SOME OBSERVATIONS ON THE PRIMARY ACCEPTOR OF *RHODOPSEUDOMONAS VIRIDIS*

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1. Introduction

Chromatophores from *Rhodopseudomonas viridis* contain two *c*-type cytochromes (designated C558 and C553 after the absorption maxima of the α bands) which undergo rapid oxidation on illumination [1]. The mid-point redox potentials (E_{m7}) of these cytochromes have been determined as 0.33 V for C558, and −0.012 V for C553 [2].

The oxidation of C558 induced by a laser flash has a half rise time of 2 μsec at 25° [1] while the oxidation of C553 is faster with a half rise time of ~ 300 nsec [3]. Thus at redox potentials above +80 mV C558 is photooxidised by a laser flash, but at redox potentials below −20 mV C553 is preferentially photooxidised [1].

When the redox potential of the suspending medium is lowered so that the primary acceptor is chemically reduced, photoreactions cease and cytochrome *c* oxidation is attenuated. We have used this attenuation of cytochrome *c* oxidation to determine the midpoint potential of the primary acceptor. We have previously shown that in the presence of ortho-phenanthroline the midpoint potential of the primary acceptor is shifted to more positive values in several species of photosynthetic bacteria including *Rps. viridis* [4]. We find that the midpoint potential of the primary acceptor in *Rps. viridis* is pH dependent. By using ortho-phenanthroline, and by varying the pH we have been able to show that both the *c*-type cytochromes donate electrons to photochemical reaction centres with the same primary acceptor.

2. Methods

Cells of *Rps. viridis* were grown for 2–3 days in batch culture. The cells were harvested and chromatophores prepared, as previously described for *Rps. spheroides* [5].

Experiments were performed in an anaerobic redox cuvette placed in a rapidly responding single-beam spectrophotometer. The cuvette was gassed with oxygen free nitrogen and redox dyes were used to mediate between the platinum electrode and the membrane bound electron transfer components. The method is essentially that of Dutton [6] described and discussed in detail in Dutton and Jackson [7]. The redox potential was changed by small additions of freshly prepared potassium ferricyanide or sodium dithionite solutions.

Activation was provided by a Nd^{3+} doped calcium tungstate laser (emitting at 1.06 μm), Q-switched by a Pockels cell giving a pulse of output 20 mjoules, and duration 15 nsec (Laser Associates, Rugby, England, Model 252B).

3. Results and discussion

Several workers have used the low potential attenuation of the laser induced cytochrome *c* photo-oxidation in *Chromatium* chromatophores to estimate the redox potential of the primary acceptor [6,8,9]. We have applied this method to *Rps. viridis* chromatophores by following the extent of the laser induced cytochrome *c* oxidation at 553 nm as a function of redox potential (figs. 1 and 2). The primary acceptor

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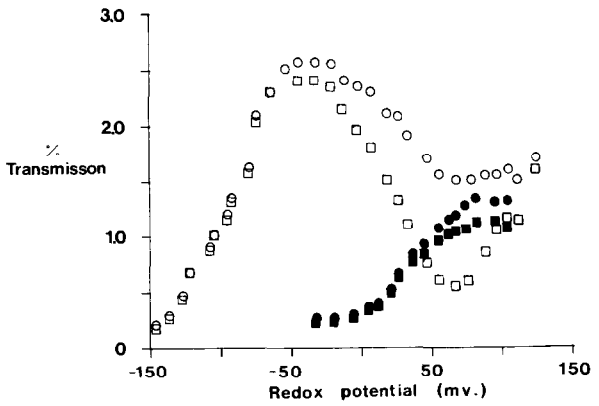

Fig. 1. The dependence on ambient redox potential of the laser induced cyt *c* oxidation at 553 nm in the presence and absence of ortho-phenanthroline. The anaerobic cuvette contained in a final volume of 7 ml, 50 mM TES, 50 mM KCl, *Rps. viridis* chromatophores (42 μg Bchl) and the following redox dyes; 10 μM diaminodurol, 10 μM phenazine methosulphate, 10 μM phenazine ethosulphate, 10 μM 2-hydroxy-1-4-napthoquinone and 7 μM pyocyanine. The medium was at pH 7, and the temperature 25°.
○,□ = no further addition;
●,■ = with 2 mM ortho-phenanthroline.
The circles show the extent of change 200 μsec after the flash, the squares the extent 2 msec after flash.

