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PHOTOSYNTHETIC MEMBRANE DEVELOPMENT STUDIED USING PICOSECOND FLUORESCENCE KINETICS

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Using measurements of the kinetics of chlorophyll *a* fluorescence emission, we have investigated the development of the photosynthetic membrane during etioplast-to-chloroplast differentiation. The chlorophyll fluorescence decay kinetics of pea chloroplasts from plants grown under intermittent (2 min light–118 min dark) and continuous light regimes were monitored with a single-photon timing system with picosecond resolution. We have associated the changes in the fluorescence yields and decay kinetics with known structural and organizational developmental phenomena in the chloroplast. This correlation provides a more detailed assignment of the origins of the fluorescence decay components than has been previously obtained by studying only mature chloroplasts. In particular, our analysis of the variable kinetics and multiexponential character of the fluorescence emission during thylakoid development focuses on the organization of photosynthetic units and the degree of communication between reaction centers in the same photosystem. Our results further demonstrate that the age of etiolated tissue is critical to plastid development.

Introduction

The development of the photosynthetic membrane is a complex, multiphasic process involving the biosynthesis and incorporation of both structural and functional components (pigments, lipids, and proteins) and their assembly and integration into active photosynthetic units. A detailed analysis of the relation of the structure, composition, and function of thylakoid membrane components is complicated by the presence of both sequential and parallel developmental phenomena. To elucidate the steps of plastid development most investigations [1–6] of chloroplast membrane development employ the light-dependent synthesis of chlorophyll as an analytical tool. Exposure of etiolated leaves to continuous light leads to the transformation of the etioplast to a normal granal chloroplast after a lag phase which depends on the age of the etiolated tissue [7–10]. The differentiation consists of the transformation of the prolamellar body of etioplasts to primary thylakoids and their subsequent growth and development into grana and stroma thylakoids. The parallel synthesis and integration of the thylakoid components into the developing membrane lead to the emergence of the characteristic features of the mature chloroplast.

The formation of the photosynthetic apparatus can be altered by a change in the mode of illumination during plant growth. Under conditions of intermittent light (e.g., 2 min light–118 min

Abbreviations: Chl, chlorophyll, PS, photosystem; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea.

dark), the etioplast is converted to a protochloroplast [11] – an intermediate stage of chloroplast development. The protochloroplast contains unstacked primary thylakoids [12] which are devoid of Chl *b* and deficient in the light-harvesting Chl *a/b* complex [1,11–13]. Intermittent light promotes the selective synthesis of Chl *a* and its organization into functional reaction centers and the closely coupled pigment-protein complexes for both photosystems. Small photosynthetic units of uniform size are formed, initially structurally isolated but later coming into contact [14]. Soon after exposure of etiolated leaves to periodic light both PS I and PS II activities appear [8,15,16], as well as excitation energy transfer between the photosystems [17]. However, the protochloroplasts do not have the capacity for cation-induced stacking or for a cation-induced rise in Chl *a* fluorescence [10]. Transfer to continuous white light transforms the protochloroplasts to chloroplasts [1], with a parallel synthesis of Chl *a* and *b* and a gradual differentiation of the primary thylakoids to stroma and grana lamellae. The extent of growth of the photosynthetic unit size depends on the length of exposure to periodic light and the age of the etiolated tissue [8,14,15,18].

In the present paper we use measurements of chlorophyll fluorescence decay kinetics to monitor the structural and organizational changes associated with thylakoid development as controlled by the mode of illumination during growth. Chlorophyll fluorescence decay kinetics in chloroplasts and green algae have been studied to determine the origin of the multicomponent steady-state Chl *a* fluorescence. The fluorescence decay of broken chloroplasts of higher plants at room temperature is generally characterized by triple-exponential character [19–22], with the amplitudes and lifetimes of the fluorescence decay components varying with such experimental factors as light intensity of excitation [19,21,22], ionic composition [23] and redox potential [24] of the chloroplast suspending medium, and the presence of electron-transport inhibitors [19,21,22]. The variable kinetics and multiexponential character of the fluorescence emission reflect the heterogeneous population of fluorescing Chl *a* molecules, the variations in organization of photosynthetic units, the degree of communication between reaction centers in the same photosystem, the extent of energy transfer between photosystems, and the state of the photochemical reaction centers [19–24]. We compare the fluorescence decay kinetics of pea protochloroplasts developed under an intermittent-light regime with that of mature chloroplasts developed under continuous light or by transfer of protochloroplasts to continuous light. We attempt to correlate the known structural and organizational development during the etioplast-to-chloroplast differentiation with the observed fluorescence lifetime and yield variations in order to construct a viable model for the photosynthetic unit and for the origin of fluorescence.

Materials and Methods

Etiolated pea leaves were grown in the dark for 6 or 8 days and then transferred directly to continuous light or preexposed to intermittent light for specific lengths of light-dark cycles (2 min light–118 min dark) as detailed in the text. Control plants were grown under a light regime of 12 h daily illumination.

Broken chloroplasts were isolated in dim light by grinding the developing pea leaves in a blender for 10 s in a medium of 0.4 M sucrose, 50 mM Hepes-NaOH, pH 7.5, and 10 mM NaCl. The filtered homogenate was centrifuged at $6000 \times g$ for 10 min. The pellet was washed with fresh grinding medium, followed by centrifugation under the same conditions. Resuspension of the pellet in a medium of 0.1 M sucrose, 10 mM Hepes-NaOH, pH 7.5, and 10 mM NaCl was followed by centrifugation at $6000 \times g$ for 10 min. The isolated chloroplasts were suspended for fluorescence measurements in a buffer solution of 0.1 M sucrose, 50 mM Hepes-NaOH, pH 7.5, $\pm$ 5 mM $MgCl_2$ at a concentration of 10 μg Chl $\cdot$ ml^{-1}.

