Biochimica et Biophysica Acta 894 (1987) 524–533
Elsevier

BBA 42663

Evidence for two Q_a-like quinone binding sites in the reaction centre of *Rhodopseudomonas viridis*

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(Received 21 April 1987)
(Revised manuscript received 17 August 1987)

Key words: Photosynthesis; Photosynthetic reaction centre; Quinone; Iron-quinone; (*Rps. viridis*)

The oxidation–reduction potential of the iron-quinone electron acceptors in the reaction centre of *Rhodopseudomonas viridis* has been reinvestigated. In chromatophores treated with *o*-phenanthroline to remove the secondary acceptor Q_b, two steps were observed in the reduction of the primary electron acceptor Q_a with $E_m \approx -100$ and ≈ -330 mV. In isolated reaction centres only one step was observed in the reduction of Q_a with $E \approx -150$ mV. Reconstitution of the reaction centres with additional menaquinone resulted in an increase in the Q_a EPR signal and reconstitution of the low-potential step in the oxidation-reduction titration. Reconstitution with ubiquinone resulted in the recovery of the secondary quinone Q_b. The addition of ubiquinone did not reconstitute the low-potential step of Q_a reduction, or affect the reconstitution of this step by menaquinone. It is concluded that menaquinone can bind to two sites on the reaction centre. Both have properties of the Q_a site but with different pK values. It is unlikely that either is the same as the Q_b site.

Introduction

The initial electron-transfer event in photosynthesis is the oxidation of the reaction centre chlorophyll and the transfer of the electron through an intermediary electron acceptor to a 'stable' electron acceptor complex. In Photosystem II and in purple bacteria these acceptors are thought to be quinones bound to the reaction centre and associated with a ferrous iron atom. The classical model of this quinone complex involves two quinones [1]. One (Q_a) is tightly bound to the reaction centre and acts as a one-electron carrier cycling between the oxidised and semiquinone state. The second quinone (Q_b) is reduced in the microsecond time range by Q_a. It acts as a two-electron gate, the semiquinone is very stable and tightly bound to the reaction centre. On double reduction the Q_b is released to the quinone pool and replaced by an oxidised quinone. Q_a and Q_b can be detected by EPR spectrometry. They have characteristic spectra around $g = 1.82$ which are thought to reflect the magnetic interaction between the semiquinone radical and the iron atom [2]. Q_a and Q_b can be distinguished by the line width of the signal [3,4]. In *Rhodopseudomonas viridis* Q_b is narrower than Q_a. In samples prepared with Q_a oxidised, and the reaction centre chlorophyll reduced, illumination at temperatures

Abbreviations: Q_a and Q_b, primary and secondary bound quinone electron acceptors; I, the pheophytin intermediary electron carrier; EPR, electron paramagnetic resonance; P, reaction centre chlorophyll; LDAO, lauryldiethylamine *N*-oxide.

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as low as 4 K results in reduction of Q_a. In purple bacteria with *c*-type cytochromes bound to the reaction centre, such as *Rps. viridis*, this reduction is irreversible if the cytochromes are initially reduced. The reaction centre chlorophyll is then rereduced by the cytochrome. There are two types of cytochrome *c* haems attached to the *Rps. viridis* reaction centre, one is high potential with $E_m \approx$ 380mV and 310 mV [5] and the other low potential with $E_m \approx -12$ mV. Both can reduce the reaction centre chlorophyll at low temperature but complete reduction of Q_a is only seen when the low-potential cytochrome is reduced. Electron donation also occurs at low temperature in Photosystem II although the electron donors are different [6]. Low-temperature reduction of Q_b is not normally observed. Photoreduction of the pheophytin intermediary electron acceptor (I) can also be observed [7–9]. If samples are prepared with Q_a reduced, illumination at 200 K results in rapid reduction of I. I can be detected by the appearance of a 1.4 mT wide symmetrical EPR spectrum around $g = 2.00$, or more diagnostically if measurements are made below 10 K, by the characteristic split signal arising from magnetic interaction between I^-, Q_a^- and the iron atom. This signal also appears slowly if the sample is illuminated at 6 K. The electron-transfer sequence can be summarised as follows:

$$\text{cytochrome } c \rightarrow P \rightarrow I \rightarrow Q_a \rightarrow Q_b$$

I reduction can be observed in the same way in Photosystem II [10,11]. There is, however, extensive evidence for the presence of additional electron acceptors in the Photosystem II reaction centre from titration of fluorescence yield [12–14] identifying two acceptors Q_H and Q_L. Using EPR measurements we recently presented evidence that both Q_H and Q_L reflect steps in the reduction of the iron-quinone complex, suggesting there are two quinones [15]. The ability to induce the reduction of I was mainly dependent on the prereduction of Q_L. Both waves of the titration had the same spectrum and the preparation used was thought to be free of Q_b [16]. This experiment suggests either that there are two Q_a binding sites on the reaction centre or that an unknown magnetic component is interacting with the system. We also found evidence for an additional acceptor when determining the potential dependence of reaction centre triplet formation [17]. It is important to know the number of components and their function to determine the mechanism of electron transport.

