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SOME EFFECTS OF *o*-PHENANTHROLINE ON ELECTRON TRANSPORT IN CHROMATOPHORES FROM PHOTOSYNTHETIC BACTERIA

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SUMMARY

The midpoint potentials of the primary electron acceptors in chromatophores from *Rhodopseudomonas spheroides* and *Chromatium* have been studied by titrating the laser-induced P605 and cytochrome *c* oxidations, respectively. Both midpoint potentials are pH dependent (60 mV/pH unit).

o-Phenanthroline shifts the midpoint potentials of the primary acceptors, by +40 mV in *Rps spheroides* and +135 mV in *Chromatium*. A similar though less extensive change in midpoint potential was observed in the presence of *batho*-phenanthroline, but not with 8-hydroxyquinoline. The shifted midpoints retain the same dependence on pH.

Some of the effects of *o*-phenanthroline can be explained by assuming that it chelates the reduced form of the primary electron acceptor. This suggests the presence in the primary electron acceptor of a metal chelated by *o*- and batho-phenanthroline.

In *Rps spheroides* chromatophores *o*-phenanthroline inhibits the laser- and flash-induced carotenoid shift at all redox potentials, stimulates the laser-induced P605 oxidation at redox potentials between +350 and +420 mV and slows the decay of the laser-induced cytochrome *c* oxidation below +180 mV. These effects show that *o*-phenanthroline may have more than one site of action.

INTRODUCTION

Light absorption by the pigments of photosynthetic bacteria leads to the rapid transfer of an electron within an excited reaction centre complex from a primary electron donor to a primary electron acceptor. Subsequently, one or both of two mechanisms may operate: (i) the "hole" left in the reaction centre complex is filled by the rapid electron transfer from an adjacent cytochrome; (ii) the electron in the primary acceptor is transferred into a pool of secondary electron acceptors. Failure of these mechanisms may result in reversal of electron flow in the reaction centre, with or without re-emission of some of the energy as delayed fluorescence. Recent thermodynamic[1–4] and kinetic[5,6] studies of early donor and acceptor pools of the photosynthetic bacteria have led to a clearer understanding of some of the reactions involved.

o-Phenanthroline has been shown[1] to act as an inhibitor of electron flow at a site between the primary and secondary acceptors. We now find that *o*-phenanthroline induces a positive shift in the apparent midpoint potential of the primary electron acceptor in chromatophores from *Rhodopseudomonas spheroides*, *Rhodopseudomonas viridis*, *Rhodopseudomonas capsulata* (Evans, E. H. and Cogdell, R. J., unpublished) and *Chromatium*. Other effects of this compound on the kinetics of chromatophore electron transport reactions are not easily explained and may indicate secondary sites of inhibition.

METHODS

Cells of *Rps spheroides* (Ga mutant) and *Chromatium* were grown in batch culture and chromatophores isolated after French press treatment at 10 tons/inch^2 (ref. 7). The chromatophores were suspended in 100 mM choline chloride, 5 mM 2-(*N*-morpholino)ethane sulphonate, pH 6.5, and the concentration of bacteriochlorophyll was determined using the *in vivo* extinction coefficient of Clayton[8].

Absorbance changes of the reaction centre bacteriochlorophyll (P605) and the carotenoid shift were monitored in a rapidly responding single-beam spectrophotometer and recorded on a Tektroni storage oscilloscope (Type 564), as described by Jackson and Crofts[9]. Cytochrome oxidation–reduction reactions were followed with a two-photomultiplier double beam-spectrophotometer of 10-μs minimum response time for *Rps spheroides* and with the single beam instrument for *Chromatium*.

Actinic flashes were provided by either a Q-switched ruby laser (Laser Associates Ltd, Slough, England; Type 213, half-pulse width 20 ns) or a xenon flash (Mecablitz 182, Metz, Germany; half-pulse width of 200 μs).

The experiments were performed in an anaerobic redox cuvette similar to that designed by Dutton[10]. The cuvette was equipped with a magnetic stirrer, gas inlet and outlet tubes for oxygen-free nitrogen, platinum and calomel electrodes and rubber septum, through which additions could be made. The potential difference between the two electrodes was monitored with a Radio-meter 22 pH meter.