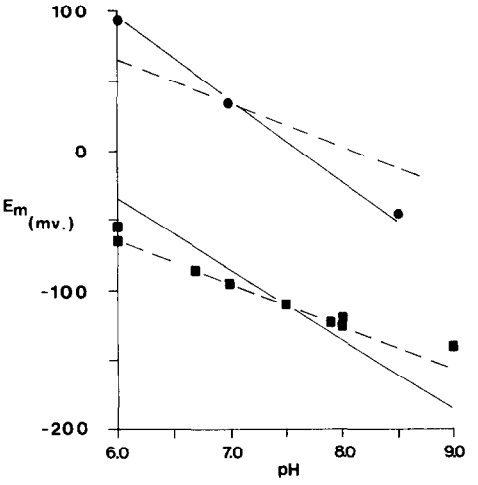

Fig. 2. The pH dependence of the midpoint potential of the primary electron acceptor in the presence and absence of ortho-phenanthroline. Conditions as for fig. 1 except that the following buffers were used: at pH 6.0 and 6.5, 50 mM MES; at pH 7.5, 50 mM TES; and at pH 8.0, 8.5 and 9.0, 50 mM tricine.
■ = no additions; ● = with ortho-phenanthroline.

had a midpoint potential of −95 mV at pH 7.0 and showed a pH dependency of 30 mV/pH unit.

By plotting spectra of the laser induced changes we have been able to confirm that above +80 mV cytochrome *c* 558 is photo-oxidised, while below −20 mV cytochrome *c* 553 is photo-oxidised. The two cytochromes crossed over between +60 mV and −20 mV, the change being half complete at a potential of ~ 20 mV. Cytochrome *c* 553 has a larger extinction coefficient at 553 nm than cytochrome *c* 558 so that the region of crossover can be seen in fig. 1 as an increase in extent of cytochrome oxidation. The potential at which this crossover occurred was essentially independent of pH between pH 6 and pH 9, and in this respect the change was similar to that observed in *Chromatium* chromatophores [8].

The change in the extent of cytochrome *c* oxidation 2 msec after the laser flash showed that at potentials between +130 mV and +50 mV a rapid decay appeared (fig. 1). The appearance of the decay was half complete at +100 mV, and this value was essentially independent of pH. An analogous decay of cytochrome oxidation was also seen in *Chromatium* chromatophores over this potential region [10]. The appearance of the decay suggests that in both species a component of E_{m7} ~100 mV is present, which has not been previously identified. The rapid decay of cytochrome oxidation in *Rps. viridis* chromatophores was only seen when C558 underwent photo-oxidation, and disappeared after the cytochromes crossed over.

Ortho-phenanthroline inhibited the extent of cytochrome *c* photo-oxidation by ~ 10%. In the presence of ortho-phenanthroline the midpoint potential of the primary acceptor in *Rps. viridis* was shifted by +130 mV at pH 7.0 (figs. 1 and 2). The shifted midpoint potential showed a pH dependency of 60 mV/pH unit. The reason for this change in pH dependence in the presence of ortho-phenanthroline is not clear, but as a consequence the degree of the ortho-phenanthroline induced shift in midpoint potential varied with pH.

Since the crossover between the two cytochromes was independent of pH, it was possible by choosing a

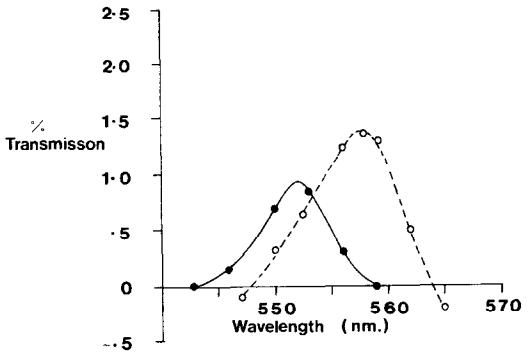

Fig. 3. Spectra of the laser-induced cytochrome oxidation in the presence of ortho-phenanthroline. Conditions as for figs. 1 and 2. (●—●—●): This spectrum was obtained with the medium at pH 8.5 and at a redox potential of –40 ± 5 mV. (○—○—○): This spectrum was obtained with the medium at pH 6.0 and at a redox potential of +100 ± 5 mV.

suitable pH, and by making use of the ortho-phenanthroline induced shift of the midpoint potential of the primary acceptor to show that both cytochromes feed reducing equivalents to reaction centres with the same primary acceptor. Thus, at pH 8.5 in the presence of ortho-phenanthroline the primary acceptor had a midpoint potential of –46 mV. The spectrum of the laser induced change under these conditions and at –40 mV was that of C553 (fig. 3.). However, at pH 6.0 in the presence of ortho-phenanthroline, the midpoint potential of the primary acceptor was +95 mV. Under these conditions the spectrum of the laser induced change showed that it was C558 which was undergoing photo-oxidation (fig. 3.). Since the midpoint potentials of the primary acceptor, at the values of pH chosen, were on the same theoretical curve and showed the same response to ortho-phenanthroline, it seems very likely that both cytochromes donated electrons to photochemical reactions with the same primary acceptor.

In several species of photosynthetic bacteria it has been suggested [11–13] that there is more than one type of reaction centre present. This idea has been challenged on the basis of observations with *Chromatium* chromatophores [14,15], where it has been shown kinetically that both cytochrome C552 and C555 donate electrons to the same P870. These results can be given further support by our own observation reported above. Furthermore, by using the method described above for *Rps. viridis*, it has been possible to show that in *Chromatium* chromatophores as well, both the *c*-type cytochromes feed electrons to photochemical reactions with a common primary acceptor [10].

Acknowledgements

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