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FLUORESCENCE PROPERTIES OF CHLOROPLASTS FROM MANGANESE DEFICIENT AND MUTANT TOBACCO

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SUMMARY

1. The kinetics of the fluorescence induction are described for chloroplasts from normal green tobacco, from the aurea tobacco mutant Su/su, from the photosynthetically inactive yellow patches of a variegated tobacco, and from tobacco plants grown in absence of manganese. The first two types display the well-known biphasic induction, but the Su/su chloroplasts have a distinctly slower rise time. Manganese deficient chloroplasts show a significantly higher fluorescence yield than any other type of chloroplasts studied. The kinetics of their fluorescence, on the other hand, are similar to those observed with the inactive chloroplasts from the variegated tobacco: the fluorescence rise is small, and the fluorescence yield is not changed very much by the addition of a reducing agent like hydrosulfite, or by addition of an oxidant like ferricyanide, or by an inhibition of the electron flow in Photosystem II with 3(3,4-dichlorophenyl)-1,1-dimethylurea.

2. Determinations of the amount of the primary electron acceptors associated with Photosystem II point to a 2- to 3-fold larger electron acceptor pool in chloroplasts of young Su/su plants than in chloroplasts of old Su/su plants and of various green leaves, including those from green tobacco. This finding agrees with recently published data on the size of the photosynthetic unit in tobacco mutants and normal green plants.

3. The different fluorescence characteristics of all four types of chloroplasts under study are discussed on the basis of their structure and their activity in photosynthetic O_2 evolution.

INTRODUCTION

During the past few years our laboratory has been investigating how the photosynthetic activity of chloroplasts from higher plants varies with genetic changes of their structure and composition. The work by SCHMID AND GAFFRON[1,2] has emphasized the role of the arrangement of the chloroplast lamellae not only as determinant for the photosynthetic activity, but also for the apparent size of the so-called 'photo-

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Abbreviations: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; NC var.y., yellow patches of the leaves of the variegated tobacco mutant derived from the normal green NC 95; JWB, green tobacco variety 'John Williams Broadleaf'.

synthetic unit'. Comparative studies on partial photosynthetic reactions using isolated chloroplasts from various tobacco mutants have indicated that Photosystem-I reactions occur in unfolded frets, whereas System-II activity, *i.e.*, O_2 evolution, may require partitions as a structural prerequisite[3]. The relation of the areas of the partitions to the areas of the frets, therefore, is one possible parameter determining the necessary balance between System-II and System-I reactions in the overall photosynthetic process.

Our data on the quantum efficiency of cyclic photophosphorylation in red and far-red light suggested that the unfolded frets in the chloroplasts from the yellow leaf areas of the variegated tobacco NC 95 lacked System-II activity in spite of the presence of pigments not related to System I (ref. 3). Since the fluorescence of chloroplasts at room temperature originates nearly exclusively from the pigment System II (ref. 4), it was hoped that comparative fluorescence studies might give more information about the state of the photosynthetic pigment Complex II in chloroplasts of mutants with an exceptionally active O_2-evolving apparatus, and chloroplasts in which the system is impaired by either a structural deficiency or lack of manganese. Such studies have now confirmed the presence of a fluorescent pigment system in the mutant chloroplasts of the variegated NC 95, and its similarity to that of manganese deficient plants.

After a period of darkness or an illumination with far-red light, the fluorescence emission of chloroplasts increases from an initial low yield to a steady-state level[4,5]. This fluorescence rise has been interpreted as reflecting the progress of reduction of an internal electron acceptor pool Q + A (refs. 4, 6). As long as this pool is oxidized, the fluorescence is at its minimum due to a suppression of the fluorescence by the 'quencher Q' (JOLIOT's 'E') (ref. 7). The reduced Q (Q^-), in contrast, does not quench, and allows a maximal emission of fluorescence[4]. The kinetics of the fluorescence rise can be analyzed quantitatively[8] and used to determine the size of the pool of primary electron acceptors associated with Photosystem II (ref. 9). Using this method, we have obtained evidence that the chloroplasts of our aurea mutant Su/su, which are characterized by a highly active O_2-evolving apparatus, may contain an unusually large amount of primary oxidants.

