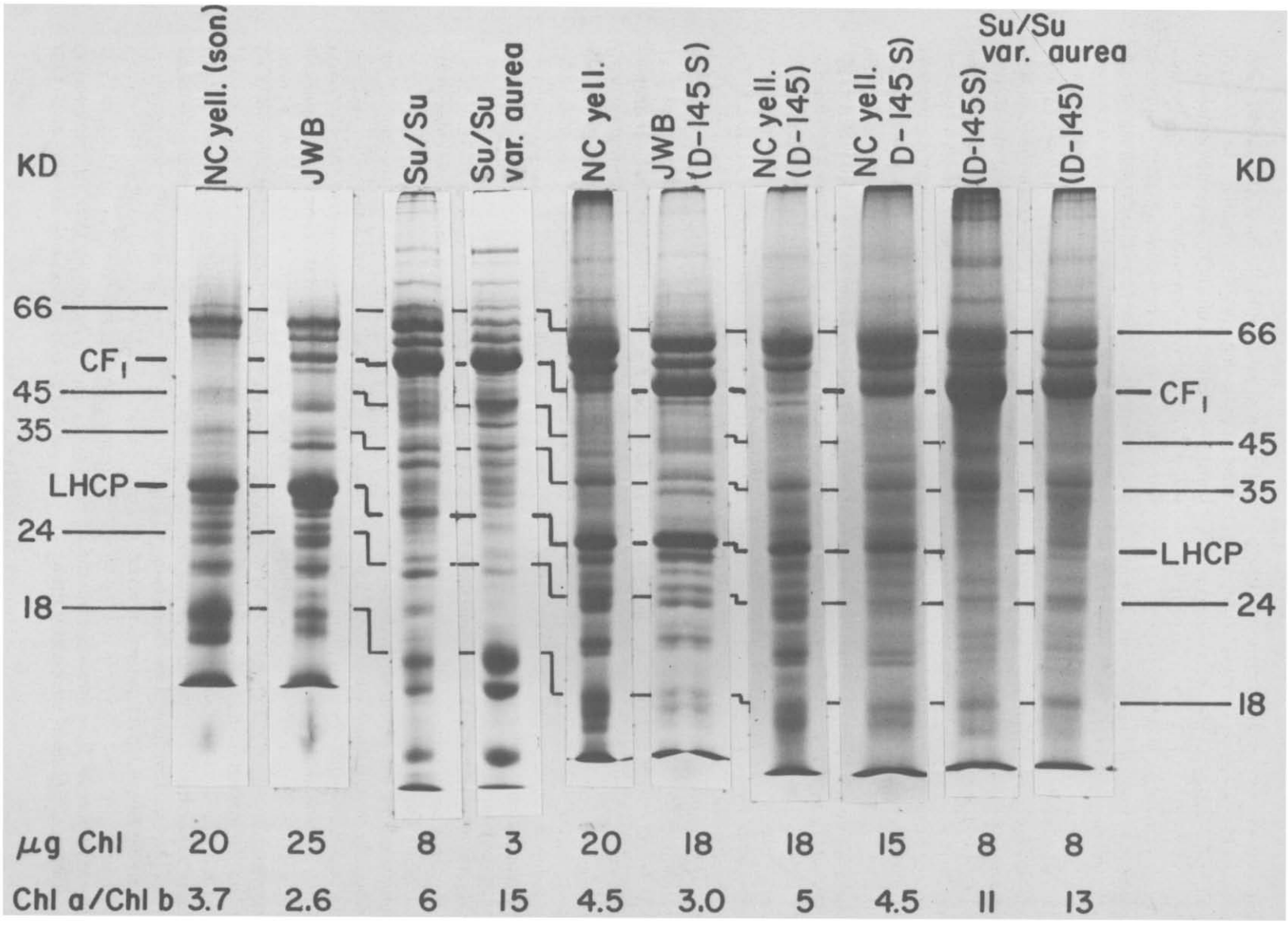

Fig. 6. SDS-polyacrylamide gel electrophoresis pattern of thylakoid polypeptides from various preparations. Solubilization and electrophoresis at room temperature in the presence of 5 M urea and 2% mercaptoethanol. Molecular weight standards used in the slabs from which above lanes were excised: β-lactoglobulin, 18.4 kdaltons; trypsinogen, 24.0 kdaltons; pepsin, 34.7 kdaltons; egg albumin, 45 kdaltons; bovine plasma albumin, 66 kdaltons, all from Sigma Chemical Co. kit (for discrepancies between apparent molecular weights of thylakoid polypeptides in these lanes, and published values, see text). From NC yellow (NC yell.), CF_1 had been removed by sonicating (son.) and washing the originally frozen preparation. KD, kilodaltons.

worthy finding was the extensive separation of free pigments from LHCP-deficient thylakoids during polyacrylamide gel electrophoresis. This paralleled the liberation of large amounts of very small particles from the same material during digitonin treatment.