The fluorescence excitation source was a Spectra Physics synchronously pumped mode-locked dye laser (SP 171 argon ion laser, SP 362 mode locker, and modified SP 375 dye laser). Chloroplast samples were excited with pulses of 8 ps half-maximum full-width duration at 620 nm. The laser pulse intensity (10^6 photons $\cdot$ cm^{-2}) was attenuated with neutral density filters for intensity dependence experiments. Fluorescence was detected at right angles at 680 nm. The single-photon

timing system and numerical analysis methods have been described previously [19,25–27]. All fluorescence decay data were resolved into a sum of exponential decays with a lifetime resolution limit of 25 ps.

Results

Plastids from 6-Day-Old Etiolated Pea Leaves Exposed to 24 Light-Dark Cycles

Fig. 1 presents the effect of continuous light on the lifetimes of the three exponential fluorescence decay components of these protochloroplasts suspended in Mg^{2+}-enriched (5 mM) suspension buffer. Measurements were made at the F_0 level of fluorescence (open reaction centers, low light excitation intensity) and at the F_{max} level (closed reaction centers, high light excitation intensity). At 0 h continuous light the protochloroplasts enhibit fluorescence decay components with lifetimes of 2600, 450 and 100 ps at F_0 and F_{max}. Under continuous illumination the lifetime of the slow decay component, τ_{slow}, decreases to 1400 ps (6 h continuous light) at F_0 and F_{max}. The middle decay component lifetime, τ_{middle}, displays more complex behavior after transfer to continuous light; at F_0, τ_{middle} gradually decreases to 400 ps (6 h continuous light), and at F_{max}, increases to 600 ps (24 h continuous light). The lifetime of the fast decay component, τ_{fast}, is independent of both excitation intensity and continuous light treatment and remains at approx. 100 ps. After 48 h continuous light the lifetimes of the decay components are comparable to those of the control chloroplasts isolated in the absence of added Mg^{2+}.

The effect of continuous illumination on the relative amplitudes of the fluorescence decay components is plotted in Fig. 2. At F_0 the amplitudes of the slow decay component, α_{slow}, and the fast decay component, α_{fast}, decrease steadily during the first 6 h continuous light and reach a plateau after 24 h continuous light. In parallel over the same time interval, the middle decay component, α_{middle}, increases and becomes the dominant contribution. At the F_{max} level of fluorescence, α_{slow}

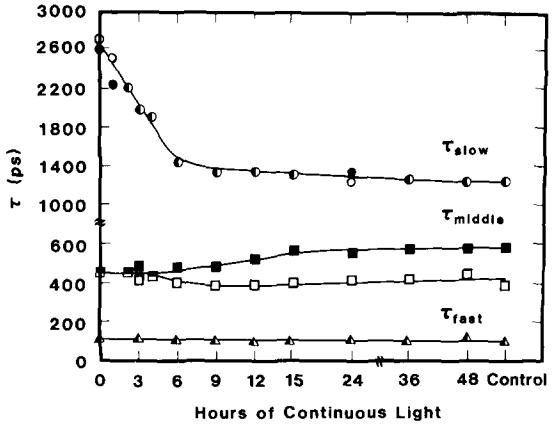


Fig. 1. Lifetimes of the components of the fluorescence decay in plastids from 6-day-old etiolated pea leaves subsequently exposed to 24 light-dark cycles and then continuous light, in the presence of 5 mM Mg^{2+}; (○) lifetime of the slow phase, (□) lifetime of the middle phase, and (△) lifetime of the fast phase. Open symbols, lifetimes measured at F_0; closed symbols, lifetimes measured at F_{max}.

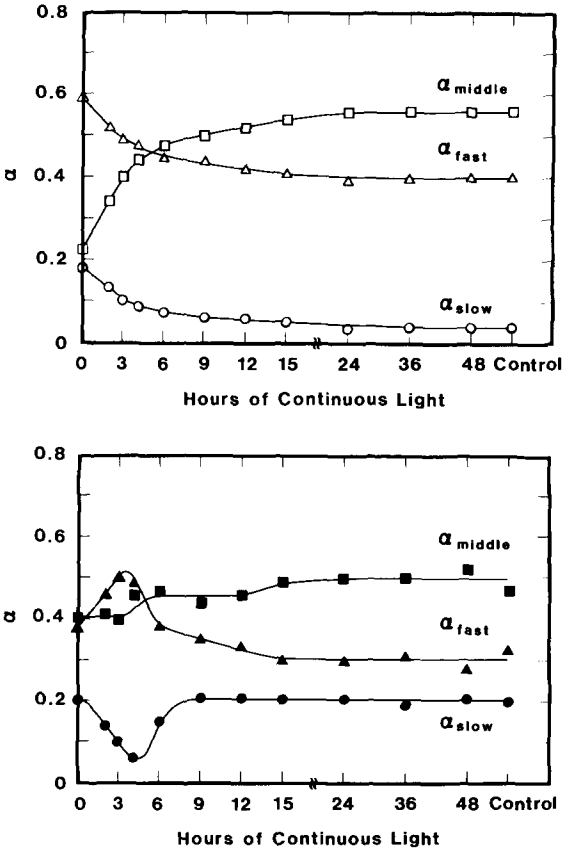


Fig. 2. Amplitudes of the components of the fluorescence decay in plastids of Fig. 1. Amplitudes are normalized such that $\Sigma\alpha = 1$ at each time point. The symbols are defined as in Fig. 1. Upper diagram, F_0; lower diagram F_{max}.