Our studies also suggest that structural parameters of the chloroplasts may be decisive in determining the degree of coupling of the fluorescent pigment aggregate to the pool of internal electron acceptors.

MATERIALS AND METHODS

The plant material and its growth in either soil or in liquid culture medium has been described earlier[1,10,11]. Chloroplasts were prepared in 0.4 M sucrose containing 50 mM Tris–HCl (pH 7.3), 10 mM NaCl, 2 mM EDTA and 20 mM ascorbate[3]. EDTA and ascorbate were omitted in the medium used for washing and resuspending the chloroplasts. When the chloroplasts were to be used for determinations of the electron acceptor pool[9], only 100 μM ascorbate was present during homogenization of the leaves and the chloroplasts were washed twice instead of once. In order to obtain a homogeneous chloroplast suspension the final suspension was spun for 1 min in a clinical centrifuge at full speed (including the time of acceleration). The supernatant was stored at 0° in the dark.

During the fluorescence measurements, the rectangular glass cuvette (1 cm × 1 cm × 4 cm) with 3 ml of a chloroplast suspension was kept at a temperature of 16° ± 1°. The exciting light ($\lambda_{\max.} = 570$ nm, half-band width 11 nm, maximal intensity 7.5 nEinsteins·sec^{-1}·cm^{-2}) was isolated from a Lucalox lamp (General Electric) with a green and a yellow plastic filter, in combination with a dilute $CuSO_4$ solution. The emitted light was detected by a potted photomultiplier tube 1P28, which was located at a right angle to the exciting light beam, behind a Schott and Gen. interference filter $\lambda_{\max.} = 684$ nm. The signal was fed into an Aminco photomultiplier-microphotometer and recorded with an Aminco X-Y recorder. Quick 'restoration' of the fluorescence rise was achieved by a 1-min irradiation with 1.4 nEinsteins·sec^{-1}·cm^{-2} far-red light ($\lambda_{\max.} = 717$ nm, half-band width 5 nm) which was obtained by passing light of a Sylvania Sungun lamp through a series of colored glass and Schott and Gen. interference filters. This far-red light entered the cuvette chamber from the side opposite to the exciting light beam.

In a typical experiment we diluted an aliquot of our concentrated chloroplast suspension with usually 0.4 M sucrose containing 50 mM Tris–HCl (pH 7.3) and 10 mM NaCl, and irradiated with 717-nm light for 1 min after temperature equilibration (3 min). Subsequently 570-nm light was admitted by a manually operated shutter and the fluorescence recorded. Any addition to the chloroplast suspension was made in darkness after a second far-red illumination. Hydrosulfite was added as a small amount of crystals rather than from a stock solution. When 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) had been added, we waited for 0.75–1 min with the recording of the fluorescence to assure that DCMU had actually diffused to its site of inhibition.

For the determination of the size of the electron acceptor pool, a new sample of chloroplasts was used for each addition of ferricyanide. In this way the progress of an eventual decay of the chloroplast activity[3] could be deduced from any changes in the shape of the control trace before addition of ferricyanide.

The actual 'titration' of the electron acceptor pool Q + A was performed according to MALKIN AND KOK[9]. It was impractical to use chlorophyll concentrations as high as those used by these authors, because this would have resulted in extremely dense and therefore strongly scattering suspensions of the chlorophyll deficient chloroplasts from the aurea mutant Su/su. This limitation, together with the low absorptivity of chloroplasts in the green part of the spectrum around 570 nm, also precluded determinations of the absorbed light intensity. For the calculation of our data, we used a simplified approach. When the area $^{\infty}\!\int_0 f\mathrm{d}t$ over the induction curve is called A, the absorbed number of quanta described as αI_0, and that portion absorbed by System II as $\alpha\beta I_0$, the quantum yield for the reduction designated as Φ, and the number of equivalents of internal oxidants and externally added oxidants given as n and n' respectively, we can rewrite the expression (4a) of MALKIN AND KOK[9]:

$$n + n' = \beta\alpha\Phi I_0 \cdot A$$

For a given chloroplast preparation $\alpha\beta\Phi I_0$ is a constant C. The equation can, therefore, be written

$$A = 1/C\ (n + n')$$

A plot of the area A, as defined by MALKIN AND KOK[9], *vs.* n' should give a straight line which intercepts the abscissa at $-n' = n$, if the ferricyanide reduction is not limited by System I, and if the electron transport in System II to n, or to $n + n'$, occurs with the same quantum efficiency. Using 5.5 μM chlorophyll, linearity was observed up to about three chlorophyll per ferricyanide.

RESULTS

General characteristics of the 'fluorescence rise' curves

Preliminary experiments showed that the position of the main peak of the emission spectrum is the same for normal chloroplasts, for chloroplasts from the aurea mutant Su/su, and for the chloroplasts from the yellow areas of the variegated NC 95 (NC var.y.), which are nearly devoid of System-II activity (Fig. 1). Since the fluorescence yield of the inactive chloroplasts of NC var.y. is comparable to that of the normal green chloroplasts (Table I), it appears that a considerable amount of pigments related to Photosystem II is present on the unfolded thylakoid of the mutant chloroplasts. Surprisingly, a second peak at about 635 nm was observed

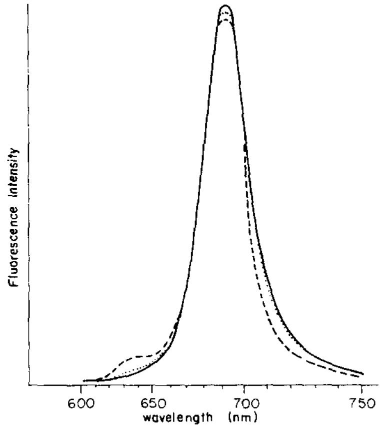

Fig. 1. Fluorescence emission spectra at 25° of three types of tobacco chloroplasts. The chloroplast concentration in 0.4 M sucrose with 50 mM Tris–HCl (pH 7.3) and 10 mM NaCl was adjusted to give an approximately equal height of the main emission peak. The spectrum was taken with an Aminco-Keirs spectrofluorimeter using 430-nm exciting light. ———, normal green chloroplasts from JWB; -------, chloroplasts from the aurea mutant Su/su; ··········, chloroplasts from NC var.y.

TABLE I

RELATIVE FLUORESCENCE YIELD (F_∞ AT 684 nm) OF VARIOUS TOBACCO CHLOROPLASTS

Chloroplasts in 0.4 M sucrose containing 50 mM Tris–HCl (pH 7.3) and 10 mM NaCl to give a chlorophyll concentration of 5.5 μM. Incident light $\lambda_{max.}$ = 570 nm, 7.5 nEinsteins·sec^{-1}·cm^{-2}. The fluorescence yield is given in arbitrary units with standard deviation; number of determinations > 5. Any distortion of the values due to a different degree of scattering of the various chloroplast preparations was found to be negligible under the conditions used.

Source of chloroplasts	*Green control (JWB or NC 95)*	*Aurea mutant (Su/su)*	*NC 95 var.y.*	*Manganese deficient (NC 95)*
Fluorescence yield (F_∞)	32 ± 8	33 ± 7	27 ± 2	59 ± 16

consistently in the fluorescence spectrum of Su/su chloroplasts. The most likely candidate for this emission is protochlorophyll[12]. Small amounts of this precursor of chlorophyll *a* occur in all green plants, and larger concentrations relative to the amount of chlorophyll *a* and *b* may be the reason why it can be easily detected in fluorescence spectra in our chlorophyll deficient mutant chloroplasts. The small hump in the spectrum of the NC var.y. chloroplasts at 635 nm may be real and indicate measurable amounts of protochlorophyll also in this chlorophyll deficient mutant.

