THE EFFECT OF OXIDATION-REDUCTION POTENTIAL ON THE FLUORESCENCE YIELD OF SPINACH CHLOROPLASTS AT 77°K

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1. Introduction

Changes of fluorescence yield in green plants have been widely assumed to be controlled directly by changes in the oxidation state of the primary electron acceptor of Photosystem II. The hypothesis of Duysens and Sweers [1] that the primary acceptor, designated Q, quenches chlorophyll fluorescence in the oxidized but not in the reduced state has been used successfully to correlate fluorescence yield changes with electron-transfer reactions. Recently, it has become apparent that the chlorophyll fluorescence yield is not solely determined by the oxidation state of the primary electron acceptor of Photosystem II. Mauzerall has demonstrated that at least six fluorescence states must be postulated to explain the fluorescence yield of *Chlorella* cells over the time interval from 10^{-8} to 10^{-2} sec after a light flash at room temperature [2]. Okayama and Butler have shown that in spinach chloroplasts the maximum light-induced fluorescence yield at liquid-nitrogen temperature depends on the oxidation state of cytochrome b_{559} prior to illumination [3]. Butler and co-workers have also shown [4,5] that at liquid-nitrogen temperature the kinetics of the light-induced fluorescence increase follow those of cytochrome b_{559} photooxidation rather than the kinetics of the photoreduction of the primary acceptor of Photosystem II. On the basis of these observations it has been concluded [3–6] that the fluorescence yield of Photosystem II reflects not only the oxidation state of the primary acceptor but also the oxidation state of P_{680} (the reaction-center chlorophyll of Photosystem II) and other components on the oxidizing side of Photosystem II. In the light of these observations, it became of interest to determine if a newly discovered component involved in the low-temperature reactions of Photosystem II [7,8] might also affect the fluorescence yield of Photosystem II. This component, of unknown chemical nature, was shown to have a midpoint oxidation-reduction potential of +475 mV by titrations of light-induced free-radical signals at low temperature [8].

2. Methods and materials

Washed, broken spinach chloroplasts [9] were suspended in 50 mM potassium phosphate buffer (pH 7.6) + 20 mM NaCl. The initial oxidation-reduction potential was adjusted to approximately +600 mV by addition of potassium ferricyanide to a final concentration of 5 mM. The oxidation-reduction potential, monitored with a Radiometer PK-149 combined platinum–calomel electrode, was lowered by the addition of small aliquots of 0.5 M sodium ascorbate. Samples were allowed to equilibrate in the dark for 5 min at defined oxidation-reduction potentials and were then transferred to a 2 mm pathlength cuvette and frozen in liquid nitrogen.

Fluorescence emission at liquid-nitrogen temperature was measured in the narrow spectral region around 695 nm to maximize the fluorescence of variable yield from Photosystem II and to minimize contributions from Photosystem I fluorescence [3,10,11]. Fluorescence was detected with an EMI 9558 phototube shielded by a Corning 2-64 filter and a Baird-Atomic 695 nm interference filter (10 nm half-band width). The fluorescence was excited by a weak, chopped (390 cps) monochromatic beam (480 nm) from a Bausch and Lomb Model 33-86-02 monochrometer

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and was measured with a PAR Model Hr-8 lock-in amplifier. The sample could also be illuminated with steady blue actinic light from a microscope lamp (100 $\mu W/cm^2$) shielded by 2 Corning 4-96 filters.

C-550 photoreduction at liquid-nitrogen temperature was measured with a dual-wavelength spectrophotometer as described previously [12].

3. Results and discussion

Fig. 1 shows the fluorescence yield, F_0, of dark-adapted chloroplasts and the maximum fluorescence yield, F_M, produced by strong actinic (act.) light at 77°K under conditions where cytochrome b_{559} and the +475 mV component were either both reduced prior to freezing (preincubation with 5 mM sodium ascorbate) or both oxidized prior to freezing (preincubation with 5 mM potassium ferricyanide). The primary acceptor of Photosystem II is oxidized and P_{680} is reduced prior to freezing in both samples. The

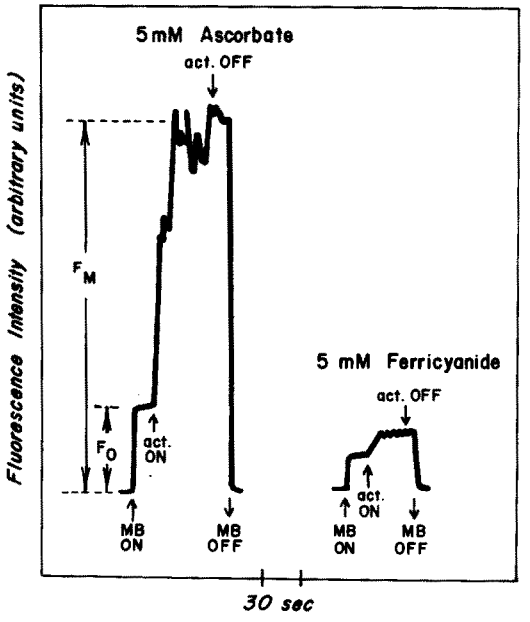

Fig. 1. Effect of ascorbate or ferricyanide on Photosystem II fluorescence at 77°K. The reaction mixture contained (per 1.0 ml) spinach chloroplasts (equivalent to 17 μM chlorophyll) and the following (μmoles): potassium phosphate buffer (pH 7.6), 50; NaCl, 20; and, where indicated, sodium ascorbate, 5, or potassium ferricyanide, 5.