It is difficult to make these measurements on Photosystem II preparations, because the preparations are unstable and the signal sizes small. We had earlier investigated the redox properties of the electron-acceptor complex of *Rps. viridis*. The results were largely interpreted in terms of the classical model, but the possibility that this reaction centre has a more complex acceptor system could not be excluded [18]. This reaction centre is now very well characterised with X-ray crystal structure available [19] and extensive biochemical and biophysical analysis. It is thought to provide a good model for the Photosystem II reaction centre.

We made an extensive study of the redox properties of Q_a, Q_b and I in *Rps viridis* chromatophores [18]. Titrations were carried out between 100 mV and -600 mV, measuring the extent of reduction of a high-potential iron quinone, Qa, and indirectly I. The ability to reduce I by 200 K illumination was also determined. Although these experiments were interpreted in terms of the classical model, there were some difficulties: particularly that the potential of Q_a was lower than expected and at alkaline pH, above the pK of Q_a, two waves were seen on the titration of Q_a and I^- induction. When the high-potential iron quinone was reduced and Q_a was expected to be reduced, the EPR signal size was smaller than expected. This was thought to reflect magnetic interactions between the two, similar to those seen when Q_a is photoreduced in the presence of Q_b. It was not clear at that time if the high-potential iron-quinone was Q_b. Alternative explanations were therefore proposed for the two waves on the Q_a titration suggesting either that they reflected interactions with Q_b, which could not be detected directly, or with an unknown component. Subsequently, it was shown that the high-potential iron-quinone is Q_b [20]. The suggestion that an additional component is present therefore seems to be correct.

The redox properties of Q_a and I have now been reinvestigated in chromatophores treated with *o*-phenanthroline to displace Q_b, and in purified

reaction centres. The results suggest that this reaction centre has two Q_a like binding sites for menaquinone in addition to the uniquinone binding Q_b site.

Materials and Methods

Rps. viridis was grown in modified Hutner's medium and chromatophores were prepared as described previously [18], exept that sonication was substituted for French Press treatment in some preparations. Reaction centres were prepared by LDAO extraction and hydroxylapatite chromatography, essentially as described by Clayton and Clayton [21]. LDAO-free reaction centres were prepared by binding reaction centres from the hydroxylappatite column to a DEAE cellulose column (Whatman DE32) washing with 20 mM Tris-HCl (pH 8.0) to remove excess LDAO and then with $Na_2S_2O_4$ 1 mg/ml in 50 mM Tris-HCl (pH 9.0). LDAO is reduced by this treatment and removed. Excess $Na_2S_2O_4$ was removed by washing with 20 mM Tris-HCl (pH 8.0) with 0.1 M NaCl. The reaction centres were then eluted with 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.2% Triton X-100. At this stage the reaction centres still retained about 30–50% of the initial Q_b, which was then removed by precipitation with ammonium sulphate, the reaction centres were resuspended in 50 mM Tris-HCl (pH 8.0). The preparation was then free of Q_b as determined by EPR spectrometry. The reaction centres were used in redox titrations at a concentration of about 10 μM based on the content of cytochrome *c*-552, assuming 2 mol of this cytochrome per reaction centre.