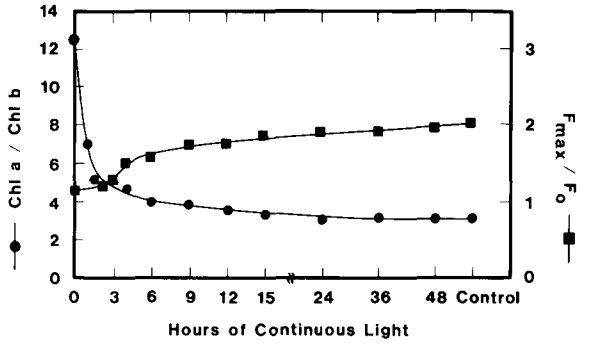


Fig. 3. The ratios of Chl a/Chl b (●) and F_{max}/F_0 (■) in plastids, as in Fig. 1.

decreases from 0 to 4 h continuous light, increases from 4 to 9 h continuous light and remains constant thereafter. The exact opposite dependence is observed for α_{fast}. During 0–3 h continuous light α_{middle} is constant, increasing somewhat at 4 h continuous light and slightly more from 24 to 48 h continuous light. The overall fluorescence yields at F_0 and F_{max} increase with increasing amounts of continuous light, reaching a plateau after approx. 9 h continuous light.

Fig. 3 shows the effect of continuous light on the Chl a/Chl b ratio and on the F_{max}/F_0 ratio. The most dramatic changes in these ratios occur during the first 6 h continuous light.

Plastids from 8-Day-Old Etiolated Pea Leaves Exposed to 24 Light-Dark Cycles

The effect of continuous light on the fluorescence decay kinetics of these protochloroplasts isolated in Mg^{2+}-depleted buffer is presented in Figs. 4 and 5. Three exponential decay components are resolved as for the 6-day-old etiolated leaves. During greening in continuous light no difference in τ_{slow} is detected at F_0 and F_{max}, but τ_{slow} decreases with continuous light from 2200 to 1400 ps, reaching a plateau after approx. 10 h continuous light. At the onset of illumination $\tau_{middle} = 500$ ps, decreasing to 400 ps at F_0 with 10 h continuous light and remaining at 500 ps at F_{max}. No effect of excitation intensity or continuous light on τ_{fast} is observed, τ_{fast} remaining constant at 100 ps. The lifetimes of the decay components after 36 h continuous light are the same as those measured for the control chloroplasts in the absence of Mg^{2+}.

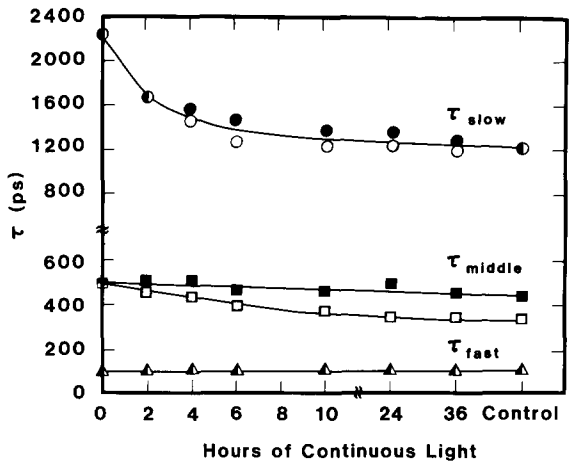


Fig. 4. Lifetimes of the components of the fluorescence decay in plastids from 8-day-old etiolated pea leaves subsequently exposed to 24 light-dark cycles and then continuous light, in the absence of 5 mM Mg^{2+}; (○) lifetime of the slow phase, (□) lifetime of the middle phase, and (△) lifetime of the fast phase. Open symbols, lifetimes measured at F_0; closed symbols, lifetimes measured at F_{max}.

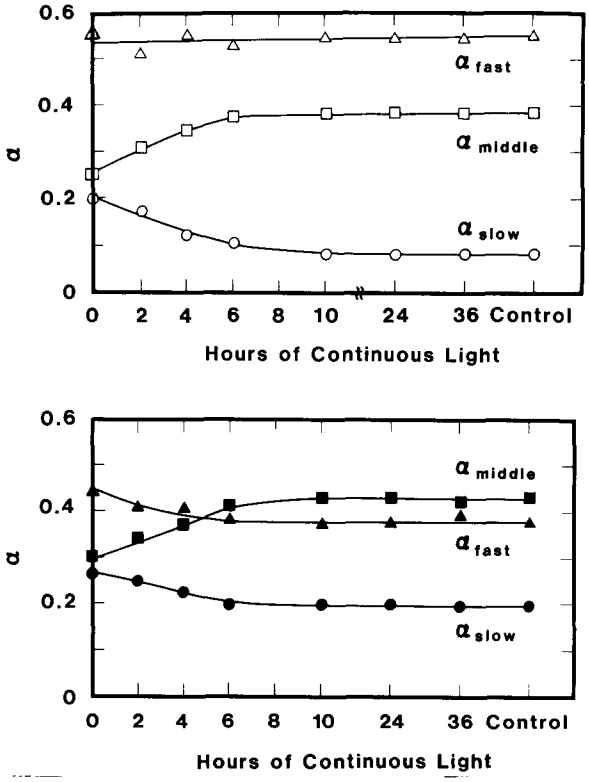


Fig. 5. Amplitudes of the components of the fluorescence decay in plastids as in Fig. 4. Amplitudes are normalized such that $\Sigma\alpha = 1$ at each time point. The symbols are defined as in Fig. 4. Upper diagram, F_0; lower diagram, F_{max}.