After these findings on the characteristics of the fluorescence emission of our chloroplasts, we wanted to know something about the organization of their fluorescent pigment aggregates. We, therefore, turned to studies on the kinetics of the induction phenomena of the fluorescence at 684 nm. Figs. 2–5 show traces of the 'fluorescence rise curves' of chloroplasts from normal green tobacco, from the aurea mutant Su/su, from NC var. y., and from manganese deficient green chloroplasts, at two light intensities.

The recording for the normal chloroplasts (Curve A in Fig. 2a) shows all the typical traits of the often described fluorescence induction[6,7,9,13]: an initial fast rise to an intermediate value F_0, and a slower biphasic rise to a final level F_∞. Su/su chloroplasts, which we have demonstrated to possess an unusually high System-II

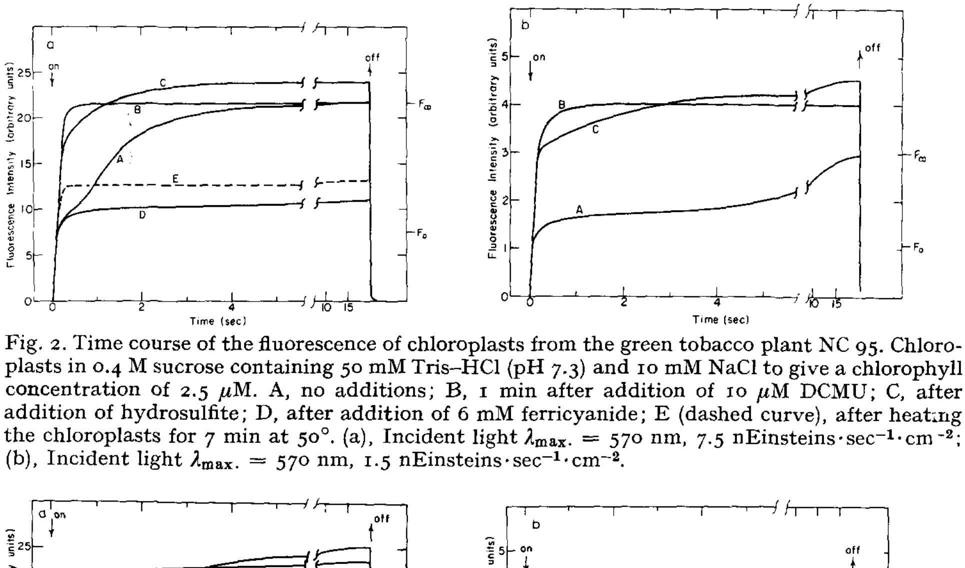

Fig. 2. Time course of the fluorescence of chloroplasts from the green tobacco plant NC 95. Chloroplasts in 0.4 M sucrose containing 50 mM Tris–HCl (pH 7.3) and 10 mM NaCl to give a chlorophyll concentration of 2.5 μM. A, no additions; B, 1 min after addition of 10 μM DCMU; C, after addition of hydrosulfite; D, after addition of 6 mM ferricyanide; E (dashed curve), after heating the chloroplasts for 7 min at 50°. (a), Incident light $\lambda_{max.}$ = 570 nm, 7.5 nEinsteins·sec^{-1}·cm^{-2}; (b), Incident light $\lambda_{max.}$ = 570 nm, 1.5 nEinsteins·sec^{-1}·cm^{-2}.

