FLASH-INDUCED REDOX CHANGES OF P700 AND PLASTOCYANIN IN CHLOROPLASTS SUSPENDED IN FLUID MEDIA AT SUB-ZERO TEMPERATURES

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1. Introduction

Following a short flash of light, the reaction centre chlorophyll of photosystem I, P700, becomes oxidised. Measurement of the dark reduction of this oxidised species generated by a single turnover give information about the mechanism of electron donation. Studies of this type have shown that the kinetics of $P700^+$ reduction do not follow a single exponential [1–5]. As a result of this both plastocyanin and cytochrome *f* have been implicated as direct donors to $P700^+$ [1,2,5,6] although a claim has also been made for another more primary donor [4]. Part of the reason for the lack of clear understanding of electron donation on the oxidising side of photosystem I is due to the speed of the decay of the $P700^+$ signal and complications associated with detecting it spectrophotometrically. One possible way to simplify the analysis of the signal is to measure its kinetics at low temperatures where the electron-transfer rates are likely to be slowed down. It has convincingly been shown that all properties of photosynthetic electron transport and associated proton translocation are preserved when chloroplasts are suspended in suitable aqueous/organic media at subzero temperatures [7–12].

Here, we have made use of this low temperature approach to investigate the flash-induced redox changes of P700 and plastocyanin. It has been found that the electron-transfer reaction involving these components are qualitatively similar to those observed at room temperature but 100-fold slower. This has allowed us to carry out a detailed kinetic analysis of the processes involved. We conclude that the simplest interpretation of the results is that plastocyanin acts as the primary donor to P700 in a bimolecular reaction.

2. Materials and methods

Intact chloroplasts were prepared from peas as in [13]. The chloroplasts were osmotically shocked in distilled water followed by addition of an equal volume of double strength wash medium to obtain a stock suspension containing 2.5–3.0 mg chl . ml^{-1} and 0.33 M sorbitol, with the pH adjusted to 7.6 with Tris base. The broken chloroplasts were suspended in a medium consisting of a 1:1 (v/v) mixture of ethanediol:water and containing 0.33 M sorbitol, 100 μM methyl viologen and 5 mM potassium phosphate pH* 8.0 (pH* = effective pH [14]). Further additions were, unless otherwise stated, 5 mM sodium ascorbate and 50 μM diaminodurene in order to get the high potential electron carriers, cytochrome *f*, plastocyanin and P700 reduced in the dark period between flashes.

Absorbance changes were measured with a single-beam flash spectrophotometer (Applied Photophysics, London) using a 10 × 10 mm cuvette. Saturating light flashes were provided with a xenon flash tube (10 μs width at half height). The flashes were filtered through either a 4 mm Corning 4-96 filter for measurements of P700 or a 2 mm Schott RG 665 filter when measuring plastocyanin. The photomultiplier was protected either by a 2 mm Schott RG 695 filter for measurements of P700 or a 4 mm Corning 4-96

Abbreviations: P700, reaction centre chlorophyll of photosystem I; chl, chlorophyll

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filter for measurements of plastocyanin. The signal from the photomultiplier was passed to a Nuclear Measurements signal averager (model 546 C). The time constant of the apparatus was 0.3 ms unless otherwise indicated. The apparatus has been described in detail in [15].

Temperature control was obtained using a Grant model LE 8 low temperature bath circulating odourless kerosene to the sample holder. The temperature was measured with a copper–constantan thermocouple inserted into the sample.

P700 was measured at 703 minus 720 nm by accumulating *n* sweeps at the measuring wavelength and subtracting the same number of sweeps at the reference wavelength. Plastocyanin was measured at 584 nm [6]. Chloroplhyll was measured according to [16].