Fig. 5 displays the effects of continuous light on the amplitudes of the fluorescence decay components of the chloroplasts depleted of Mg^{2+}. The changes in α_{slow} and α_{middle} at F_0 with continuous light are in opposite directions, α_{slow} decreasing during the first 6 h continuous light and remaining constant thereafter and α_{middle} increasing with 6 h continuous light and leveling off from 10 to 36 h continuous light. The amplitude of the fast decay component is constant with continuous light. At F_{max} continuous light causes α_{slow} and α_{fast} to decrease during the first 6 h continuous light, with α_{middle} increasing during this time interval. The total fluorescence yield at F_0 decreases during the first 10 h continuous light, while that at F_{max} increases throughout this time period.

Figs. 6 and 7 present the effect of continuous light on the lifetimes and amplitudes of the fluorescence decay components of chloroplasts incubated in Mg^{2+}-enriched (5 mM) suspension buffer. The lifetime of the slow decay component is the same in the F_0 and F_{max} states during 6 h continuous light; during this interval τ_{slow} decreases from 2200 to 1400 ps. A variation with excitation intensity is noted for τ_{slow} after 6 h continuous light. The value of τ_{slow} decreases to

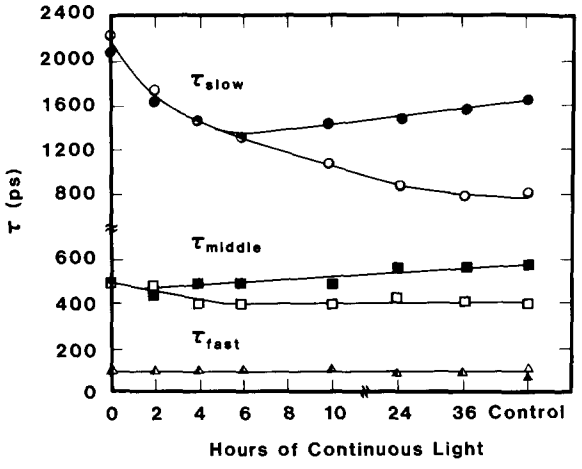


Fig. 6. Lifetimes of the components of the fluorescence decay in plastids from 8-day-old etiolated pea leaves subsequently exposed to 24 light-dark cycles and then continuous light, in the presence of 5 mM Mg^{2+}; (○) lifetime of the slow phase, (□) lifetime of the middle phase, and (△) lifetime of the fast phase. Open symbols, lifetimes measured at F_0; closed symbols, lifetimes measured at F_{max}.

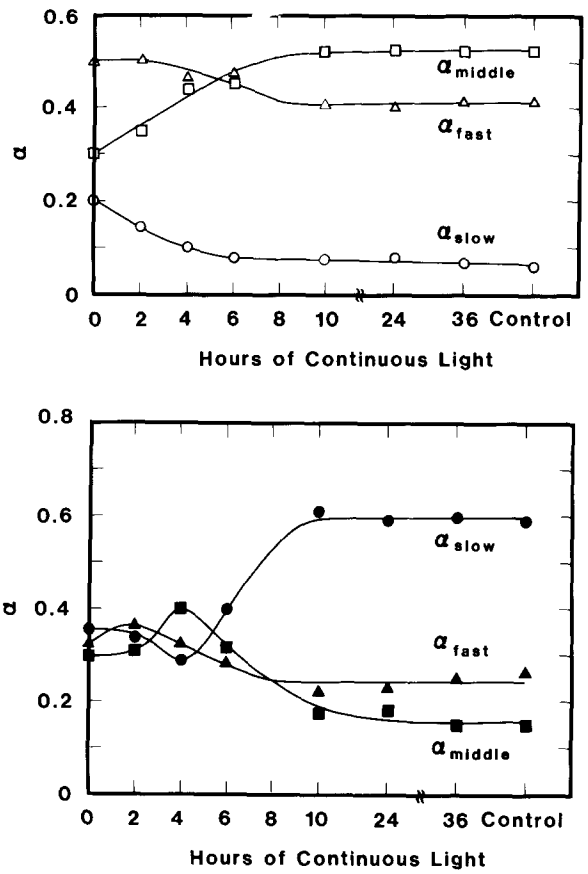


Fig. 7. Amplitudes of the components of the fluorescence decay in plastids as in Fig. 6. Amplitudes are normalized such that $\Sigma\alpha = 1$ at each time point. The symbols are defined as in Fig. 6. Upper diagram, F_0; lower diagram, F_{max}.

