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RELATIONSHIP BETWEEN P_{700} TURNOVER (m SECOND) AND NADP REDUCTION AS A FUNCTION OF FERREDOXIN CONCENTRATION IN ISOLATED BROKEN CHLOROPLASTS

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Summary

Steady-state electron flux through P_{700} (t 1/2 20 msec) and concomitant rate of NADP reduction have been measured under weak actinic illumination as a function of concentration of ferredoxin added to broken chloroplasts isolated from peas. At suboptimal concentrations of ferredoxin this P_{700} is not sufficient to account for the NADP reduction. At high concentrations ferredoxin inhibits the rate of NADP reduction without affecting the P_{700} flux under short wavelength illumination. Under far red illumination P_{700} flux is also inhibited by ferredoxin at high concentrations. Addition of 5 mM Mg^{++} increases the rate of NADP reduction at all concentrations of ferredoxin under both kinds of illumination, while P_{700} flux is inhibited under short wavelength illumination and remains unchanged under far red illumination. The results indicate that the observed (20 msec) P_{700} is not involved in NADP reduction.

Introduction

Kinetic studies on P_{700} have revealed three major reduction components (1, 2 for review). The usually observed component has t 1/2 around 20 msec, and for the others around 200 μsec and 20 μsec. Although these components have been attributed in terms of the series formulation (Z-scheme) of electron transport to the reduction of P_{700} by electrons from plastocyanin and cytochrome f (μsec components) and from plastoquinone (msec components), their functional relationship with

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terminal electron transport (NADP reduction, for instance), cyclic electron transport, photophosphorylation, oxidation of water and artificial electron donors, etc. has not yet been established. In fact, the basic question, whether the components arise from parallel photosystems, is also open. No conclusion is possible at this moment because very limited data are available on the role of the individual components of P_{700} in various functions stated above. In this communication a P_{700} component ($\sim$ 20 msec) has been described that is sensitive to the amount of ferredoxin added to isolated broken chloroplasts and is not parallel to NADP reduction.

Materials and Methods

Class II chloroplasts were isolated as described earlier (3). P700 flux and its relaxation time were measured using the steady-state relaxation spectrophotometer (4, 5). The extinction coefficient for P_{700} was taken as 65 $mM^{-1}cm^{-1}$ (6). The rate of NADP reduction concomitant with P_{700} flux was measured by the relaxation spectrophotometer by continuously recording the light-dependent change in transmission at 340nm. All measurements were made under weak illumination of 3×10^3 ergs cm^{-2} sec^{-1}. Short wavelength illumination was provided by filtering the actinic light through a broad band interference filter (Baird Atomic) transmitting between 530 and 640nm For far red illumination a narrow band interference filter with transmission around 696 nm was used. NADP and spinach ferredoxin were purchased from Sigma Chemicals Inc., USA. Crude ferredoxin (PPNR) was prepared from spinach chloroplasts following Selman (7).

Results

Figure 1 shows that the rate of NADP reduction (V_{340}) reached an optimum value at around 15 μl of ferredoxin and above this concentration V_{340} decreased. At 75 μl V_{340} decreased by 40%. The electron flux through P_{700} (V_{700}) followed a different function of ferredoxin concentration: at low concentrations it increased at a lower rate than that