Redox titrations were carried out as described previously [7,18] by the method of Dutton [22]. The following compounds were used as mediators; methyl viologen, benzyl viologen, neutral red, saffranine O and T, phenosaffranine, indigodisulphonate, indigotetrasulphonate, janus green, methylene blue, thionine, dichlorophenolindophenol. All mediators were used at 20 or 50 μM, and possible artefacts due to interaction with specific mediators were tested by omitting individual compounds from some titrations. All quinones and quinone derivatives were omitted as many interact with the quinone binding site(s). Titrations were normally done in the reductive direction, the transitions measured in these experiments have previously been shown to be reversible [3], and this was confirmed during the present series of experiments. All the titrations presented were done at pH 10.0 in 0.1 M glycine-HCl buffer. This pH was chosen as it was more alkaline than the pK of Q_a determined by Prince et al. [23] and in our previous experiments, so that no pH dependence would be expected for the potentials measured. Reconstitution of the reaction centres with quinones was carried out by dissolving the quinones in propan-2-ol (with warming if necessary). The quinone solution was added to the reaction centre preparation which was oxidised to +350 mV before incubation for 30 or 60 min in the dark prior to the titration. Propan-2-ol was used as solvent as the menaquinones were insufficiently soluble in ethanol, and cyclohexane solutions were not effective in reconstitution. No effect of increased preincubation time on reconstitution was observed. The following quinones were used, menaquinone-9 and ubiquinone-9 isolated from *Rps. viridis* by Dr. R. Powls, menaquinone-10, a gift from Dr. P. Rich, ubiquinone-10, ubiquinone-1, phytylmenaquinone (Vitamin K-1) and menadione from Sigma Chemical Co.

EPR spectra were recorded using a JEOL FE1X spectrometer with an Oxford instruments ESR 9 cryostat as described previously [18]. Samples were illuminated in the cryostat using a 150 W halogen lamp with fibre optic light guide to the cavity. Samples were illuminated at 200 K in an ethanol/solid CO_2 bath in an unsilvered dewar with a slide projector for 10 min. Maximum signal sizes were attained after 1–2 min, the longer period was used routinely to avoid any variations due to sample position in the bath when a number of samples were illuminated together. Signal intensities were measured as peak to trough heights of the $g = 1.82$ signal (difference between $g \approx 1.84$ and $g \approx 1.81$) and of the high field part of the trapped I^- doublet at $g \approx 1.97$.

Results

*Ortho*phenanthroline inhibits electron flow from the reaction centre to the quinone pool in intact chromatophores. It is thought to act by

binding at or close to the Q_b site displacing Q_b. In the absence of Q_b it might be expected that titration of the iron-quinone complex would show a single wave due to Q_a. Fig. 1 shows the EPR spectra of chromatophores posed at different potentials in the presence and absence of *o*-phenanthroline. At +75 mV there is essentially no signal in the dark, illumination at 6 K causing the reduction of Q_a. In untreated chromatophores Q_b is reduced at −100 mV. Illumination then results in a decrease in signal size as Q_a is photoreduced. As expected no Q_b spectrum is observed in the presence of *o*-phenanthroline. However, a Q_a spectrum is seen at −100 mV. Illumination results in an increase in the size of the Q_a signal. At −400 mV Q_a is reduced in both samples and illumination has no effect.

The titration of Q_a in the *o*-phenanthroline-treated sample shows two waves with $E_m \approx -100$ and −340 mV (Fig. 2a). This result is similar to that obtained in Photosystem II but not that which would be expected if *Rps. viridis* reaction centres contain a single Q_a, when presumably only a single wave should be seen in the titration. It also differs from the result of indirect titrations of Q_a [24] in which only a single wave was observed.

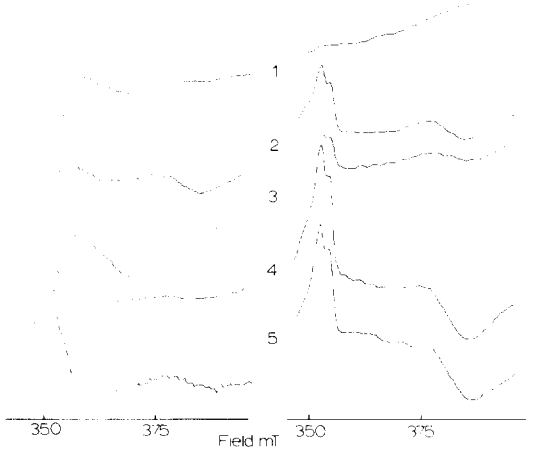

Fig. 1. The effect of *o*-phenanthroline on the iron-quinone electron acceptors in chromatophores of *Rps. viridis*. Left: Untreated chromatophores. Right: Chromatophores incubated with 10 mM *o*-phenanthroline at +340 mV before poising at the appropriate potentials. Samples were taken from an oxidation – reduction potential titration as described in the methods section. (1) 75 mV dark; (2) 75 mV after 30 s illumination at 6 K; (3) −100 mV dark; (4) −100 mV after 30 s illumination at 6 K; (5) −400 mV dark and following 30 s illumination at 6 K. EPR conditions: temperature, 6 K; frequency, 9.05 GHz; microwave power, 25 mW; modulation amplitude, 10 mT; gain, 500.