The following dyes were used to mediate the redox potential between the platinum electrode and the redox components in the chromatophores; potassium ferricyanide ($E_{m,7.0} = +430$ mV), phenazine methosulphate ($E_{m,7.0} = +80$ mV), phenozine ethosulphate ($E_{m,7.0} = +55$ mV), pyocyanine ($E_{m,7.0} = -34$ mV) and 2-hydroxy-1,4-naphthoquinone ($E_{m,7.0} = -145$ mV). The redox dyes must be sufficiently concentrated to catalyse efficient mediation between the electrodes and chromatophore electron transport components without interfering with the kinetics of electron transport immediately following the exciting flash[10]. The dyes were used at concentrations which best satisfied these requirements. Small volumes of freshly prepared potassium ferricyanide and sodium dithionite solution in buffer were used as oxidant and reductant. For details of the titration procedure see ref. 4.

RESULTS AND DISCUSSION

Dutton and Jackson[4] have shown recently that the flash induced spectral changes associated with reaction centre bacteriochlorophyll, carotenoids and cyto-

chromes *b* and *c* in *Rps spheroides* chromatophores attenuate as the redox potential of the suspension is lowered through a midpoint value (at pH 7) of −20 mV, associated with chemical reduction of the primary electron acceptor. We have repeated these experiments at several pH values and in the presence and absence of *o*-phenanthroline (Figs 1 and 2). *o*-Phenanthroline raised the apparent midpoint potential of the primary acceptor by approximately 40 mV. The E_m values both in the presence and absence of *o*-phenanthroline varied by 60 mV/pH unit indicating a stoichiometry of 1 H^+ per electron for the reduction of the primary acceptor. Similar results were obtained when the primary acceptor midpoint potential was estimated from the fast phase of the flash induced carotenoid shift. This is in contrast to the result of Reed *et al.*[11] who related the fluorescence yield of *Rps spheroides* reaction centre preparations to the redox state of the primary acceptor and found the E_m to be pH independent. We are unable to account for this difference in pH dependence for the primary acceptor in coupled chromatophores and isolated

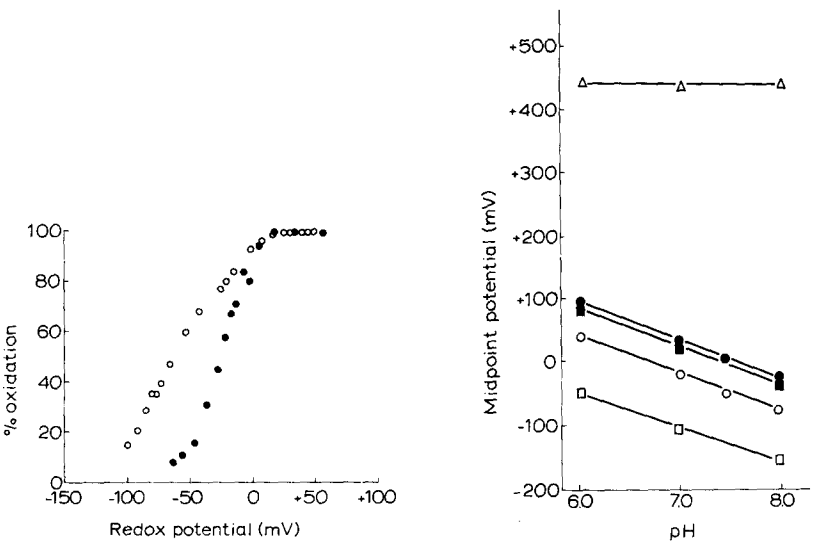

Fig. 1. The redox titration of the primary electron acceptor in chromatophores from *Rps spheroides* at pH 8.0 with or without *o*-phenanthroline. The midpoint potential of the primary electron acceptor was estimated from the laser-induced extent of P605 oxidation. The cuvette contained 7 cm^3 of 50 mM KCl., 50 mM Tricine, pH 8.0, 0.26 mg/ml bacteriochlorophyll and 10 μM pyocyanine, 10 μM phenazine methosulphate and 10 μM phenazine ethosulphate. ○, no additions; ●, with 2 mM *o*-phenanthroline.