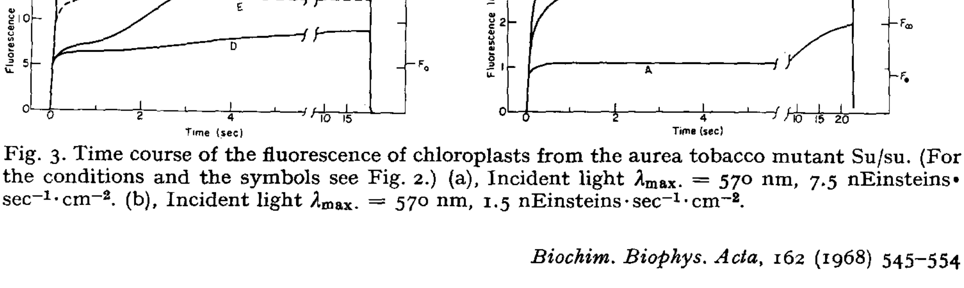

Fig. 3. Time course of the fluorescence of chloroplasts from the aurea tobacco mutant Su/su. (For the conditions and the symbols see Fig. 2.) (a), Incident light $\lambda_{max.}$ = 570 nm, 7.5 nEinsteins·sec^{-1}·cm^{-2}. (b), Incident light $\lambda_{max.}$ = 570 nm, 1.5 nEinsteins·sec^{-1}·cm^{-2}.

activity[3] show the same basic fluorescence kinetics, but the final level is reached after about 20 instead of 8 sec (Fig. 3a, Curve A). In normal chloroplasts such a slow rise curve can be obtained by lowering the intensity of the incident light[9]. Retarded fluorescence kinetics and a relatively low steady-state emission are also typical for chloroplasts in which the quantum efficiency for O_2 evolution is low, for example due to aging. Consequently, the area between the induction curve and the final level F_∞ varies widely with the quality of the chloroplast preparation. The difference in the induction curves, however, between chloroplasts from normal green tobacco leaves and chloroplasts from Su/su leaves was confirmed by many experiments.

Figs. 2 and 3 also show the effect of various chemicals on the fluorescence curve. A block of System II by DCMU (Curves B) or a chemical reduction of the electron acceptor pool by hydrosulfite (Curves C) nearly completely abolish the induction phenomena, and, consequently, give immediately high fluorescence yields. A large difference between these high fluorescence levels and that reached by the chloroplasts in the absence of DCMU or hydrosulfite indicates that the System-II mediated reduction of the primary oxidants cannot compete with their reoxidation, *e.g.*, by a Mehler reaction[14]. This is generally observed in weak light, and, with Su/su chloroplasts, also at higher light intensities (Figs. 2b, 3a, b).

The addition of an oxidant like ferricyanide suppresses the fluorescence to a

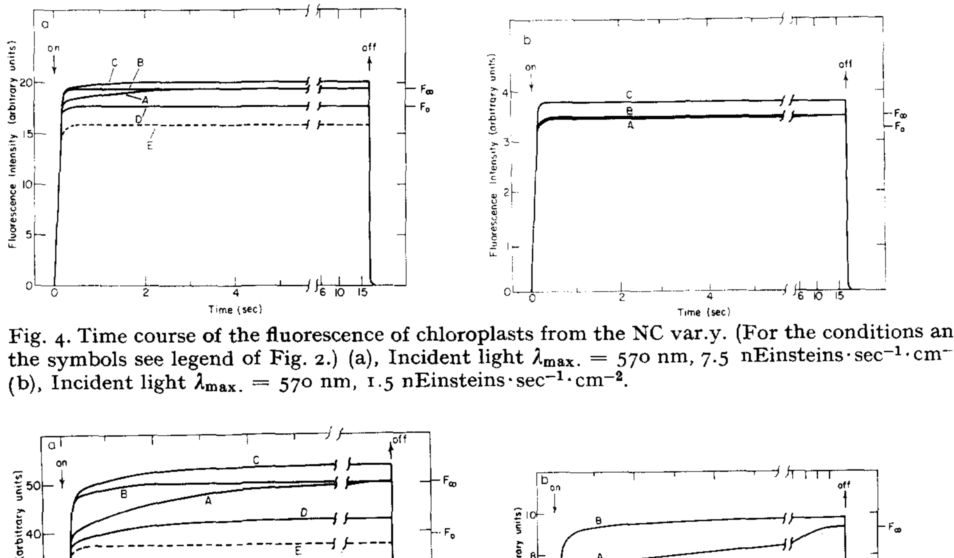

Fig. 4. Time course of the fluorescence of chloroplasts from the NC var.y. (For the conditions and the symbols see legend of Fig. 2.) (a), Incident light $\lambda_{max.}$ = 570 nm, 7.5 nEinsteins·sec^{-1}·cm^{-2}. (b), Incident light $\lambda_{max.}$ = 570 nm, 1.5 nEinsteins·sec^{-1}·cm^{-2}.