No attempts were made to identify all proteins separated by polyacrylamide gel electrophoresis. Assignments of the main bands were based on comparisons with PS I- and PS II-enriched particles prepared from normal control chloroplasts, and with the band patterns obtained by Süss [28] and others [14,27]. Representative reproductions of Coomassie blue-stained gels are shown in Fig. 6. One of the most abundant proteins was the coupling factor CF_1, especially in the grana-deficient *Su/su* var. *aurea* thylakoids and even more in their digitonin-145S fraction. As expected, LHCP was essentially absent from *Su/su* var. *aurea* chloroplasts with a high Chl *a/b* ratio, but was clearly evident in the plastids of chlorotic Mn^{2+}-deficient leaves (not shown), in NC yellow thylakoids, and in the digitonin-145S fraction of control chloroplasts after fractionation in low-salt media.

Peculiarly, with our urea-denatured preparations, the main component of LHCP always banded well behind the molecular weight standards trypsinogen (24 kdaltons, Sigma Chemical Co.) or chymotrypsinogen 25.7 kdaltons, Calbiochem) at the 29 kdalton position even though others [13,20,23] assigned a 25 kdalton size to it. All our size values, therefore, must be taken with this discrepancy in mind.

As was already indicated by the polyacrylamide gel electrophoresis analysis of NC yellow chloroplasts performed by Miller and Cushman [29], the main alterations in the protein content of these PS II-deficient mutant plastids are in the region between 50 and 30 kdaltons. In the PS II-deficient mutant F-34 of *Chlamydomonas,* Wollman et al. [30] found strongly diminished amounts of three polypeptides having estimated sizes of 50, 47 and 32 kdaltons. While we encountered some unexpected variability in the protein bands separated by SDS-polyacrylamide gel electrophoresis of NC yellow thylakoids, this variability involved apparently the same three polypeptides of which a 33 and 43 kdalton protein were most consistently missing (Fig. 6). All three polypeptides are thought to be part of the PS II reaction center complex [30] and, accordingly, were enriched in the digitonin-10 fraction of control chloroplasts fractionated in the presence of Mg^{2+}. They also remained prominent in the digitonin-145S fraction when prepared under low-salt conditions. Of this group of three proteins, a 45 kdalton polypeptide was especially conspicuous in the gel lanes of SDS-polyacrylamide gel electrophoresis-separated proteins from thylakoids of *Su/su* var. *aurea* (Fig. 6).

Polypeptide constituents of PS II- and PS I-enriched subchloroplast particles are well studied and have been analyzed in this study for use in the identification of polypeptide bands seen in our electrophoresis gels. Relatively little is known about the composition of small particles obtained under the unstacking influence of low-salt media. Some results from our preliminary analyses of these particles are depicted in the gel photographs of Fig. 6. It can be seen that the small thylakoid fragments were invariably enriched in the coupling factor. While the digitonin-145S fraction of the control chloroplasts contained most of the polypeptides visible in the gels of the unfractionated thylakoids, there appeared to have occurred a significant depletion in proteins during the preparation of the small *Su/su* particles. On the other hand, the SDS-polyacrylamide gel electrophoresis analysis of particles prepared from NC yellow occasionally revealed a striking enrichment of polypeptides, e.g., a 51 kdalton protein in the gel of the digitonin-145 fraction shown in Fig. 6.

Discussion

A physical separation of PS I from PS II by a fractional centrifugation of digitonin-treated chloroplasts requires that they contain LHCP. This has been shown by many authors and also explains most of our data. We have confirmed that a successful fractionation depends on the composition of the suspension medium in the same way as the assembly of thylakoids into stacks [18]. However, we again show, as in an earlier study [6], that a native granal structure is no precondition. As an explanation for the successful fractionation of our grana-deficient NC yellow thylakoids, we suggest that LHCP-containing thylakoid particles may aggreate under appropriate conditions and, therefore, sediment at low *g* values. This concept is supported by the known ability of LHCP to cause an aggregation of liposomes after its incorporation [31], and may also explain why Jennings et al. [32]

found unexpectedly large amounts of heavy pellet under certain conditions that prevented normal grana formation.