Fig. 2. The pH dependence of the midpoint potentials of the primary donor and primary acceptor. △, midpoint potential of the primary donor of *Rps spheroides* chromatophores, estimated from the high potential attenuation of the laser-induced P605 change. 0.3 mg/ml bacteriochlorophyll, 500 μM potassium ferricyanide. ○—○, the midpoint potential of the primary acceptor of *Rps spheroides* chromatophores, estimated from the low potential attenuation of the laser-induced P605 change. 0.26 mg/ml bacteriochlorophyll, 10 μM phenazine methosulphate, 10 μM phenazine ethosulphate and 7 μM pyocyanine. ●—●, the same conditions as ○—○, but with 2 mM *o*-phenanthroline. □—□, midpoint potential of the primary acceptor of *Chromatium* chromatophores, estimated from the low-potential attenuation of the laser-induced cytochrome *c* oxidation monitored at 552 nm, 60 μg/ml bacteriochlorophyll, 10 μM phenazine methosulphate, 10 μM phenazine ethosulphate, 10 μM 2-hydroxy-1,4-naphthoquinone and 7 μM pyocyanine. ■—■, the same conditions as for □—□ but with 2 mM *o*-phenanthroline.

reaction centres. The titrations shown in Fig. 1 suggest that in the presence of *o*-phenanthroline the curve may have changed from that of a 1-electron to that of a 2-electron carrier. The accuracy of our measurements was not sufficient to clearly distinguish between these possibilities.

batho-Phenanthroline, the 4,7-biphenyl derivative of *o*-phenanthroline, also gave rise to a shift in midpoint potential of the primary acceptor but 8-hydroxyquinoline, another metal chelating agent, did not. *batho*-Phenanthroline acted at lower concentrations than *o*-phenanthroline, but the maximum degree of shift in midpoint potential was less (+25 mV at 16 μM).

The midpoint potential of the primary electron donor in the reaction centre of *Rps spheroides* chromatophores, estimated from the attenuation of the laser-induced 605-nm change ((P605) and the carotenoid shift at high redox potentials[4], was independent of pH, in agreement with previous determinations[12,13], and was unaffected by *o*-phenanthroline (Fig. 2).

The midpoint potential of the primary electron acceptor of *Chromatium* chromatophores has been estimated from the redox potential dependence of cytochrome photooxidation[3,10], as shown in Fig. 2. The midpoint potential of the primary acceptor was shifted by approximately +135 mV following addition of *o*-phenanthroline. The midpoint in the presence or absence of *o*-phenanthroline displayed the same dependence on pH (60 mV/pH unit) as was found with *Rps spheroides* chromatophores. These values are in agreement with those reported elsewhere[13–16] although Case and Parson[3] using a rather high concentration of redox dyes found a dependence on pH of nearer 30 mV/pH unit.

o-Phenanthroline also shifted the midpoint potential of the primary electron acceptor of *Rps viridis* (by +130 mV) and of *Rps capsulata* (by +45 mV) (unpublished observations in collaboration with E. H. Evans).

The shift in midpoint of the primary acceptor induced by *o*-phenanthroline is of interest in the light of the recent low-temperature ESR studies of Leigh and Dutton[17]. These workers suggested that the primary electron acceptor of bacterial and green plant photosynthesis is an iron–sulphur protein. In complexing iron *o*-phenanthroline binds preferentially to the ferrous state so that the following equilibria may be expected.

$$[Fe^{3+}\text{–S}] \underset{-e^-}{\overset{e^-}{\rightleftharpoons}} [Fe^{2+}\text{–S}] \underset{-o\text{-Phenanthroline}}{\overset{o\text{-Phenanthroline}}{\rightleftharpoons}} [Fe^{2+}\text{–S}]o\text{-phenanthroline}$$

Such a reaction could result in a change in midpoint, and apparent affinity constants of *o*-phenanthroline for the Fe^{2+} form of the primary acceptor can be calculated from the shift in midpoint potential observed assuming that the reactions above occur. Values for log K of 0.68 for the 40-mV shift in *Rps spheroides* and 2.2 for the 135-mV shift in *Chromatium* are found. These values compare with log $K=5.9$ for *o*-phenanthroline binding to Fe^{2+} in aqueous solution[18].

This explanation for the mechanism by which *o*-phenanthroline shifts the midpoint potential of the primary electron acceptor, receives some support from the fact that submaximal concentrations of *o*-phenanthroline (or of *batho*-phenanthroline) produce less of a shift of E_m than was observed at maximal concentrations. This is illustrated in Fig. 3 for the effect of various concentrations of *o*-phenan-

throline on the midpoint potential of the primary electron acceptor in *Chromatium* chromatophores. This type of concentration dependence, where submaximal concentrations of *o*-phenanthroline produce less of a shift, is similar to that described by Clark[19] for a reaction where a ligand or a chelating agent shifts the equilibrium of a redox reaction by preferentially binding to either the oxidised or reduced form of the couple. At all the concentrations of *o*-phenanthroline used the titration curve was continuous and showed no indication that more than one species was undergoing titration.