In an attempt to resolve the question of whether there are two Q_a sites in reaction centres it seemed worthwhile to determine the properties of the iron-quinone complex in isolated reaction centres. Extensive processing of the reaction centres was required to remove LDAO and Q_b. Following this procedure the reaction centres retained activity showing Q_a photoreduction at low temperature. Titration of Q_a showed a single wave with $E_m \approx -160$ mV (Fig. 2b). Addition of ubiquinone to the preparation at 1 : 1 to 1 : 2 mol ratio resulted in the reconstitution of an EPR signal with the line shape and *g*-value of Q_b, although the signal intensity was smaller, compared to the Q_a signal, than in chromatophores. Titration of these samples showed a quinone-semiquinone-quinol titration of Q_b, and a single wave of Q_a reduction as in the reaction centres without added quinone. (Fig. 2c), although the E_m is shifted slightly to −200 mV. Addition of a 10 : 1 excess of ubiquinone resulted in loss of the Q_b signal. This was unexpected in that the Q_b semiquinone is stable in chromatophores in the presence of the quinone pool. It may be that the site is more accessible and less effective in stabilising the semiquinone against dismutation by soluble electron carriers in the reaction centres. This is also suggested by the lower intensity of the signal in the titrations compared to those in chromatophores. Addition of other quinones such as dimethylbenzoquinone or menadione also resulted in formation of stable high potential iron-quinone signals, all quinones were therefore excluded from the mediators used. Addition of *o*-phenanthroline in the presence of ubiquinone removed the Q_b signal and a single wave titration of Q_a identical to that seen in the original reaction centre preparation was observed.

Addition of menaquinone-9 or -10 or phytylmenaquinone (vitamin K-1) did not reconstitute a high-potential iron quinone. However, it did reconstitute a low-potential wave on the Q_a titration (Fig. 2d). The two waves had an $E_m \approx -150$ mV and ≈ -300 mV. Addition of *o*-phenanthroline decreased the −300 mV wave, but did not