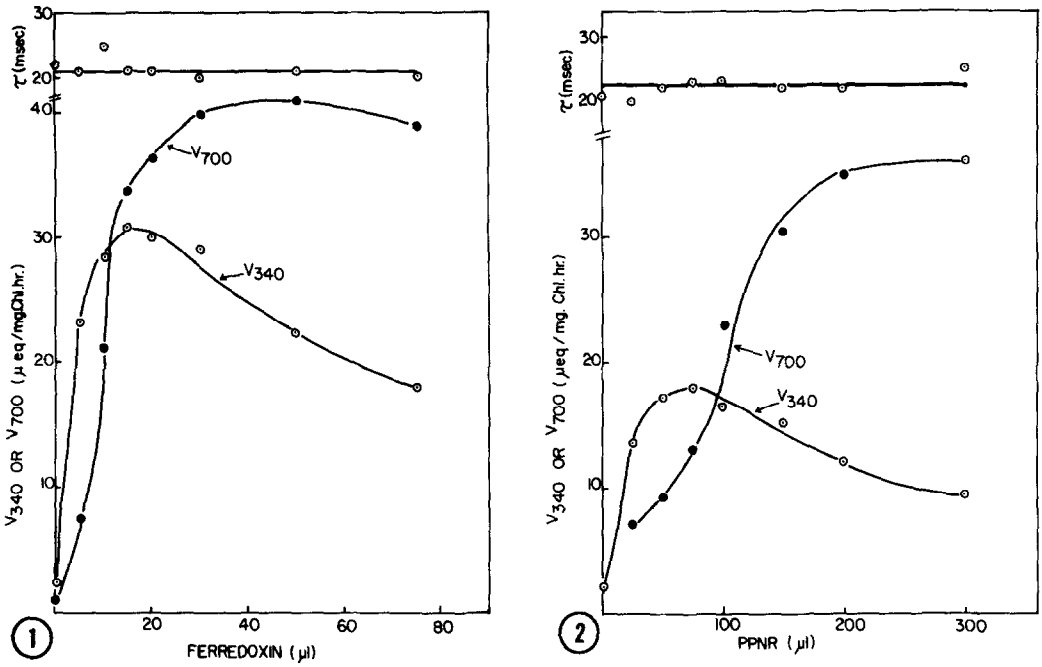

Fig.1 V_{340} and V_{700} as a function of ferredoxin concentration under weak short wavelength illumination. The reaction mixture (2.0 ml) contained 15 mM Tricine-NaOH (pH 7.5), 50 mM sucrose, 20 mM NaCl, 0.25 NADP, 2.5 mM NH_4Cl, chloroplasts of 40 μg chlorophyll equivalent and varied amounts of ferredoxin (stock concentration 2.8 mg/ml). τ represents relaxation time of P_{700}

Fig.2 V_{340} and V_{700} as a function of crude ferredoxin (PPNR) under weak short wavelength illumination. Reaction conditions same as in Fig.1 except PPNR replaced ferredoxin

of V_{340}, attained the maximum value at a concentration about three fold higher than that required for V_{340}, and remained practically unchanged at 75 μl. In the low concentration range V_{340} was considerably higher than V_{700}, and in the high concentration range V_{700} was considerably higher than V_{340}. The relaxation time (τ) of P_{700} remained practically unchanged (20-25 msec). The commercial ferredoxin used contained high salt in the solution (150 mM Tris). To avoid secondary effects, if any, of Tris, crude ferredoxin (PPNR)

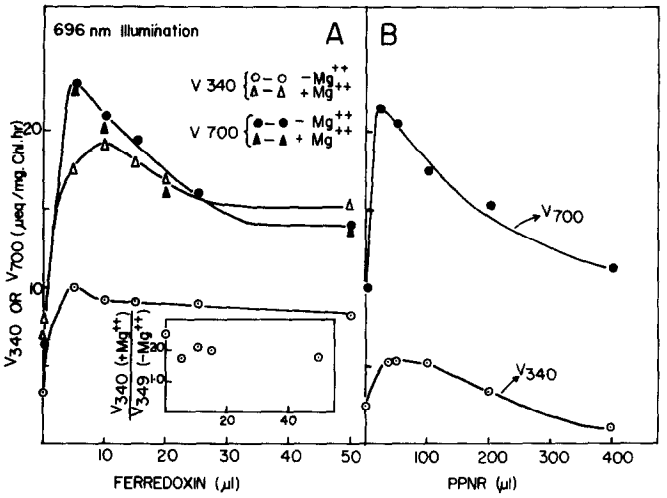

Fig.3 V_{340} and V_{700} as a function of ferredoxin (A) and PPNR (B) under weak 696 nm illumination. Reaction conditions same as in Fig.1. Mg^{++} was added as 5 mM $MgCl_2$

was prepared from spinach in the laboratory. With PPNR the results obtained were qualitatively similar (Fig.2). The amount of PPNR required for optimum V_{340} was 75 μl; at 300 μl the inhibition was 50%. V_{700} reached the maximum value at 200 μl, and remained practically unchanged at 300 μl. The relaxation time was also independent of PPNR concentration.