While fluorescence spectra have frequently been used as criteria for the composition and properties of detergent-generated thylakoid particles, the interpretation of our measurements was complicated by the modifying effects of the detergent and the ionic milieu on the relative emission intensities. Gasanov and Govindjee [33] have previously discussed these and other aspects of the fluorescence spectra. In this paper we paid special attention to the contribution of LHCP. Unfortunately, no agreement exists concerning the location of the fluorescence peak of functional, thylakoid-bound LHCP. Our own determinations concur with the claim of others [1,22] that isolated LHCP fluoresces maximally at about 680 nm. This emission is shifted a few nanometers upwards in certain LHCP aggregates [31]. Our data confirm the conclusion of Vernotte et al. [34] that, usually, no correlation exists between the LHCP content of chloroplasts and their fluorescence between 675 and 685 nm. However, such a correlation was found for our chloroplasts that lacked PS II activity while containing LHCP. They displayed a distinct F_{680} component just as LHCP-containing normal chloroplasts after digitonin treatment. Cognizant of the finding by Rijgersberg et al. [3] that F_{680} appears in normal chloroplasts only after cooling them below 77 K, we suggest that LHCP does not significantly contribute to the fluorescence spectrum provided that the pigments around the PS II center and PS I function as efficient excitation sinks. Very low temperatures, disruptions of pigment interactions by detergents, and an absence of PS II as in our NC yellow chloroplasts and in certain *Chlamydomonas* mutants [23,24], therefore, would be expected to cause the appearance of F_{680}. As is evident from the observed increase in the fluorescence emission after detergent treatment of LHCP-deficieint chloroplasts, the utilization of excitation energy in the F_{685} pigments can similarly be impaired by detergents. Maintenance of these detergent-effected interruptions of pigment interactions may involve electrical forces as indicated by the ability of Mg^{2+} to partially restore a low fluorescent state in detergent particles [19,25].

Our view is in good agreement with the concept of a hierarchical pigment organization on chloroplast thylakoids, and an efficient excitation coupling between LCHP and especially PS II. While it could be argued that F_{685} originates from aggregated, functional LHCP, and F_{680} from LHCP disassociated from its normal environment [23], the apparent linkage of F_{680} to the Mg^{2+}-controlled pattern of excitation distribution in our mutant and that of Wollman and Diner [24] speaks against such an explanation.

The similarities between the *Chlamydomonas* mutant F-34 which has been studied extensively by Chua and Bennoun [14], and our PS II-deficient NC yellow mutant were evident also in the SDS-polyacrylamide gel electrophoresis analyses. In both cases, a variable degree of deficiency was noted for three polypeptides in the size class 30–50 kdaltons. Their attenuation was coupled with an absence of F_{685} even in the smallest particles of NC yellow in which our experience with *Su/su* chloroplasts would have predicted a detergent-enhanced F_{685}. It appeared, therefore, that the Chl *a* protein responsible for F_{685} is identical with one of the deficient polypeptides. Additional support for this contention came from the observed simultaneous occurrence of an intense F_{685} and a prominent 45 kdalton polypeptide in chloroplasts of *Su/su* var. *aurea*, suggesting that it may be the apoprotein of a pigment aggregate at the PS II center. This had been proposed previously by Henriques and Park [35], and was confirmed recently by Delepelaire and Chua [36].

Two of the unique features of *Su/su* thylakoids were their high coupling factor content, and the sensitivity of their F_{685} pigment to digitonin. The former observations correlated with the availability of extended stroma-exposed surfaces on which the coupling factor can be assembled, and the latter finding may indicate some stabilizing influence of LHCP on the F_{685} pigment in normal chloroplasts. The functional significance of these properties will be subject to further studies. Presently we are exploring in more depth the correlation between composition, organization and photochemical properties of the small particles which one obtains in low-salt media. This material is of interest because it is susceptible to the regulatory effect of ions [25] and retains polypeptides and activities of both photosystems, especially when prepared from LHCP-deficient thylakoids.

