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THE EFFECT OF *o*-PHENANTHROLINE ON THE MIDPOINT POTENTIAL OF THE PRIMARY ELECTRON ACCEPTOR OF PHOTOSYSTEM II

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Summary

The primary electron acceptor of Photosystem II has a midpoint oxidation-reduction potential of +95 mV at pH 7.0 in Photosystem II chloroplast fragments prepared by digitonin treatment. The midpoint potential of the acceptor has a pH dependence of −60 mV/pH unit. At concentrations that inhibit oxygen evolution, *o*-phenanthroline shifts the midpoint potential of the primary acceptor by +70 mV. The shifted potential retains the same dependence on pH. The effect of *o*-phenanthroline suggests that it interacts directly with the primary electron acceptor of Photosystem II in a manner similar to that reported previously for the primary electron acceptor in purple photosynthetic bacteria.

o-Phenanthroline is an inhibitor of oxygen evolution by plant chloroplasts [1]. It appears to act by blocking the oxidation of the photoreduced primary acceptor of Photosystem II by a secondary electron acceptor. Evidence for such an inhibitory site was obtained by Knaff and Arnon, who showed that *o*-phenanthroline had no effect on the photoreduction of the primary electron acceptor of Photosystem II (monitored by the C-550 absorbance change) but did inhibit the rate of oxidation of the photoreduced acceptor [2]. *o*-Phenanthroline also inhibits electron flow at a similar site in the electron transport chain of purple photosynthetic bacteria. Studies of cytochrome photooxidation [3] and reaction-center bacteriochlorophyll photooxidation [4] purple sulfur bacterium *Chromatium*, have demonstrated that *o*-phenanthroline blocks the oxidation of the photoreduced primary electron acceptor by a secondary acceptor. Similar conclusions have been reached concerning the site of *o*-phenanthroline inhibition in the purple non-sulfur bacteria *Rhodopseudomonas spheroides* [5,6] and *Rhodospirillum rubrum* [6].

In photosynthetic bacteria, *o*-phenanthroline appears to interact

directly with the primary electron acceptor. This interaction is manifested by a shift in the midpoint oxidation-reduction potential of the primary electron acceptor on addition of *o*-phenanthroline. In *Rps. spheroides* the midpoint potential is shifted by +40 mV [7,8]; in *Chromatium*, by +135 mV [7,9]. It was of interest to see if *o*-phenanthroline had a similar effect on the midpoint oxidation-reduction potential of Photosystem II in chloroplasts. Titrations of the primary electron acceptor, as monitored by the C-550 absorbance change, in Photosystem II chloroplast fragments (D-10) show that *o*-phenanthroline raises the midpoint potential of the acceptor by 70 mV.

Photosystem II chloroplast fragments (D-10) were prepared by digitonin treatment of spinach chloroplasts according to the procedure of Anderson and Boardman [10]. Optical absorbance measurements at defined oxidation–reduction potentials were performed as described previously [11]. The samples were titrated reductively by the addition of small amounts of 0.01 M $Na_2S_2O_4$. Although experiments with K_2IrCl_6-treated chloroplasts have indicated that C-550 is not the actual primary acceptor of Photosystem II [12], the C-550 absorbance change has been shown to be a reliable indicator of the oxidation state of the primary acceptor of Photosystem II [13]. C-550 was monitored at 548 nm minus 538 to minimize absorbance changes caused by the reduction of low-potential *b*-type cytochromes [11]. The phototube was shielded to eliminate any contribution from the changes in chlorophyll fluorescence yield that accompany reduction of the primary acceptor of Photosystem II [14].

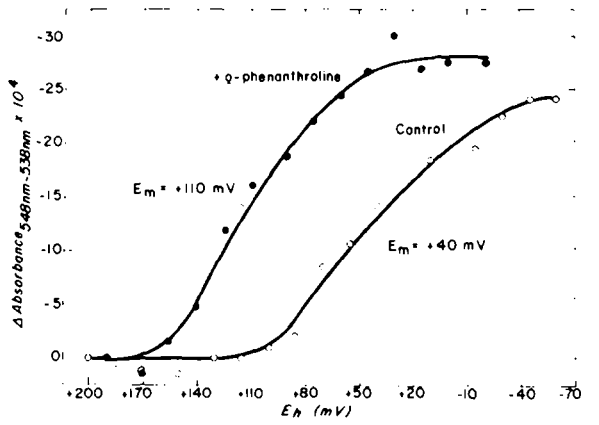
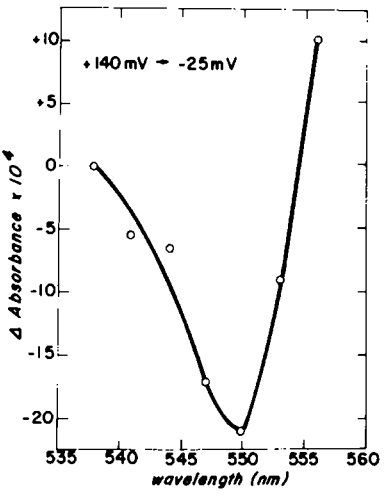


Fig.1. The effect of *o*-phenanthroline on the midpoint oxidation-reduction potential of C-550. The reaction mixture contained D-10 fragments (chlorophyll concentration, 50 μM), 100 mM Tris buffer at pH 7.8, and the following oxidation-reduction mediators: 20 μM 2,5-dimethylbenzoquinone; 10 μM 1,2-naphthoquinone; 10 μM 1,4-naphthoquinone; 10 μM duroquinone; and 2.5 μM pyocyanine. *o*-Phenanthroline was present at a concentration of $2.5 \cdot 10^{-4}$ M where indicated.

