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REDOX TITRATION OF THE PRIMARY ELECTRON ACCEPTOR OF PHOTOSYSTEM I IN SPINACH CHLOROPLASTS

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SUMMARY

The midpoint potential of the primary electron acceptor of Photosystem I in spinach chloroplasts was titrated using the photooxidation of P_{700} at −196 °C as an index of the amount of primary acceptor present in the oxidized state. The redox potential of the chloroplast suspension was established by the reducing power of hydrogen gas (mediated by clostridial hydrogenase and 1,1′-trimethylene-2,2′-dipyridylium dibromide) at specific pH values at 25 °C. Samples were frozen to −196 °C and the extent of the photooxidation of P_{700} was determined from light-minus-dark difference spectra. This titration indicated a midpoint potential of −0.53 V for the primary electron acceptor of Photosystem I.

INTRODUCTION

Knowledge of the redox potentials generated by the primary photochemical reactions of photosynthesis is essential for our understanding of the bioenergetics of photosynthesis. Several laboratories [1–3] have examined the reducing power of Photosystem I by determining the ability of chloroplasts to photoreduce low potential electron acceptor compounds with midpoint potentials in the range of −0.3 to −0.7 V. Estimates of the redox potential of the primary electron acceptor of Photosystem I from these kinds of experiments range from −0.5 [1] to −0.7 V [2]. Ke [4] employed a direct potentiometric titration in which he measured the light-induced absorbance change at 430 nm at room temperature which was due to both the photoreduction of the primary electron acceptor, P_{430}, and the photooxidation of the primary donor, P_{700}, as a function of the redox potential of the medium. Ke reported a midpoint potential of −0.47 V from these titration experiments but he has more recent data, including titration experiments with EPR measurements at low temperature, which indicate a somewhat lower potential [5].

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The purpose of this communication is also to report a redox titration of the primary electron acceptor of Photosystem I. H_2 was used as the reductant and specific redox potentials were adjusted by setting the pH of the medium. Chloroplast fragments, after redox equilibration at 25 °C, were frozen to −196 °C. The photooxidation of P_{700} at −196 °C was used as an index of the amount of the primary electron acceptor present in the oxidized state. A value of −0.53 V was obtained for the midpoint of the primary electron acceptor of Photosystem I.

MATERIALS AND METHODS

Spinach chloroplasts were prepared as previously described [6] and broken by suspension in distilled water for 1.5 h at 0 °C. Larger fragments were removed by centrifugation at $1000 \times g$ for 5 min and smaller fragments, collected by centrifugation at $10\,000 \times g$ for 10 min, were suspended in a small volume of distilled water and stored under hydrogen.

Redox potentials were established by incubating samples under 1 atm of H_2 (Matheson) at specific pH values at 25 °C. Borate buffers at various pH values were prepared according to Bates and Bower [7] except that pH values were measured with a Radiometer Model 26 pH meter standardized with pH-7.0 and pH-10.0 buffers (Mallinckrodt BuffAR). Triquat (1,1′-trimethylene-2,2′-dipyridylium dibromide) and hydrogenase were used as redox mediators. The hydrogenase was isolated from *Clostridium kluverii* according to the procedure of Nakos and Mortenson [8].

Triquat reduction at 25 °C and P_{700} photooxidation at −196 °C were measured with the scanning single beam spectrophotometer described by Butler and Hopkins [9]. Our normal vertical cuvette [10] was modified for anaerobic work by adding an 0-ring sealed lucite window at the top and a side arm fitted with a silicon rubber septum. For each pH value 0.5 ml of the appropriate buffer containing 20 μM triquat was bubbled with H_2 admitted through a syringe needle penetrating the septum with a second needle present to allow exit of the gas and maintenance of atmospheric pressure. After 10 min, both syringes were removed and 10 μl of the chloroplast suspension (containing 20 μg chlorophyll) and 10 μl of hydrogenase were added by syringe. Absorption spectra were measured in the 370–430-nm region to determine the concentration of reduced triquat and when the triquat absorbance reached a stable value (after about 10 min at 25 °C) the sample was frozen to −196 °C in the dark by immersion of the cuvette into liquid N_2. The thickness of the frozen sample was about 3 mm. Absorption spectra of the frozen sample were measured in the 670–730-nm region before and after a saturating far-red actinic irradiation (735 nm, 2.0 mW · cm^{-2}, 1.5 min) and the extent of P_{700} photooxidation was determined from the difference spectrum. The top of the cuvette was then removed, the sample was thawed and refrozen under air and the light-minus-dark difference spectrum again measured.