Figure 3 illustrates the dependency of V_{340} and P_{700} on ferredoxin and PPNR concentration under far red illumination. As compared to the observations with short wavelength illumination, the following major differences are to be noted: 1. V_{700} was inhibited at the higher concentrations of ferredoxin and PPNR; 2. the concentration of ferredoxin or PPNR required for optimum V_{340} or V_{700} was significantly less, the effect being more predominant with V_{700}; 3. V_{700} was always significantly higher than V_{340}. Other differences with respect to effects of Mg^{++} are described below:

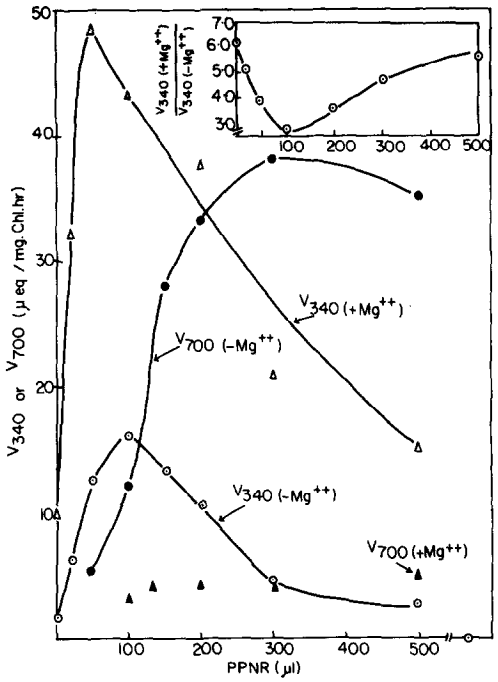

Fig.4 Effects of Mg^{++} on V_{340} and V_{700} as a function of PPNR under weak short wavelength illumination. Reaction conditions same as in Fig.1. $MgCl_2$ was added at 5 mM

Photosynthetic electron transport is known to be sensitive to the absence of cations (8 for review). Figure 4 and Figure 3A describe some interesting effects of Mg^{++} on V_{340} and V_{700} as a function of ferredoxin and PPNR concentrations under short wavelength and far red illumination respectively. Under short wavelength illumination (Fig.4) V_{340} was stimulated as much as six-fold at the limiting concentrations of PPNR; at optimum concentration the stimulation reached a minimum of about two-fold, which increased to five-fold at high inhibitory concentration. V_{700}, on the other hand, decreased four to six-fold by Mg^{++} in the range of PPNR used. Rurainski et al., (9) and Bose (10) have also observed a two-fold increase of V_{340} and about five-fold decrease of V_{700}

by Mg^{++} under short wavelength illumination. Under far red illumination (Fig.3A), the magnitude of stimulation of V_{340} by Mg^{++} was around two-fold and was much less sensitive to ferredoxin concentration. More interestingly, V_{700} under far red illumination was practically insensitive to Mg^{++}.

Discussion

Rurainski et al., (9) and Rurainski and Hoch (11) have observed that NADP reduction was not parallel to the observed (20 msec) P_{700} turnover as a function of cation concentration in isolated chloroplasts. In fact, they found, upon addition of cations, a stoichiometry between the increase in the yidld of NADP reduction and the decrease in the yield of P_{700} turnover. They proposed that the observed (20 msec) P_{700} was in competition with the reduction of NADP and that the divalent cations switched quanta from one photosystem to the other. Following Haehnel's observation (1) that P_{700} is reduced after a strong actinic flash with two fast reduction components ($\sim 20\,\mu$sec and $\sim 200\,\mu$sec) along with the usually observed (~ 20 msec) component, it has been further proposed (2) that the μsec components, not the msec one, are involved in NADP reduction.

The above observations indicate that the role of P_{700} has become an open question. Because of lack of sufficient data no conclusion can be reached at this moment. This communication provides several informative data regarding the behaviour of 20 msec P_{700} as a function of ferredoxin concentration under varied condition of illumination and cation concentration. The observations that at limiting concentrations of ferre-

doxin V_{700} was not sufficient to account for V_{340} and at high concentrations of ferredoxin V_{700} was not parallel to V_{340} (Fig.1 and 2) give strong support to the msec P_{700} being not involved in NADP reduction. The proposal that these two photosystems (NADP reduction and 20 msec P_{700}) are different is supported by the observation that the concentrations required for optimal V_{700} and V_{340} were significantly different.

The observations with far red illumination suggest further that the sensitization characteristics of the proposed photosystems are not identical, because 1. the concentrations of ferredoxin required for optimum yield of either V_{700} or V_{340} were different in two kinds of illumination, 2. V_{700} as a function of ferredoxin concentration behaved differently in two kinds of illumination, 3. Mg^{++} stimulation obeyed different functions of ferredoxin concentration (insets of Fig.4 and Fig.3A) and (4) Mg^{++} effects on V_{700} were quite different in two kinds of illumination.

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