Fig. 1 shows the results of a titration at pH 7.8 of the C-550 absorbance change in D-10 fragments in the presence and absence of *o*-phenanthroline. The curves drawn through the experimental points are theoretical 1-electron curves with midpoint potentials of +40 mV and +110 mV, respectively.

Five titrations gave values of +55 ± 10 mV for the midpoint oxidation-

reduction potential of C-550 in the absence of *o*-phenanthroline and +110 ± 10 mV for the midpoint potential in the presence of $2.5 \cdot 10^{-4}$ M *o*-phenanthroline at pH 7.8. No effect of *o*-phenanthroline on the midpoint potential of C-550 was observed at *o*-phenanthroline concentrations less than $1.0 \cdot 10^{-5}$ M, and the shift in midpoint potential became maximal at approx. $1.0 \cdot 10^{-4}$ M *o*-phenanthroline. The range of *o*-phenanthroline concentrations over which the shift in the midpoint potential becomes apparent is the same range over which oxygen evolution [1] and C-550 oxidation [2] are inhibited. Although 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) inhibits Photosystem II reactions in a manner similar to that observed with *o*-phenanthroline [1,2,15], no effect of DCMU on the midpoint potential of C-550 was observed (three titrations at pH 7.8 gave a value of +45 ± 10 mV).



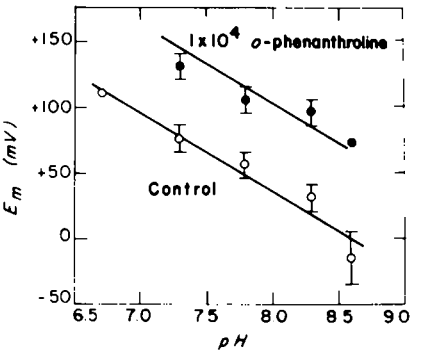


Fig.2. The reduced minus oxidized difference spectrum of C-550. Reaction conditions as in Fig.1. Reference wavelength, 538 nm.

Fig.3. The effect of pH and *o*-phenanthroline on the midpoint potential of C-550. The midpoint potentials were determined from titration curves similar to those of Fig.1. The oxidation-reduction mediators were those listed in the legend to Fig.1 supplemented with 20 μM benzoquinone and 10 μM 2-hydroxy-1,4-naphthoquinone. The pH buffers were: 100 mM morpholine ethane sulfonate (pH 6.7); 100 mM Tris buffer (pH 7.3—8.3); and 100 mM Tricine buffer (pH 8.6). Where multiple determinations were made, error brackets indicate the average deviation of the measurements.

Fig. 2 shows the spectrum of the absorbance change produced in the absence of *o*-phenanthroline as the oxidation-reduction potential is lowered from +140 mV to −25 mV and the component being titrated in Fig. 1 goes from the completely oxidized state to the completely reduced state. The absorbance decrease on reduction centered at 550 nm (C-550) is indistinguishable from that observed on photoreduction of the primary electron acceptor of Photosystem II [2]. The absorbance increase on reduction at wavelengths greater than 555 nm is caused by the reduction of low-potential *b*-type cytochromes [11]. An essentially identical spectrum was obtained in the presence of *o*-phenanthroline.

Fig. 3 shows the dependence of the midpoint potential of C-550 on pH. The lines are theoretical ones for a component that takes up $1H^+$/electron

on reduction (60 mV/pH unit). *o*-Phenanthroline ($1 \cdot 10^{-4}$ M) had no effect on the pH dependence of the midpoint potential. A similar dependence on pH of the midpoint potential of the primary electron acceptor of Photosystem II was observed by Cramer and Butler [14] in the course of titrations of the chlorophyll fluorescence yield in untreated chloroplasts.

The midpoint potential of the primary acceptor of Photosystem II in D-10 fragments in the absence of *o*-phenanthroline is +95 mV at pH 7.0. This value is significantly more positive than the value in the range from +25 mV to −25 mV obtained by Butler and co-workers [14,16] using untreated chloroplasts. The more positive midpoint potential observed in the experiments reported above may result from the treatment of the chloroplasts with digitonin. The lower signal:noise ratio attainable in titrations with untreated chloroplasts made it impossible to obtain reliable values for the midpoint potential of C-550. However, the hypothesis that the midpoint potential of the Photosystem II primary acceptor is more positive in D-10 fragments than in untreated chloroplasts is supported by the finding (based on C-550 and fluorescence measurements in the presence of DCMU at 77° K) that the primary acceptor can be reduced by 100 mM ascrobate in D-10 fragments but not in untreated chloroplasts.