900 ps for open reaction centers and increases to 1600 ps for closed reaction centers with 36 h continuous light. For the first 2 h continuous light $\tau_{middle} = 500$ ps for both F_0 and F_{max}, but a differ-

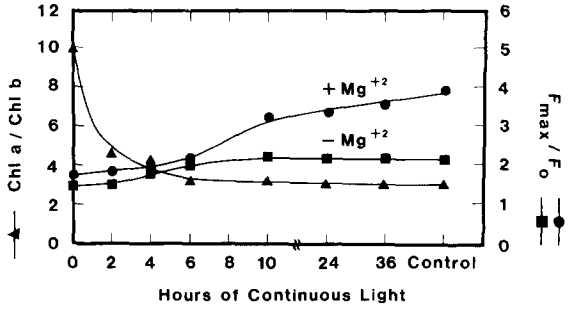


Fig. 8. The ratios of Chl a/Chl b (▲) and F_{max}/F_0 (■, ●) of plastids described in Figs. 4 (■) and 6 (●).

entiation between the F_0 and F_{max} fluorescence levels after 2 h continuous light leads to a decrease in τ_{middle} to 350 ps for open reaction centers and an increase to 550 ps for closed reaction centers after 36 h continuous light. τ_{fast} is constant at about 100 ps during 36 h continuous light for both open and closed reaction centers. The lifetimes after 36 h continuous light are the same as those measured for the Mg^{2+}-enriched control chloroplasts.

As depicted in Fig. 7, at F_0 α_{slow} decreases during the first 6 h continuous light, α_{middle} increases with 8 h continuous light, and α_{fast} decreases for the first 8 h continuous light. At F_{max}, α_{slow} initially decreases with 4 h continuous light, then increases to a maximum and constant level after 10 h continuous light. The exact opposite dependence is noted for α_{middle}. The behavior of α_{fast} with continuous light is to increase slightly with 2 h continuous light, then decrease to a minimum and constant level after approx. 10 h continuous light. The F_0 level of fluorescence decreases with increasing amounts of continuous light, while the F_{max} level of fluorescence reaches a maximum after approx. 10 h continuous light.

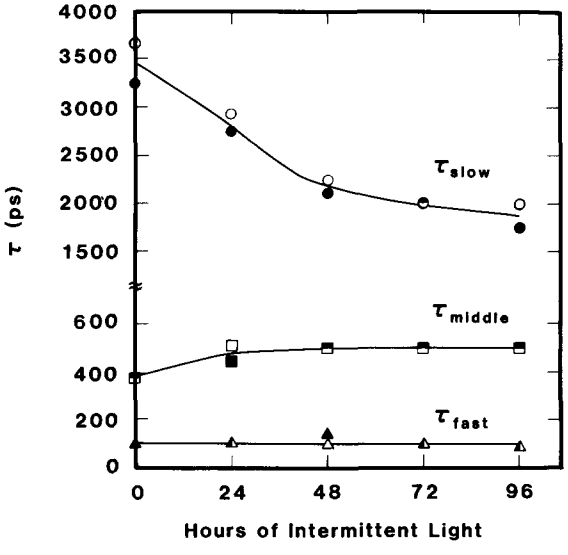


Fig. 9. Lifetimes of the components of the fluorescence decay in plastids from 8-day-old etiolated pea leaves subsequently exposed to variable numbers of light-dark cycles, in the presence of 5 mM Mg^{2+}; (○) lifetime of the slow phase, (□) lifetime of the middle phase, and (△) lifetime of the fast phase. Open symbols, lifetimes measured at F_0; closed symbols, lifetimes measured at F_{max}.

The changes in the ratio of Chl *a*/Chl *b* and in the ratio of fluorescence yield at F_{max} to F_0 are presented in Fig. 8 for chloroplasts in the presence and absence of Mg^{2+}. The significant effect of Mg^{2+} on the ratio F_{max}/F_0 occurs after 6 h continuous light, with F_{max}/F_0 constant in the absence of added Mg^{2+} but continuing to increase to a maximum at 36 h continuous light in the presence of Mg^{2+}. Changes in the Chl *a*/Chl *b* ratio are complete after 6 h continuous light.

Plastids from 8-Day-Old Etiolated Pea Leaves Exposed to Variable Numbers of Light-Dark Cycles

The effect of the fluorescence decay kinetics of variable numbers of light-dark cycles for protochloroplasts in the presence of Mg^{2+} is shown in Fig. 9. No variation with excitation intensity is observed for the resolved lifetimes. With increasing hours of intermittent light, τ_{slow} decreases from approx. 3400 to 2400 ps, τ_{middle} increases from 400 to 500 ps, and τ_{fast} is constant at 100 ps. Fig. 10 presents the effect of the number of hours of intermittent light on the amplitudes of the fluorescence decay components. At F_0 α_{slow} and α_{middle} show opposite dependence on intermittent light, and at F_{max} α_{slow} and α_{fast} change in antiparallel fashion. The F_{max}/F_0 ratio increases from 0.8 at 0 h intermittent light to 1.3 at 24 h intermittent light and to 1.7 after 96 h intermittent light (results not shown).

Chloroplasts from 8-Day-Old Etiolated Pea Leaves Exposed to Continuous Light

Changes in the fluorescence decay kinetics of etioplasts in Mg^{2+}-enriched buffer with variable hours of continuous light are shown in Fig. 11. For the first 7 h continuous light no difference is observed in the lifetimes measured at F_0 and F_{max}. During this interval τ_{slow} decreases from 3400 to 1600 ps, τ_{middle} is constant at 500 ps, and τ_{fast} is constant at 100 ps. From 7 to 48 h continuous light, τ_{slow} decreases to 900 ps for open reaction centers and to 1300 ps for closed reaction centers, τ_{middle} decreases to 400 ps for open reaction centers and remains at 500 ps for closed reaction centers. In Fig. 12 for fluorescence decays measured at F_0, α_{slow} and α_{fast} decrease and α_{middle} increases with lengthening exposure to continuous light. At F_{max},