RESULTS AND DISCUSSION

The light-induced difference spectra of the chloroplast samples frozen to —196 °C at various pH values are shown in Fig. 1. Difference spectra of the samples frozen under reducing conditions at the indicated pH values are shown on the left;

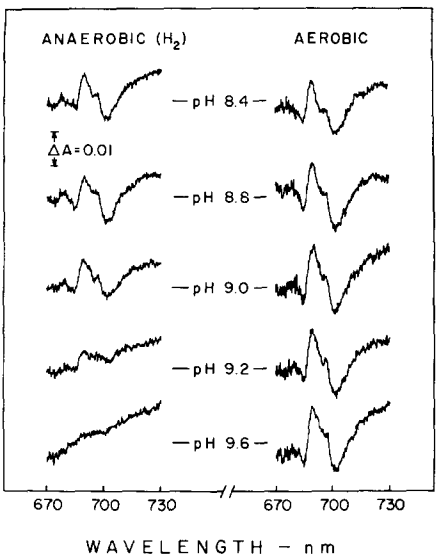

Fig. 1. Far-red irradiated-minus-dark difference spectra of chloroplast samples at −196 °C containing 20 μg chlorophyll, 10 nmoles of triquat and 10 μl of clostridial hydrogenase in 0.5 ml of a 0.0125 M borate buffer at the pH indicated. The spectra on the left side were obtained from samples frozen under H_2; the spectra on the right were obtained from the same samples after thawing and refreezing to −196 °C in contact with air.

difference spectra of the same samples after thawing and refreezing in contact with air are shown on the right. It is apparent that the amplitude of the light-induced absorbance change decreases as the redox potential of the medium becomes more negative (higher pH values) whereas the amplitude is constant under aerobic conditions. Strict anaerobic conditions are required to achieve and maintain the low redox potentials used in this titration.

The absorbance change, ΔA, indicating the amount of P_{700} oxidized was measured between the minimum at 702 nm and the maximum at 690 nm. The absorbance increase at 690 nm is isomorphic with the bleaching at 702 nm and probably is due to a 690-nm absorbing form of chlorophyll which senses the redox state of P_{700} [11]. The same results are obtained if P_{700} is measured as the bleaching at 702 nm relative to an estimated baseline.

The analysis assumes a 1 : 1 stoichiometry between the oxidation of the primary electron donor and the reduction of the primary electron acceptor in the low temperature photoreaction. Any chemical reduction of the primary acceptor prior to the low temperature photoreaction should result in a proportionate decrease in the photooxidation of P_{700}. Thus, ΔA is taken as an index of the amount of the primary acceptor present in the oxidized form (and therefore capable of being photoreduced) and $\Delta A_{max} - \Delta A$, the amount in the reduced state at the particular redox potential of the experiment. A Nernst plot of the data from Fig. 1 is shown in Fig. 2. Theoretical lines are also shown for Nernst plots of a one-electron and a two-electron change with midpoint potentials of −0.53 V. Although we would expect the primary photochemical reaction to be a one-electron transfer the data are not sufficiently precise to establish that experimentally.

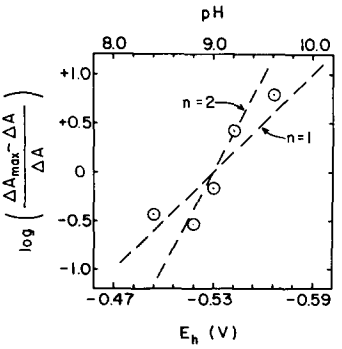

Fig. 2. Nernst equation plot of the data in Fig. 1. ΔA was measured as the absorbance difference between the minimum at 702 nm and the maximum at 690 nm at the various pH values from the spectra on the left side of Fig. 1. ΔA_{max} is the average of the aerobic ΔA values obtained from the spectra on the right side of Fig. 1. The scale on the abscissa is marked both for pH and redox potential on the standard hydrogen scale.

Takamiya and Nishimura [12] reported that dithionite did not prevent the photooxidation of P_{700} at room temperature and concluded it was not a sufficiently strong reductant to reduce the primary electron acceptor of Photosystem I. Dithionite is thermodynamically capable of reducing the primary acceptor but the acceptor does not appear to be accessible to this reductant; a mediator such as a viologen type dye is needed to transmit the reducing power of dithionite to the primary acceptor. We also carried out titration experiments using small aliquots of dithionite as the reductant in the presence of triquat with a platinum electrode to measure the redox potential. Chloroplast samples at various potentials were frozen to −196 °C and the extent of the photooxidation of P_{700} measured. These experiments (data not shown) gave essentially the same results that are reported here but the titration appeared less reliable than that obtained with the H_2–hydrogenase system.

The titration with H_2 at different pH values assumes that the redox potential of the acceptor is not pH dependent. The results of the titration using dithionite at constant pH support this assumption.

Our value of −0.53 V for the midpoint potential of the primary acceptor is more negative than the value reported by Ke [4] but is consistent with the more recent data of Ke et al. [5] and with the indirect estimates of Zweig and Avron [1] and Black [3]. The question can be raised as to whether the redox state established at 25 °C is altered on freezing to −196 °C. We believe that any discrepancy due to cooling is negligible, however, because the redox equilibration of the system is relatively slow (and will become even slower as the temperature decreases) and the rate of cooling is relatively rapid. A major advantage in using the assay based on the photoreaction at −196 °C is that secondary reactions which may complicate a room temperature titration are frozen out. The assay also insures that the species titrated is photochemically active.

After these titration experiments were completed, we became aware [11] that only about half of the P_{700} was stable in the oxidized form after an irradiation at −196 °C; the other half participates in a backreaction with the primary acceptor and thus does not appear in the stable light-minus-dark difference spectra at −196 °C.

We don't believe that the primary electron acceptor is different for the reversible and irreversible P_{700} changes at -196 °C (see model proposed in [11]) but this question should be settled experimentally.

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