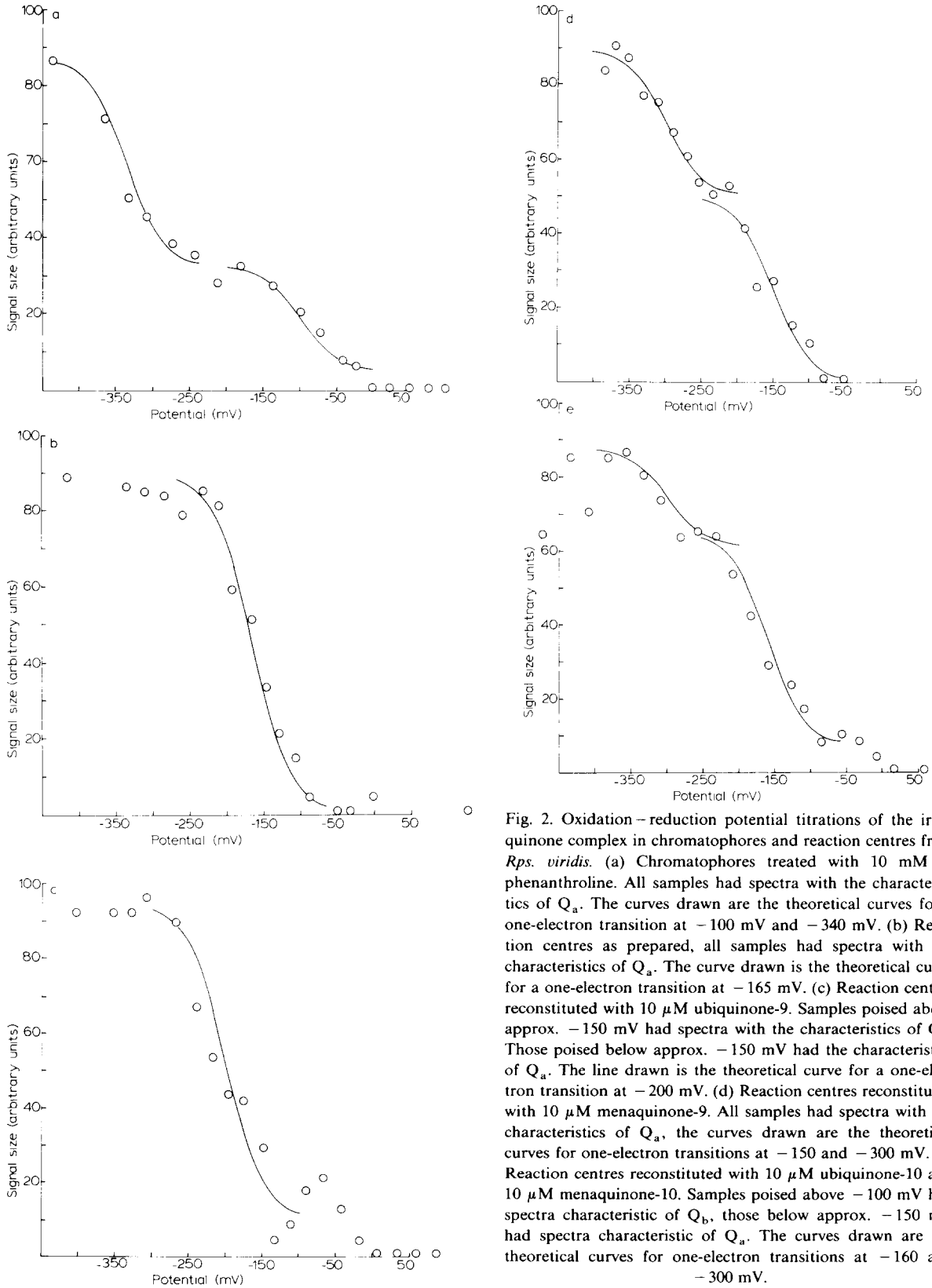

Fig. 2. Oxidation–reduction potential titrations of the iron-quinone complex in chromatophores and reaction centres from *Rps. viridis*. (a) Chromatophores treated with 10 mM *o*-phenanthroline. All samples had spectra with the characteristics of Q_a. The curves drawn are the theoretical curves for a one-electron transition at −100 mV and −340 mV. (b) Reaction centres as prepared, all samples had spectra with the characteristics of Q_a. The curve drawn is the theoretical curve for a one-electron transition at −165 mV. (c) Reaction centres reconstituted with 10 μM ubiquinone-9. Samples poised above approx. −150 mV had spectra with the characteristics of Q_b. Those poised below approx. −150 mV had the characteristics of Q_a. The line drawn is the theoretical curve for a one-electron transition at −200 mV. (d) Reaction centres reconstituted with 10 μM menaquinone-9. All samples had spectra with the characteristics of Q_a, the curves drawn are the theoretical curves for one-electron transitions at −150 and −300 mV. (e) Reaction centres reconstituted with 10 μM ubiquinone-10 and 10 μM menaquinone-10. Samples poised above −100 mV had spectra characteristic of Q_b, those below approx. −150 mV had spectra characteristic of Q_a. The curves drawn are the theoretical curves for one-electron transitions at −160 and −300 mV.