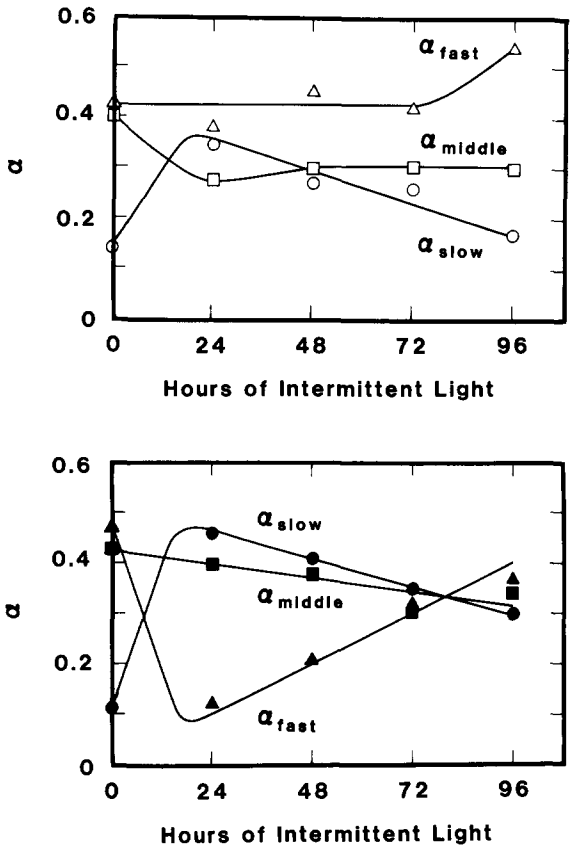
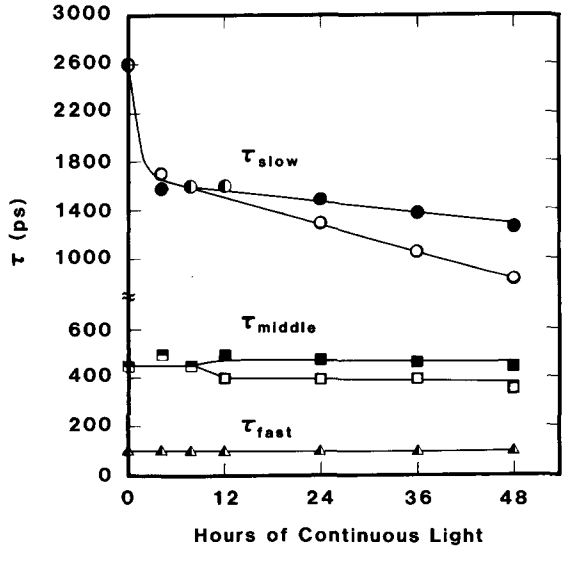
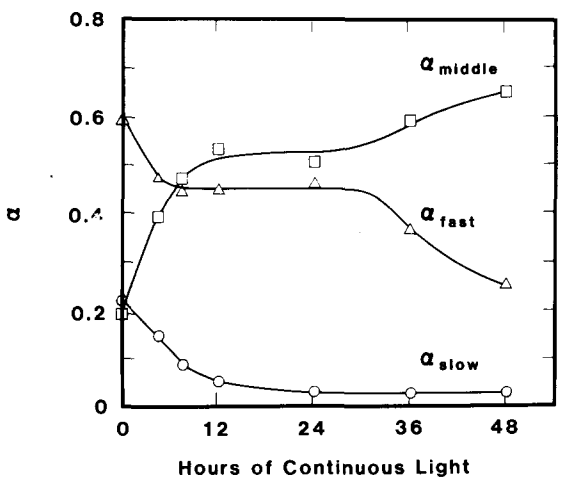


Fig. 10. Amplitudes of the components of the fluorescence decay in plastids of Fig. 9. Amplitudes are normalized such that $\Sigma\alpha = 1$ at each time point. The symbols are defined as in Fig. 9. Upper diagram, F_0; lower diagram, F_{max}.





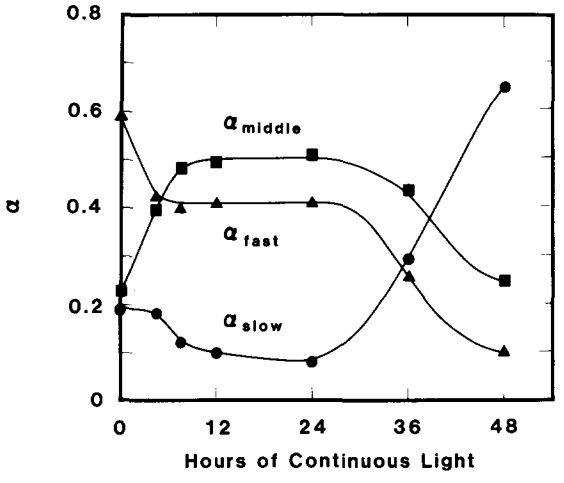


Fig. 12. Amplitudes of the components of the fluorescence decay in plastids of Fig. 11. Amplitudes are normalized such that $\Sigma\alpha = 1$ at each time point. The symbols are defined as in Fig. 11. Upper diagram, F_0; lower diagram, F_{max}.

α_{fast} decreases while α_{slow} and α_{middle} change simultaneously in opposite directions with increasing continuous light (α_{slow} first decreasing to a minimum after 24 h continuous light, then increasing to a maximum).