The positive shifts produced by *o*-phenanthroline in the midpoint potentials of the primary electron acceptors of both plant Photosystem II and the purple photosynthetic bacteria suggest that *o*-phenanthroline interacts directly with the acceptor in these two different photosynthetic systems. In addition to this interaction, other similarities exist in the properties of the primary electron acceptors in these two systems. The primary electron acceptor of plant Photosystem I appears to have quite different properties. (Reactions in chlorplasts that involve only Photosystem I are not inhibited by *o*-phenanthroline [1].) The midpoint potentials of the primary electron acceptors of purple photosynthetic bacteria range from −20 mV to −100 mV [7—9, 17—19], similar to that of the primary electron acceptor of Photosystem II in untreated chloroplasts [13,16]. The primary electron acceptor of Photosystem I has a much more negative midpoint potential, −530 mV [20,21]. The primary electron acceptors of purple photosysnthetic bacteria and plant Photosystem II both have midpoint potentials that have a −60 mV pH unit dependence [7—9, 19], while the primary acceptor of plant Photosystem I appears to be pH independent [20,21].

The primary electron-accepting site of the purple photosynthetic bacteria appears to involve an iron protein [8,9,22,23] and ubiquinone [24—26] Recent experiments have suggested that plastiquinone functions as the primary acceptor of chloroplast Photosystem II [27,28]. The similarities in behavior of the primary acceptors in Photosystem II and purple photosynthetic bacteria raise interesting questions about possible chemical similarities between the two acceptors.

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References

1 Arnon, D.I., Tsujimoto, H.Y. and McSwain, B.D. (1967) Nature 214, 562—566
2 Knaff, D.B. and Arnon, D.I. (1969) Proc. Natl. Acad. Sci. U.S. 63, 963—969
3 Parson, W.W. and Case, G.D. (1970) Biochim. Biophys. Acta 205, 232—245
4 Ke, B., Garcia, A.F. and Vernon, L.P. (1973) Biochim. Biophys. Acta 292, 226—236
5 Clayton, R.K., Szuts, E.Z. and Fleming, H. (1972) Biophys. J. 12, 64—79
6 Hsi, E.S.P. and Bolton, J.R. (1974) Biochim. Biophys. Acta 347, 126—133
7 Jackson, J.B., Cogdell, R.J. and Crofts, A.R. (1973) Biochim. Biophys. Acta 292, 218—225
8 Dutton, P.L., Leigh, J.S. and Wraight, C.A. (1973) FEBS Lett. 36, 169—173
9 Evans, M.C.W., Lord, A.V. and Reeves, S.G. (1974) Biochem. J. 138, 177—183
10 Anderson, J.M. and Boardman, N.K. (1966) Biochim. Biophys. Acta 112, 403—414
11 Knaff, D.B. and Malkin, R. (1973) Arch. Biochem. Biophys. 159, 555—562
12 Malkin, R. and Knaff, D.B. (1973) Biochim. Biophys. Acta 325 336—340
13 Butler, W.L. (1973) Acc. Chem. Res. 6, 177—184
14 Cramer, W.A. and Butler, W.L. (1969) Biochim. Biophys. Acta 172, 503—510
15 Duysens, L.N.M. and Sweers, H.E. (1963) in Studies on Microalgae and Photosynthetic Bacteria (Miyachi, S., ed.), pp. 353—372, University of Tokyo Press, Tokyo
16 Erixon, K. and Butler, W.L. (1971) Biochim. Biophys. Acta 234, 381—389
17 Cramer, W.A. (1969) Biochim. Biophys. Acta 189, 54—59
18 Dutton, P.L. (1971) Biochim. Biophys. Acta 226, 63—80
19 Case, G.D. and Parson, W.W. (1971) Biochim. Biophys. Acta 253, 187—202
20 Ke, B., Hansen, R.E. and Beinert, H. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2941—2945
21 Lozier, R.H. and Butler, W.L. (1974) Biochim. Biophys. Acta 333, 460—464
22 Dutton, P.L., Leigh, J.S. and Reed, D.W. (1973) Biochim. Biophys. Acta 292, 654—664
23 Dutton, P.L. and Leigh, J.S. (1973) Biochim. Biophys. Acta 314, 178—190
24 Loach, P.A. and Hall, R.L. (1972) Proc. Natl. Acad. Sci. U.S. 69, 786—790
25 Feher, G., Okamura, M.Y. and McElroy, J.D. (1974) Biochim. Biophys. Acta 267, 222—226
26 Cogdell, R.J., Brune, D.C. and Clayton, R.K. (1974) FEBS Lett. 45, 344—347
27 Witt, K. (1973) FEBS Lett. 38, 116—118
28 VanGorkom, H.J. (1974) Biochim. Biophys. Acta 347, 439—442