

dominate in the ensuing oxidation. Taking this into consideration it does appear that the membrane imposes a limitation on the rates of oxidation of *cis*-aconitate and isocitrate by rat liver mitochondria under these conditions, even at saturating L-malate concentrations. This limitation, it must be emphasised would probably have little bearing on the situation *in vivo* where mitochondrial NADP⁺ is substantially reduced and the NAD-isocitrate dehydrogenase would predominate in the oxidation of isocitrate derived from citrate.

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The active chlorophyll α_{II} in suspensions of lyophilized and Tris-washed chloroplasts

In photosynthesis, light absorbed in two different pigment systems is used for two different light reactions^{1,2}. The reaction center for System I (the P700) was discovered by KOK³. Suggestions for the existence of a trap in System II came first from EMERSON AND RABINOWITCH⁴. Further hints came from fluorescence studies at low temperatures⁵⁻⁷. Recently DÖRING *et al.*⁸ have discovered absorbance changes due to chlorophyll *a* (Chl *a*) in System II with peaks at 435 nm and 682-690 nm⁹ and a half-life a 100 times shorter than that of P700.

Abbreviations: Chl, chlorophyll; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

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We have investigated suspensions of (a) lyophilized chloroplasts, (b) the same after extraction with 'dry' heptane or 'wet' heptane (extraction was performed after chloroplasts had been wetted with a few drops of water¹⁰) and (c) Tris-washed chloroplasts¹¹ to see if Chl a_{II} absorbance changes were present in them.

The techniques for measuring absorbance changes were described by DÖRING *et al.*^{8,9}. Repetitive flashes (8192) of 380–550-nm light of 20- μ sec duration were used for excitation. The samples contained 50 μ g of Chl per ml. Lyophilized chloroplasts were suspended in 0.1 M Tris-maleate buffer, pH 6.6. Tris-washed chloroplasts were suspended in 50 mM Tris buffer, pH 7.2.

Buffered suspensions of lyophilized chloroplasts from maize containing ferricyanide (0.5 mM) showed an absorbance change of $64 \cdot 10^{-6}$ at 690 nm. These changes, which decayed rapidly to the 'dark' value, were plotted as a function of time on 2-cycle semi-log paper and the slow changes were separated from the fast changes (Fig. 1). At 690 nm, the half-times of decay ($t_{1/2}$) were 6.4 msec for the slow change and 0.25 msec for the fast change. In unextracted chloroplasts from both spinach and maize, the faster decay times ranged from 0.08 to 0.29 msec and the slower from 3.0 to 6.5 msec. The latter are only 20–40 times slower than the Chl a_{II} changes, and they appeared to be related to P700 (Chl a_I). It is not clear whether lyophilization has caused an increase in the decay rate of P700 or indeed, whether there is an additional fast P700 change. G. DÖRING (unpublished) has shown that the fast component of P700 disappears after irradiation with far-red light (720 nm).

The fast component ($t_{1/2} = 0.08$ –0.29 msec) is, in all likelihood, due to Chl a_{II} because of the similarity of the rate of decay with those observed earlier by DÖRING *et al.*^{8,9}. This assignment was confirmed by the difference spectra of this component. They were similar to those published by DÖRING *et al.*¹².

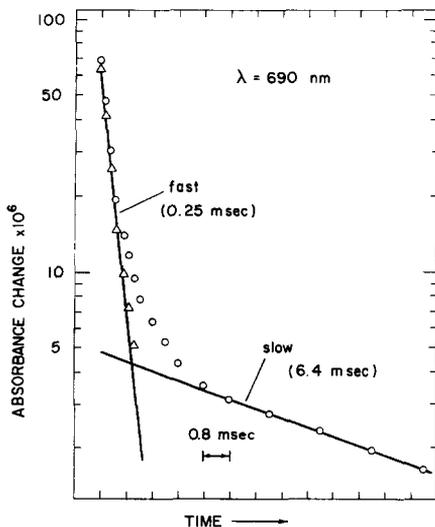


Fig. 1. Absorbance change (following a flash) at 690 nm plotted (on a log scale) as a function of time. Sample: suspension of lyophilized chloroplasts from maize; 50 μ g Chl per ml; 0.1 M Tris-maleate buffer, pH 6.6; electron acceptor: ferricyanide, 0.5 mM; 8192 light flashes; duration of each flash, 20 μ sec; wavelength of excitation, broad-band blue light (380–550 nm). The 'fast' change (Δ , $t_{1/2}$ approx. 0.25 msec) was obtained by subtracting the slow change ($t_{1/2}$ approx 6.4 msec) from the total change (\circ). The fast change belongs to the Chl a_{II} .