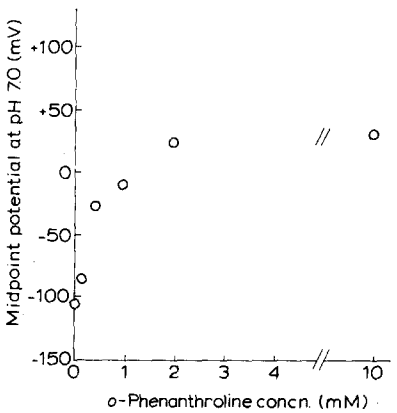

Fig. 3. Dependence on the concentration of *o*-phenanthroline of the shift in midpoint potential of the primary electron acceptor in *Chromatium* chromatophores. The conditions were as described in Fig. 1 except that the concentration of the bacteriochlorophyll was 47 μg/ml. The medium had a pH of 7.0 and the concentrations of *o*-phenanthroline added were as indicated in the figure.

The inhibition of chromatophore electron transport by *o*-phenanthroline could be explained in terms of the weaker reducing power of the primary acceptor relative to the secondary acceptor resulting from such an effect. This mechanism is consistent with the observation that *o*-phenanthroline is a more potent inhibitor of Site I H^+ binding in *Chromatium* chromatophores (unpublished observations) than in *Rps spheroides* chromatophores[20,21]. Back reaction from the primary acceptor to the strongly oxidising P870 would be less affected by the midpoint potential shift as long as the activation barrier was low. However, this interpretation does not explain the stimulation of delayed fluorescence in the presence of *o*-phenanthroline reported by Fleischman and Clayton[22], since emission would depend on reexcitation to the singlet level, and the activation barrier would be considerably greater for electrons from the acceptor shifted to the higher potential.

Clayton and his collaborators have shown that *o*-phenanthroline stimulates P870 fluorescence and dark re-reduction after flash excitation of "reaction-centre" preparations from *Rps spheroides*[23]. There are several lines of evidence to suggest that the bacteriochlorophyll absorption change centred around 600 nm (P605) is associated with P870[4]. However, we have found that at high redox potentials (*i.e.* where cytochrome *c* is chemically oxidised before the flash) the apparent extent of laser-induced P605 oxidation was stimulated in the presence of *o*-phenanthroline (Fig. 4B). Dutton and Jackson[4] found that the decay of the laser-induced P605