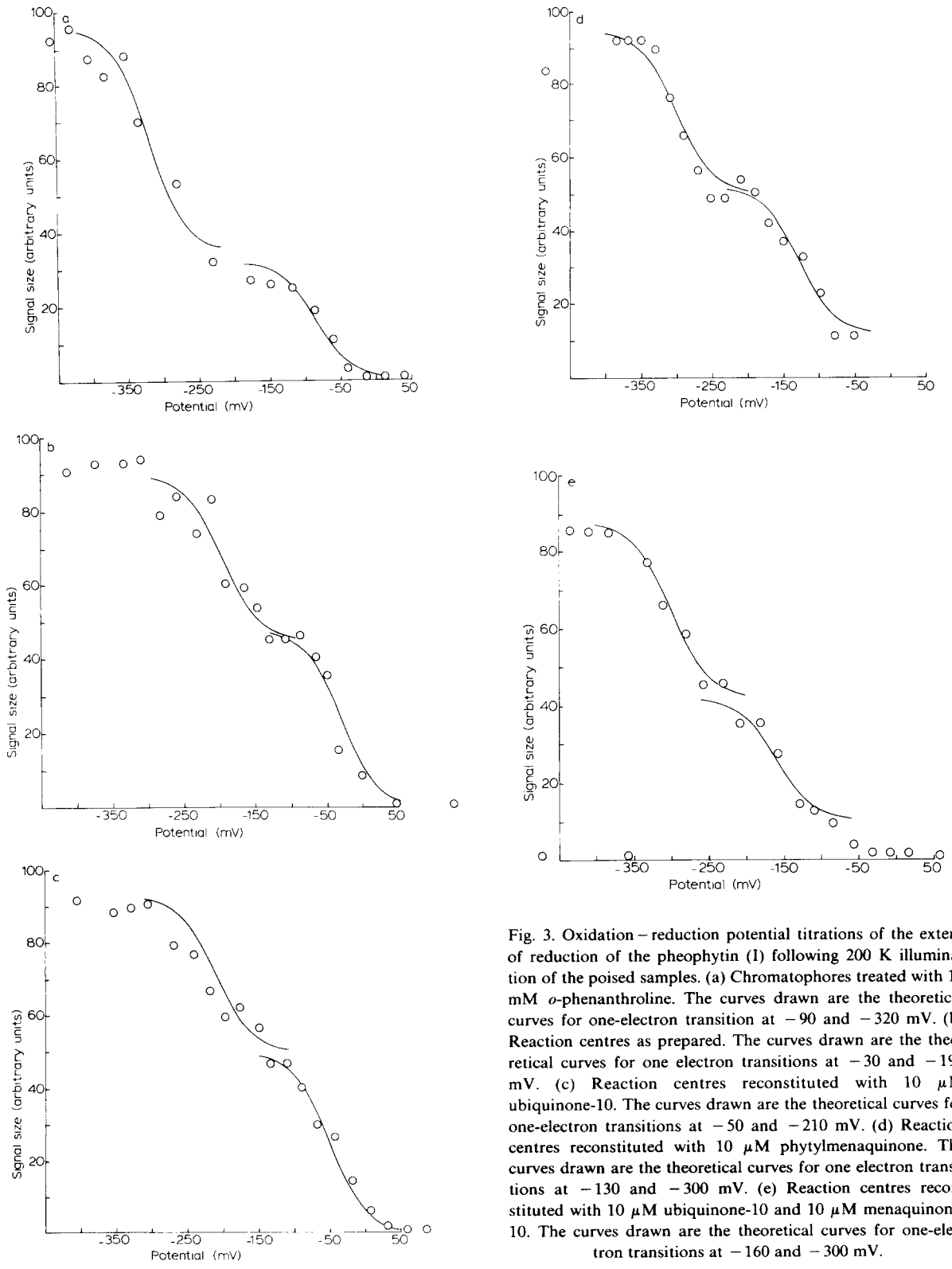

Fig. 3. Oxidation – reduction potential titrations of the extent of reduction of the pheophytin (I) following 200 K illumination of the poised samples. (a) Chromatophores treated with 10 mM *o*-phenanthroline. The curves drawn are the theoretical curves for one-electron transition at −90 and −320 mV. (b) Reaction centres as prepared. The curves drawn are the theoretical curves for one electron transitions at −30 and −195 mV. (c) Reaction centres reconstituted with 10 μM ubiquinone-10. The curves drawn are the theoretical curves for one-electron transitions at −50 and −210 mV. (d) Reaction centres reconstituted with 10 μM phytylmenaquinone. The curves drawn are the theoretical curves for one electron transitions at −130 and −300 mV. (e) Reaction centres reconstituted with 10 μM ubiquinone-10 and 10 μM menaquinone-10. The curves drawn are the theoretical curves for one-electron transitions at −160 and −300 mV.

remove it, the titration being similar to that seen in chromatophores with *o*-phenanthroline. The titration of the reaction centres in the presence of both ubiquinone and menaquinone showed the presence of a Q_b signal and the two waves of the Q_a reduction; that is the effect of both quinones was seen. The presence of one did not prevent the binding of the other (Fig. 2e) or cause any obvious change in the signal intensity or redox potential of the signals.

The size of the Q_a signal in the preparation reconstituted with menaquinone was greater than in the depleted reaction centres. Illumination at 6 K of a sample poised at −50 mV, resulted in photoreduction of Q_a and the appearance of a signal as large as the maximum chemically induced signal. However, this ability to increase the size of the Q_a signal by low temperature illumination was lost during the first wave of the titration. At potentials below −250 mV there was no increase and in some cases a decrease in the size of the Q_a signal on illumination. This may reflect reduction of the pheophytin which occurred to some extent even at 6 K. When the pheophytin was fully reduced by illumination at 200 K the Q_a signal disappeared, probably as a result of magnetic interactions between the components. The measured Q_b signal size was the same in the presence or absence of menaquinone, the apparently small size of the Q_b peak in fig. 2e reflects the increased total Q_a signal in the presence of menaquinone. The signal sizes were normalised to the maximum Q_a signal for presentation.