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References

1 Butler, W.L. (1978) Annu. Rev. Plant Physiol. 29, 345–378
2 Lavorel, J. and Etienne, A.-L. (1977) in Primary Processes in Photosynthesis (Barber, J., ed.), pp. 203–268, Elsevier, Amsterdam
3 Rijgersberg, C.P., Amesz, J., Thielen, A.P.G.M. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473–482
4 Gasanov, R., Abilov, Z.K., Gazanchian, R.M., Kurbonova, U.M., Khanna, R. and Govindjee (1979) Z. Pflanzenphysiol. 95, 149–169
5 Menke, W. and Schmid, G.H. (1980) Z. Naturforsch. 35c, 461–466
6 Salin, M.L. and Homann, P.H. (1977) Photosynthetica 11, 5–10
7 Homann, P.H. and Schmid, G.H. (1967) Plant Physiol. 42, 1619–1632
8 Okabe, K., Schmid, G.H. and Straub, J. (1977) Plant Physiol. 60, 150–156
9 Homann, P.H. (1967) Plant Physiol. 42, 997–1007
10 Liu, Y.-Y. (1980) Thesis, Florida State University,
11 Homann, P.H. (1972) Biochim. Biophys. Acta 256, 336–344
12 Sayre, R.T. and Homann, P.H. (1979) Arch. Biochem. Biophys. 196, 525–533
13 Hoyer-Hansen, G., Machold, O. and Kahn, A. (1976) Carlsberg Res. Commun. 41, 349–357
14 Chua, N.H. and Bennoun, P. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 2175–2179
15 McKinney, G. (1941) J. Biol. Chem. 140, 315–322
16 Boardman, N.K. and Thorne, S.W. (1971) Biochim. Biophys. Acta 253, 222–231
17 Boardman, N.K., Thorne, S.W. and Anderson, J.M. (1966) Proc. Natl. Acad. Sci. U.S.A. 56, 586–593
18 Sculley, M.J., Duniec, J.T., Thorne, S.W., Chow, W.S. and Boardman, N.K. (1980) Arch. Biochem. Biophys. 201, 339–346
19 Prochaska, L.J. and Gross, E.L. (1977) Arch. Biochem. Biophys. 181, 147–154
20 Argyroudi-Akoyunoglou, J.H. (1976) Arch. Biochem. Biophys. 176, 267–274
21 Davis, D.J., Armond, P.A., Gross, E.L. and Arntzen, C.J. (1976) Arch. Biochem. Biophys. 175, 64–70
22 Melis, A. and Thielen, A.P.G.M. (1980) Biochim. Biophys. Acta 589, 275–286
23 Burke, J.J., Steinback, K.E., Ohad, I. and Arntzen, C.J. (1978) in Chloroplast Development (Akoyunoglou, G. and Argyroudi-Akoyunoglou, J.H., eds.), pp. 413–418, Elsevier/North-Holland, Amsterdam
24 Wollman, F.A. and Diner, B.A. (1980) Arch. Biochem. Biophys. 201, 646–659
25 Bennoun, P. (1980) in Proceedings of the 5th International Congress on Photosynthesis (Akoyunoglou, G., ed.), International Science Services, in the press
26 Satoh, K. (1980) FEBS Lett. 110, 53–56
27 Mullet, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 823–827
28 Süss, K.-H. (1980) FEBS Lett. 112, 255–259
29 Miller, K.R. and Cushman, R.A. (1979) Biochim. Biophys. Acta 456, 481–497
30 Wollman, F.A., Olive, J., Bennoun, P. and Recouvreur, M. (1980) J. Cell Biol. 87, 728–735
31 Mullet, J.E. and Arntzen, C.J. (1980) Biochim. Biophys. Acta 589, 100–117
32 Jennings, R.C., Gerola, P.D., Garlaschi, F.M. and Forti, G. (1980) FEBS Lett. 115, 39–42
33 Gasanov, R.A. and Govindjee (1974) Z. Pflanzenphysiol. 72, 193–202
34 Vernotte, C., Briantais, J.M. and Remy, R. (1976) Plant Sci. Lett. 6, 135–140
35 Henriques, F.A. and Park, R.B. (1978) Biochem. Biophys. Res. Commun. 81, 1113–1118
36 Delepelaire, P. and Chua, N.H. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 111–115