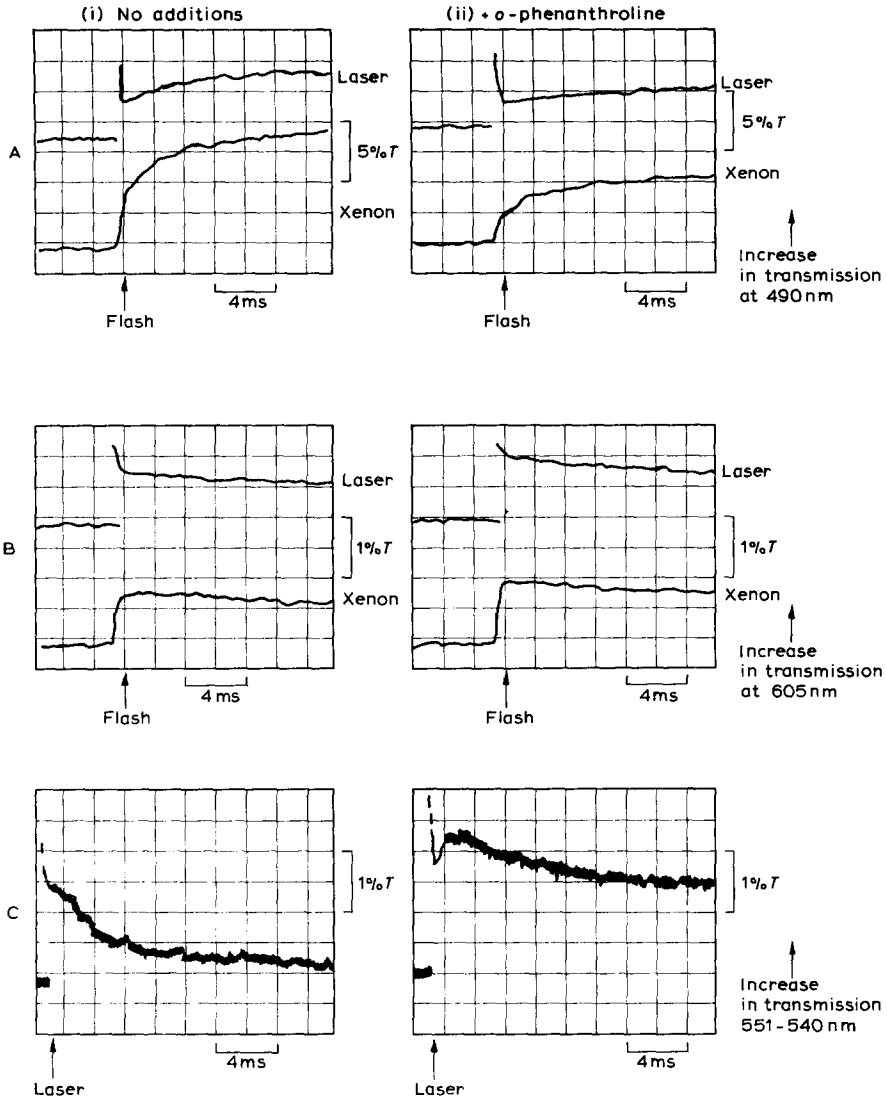

Fig. 4. Effects of *o*-phenthroline on the kinetics of some electron flow reactions in *Rps spheroides* chromatophores. (A) The effect on the laser- and xenon-induced carotenoid shift at +160 mV: (i) 0.1 mg/ml bacteriochlorophyll, 10 μM phenazine methosulphate, and 10 μM diaminodurol, pH 7.0; (ii) the same condition as (i) but in the presence of 2 mM *o*-phenanthroline. (B) The effect on the laser- and xenon-induced P605 change at +384 mV; (i) 0.3 mg/ml bacteriochlorophyll, 200 μM potassium ferricyanide, pH 7.0; (ii) the same condition as (i) except 2 mM *o*-phenanthroline is present. (C) The effect on the laser-induced cytochrome oxidation at +104 mV: (i) 0.2 mg/ml bacteriochlorophyll, 10 μM phenazine methosulphate, pH 7.0; (ii) the same conditions as (i) but in the presence or 2 mM *o*-phenanthroline.

change was faster when cytochrome *c* was chemically reduced before the flash (indicating an electron transport role for P605) but that the rate was too slow ($t_{1/2} \simeq 2$–3 ms) to match the initial phase of cytochrome *c* oxidation ($t_{1/2} \simeq 100$ μs).

The anomalous characteristics of cytochrome and P605 kinetics reported here and in ref. 4 are under further investigation.

The fast phase of the laser-induced carotenoid spectral shift has been related to a trans-membrane charge separation during the early photoreactions of *Rps spheroides* chromatophores[9]. Fig. 4A shows that *o*-phenanthroline partially inhibited this change. There is no evidence to suggest that this was the result of a rapid decay of the electrical field, as might be expected from the rapid return of an electron from reduced primary acceptor to oxidised P870. Nor does it appear to be a "deamplification" of the electrochromic shift induced by *o*-phenanthroline, since the inhibitor was without effect on carotenoid shifts induced by ion gradients in the dark (Cogdell, R. J., unpublished). This effect may be related to the partial inhibitory effect of *o*-phenanthroline on flash-induced P870 oxidation in *Rps spheroides* reaction centre preparations[23]. A further possibility is that *o*-phenanthroline interferes with the as yet unknown mechanism by which electric fields perturb the carotenoid absorption spectrum[22].

The data shown in Fig. 4C suggest that *o*-phenanthroline may not be a specific inhibitor of the low-potential electron transport reactions of *Rps spheroides* chromatophores. The decay of the laser-induced cytochrome *c* oxidation is evident when a component $E_m \simeq 150$ mV is reduced before the flash[4]. *o*-Phenanthroline partly inhibited this decay (Fig. 4C), but was without effect on cytochrome *c* kinetic changes when the 150-mV component was chemically oxidised before excitation (not shown). It is probable therefore that in addition to effects on the primary acceptor, *o*-phenanthroline also inhibits electron flow at a second site close to that of antimycin inhibition[4,20]. This would be consistent with the partial inhibitory effect of *o*-phenanthroline on the slow phase of the laser-induced carotenoid shift (Fig. 4A), and this may suggest the involvement of a second "non-haem iron" component in *Rps spheroides* photosynthetic electron transport.

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