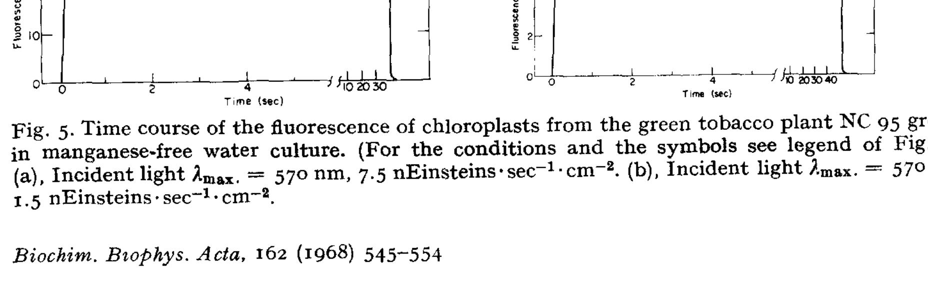

Fig. 5. Time course of the fluorescence of chloroplasts from the green tobacco plant NC 95 grown in manganese-free water culture. (For the conditions and the symbols see legend of Fig. 2.) (a), Incident light $\lambda_{max.}$ = 570 nm, 7.5 nEinsteins·sec^{-1}·cm^{-2}. (b), Incident light $\lambda_{max.}$ = 570 nm, 1.5 nEinsteins·sec^{-1}·cm^{-2}.

level close to F_0 (Curves D). A similar low fluorescence is obtained when System II has been inactivated by heating the chloroplasts 7 min at 50° (dashed Curves E). These effects have been described already by MALKIN AND KOK[9] and by MURATA, NISHIMURA AND TAKAMIYA[13]. They are shown here again to allow a comparison with the responses to the same treatments of the chloroplasts from NC var.y. and from manganese deficient plants. Figs. 4 and 5 give typical fluorescence recordings obtained with these chloroplasts.

Both the chloroplasts from the photosynthetically inactive leaves of manganese deficient plants and of the inactive leaf patches of NC var.y. almost completely lack the induction phase. A striking peculiarity of the manganese deficient chloroplasts is their much higher fluorescence yield (Table I, Fig. 5). In contrast to the observations with normal chloroplasts, we found that the effects of an addition of hydrosulfite, DCMU and ferricyanide, and of a heat treatment, are markedly smaller or negligible in both types of chloroplasts with an impaired System II.

The size of the internal electron acceptor pool

In order to gain some insight into the composition of the highly active O_2 evolving system of the aurea mutant Su/su, we decided to determine the size of its primary electron acceptor pool on the reducing side of System II. 18 mM methylamine was routinely added for the estimation of the internal electron acceptor pool in order to avoid the electron flow to ferricyanide being limited by factors other than the absorbed light energy. It can be seen from our results shown in Table II that an average value of about fifteen chlorophylls per one acceptor molecule was found for chloroplasts from various green plants and for chloroplasts of old Su/su leaves, whereas in chloroplasts of young Su/su there appears to be a pool which is about two to three times as large. The correctness of this finding can possibly be questioned on grounds of the special fluorescence characteristics of Su/su chloroplasts (an extreme case is shown in Fig. 3), which may preclude the application of the equations developed by MALKIN AND KOK[9]. It is in good agreement, however, with the data of SCHMID AND GAFFRON[2] on the size of the photosynthetic unit in leaves of the aurea mutant and normal green leaves. These authors found that the chlorophyll in young Su/su leaves fixed about two to three times more CO_2 per flash than that of green leaves or old