weak, chopped beam (MB) does not itself produce any measurable change in the fluorescence yield over the course of the measurement, as indicated by the constant level of the fluorescence prior to actinic illumination. The ratio of the fluorescence yields, F_M/F_0, ranged from 3.8 to 4.5 in the presence of ascorbate and from 1.6 to 2.2 in the presence of ferricyanide. An oxidation-reduction titration of F_M/F_0 gave a midpoint potential of +350 mV (identical to the midpoint potential of cytochrome b_{559} [3,13]) for the transition from $F_M/F_0 = 4$ to $F_M/F_0 = 2$. These results are essentially identical to those obtained by Okayama and Butler [3]. No effects on the F_M/F_0 ratio other than that accompanying the oxidation of cytochrome b_{559} were observed in the potential range between +200 mV and +600 mV. No effect of oxidation–reduction potential on photoreduction of the primary acceptor of Photosystem II (monitored by the C-550 absorbance change [4–6,12]) was observed at liquid-nitrogen temperature over the range from +200 mV to +600 mV. Thus the effect of potential on the F_M/F_0 ratio cannot be attributed to an effect on the extent of photoreduction of the Photosystem II primary acceptor.

The fluorescence yield of dark-adapted chloroplasts, F_0, is also affected by oxidation-reduction potential, as may be seen in fig. 1. The ratio of F_0 at low potential to that at high potential ranged from 1.5 to 2.3, with values near 2.0 most typical. Fig. 2 shows the oxidation–reduction dependence of F_0 in more detail. The data are consistent with the hypothesis that a one-electron component in spinach chloroplasts with a midpoint oxidation-reduction potential of +455 mV quenches fluorescence in the oxidized state. Three titrations gave a value of +455 mV ± 10 mV with an n value of 1.0 ± 0.2 for this component.

The midpoint oxidation-reduction potential of this fluorescence-quenching component (+455 mV) is indistinguishable within experimental error from that of the secondary donor to Photosystem II (+475 ± 20 mV) discovered by Malkin and Bearden in the course of low-temperature electron paramagnetic resonance (EPR) measurements [7,8], suggesting that the two components may be identical. This possibility is strengthened by the fact that no other components associated with Photosystem II are known to have midpoint oxidation-reduction potentials in this range.

The +455 mV component is the first chloroplast

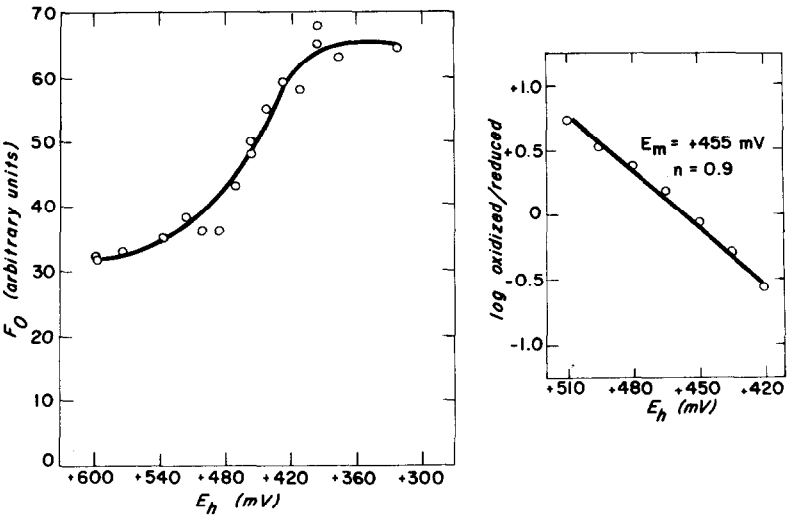

Fig. 2. Effect of oxidation–reduction potential on fluorescence of dark-adapted chloroplasts at 77°K. The reaction mixture was as in fig. 1 except that 5 mM potassium ferricyanide was present. The oxidation–reduction potential was adjusted to the indicated level by the addition of small aliquots of sodium ascorbate.

component on the oxidizing side of Photosystem II known to affect the F_0 level of Photosystem II fluorescence at low temperature. Cytochrome b_{559} does not affect fluorescence directly, as indicated by the absence of any change in fluorescence yield when cytochrome b_{559} is chemically oxidized or reduced (see above and [3]). An understanding of the mechanism by which the +455 mV component affects Photosystem II fluorescence will depend on the identification of the chemical nature of this component. If, as seems likely, this component is identical with the +475 mV component discovered by Bearden and Malkin [7]. The fluorescence effects may result from its location in proximity to the reaction center of Photosystem II.

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