The observed Chl a_{II} changes appear to be related to the electron transport in photosynthesis because 50 μM 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), the well-known inhibitor of O_2 evolution, abolished these changes. Also, when lyophilized chloroplasts were extracted with dry heptane and the chloroplasts then suspended in buffer, absorbance changes due to Chl a_{II} were absent. Heptane is known to extract quinones¹³; a loss of electron transport occurs and the variable fluorescence disappears¹⁰. In these preparations, the O_2 spike is also absent and the fluorescence level is low, close to that of the 'O' level¹⁰. We could not restore these changes by adding a Hill oxidant like ferricyanide (50 μM) nor by adding sodium ascorbate (100 μM) plus phenylenediamine (10 μM) (see ref. 11).

When lyophilized chloroplasts were extracted with heptane after they had been wetted with a few drops of water, Chl a_{II} changes were distinctly present; the slow absorbance changes, however, had almost been eliminated. In these preparations, the O_2 spike is absent and the fluorescence level is high, close to the 'P' level¹⁰. Chl a_{II} changes ($30 \cdot 10^{-6}$) were present even when no Hill oxidant was added, but the addition of ferricyanide doubled the absorbance change ($58 \cdot 10^{-6}$). Contrary to the results with DCMU, this suggested that the net electron transfer was not necessary for observing changes in Chl a_{II} ; this agrees with results obtained by DÖRING *et al.*¹². The two components in the heptane-extracted maize chloroplasts had half-times of 0.1 and 0.5 msec in the presence of 50 μM ferricyanide and 0.16 and 0.4 msec in the absence of ferricyanide. Thus, the slow component was faster than that observed in the unextracted chloroplasts as noted above.

Tris-washed spinach chloroplasts¹¹ also showed Chl a_{II} changes, even in the absence of a Hill oxidant. It is known that in Tris-washed chloroplasts there is no O_2 evolution and no electron transport¹¹ unless H donors are added. Absorbance changes at 690 nm showed two components ($t_{1/2}$ of fast component = 0.1–0.2 msec; $t_{1/2}$ of slow component = 1–2 msec) under widely different treatments: (a) 0.1 mM benzyl viologen plus 1 mM NH_4Cl ; (b) 1 mM sodium ascorbate; (c) 1 mM sodium ascorbate plus 10 μM phenylenediamine; (d) 1 mM sodium ascorbate plus 30 μM phenylenediamine, and 0.1 mM benzyl viologen; and (e) 50 μM phenazine methyl sulfate.

In summary, our experiments confirm the existence of fast absorbance changes ($t_{1/2} = 0.1\text{--}0.2$ msec) centered around 690 nm (Chl a_{II}) in suspensions of lyophilized maize and spinach chloroplasts, in lyophilized maize chloroplasts after extraction with heptane (in the presence of water) and in Tris-washed spinach chloroplasts. However, treatment with DCMU and extraction with dry heptane abolished Chl a_{II} changes.

Our finding that Chl a_{II} is fully active in Tris-washed and 'wet' heptane-extracted chloroplasts supports the suggestion of DÖRING *et al.*¹² that the net electron transport is not necessary for observing changes in Chl a_{II} . On the other hand, in untreated chloroplasts the Chl a_{II} absorbance changes show the same dependence on the intensity of the exciting flash⁸ and on the concentration of DCMU¹² as the electron transport. These results suggest that there is a causal relation between the Chl a_{II} activity and the electron transport. DÖRING¹⁴ found that it is possible to block the electron transport without deactivation of Chl a_{II} , but it is not possible to deactivate the Chl a_{II} without blocking the electron transport. We consider here two alternative interpretations: (1) The Chl a_{II} acts as a 'sensitizer' not engaged in a redox reaction in the electron transfers (in contrast to Chl a_I)^{12, 14}. In this case, the energy necessary for the electron transfer from water to 'Q' (the electron acceptor of System II) comes from the

excited state of Chl a_{II} . (An unknown energy acceptor may transform this excitation energy into electronic energy.) (2) When the linear electron flow from water to NADP⁺ is blocked in Tris-washed and 'wet' heptane-extracted chloroplasts, there operates a 'mini cycle' around Chl a_{II} which does not include 'Q': Chl $a_{II} + h\nu \rightarrow$ Chl a_{II}^* ; Chl $a_{II}^* \rightarrow$ Chl $a_{II} +$ energy. The second possibility does not seem to be very probable because in the presence of artificial electron donor systems there is a linear electron flow from the donor to NADP⁺ as shown by YAMASHITA AND BUTLER¹⁵. We must, however, emphasize that the real mechanism of the reaction center II is unknown, but the assumptions made above in (1) are the most likely ones; it is clear from the available data that the Chl a_{II} is not acting directly in a redox reaction.

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