Q_a was photoreduced by illumination at 6 K in all of the preparations used. It is clear, however, that there is heterogeneity in the behaviour of the cytochromes as electron donors. In all preparations part of the Q_a is photoreduced irreversibly at potentials above 100 mV when the high potential cytochromes must act as a donor; however, complete reduction of Q_a is only observed below 0 mV when the low-potential cytochrome is also reduced. This donor heterogeneity means that it is uncertain how many electrons may be available for transfer through the reaction centre. These observations were made using 6 K illumination. It is likely that this heterogeneity also exists in 200 K illumination experiments as all the redox changes are seen at both temperatures, although some are slower at 6 K. Determination of the potential dependance of the ability to induce the reduction of the pheophytin (I) by 200 K illumination is therefore probably not diagnostic for the state of the acceptors; however, a large shift in the potential at which I can be photoreduced may reflect a change in the number of acceptors available.

Two waves were seen in all of the titrations of the induction of I, whether I was measured as the split radical at 7 K or as the unsplit radical at 20 K, so that they must reflect increased pheophytin reduction. In untreated reaction centres or with added ubiquinone one wave is at −50 mV and the other parallels the reduction of Q_a (Fig. 3b and c). In chromatophores treated with *o*-phenanthroline one wave is at about −100 mV and the other at −300 mV. Reaction centres reconstituted with menaquinone also have a low potential wave parallel to the low-potential wave of Q_a. The high-potential wave was not clear, as in some titrations most of the signal was induced around −50 mV and in others around −150 mV (Fig. 3d). This may indicate that reconstitution is incomplete. Reconstitution with both ubiquinone and menaquinone resulted in titrations the same as those seen with menaquinone alone (Fig. 3e).

Discussion

The results described in this paper do not fit easily with the classical model of the quinone acceptor complex in purple bacteria or with the structural information available for the *Rps. viridis* reaction centre.

Treatment of chromatophores with *o*-phenanthroline removes Q_b as expected. However, titration of Q_a indicates the presence of two Q_a-like centres. The reaction centre preparation is similar to that which has been used in most studies of this reaction centre, except that LDAO has been removed. It has the expected properties for the Q_a acceptor. It is photoreducible at low temperature and there is a single component seen in the redox titrations. Reconstitution of this preparation with ubiquinone also occurs as expected with the appearance of Q_b, although the EPR signal is less intense than in the chromatophores or LDAO-containing reaction centres. The reduction of Q_b occurs at the same potential as in chromatophores,

the double reduction clearly occurs in a classical quinone-semiquinone-hydroquinone manner over a narrow potential range. Although we did not consider it at the time, a possible explanation of our earlier results [18] might have been that at pH 10 the semiquinone of Q_b was stable with double reduction occurring around −300 mV. There is no indication that the semiquinone is stable over a wide potential range. It seems likely therefore that double reduction also occurs over a narrow range in the chromatophore. There is no indication in the reaction centres reconstituted with ubiquinone of the two steps seen in the titration of Q_a in chromatophores at alkaline pH or after *o*-phenanthroline treatment. If the reaction centres are reconstituted with menaquinone-9 or -10 no Q_b reconstitution is seen but there is an increase in the extent of the photoreducible Q_a and a second low-potential wave is seen on the titration of Q_a. Reconstitution with both ubiquinone and menaquinone results in a titration qualitatively similar to that in untreated chromatophores although the intensity of the Q_b signal is low.

The ability to reduce the pheophytin by 200 K illumination also shows two potential dependent steps. In chromatophores these paralleled the two steps on the Q_a titration. In reaction centres one parallels Q_a, while the other is at more oxidised potential. This high potential step probably reflects the reduction of the low-potential cytochrome *c*. Reconstitution with ubiquinone does not affect this, whereas reconstitution with menaquinone results in the appearance of a low-potential step and, perhaps because of incomplete reconstitution, a partial disappearance of the high-potential step. These results suggest that the menaquinone can function as an electron acceptor at both 6 and 200 K. The ubiquinone cannot accept electrons under these conditions as it would be reduced before the cytochrome.

In our original work on *Rps. viridis* we suggested that the two steps seen in the titration of Q_a and the photoreduction of I reflected magnetic interactions with Q_b or an unknown component. The identification of the high-potential iron-quinone as Q_b made it unlikely that interaction with Q_b was involved. The experiments presented here show clearly that a different quinone is reduced at the low-potential step. Two steps are seen in the Q_a titration only when excess menaquinone is present, but there is no requirement for ubiquinone. Two steps are not seen when only ubiquinone is added although Q_b and Q_a are both present.