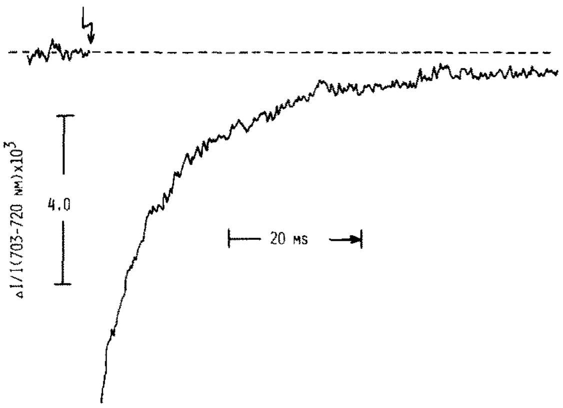

Fig.1. Kinetics of reduction of P700 following a flash in chloroplasts suspended in a 1:1 (v/v) mixture of ethanediol:water at −17°C, 16 signals were averaged with a flash frequency of 0.25 Hz. Chlorophyll was 40 μM.

3. Results and discussion

Fig.1 shows the absorbance changes at 703 minus 720 nm due to P700 following a flash, measured at −17°C. The half time of reduction is 6 ms, compared to values of 20–200 μs reported at room temperature [1–6]. This slowing down of the reaction eliminates complications of fluorescence artefacts or double turnovers during the tail of the flash and facilitates an interpretation of the kinetics. The time course of the reduction could be analysed as the sum of two exponentials as shown in fig.2A, which could simply be interpreted as evidence for the involvement of two components in the electron donation process. How-

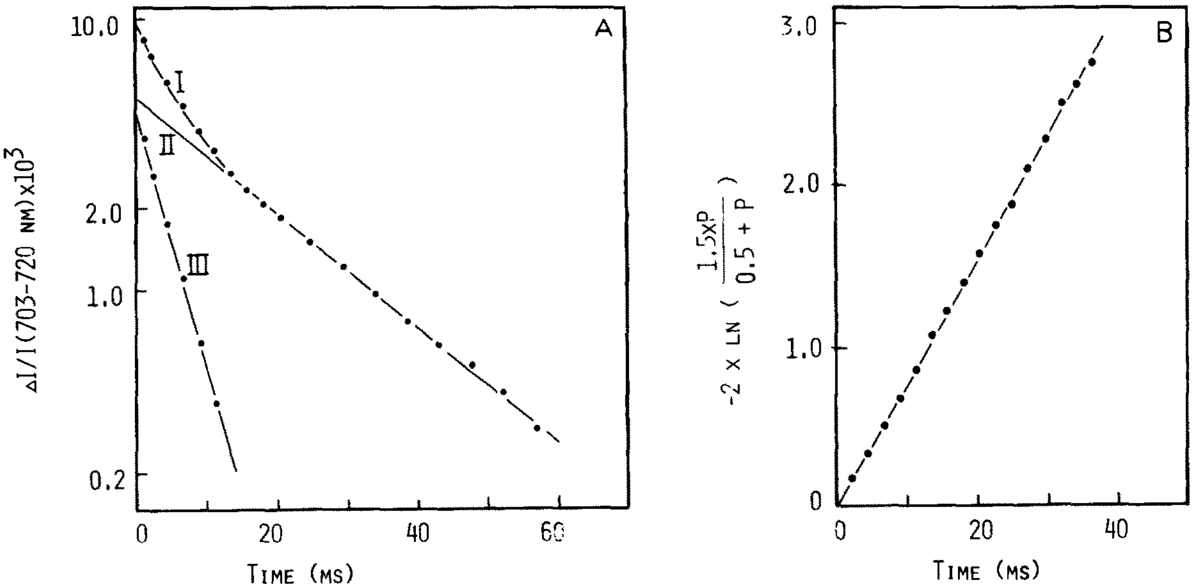

Fig.2. (A) Semilogarithmic plot of the reduction of P700$^+$ from fig.1: I, original data; II, estimated contribution of the slow phase; III, semilogarithmic plot of I minus II. (B) The same data analysed as a bimolecular reaction between P700$^+$ and its electron donor by using eq. (2) and assuming $\alpha = 1.5$. For further details see text.