TABLE II

AMOUNT OF THE PRIMARY ELECTRON ACCEPTOR IN VARIOUS CHLOROPLASTS

Suspension medium, chlorophyll concentration and light intensity as in Table I except for an additional presence of 18 mM methylamine. The data were obtained from plots of the area over the induction curves *vs.* amounts of ferricyanide added using four or more different additions of the oxidant (see MATERIALS AND METHODS) per chloroplast preparation. Young plants had no leaf longer than about 15 cm (approx. 12 weeks after germination).

Source of chloroplasts	*N. tabacum (green, JWB)*		*N. tabacum (aurea, Su/su)*		*Spinacia oleracea*	*Medicago lupulina*
	Old plants	*Young plants*	*Old plants*	*Young plants*		
Molecules chlorophyll per electron acceptor	15, 16, 17, 14	17, 19	15, 14	7, 6, 5, 7	15, 17	13, 6, 18
Average	16	18	15	6	16	12

Su/su leaves. Occasionally, they observed a predominance of small units also in green leaves. The large pool of internal electron acceptors comparable to that of chloroplasts from young Su/su leaves which was estimated for one sample of Medicago chloroplasts (Table II), therefore, must not be an experimental error.

Our values for the number of primary oxidants in green chloroplasts (Table II) suggest electron acceptor pools, which are about twice as large as those found by MALKIN AND KOK[9]. We have no explanation for this discrepancy, but a comparison reveals that values close to ours have been found by these authors in four out of eleven preparations of higher plant chloroplasts. It is obvious that an incomplete restoration of the fluorescence rise in our experiments, or a decreased quantum efficiency of the ferricyanide reduction under our conditions of low chlorophyll concentrations, should give an extrapolation to smaller, not larger, pools.

One may expect that a large pool of primary electron acceptors in chloroplasts of young Su/su plants is matched by a similarly large pool of electron donors on the oxidizing side. Our earlier data on the manganese content of Su/su chloroplasts, however, suggest that their manganese content is the same as in green chloroplasts[3]. These data, however, were obtained with plants more than 4 months old. In an earlier communication[15] it was reported that the manganese content in Su/su leaves was determined to be twice as large as in green leaves. Such values were initially found, but disregarded later as erroneous due to the probability of an incomplete removal of unbound manganese from the suspensions of these chlorophyll deficient chloroplasts[3]. Now it would appear that those values are not improbable.

DISCUSSION

An investigation of the photosynthetic activities of chloroplasts from various tobacco mutants had revealed that System-I reactions occur on unfolded thylakoids, whereas electron transfer processes involving O_2 evolution may be restricted to the partitions of lamellar overlappings or grana[3]. Furthermore, an analysis of the quantum efficiency of cyclic phosphorylation in red and far-red light had indicated that a considerable amount of light harvesting pigments not involved in System-I reactions is located on the chloroplast lamellae, independent of the presence of partitions. This other pigment aggregate was assumed to be part of an inactive Photosystem II (ref. 3). The fluorescence characteristics of the chloroplasts of NC var.y., which are described in this paper, confirm this assumption. Such a conclusion is, however, correct only when nearly all the fluorescence of chloroplasts at room temperature arises from chlorophyll of System II, as presently believed[16]. Our data on the high level of the basic fluorescence F_0 obtained with chloroplasts from manganese deficient plants, and NC 95 var.y., suggest that the fluorescence yield may not depend only on the number of pigment molecules associated with Photosystem II. Another factor may be the structural organization in the chloroplast, which is known to be affected by manganese deficiency[10,17]. It is conceivable, for example, that usually non-fluorescent chlorophyll molecules become fluorescent due to a structure-dependent exposure to the polar environment of the stroma. In chloroplasts of plants grown in absence of manganese, such side effects may strongly mask the primary effects resulting from a manganese deficiency of the electron transport chain associated with System II. Structural and other still unknown parameters in combination with a low Hill activity are possibly the reasons also for some similarities between the fluorescence of the mutant chloroplasts and those from the manganese deficient chloroplasts: the negligible fluorescence rise, and the failure of DCMU, and of a chemical reduction of the electron acceptor pool by hydrosulfite or its chemical oxidation with ferricyanide, to change the final fluorescence level significantly. If the fluorescence yield is really determined only by the redox state of the 'quencher Q', one would have to postulate that the concentration of Q is either very small, or that its influence on the fluorescence is diminished by a detachment from the pigment aggregate. It is noteworthy that an inactivation of System II by heating the chloroplast preparation for 7 min at 50° produces a fluorescence rise curve similar to that of our mutant or manganese deficient chloroplasts, but there are two differences: the initial fluorescence level F_0 is usually low, and the addition of hydrosulfite increases the yield to a variable, but markedly higher level.