Fig. 11. Lifetimes of the components of the fluorescence decay in plastids from 8-day-old etiolated pea leaves subsequently exposed to continuous light, in the presence of 5 mM Mg^{2+}. The symbols are defined as follows: (○) lifetime of the slow phase, (□) lifetime of the middle phase, and (△) lifetime of the fast phase. Open symbols, lifetimes measured at F_0; closed symbols, lifetimes measured at F_{max}.

Discussion

Analysis of the fluorescence decay kinetics from chloroplasts developed under the various light regimes can be related to known structural and functional changes during the etioplast-to-chloroplast differentiation.

Etioplast → Protochloroplast Differentiation

The greening of etioplasts under conditions of intermittent light forms protochloroplasts which are agranal, devoid of Chl *b*, and deficient in the light-harvesting Chl *a/b* complex. The high Chl *a*/Chl *b* ratio in the primary thylakoids (Figs. 3 and 8) verifies the selective synthesis of Chl *a*. The Chl *a*-protein complex CPa, associated with PS II, as well as CP I associated with PS I, are predominant [16]. A high concentration of unorganized Chl *a* is present at the beginning of greening and decreases as greening continues [14,28]. Consequently, the contribution of unorganized chlorophyll to the F_0 level decreases with the time of greening. The slow decay component of 2200–3400 ps (Figs. 1,4,6,9 and 11) is assigned to Chl *a* uncoupled to either PS I or PS II reaction centers. The decreases in τ_{slow} and α_{slow} at F_0 with increased amounts of either intermittent or continuous light are manifestations of the incorporation of Chl *a* into small photosynthetic units [14]. The increase in the ratio F_{max}/F_0 after 24 h intermittent light and after 96 h intermittent light is a result of a decrease in the contribution of uncoupled Chl *a* to the F_0 level.

The lack of cation-induced changes in initial fluorescence levels (Figs. 3 and 8) or lifetimes (Figs. 1,4 and 9) reflects the absence of the light-harvesting Chl *a/b* complex and grana [10]. The absence of any effect of excitation intensity on the fluorescence kinetics of the slow decay component (Figs. 1,4 and 9) is attributed to the presence of isolated photosynthetic units of uniform size [14].

Protochloroplast → Chloroplast Differentiation

Transfer of the periodic-light leaves to continuous light induces a parallel synthesis of Chl *a* and Chl *b*, growth of the photosynthetic unit size, and a gradual differentiation of the primary thylakoids to grana and stroma lamellae [1]. The decrease in the Chl *a*/Chl *b* ratio (Figs. 3 and 8) with increasing continuous light is evidence of the synthesis of Chl *b*. The older (8-day) etiolated leaves reach the Chl *a*/Chl *b* ratio of mature chloroplasts faster than do the 6-day-old etiolated leaves. This result is in agreement with the finding of Akoyunoglou and Argyroudi-Akoyunoglou [7] that age is a critical factor for the biosynthetic mechanism of chlorophyll in plants and that Chl *b* is synthesized at an increased rate in an older plant. As evidenced by the minimum in α_{slow} and τ_{slow} at F_0 after 6 h continuous light, the incorporation of Chl *a* into reaction centers is essentially complete after 6 h continuous light for both 6- and 8-day-old plants. Furthermore, τ_{slow} (= 1400 ps) at both F_0 and F_{max} is independent of the age of the etiolated tissue and the ion level in the suspending medium.

The cation-induced Chl *a* fluorescence rise appears after 6 h continuous light in 8-day-old etiolated leaves exposed to 48 h intermittent light (Fig. 8). The increase in F_{max}/F_0 during continuous light parallels the appearance of grana stacks and the accumulation of light-harvesting complexes [16]. The differentiation in the kinetics of the slow decay component at F_0 and F_{max} in the presence of Mg^{2+} can also be related to grana formation. The decrease in τ_{slow} at F_0 and the corresponding increase in τ_{slow} at F_{max} are indicative of the development of PS II reaction centers capable of intercommunication via energy transfer [19,23,24].

The difference in τ_{middle} at F_0 and F_{max} is independent of cation concentration and present prior to grana formation. The time scale of the changes in τ_{middle} is believed to correspond to that of the differentiation of PS II units into α- and β-centers with the attainment of the photosynthetic unit sizes and the respective photochemical rate constants of a mature chloroplast [29]. The presence of grana is not a prerequisite for the development of the two heterogeneous populations of PS II units [29].

That the age of etiolated tissue is critical to development [8,9,14] is apparent in the lack of cation-induced grana formation in the 6-day-old etiolated pea leaves exposed to 24 light-dark cycles. The typical cation-induced fluorescence rise and differentiation in τ_{slow} at F_0 and F_{max} is not apparent even after 48 h continuous light. In young etiolated tissue the structure of the etioplast is still under development [7], preventing a rapid synthe-

sis of Chl *b* [14]. The parallel increase in light-harvesting Chl *a/b* complex and grana is delayed by the less-developed structure [14]. We found (results not shown) that prolonged exposure (120 h continuous light) eventually did induce the characteristic cation-induced fluorescence rise and differentiation in τ_{slow} at F_0 and F_{max}.