ever we also attempted to interpret these data in terms of a bimolecular reaction between P700$^+$ and its primary donor as suggested in [3]:

$$D + P700^+ \xrightarrow{k} D^+ + P700$$

Provided that P700 and D are not present in equal amounts the solution of the rate equation of this type of reaction is:

$$\ln \frac{[P700^+]\ [D]_{tot}}{[D]\ [P700]_{tot}} = (|P700|_{tot} - |D|_{tot})\, k\, t \tag{1}$$

where $[P700]_{tot}$ and $[D]_{tot}$ represent the concentrations of oxidised P700 and reduced D initially present and k is the rate constant. This equation can be transformed into:

$$\ln \left(\frac{\alpha P}{(\alpha - 1) + P}\right) = (1 - \alpha)\ [P700]_{tot}\, k\, t \tag{2}$$

where $\alpha = [D]_{tot}/[P700]_{tot}$ and $P = [P700^+]/[P700]_{tot}$. Fig.2B shows a plot of $\ln(\alpha P/((\alpha - 1) + P))/(1 - \alpha)$ against t, assuming $\alpha = 1.5$. The plot is a straight line as predicted from eq. (2).

To discriminate between the possibility of two separate components or a bimolecular process the temperature dependence of the reaction was investigated. When analysed as a sum of exponentials, both apparent phases of the decay showed linear Arrhenius plot with an activation energy of 56 kJ . mol^{-1} (fig.3). This seems to suggest that a single reaction, rather than two separate reactions, is involved and supports the idea that the time course represents a second-order reaction. When analysed as a second order reaction as in fig.2B the slope of the lines also showed a linear Arrhenius plot with an activation energy of 56 kJ . mol^{-1}. The activation energy found here is close to that reported for the oxidation of exogenous plastocyanin by digitonin treated chloro

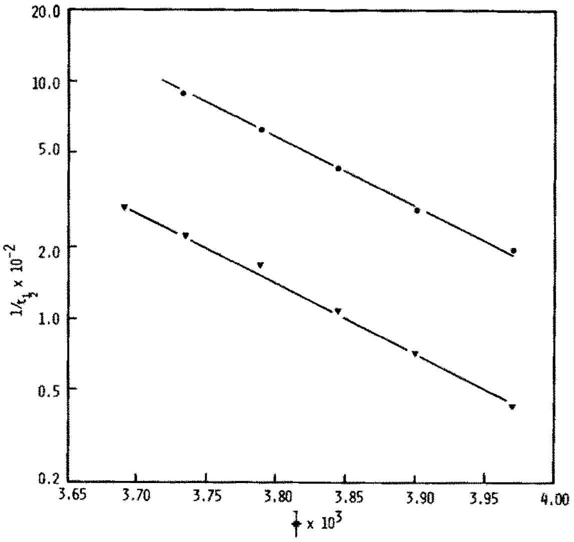

Fig.3. Plot (in a logarithmic scale) of the reciprocal of the half times of the fast and slow phases of P700$^+$ reduction, obtained from semilogarithmic plots as in fig.2A, against the reciprocal of the absolute temperature (Arrhenius plot). Other conditions as in fig.1.

with a rate constant of 6.6 . 10^4 M^{-1} . s^{-1}, comparable to that of 8.7 . 10^4 M^{-1} . s^{-1} estimated from the effect of this mediator on the rate of reduction of P700 following continuous illumination of chloroplasts inhibited with DCMU (L. F. O., unpublished). In contrast to this we found that the rate of reduction of P700 following the flash was not affected by diaminodurene over the concentration range used.

When correcting for the reduction of plastocyanin by diaminodurene the magnitude of the flash-induced change at 584 nm in fig.4A corresponded to the oxidation of 1 molecule of plastocyanin per 680 chl molecules if an extinction coefficient (ϵ) of 4.6 mM^{-1} . cm^{-1} at 584 nm is used for plastocyanin [18]. This can be compared with 1 molecule of P700 per 610 chl molecules photooxidised during the flash estimated from fig.1 using