The fluorescence induction described for our Su/su chloroplasts shows some features similar to those displayed by the chloroplasts deficient in chlorophyll *b* of BOARDMAN AND THORNE[18]. Both types of chloroplasts have a slow fluorescence rise, and both leave a considerable part of the primary pool of oxidants in its oxidized state. Initially we sought the reason for the slower induction kinetics of our Su/su chloroplasts in their larger pool of electron acceptors, but a comparison of many recordings obtained with green and Su/su chloroplasts from plants of different stages of development did not give a clear correlation. Although the size of the acceptor pool certainly does contribute to the length of the induction period, an additional reason for the slow rise could be a lower quantum efficiency of the System-II induced electron transport in the Su/su chloroplasts. This may be true for the experiments described in this paper in spite of the similar quantum yields in green and aurea chloroplasts reported for earlier experiments under different conditions, and in spite of the generally much higher saturation rates of the Hill reaction in the chloroplasts of the aurea mutant[3]. One can also assume that in the Su/su chloroplasts Photosystem I absorbs more quanta from the incident light than the pigment System II. Such an unequal distribution of quanta could occur if there were more chlorophyll molecules associated with System I, than with System II, as has been postulated for the chloroplasts lacking chlorophyll (ref. 18).

Finally we have to discuss the effect of hydrosulfite on the fluorescence kinetics. This agent is assumed to reduce the primary electron acceptor pool chemically. Consequently it should eliminate the fluorescence induction completely. Actually it was found that the fluorescence did not reach its final level instantaneously, particularly in Su/su chloroplasts with a slow normal rise curve, and at low light intensities. This had been observed already by BOARDMAN AND THORNE[18], who did not discuss it. At present it is difficult to explain this phenomenon, but one can speculate that either hydrosulfite does not reduce all of the primary oxidants, so that the rest of the pool has to be filled with electrons from water in a light-dependent reaction, or that the fluorescence kinetics in the presence of hydrosulfite reflect the progress of the activation step[7,19] in the O_2 evolving system. The task of interpreting the action of hydrosulfite is not made easier by our observation that the fluorescence yield of chloroplasts in the presence of this agent often begins to decrease steadily after about 15 to 20 sec of continuous illumination with 570-nm light of 7.5 nEinsteins $\cdot$ sec^{-1} $\cdot$ cm^{-2}, probably indicating a fast reversal of the activation reaction.

ADDENDUM

After this paper had been submitted for publication, ANDERSON AND THORNE[20] reported about the fluorescence characteristics of manganese deficient chloroplasts from spinach. While there is general agreement between most of their data and those presented in this paper, the conclusions are quite different. Studies with chloroplasts artificially depleted of 80 % of their bound manganese[21] support our concept that the fluorescence of chloroplasts from manganese deficient plants is strongly influenced by secondary effects of the deficiency. They clearly reveal, moreover, that manganese does not function on the reducing side of System II, as ANDERSON AND THORNE conclude from their data, but rather acts close to the site of O_2 evolution.

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