Two steps are always seen in the photoreduction of I, suggesting these represent either the forced reduction of both of the pheophytin molecules or donor heterogeneity, with an additional electron from cytochrome *c* available in some reaction centres. Low-temperature donation by the high-potential haems was not reported in earlier experiments with chromatophores [18,23]. The electron donor at higher potentials observed in the present experiments has not been fully characterised, but may be high potential cytochrome *c*. A role for the high-potential haem in donation to the reaction centre chlorophyll in reaction centres was suggested by a recent room-temperature study [5].

A low-potential (−300 mV) step in the photoreduction of I is seen only after reconstitution with menaquinone, suggesting an additional acceptor is present under these conditions.

These results suggest that menaquinone can bind at two sites: the classical Q_a site and a site with similar but not identical properties. The second site apparently loses menaquinone rather easily, and probably has a different pK value, as two potentials for Q_a are only seen in titrations of chromatophores as the pH becomes more alkaline. It seems simplest to assume that the two sites are on the same reaction centre. In Photosystem II there is considerable evidence for reaction-centre heterogeneity. These experiments would not show whether two different types of reaction centre exist, but no other experiments suggest that there are two types of reaction centre in purple bacteria. If both sites are on the same reaction centre there are two possibilities for the identity of the site. Either the additional menaquinone binds to the Q_b site or there is an additional quinone binding site.

The possibility that the menaquinone binds to the Q_b site seems unlikely for several reasons. The observed EPR spectrum is the same as Q_a; the reasons for the differences in the spectra of Q_a and Q_b are not clear, though they are not simply quinone dependent. In *Rhodobacter sphaeroides*

both Q_a and Q_b are ubiquinone, in *Rps. viridis* Q_a is menaquinone and Q_b is ubiquinone, but in both species the spectra are different. The additional Q_a seems to be photoreducible at 6 K, whereas ubiquinone bound to the Q_b site is not normally photoreduced at this temperature. However, the full reduction of Q_a at low temperature requires the reduction of the low-potential cytochrome. It is not possible to reduce this without reducing ubiquinone at the Q_b site. Photoreduction of a second Q_a at 6 K would require that more than one electron was transferred through the reaction centre. This may well be possible as there are four cytochromes bound to the reaction centre, and as reported here in at least some of the centres both high- and low-potential cytochromes can apparently act as donor to the reaction centre at 6 K.

The presence of both ubiquinone (Q_b) and menaquinone does not seem to result in competition for the Q_b site, and the menaquinone does not seem to be displaced by *o*-phenanthroline. The pH dependence of the site is, however, similar to that of the Q_b site which our earlier results suggested had a pK between 9 and 10. Kleinfeld et al. [25] showed that in *Rb. sphaeroides* reaction centres frozen under illumination, oxidation of the reaction centre chlorophyll at low temperature was irreversible. They suggested that this was due to electron transfer from Q_a to Q_b, facilitated by structural changes induced by illumination. They did not report EPR spectra showing that Q_b was in fact reduced. If in the experiments described here menaquinone was bound to the Q_b site it might produce similar changes allowing transfer to the second quinone. It is not possible from their data or from the ones reported here to determine whether electron transfer to a second quinone occurs through 'Q_a' or directly.

In the crystal structure of the reaction centre of *Rps. viridis* a site similar to the Q_a site has been identified, which binds terbutryn and *o*-phenanthroline [26]; it is assumed to be the Q_b site. This identification is supported by studies of the *Rb. sphaeroides* reaction centre in which a second ubiquinone is bound to this site [27]. However, it has not been possible to crystallise *Rps. viridis* reaction centres with ubiquinone specifically bound in this site; in fact it is bound rather nonspecifically to several positions on the reaction centre. It may therefore be that this is in fact a second menaquinone binding site.

The experiments described here show that two Q_a-like binding sites can be detected in reaction centre preparations of *Rps. viridis*. They do not show whether both are involved in forward electron transfer or conclusively that the second site is different from the Q_b site. This will require a different type of experiment to investigate Q_b reduction in preparations with different quinone content and investigation of the kinetics of electron transfer under these different conditions.

Acknowledgements

I am grateful to Dr. R. Powls who extracted and purified menaquinone-9 and ubiquinone-9 from *Rps. viridis* and to Dr. P. Rich for a gift of menaquinone-10, to Dr. R.J. Cogdell who suggested the procedure for removing LDAO, to Ms. L. Tilling for technical assistance and to all my colleagues for many discussions of these experiments.

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