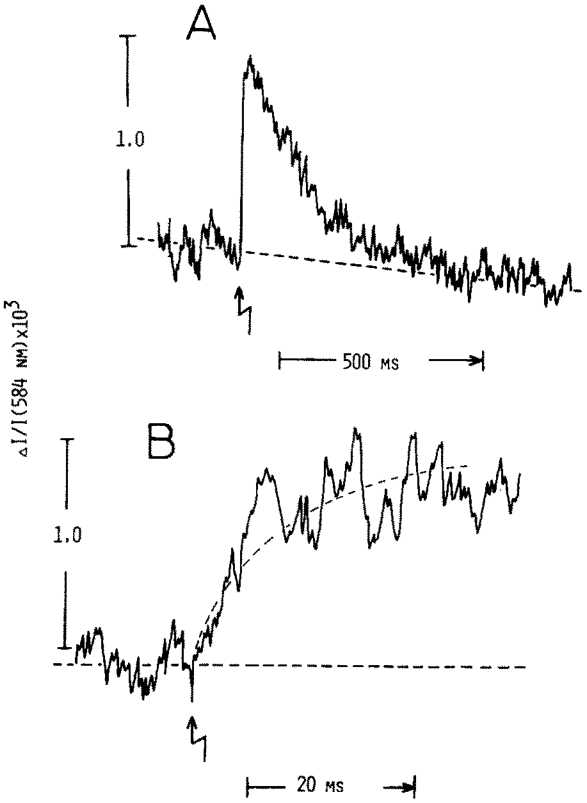

Fig.4. Flash-induced absorption changes due to plastocyanin at −18°C. (A) Dark reduction by diaminodurene (100 μM) following photooxidation: 32 signals were averaged with a flash frequency of 0.25 Hz and an instrument time constant of 10 ms. Gramicidin was present at 10 μM. Chlorophyll was 70 μM. (B) Photooxidation of plastocyanin following a flash. Conditions as in A except that the instrument time constant was changed to 0.3 ms and 128 signals were averaged. The dashed curve is the expected time course of plastocyanin oxidation, calculated using eq. (2) and the data from fig.2B, if plastocyanin is the primary electron donor to P700$^+$.

by P700. This time course is represented by the dashed curve in fig.4B.

These results can be most simply accounted for by the occurrence of a bimolecular reaction between P700$^+$ and plastocyanin, with the latter being present at ~1–2-times the concentration of the reaction centre chlorophyll. This estimate of the amount of plastocyanin per electron-transport chain is in agreement with the estimates in [6,20]. Our results do not indicate the existence of an extra electron-transfer component between plastocyanin and P700 [4].

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References

[1] Haehnel, W., Döring, G. and Witt, H. T. (1971) Z. Naturforsch. 26b, 1171–1174.
[2] Haehnel, W. and Witt, H. T. (1972) in: Proc. 2nd Int. Congr. Photosynth. (Forti, G. et al. eds) pp. 469–476, Junk, The Hague.
[3] Delosme, R., Zickler, A. and Joliot, P. (1978) Biochim. Biophys. Acta 504, 165–174.
[4] Bouges-Bocquet, B. and Delosme, R. (1978) FEBS Lett. 94, 100–104.
[5] Haehnel, W., Hesse, V. and Pröpper, A. (1980) FEBS Lett. 111, 79–82.
[6] Haehnel, W. (1977) Biochim. Biophys. Acta 459, 418–441.
[7] Cox, R. P. (1975) Biochim. Biophys. Acta 387, 588–598.
[8] Cox, R. P. (1975) Eur. J. Biochem. 55, 625–631.
[9] Cox, R. P. (1978) Biochem. Soc. Trans. 6, 689–697.
[10] Olsen, L. F. and Cox, R. P. (1979) Eur. J. Biochem. 95, 427–432.
[11] Olsen, L. F. and Cox, R. P. (1979) FEBS Lett. 103, 250–252.
[12] Olsen, L. F. and Cox, R. P. (1979) Eur. J. Biochem. 102, 139–145.
[13] Nakatani, H. Y. and Barber, J. (1977) Biochim. Biophys. Acta 461, 510–512.
[14] Douzou, P. (1977) Cryobiochemistry: An Introduction, Academic Press, London.
[15] Olsen, L. F., Telfer, A. and Barber, J. (1980) FEBS Lett. 118, 11–17.
[16] Arnon, D. I. (1949) Plant Physiol. 24, 1–15.
[17] Wood, P. M. and Bendall, D. S. (1975) Biochim. Biophys. Acta 387, 115–128.
[18] Katoh, S., Shiratori, I. and Takamiya, A. (1962) J. Biochem. 51, 32–40.
[19] Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171.
[20] Haehnel, W. (1973) Biochim. Biophys. Acta 305, 618–631.