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The oxidation–reduction potential of membrane-bound chloroplast plastocyanin and cytochrome *f*

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SUMMARY

The *in situ* oxidation–reduction potentials of plastocyanin and cytochrome *f* have been measured in the same preparation of spinach chloroplasts using electron paramagnetic resonance spectroscopy and dual-wavelength absorbance spectroscopy, respectively. A midpoint potential of 340 mV (n = 1.0) at pH 7.8 was found for the bound plastocyanin and a value of 385 mV (n = 1.0) was found for the bound cytochrome *f*.

The relationship of plastocyanin and cytochrome *f* in the chloroplast noncyclic electron transport chain remains unclear[1–9]. The identification of an electron paramagnetic resonance (EPR) signal of oxidized plastocyanin has permitted the first *in situ* characterization of this carrier in spinach chloroplasts[10] and allows direct study of this bound component. To further define the functional site of plastocyanin in the chloroplast electron transport chain and to attempt to relate this site to that of cytochrome *f*, we have measured the *in situ* oxidation–reduction potentials of these carriers in the same chloroplast preparation. Our results indicate that plastocyanin has a significantly more reducing midpoint potential than does cytochrome *f*.

Washed, broken spinach chloroplasts[10] were suspended in 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.8) *plus* 10 mM NaCl. Chlorophyll concentrations were determined by the method of Arnon[11]. Soluble spinach plastocyanin, prepared by a modification of the method of Katoh *et al.*[12], was generously provided by R. Chain. Oxidation–reduction potentials were measured with a Radiometer PK-149 combined platinum–calomel electrode in a cell similar to one previously described

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

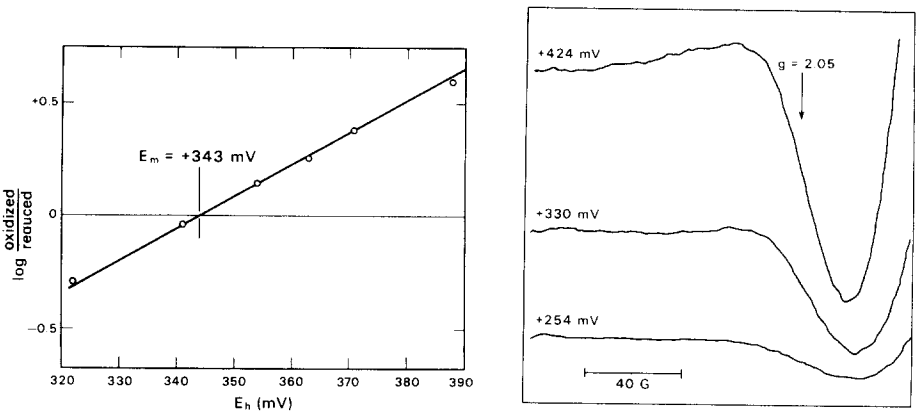

Fig.1. EPR spectra of bound chloroplast plastocyanin at defined oxidation–reduction potentials. Washed, broken chloroplasts (0.6 mg/ml) in 50 mM HEPES buffer (pH 7.8) *plus* 10 mM NaCl were incubated with 1 mM potassium ferricyanide and then titrated reductively with sodium ascorbate. Samples were removed at the indicated oxidation–reduction potentials and frozen in liquid nitrogen. First-derivative EPR spectra were recorded at 77 °K with the following instrument settings: frequency, 9.22 GHz; power, 10 mW; modulation amplitude, 10 G.

Fig.2. Oxidation–reduction potential of chloroplast plastocyanin *in situ*. Experimental conditions as in Fig. 1. The extent of plastocyanin reduction was estimated from the amplitude of the g = 2.05 signal.

by other workers[13,14]. The electrode was standardized against a saturated quinhydrone solution at pH 7.0 (E_m = 296 mV at pH 7.0, 20 °C; see ref. 15).

The EPR spectra of chloroplasts in the g = 2.05 region at three different oxidation-reduction potentials are shown in Fig. 1. This EPR signal has been previously shown to be due to the cupric form of plastocyanin *in situ*[10]. At an oxidation–reduction potential of 424 mV, bound plastocyanin is completely oxidized. When the potential is lowered to 330 mV, a significant portion of the g = 2.05 signal disappears because of the reduction of plastocyanin, while at an oxidation–reduction potential of 254 mV the disappearance of the g = 2.05 signal indicates complete reduction of plastocyanin.

By measuring the height of the g = 2.05 signal at defined oxidation–reduction potentials we have determined the midpoint oxidation–reduction potential of the bound plastocyanin. A typical Nernst plot is shown in Fig. 2, where the ratio of oxidized to reduced plastocyanin is plotted relative to the standard hydrogen electrode. The bound plastocyanin behaves as a one-electron carrier with a midpoint potential of 343 mV. In a series of measurements, we have found a midpoint potential of 340 ± 10 mV at pH 7.8 for the plastocyanin *in situ*. By these methods we have also obtained a midpoint potential of 370 ± 10 mV at pH 7.8 (n = 1.0) for isolated soluble plastocyanin. This value is similar to the midpoint potential of soluble plastocyanin from *Chlorella*[16] (E_m = 390 mV at pH 7.0), spinach[12] (E_m = 370 mV at pH 7.0), and *Chlamydomonas*[17] (E_m = 370 mV at pH 7.0), although these previous measurements were based on the optical change at 597 nm associated with reduction of plastocyanin.

Since values of 340 mV (ref. 18), 360 mV (ref. 19, 20), and 390 mV (ref. 21)

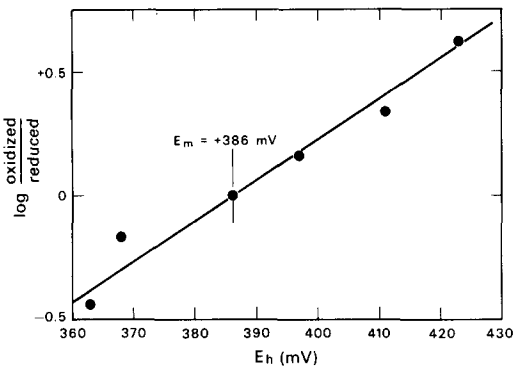

Fig.3. Oxidation–reduction potential of chloroplast cytochrome *f in situ*. Reaction mixture as in Fig. 1 except that the chlorophyll concentration was 75 μg/ml. The extent of cytochrome *f* reduction was estimated from the absorbance difference at 550 nm *minus* 540 nm (see ref. 21).

have been reported for the oxidation–reduction potential of spinach cytochrome *f in situ*, we have remeasured this midpoint potential in the same chloroplast preparations used in our plastocyanin studies. The oxidation–reduction state of cytochrome *f* was monitored at 550 nm instead of at the α band maximum of 554 nm to avoid any contribution to the absorbance from cytochrome b_{559}. No absorbance changes due to another chloroplast component, C550 (refs 21, 22), were observed in this potential region since the midpoint potential of C550 has been shown to be approximately 0 V (ref. 23). As shown in Fig. 3, cytochrome *f in situ* has a midpoint oxidation–reduction potential of 386 mV, and the results of several titrations gave a value of 385 ± 10 mV (n = 1.0) at pH 7.8.

Although in our comparison of the midpoint potentials of plastocyanin and cytochrome *f in situ* we necessarily utilized different experimental techniques, our findings indicate that plastocyanin has a significantly lower midpoint potential than the potential of cytochrome *f*. This observation is consistent with a functional site for plastocyanin on the reducing side of cytochrome *f*.

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