Conclusion

We have correlated structural and organizational changes associated with etioplast-to-chloroplast differentiation with the measured fluorescence lifetime and yield variations during plant growth. Changes in the kinetics of the slow phase of fluorescence decay are associated with the incorporation of Chl *a* into PS I and PS II reaction centers, the formation of grana, and the development of PS II reaction centers capable of intercommunication via energy transfer (α-centers). The middle phase kinetics reflect the formation of a heterogeneous population of PS II units (α- and β-centers) independent of the presence of grana. The presence of the middle decay phase even in the absence of Chl *b* indicates that this phase reflects a more complex origin than excitation lost from just the Chl *a/b* light-harvesting antenna [19]. The kinetics of the fast component of fluorescence decay, attributed to excitation lost from the Chl *a* atennae closely coupled with the PS I and PS II reaction centers [19], are independent of plant development, probably as a consequence of closely coupled Chl *a* to PS I and II reaction centers throughout plant growth. In light of these variations in the kinetic parameters of fluorescence decay with membrane development, this study prompts a future detailed analysis of the kinetic models for fluorescence emission.

Acknowledgements

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References

1 Argyroudi-Akoyunoglou, J.H. and Akoyunoglou, G (1970) Plant Physiol. 46, 247–249
2 Akoyunoglou, G., Argyroudi-Akoyunoglou, J.H., Michel-Wolwertz, M.R. and Sironval, C. (1966) Physiol. Plant. 19, 1101–1104
3 DeGreef, J., Butler, W.L. and Roth, T.F. (1971) Plant Physiol. 47, 457–464
4 Ogawa, T. and Shibata, K. (1973) Physiol. Plant. 29, 112–117
5 Henningsen, K.W. and Boardman, N.K. (1973) Plant Physiol. 51, 1117–1126.
6 Ohad, I. and Drews, G. (1982) in Photosynthesis: Development, Carbon Metabolism, and Plant Productivity, Vol. 2 (Govindjee, ed.), pp. 89–140, Academic Press, New York
7 Akoyunoglou, G and Argyroudi-Akoyunoglou, J.H. (1969) Physiol. Plant. 22, 288–295
8 Akoyunoglou, G. and Michelinaki-Maneta, M. (1975) in Proceedings of the 3rd International Congress on Photosynthesis (Avron, M., ed.), Vol. 3, pp. 1885–1896, Elsevier, Amsterdam.
9 Akoyunoglou, G. and Siegelman, H.W. (1968) Plant Physiol. 43, 66–68
10 Akoyunoglou, G. and Argyroudi-Akoyunoglou, J.H. (1978) in Photosynthetic Oxygen Evolution (Metzner, H., ed.), pp. 453–488, Academic Press, London
11 Argyroudi-Akoyunoglou, J.H. and Akoyunoglou, G. (1973) Photochem. Photobiol. 18, 219–228
12 Argyroudi-Akoyunoglou, J.H., Feleki, Z. and Akoyunoglou, G. (1971) Biochem. Biophys. Res. Commun. 45, 606–614
13 Argyroudi-Akoyunoglou, J.H., Feleki, Z. and Akoyunoglou, G. (1972) in Proceedings of the 2nd International Congress on Photosynthesis Research (Forti, G., Avron, M. and Melandri, A., eds.), Vol. 3, pp. 2417–2426, Dr. W. Junk, The Hague
14 Akoyunoglou, G. (1977) Arch. Biochem. Biophys. 183, 571–580
15 Tsakiris, S. and Akoyunoglou, G. (1981) in Photosynthesis, Vol. 5, Chloroplast Development (Akoyunoglou, G., ed.), pp. 513–522, Balaban International Science Services, Philadelphia
16 Akoyunoglou, G. (1981) in Photosynthesis, Vol. 5, Chloroplast Development (Akoyunoglou, G., ed), pp. 353–366, Balaban International Science Services, Philadelphia
17 Strasser, R.J. and Butler, W.L. (1976) Biochim. Biophys. Acta 449, 412–419
18 Akoyunoglou, G., Tsakiris, S. and Argyroudi-Akoyunoglou, J.H. (1981) in Photosynthesis, Vol. 5, Chloroplast Development (Akoyunoglou, G., ed.), pp. 523–532, Balaban International Science Services, Philadelphia
19 Haehnel, W., Nairn, J.A., Reisberg, P. and Sauer, K. (1982) Biochim. Biophys. Acta 680, 161–173
20 Gulotty, R.J., Fleming, G.R. and Alberte, R.S. (1982) Biochim. Biophys. Acta 682, 322–331

21 Magde, D., Berens, S.J. and Butler, W.L. (1982) Proc. SPIE – Int. Soc. Opt. Eng. 322, 80–86
22 Haehnel, W., Holzwarth, A.R. and Wendler, J. (1983) Photochem. Photobiol. 37, 435–443
23 Nairn, J.A., Haehnel, W., Reisberg, P. and Sauer, K. (1982) Biochim. Biophys. Acta 682, 420–429
24 Karukstis, K.K. and Sauer, K. (1983) Biochim. Biophys. Acta 722, 364–371
25 Hartig, P.R., Sauer, K., Lo, C.C. and Leskovar, B. (1976) Rev. Sci. Instrum. 47, 1122–1129
26 Leskovar, B. Lo, C.C., Hartig, P.R. and Sauer, K. (1976) Rev. Sci. Instrum. 47, 1113–1121
27 Turko, B.T., Nairn, J.A. and Sauer, K. (1983) Rev. Sci. Instrum. 54, 118–120
28 Thorne, S.W. and Boardman, N.K. (1971) Plant Physiol. 47, 252–261
29 Melis, A. and Akoyunoglou, G. (1977) Plant Physiol. 59, 1156–1160
30 Akoyunoglou, G. (1977) in Bioenergetics of Membranes (Packer, L., Papageorgiou, G.C. and Trebst, A., eds.), pp. 71–84, Elsevier, Amsterdam