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THE PHOTO-OXIDATION OF HORSE HEART CYTOCHROME *c* AND NATIVE CYTOCHROME c_2 BY REACTION CENTRES FROM *RHODOPSEUDOMONAS SPHEROIDES* R_{26}

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SUMMARY

The primary event in bacterial photochemistry is the excitation of the reaction centre bacteriochlorophyll by light, causing oxidation of the reaction centre bacteriochlorophyll, and reduction of the primary electron acceptor. Subsequently the reaction centre is re-reduced by cytochrome c_2. We have studied this latter reaction with isolated reaction centres prepared from *Rhodopseudomonas spheroides* R_{26} and cytochrome c_2 prepared from the same species, or alternatively mammalian cytochrome *c*.

The reaction with cytochrome *c* followed second-order kinetics, with a rate constant of $3.8 \pm 1 \times 10^8\ s^{-1} \cdot mole^{-1}$ at pH 7.5 in 10 mM Tris–Cl^- buffer. The rate of reaction varied with pH and ionic strength, indicating an electrostatic interaction between two oppositely charged reactants, as would be expected from the large difference between the isoelectric points of cytochrome *c* ($\geqslant 10$) and that of the reaction centre complex (6.1, measured by isoelectric focusing).

The reaction with cytochrome c_2 also followed second-order kinetics, with a rate constant of $8.2 \pm 1 \times 10^8\ s^{-1} \cdot mole^{-1}$. The rate of reaction varied with ionic strength, but not with pH over the range 4.5–11.0. In contrast to the reaction with cytochrome *c*, where low concentrations of poly-L-lysine are inhibitory, the reaction with cytochrome c_2 is stimulated by poly-L-lysine over the approximate range of 0.3–1.0 poly-L-lysine molecules per reaction centre.

The possible physiological significance of these findings are discussed, and from the equivalence of the rates of reaction centre re-reduction and cytochrome *c* oxidation, it has been possible to determine extinction coefficients for the reaction centre changes at 603 and 865 nm: $\Delta E^{red-ox}_{603\ nm} = 26.5\ (\pm 1.2)\ mM^{-1} \cdot cm^{-1}$ and $\Delta E^{red-ox}_{865\ nm} = 116\ (\pm 8)\ mM^{-1} \cdot cm^{-1}$.

Abbreviations: LDAO, lauryl dimethyl amine oxide; MES, 2-(*N*-morpholino)-ethane sulphonic acid; Tricine, *N*-tris-(hydroxymethyl)-methyl glycine.

INTRODUCTION

During the primary light reaction of bacterial photosynthesis, the excited reaction centre bacteriochlorophyll is oxidised, and the primary electron acceptor reduced. In most species, this is followed by rapid migration of the "hole" in the reaction centre to an adjacent cytochrome c_2. Purified reaction centres retain the ability to photo-oxidise added cytochrome *c* [1–3].

Ke et al. [2] have studied the interaction between reaction centres prepared from *Rhodopseudomonas spheroides* R_{26} using Triton X-100, and reduced mammalian cytochrome *c*. They concluded that the interaction was electrostatic between the positively charged cytochrome *c* (pI 10.1) and the negatively charged reaction centre; the light-induced reaction between the reduced cytochrome *c* and the reaction centre had "zero-order" kinetics. However, as they pointed out, cytochromes of the c_2 type isolated from photosynthetic bacteria have isoelectric points essentially the same as that of the reaction centre. It was therefore important to investigate the more physiological cytochrome c_2: reaction centre interaction, and compare this to the reaction with mamalian cytochrome *c*.

For this study we have used reaction centres prepared from *Rps. spheroides* R_{26} by treatment with the zwitterionic detergent, lauryl dimethyl amine oxide (LDAO) [4], and cytochrome c_2 also prepared from *Rps. spheroides*. Reaction centres prepared with LDAO contain no endogenous cytochromes, whereas those prepared using Triton X-100 contain up to one bound cytochrome c_2 per reaction centre depending on the detergent concentration (refs 5 and 6, but see also ref. 2). The photo-oxidation of cytochromes c_2 from *Rhodopseudomonas viridis*, *Rhodopseudomonas capsulata* and *Rhodospirillum rubrum* by *Rps. spheroides* reaction centres was also investigated, as were similar reactions catalysed by reaction centres from the blue green mutant (A1a *pho*$^+$) of *Rps. capsulata* [7].

METHODS

Preparation of photochemical reaction centres

Cells of *Rps. spheroides* R_{26} (the blue green mutant) were grown anaerobically in the light as previously described [8]. Chromatophores were then prepared by breaking the cells in the "French" pressure cell (16 tons/inch^2), and reaction centres were isolated using the detergent lauryl dimethyl amine oxide as described by Clayton and Wang [4]. Reaction centres from *Rps. capsulata* Ala *pho*$^+$ were isolated as previously described [7]. Reaction centres were also prepared from *Rps. spheroides* 2.4.1 using sodium dodecylsulphate by the method of Slooten [9]. The concentration of both were calculated using E_{mM} 870 nm = 113 [4].

Preparation of c_2-type cytochromes

Mammalian cytochrome *c* (Type III from horse heart) was obtained from Sigma. Bacterial cytochromes c_2 were isolated from the supernatant remaining after centrifugation of chromatophores prepared from cells grown anaerobically in the light [8]. The cytochromes were purified by column chromatography on DEAE-cellulose (Whatman DE-52) and Sephadex G-25 and G-75 [10]. In the work reported in the present paper cytochrome c_2 from the Ga mutant of *Rps. spheroides* was used.

Identical results were obtained when cytochrome c_2 from *Rps. spheroides* 2.4.1 or *Rps. spheroides* R_{26} were used instead. Similar results were obtained with cytochrome c_2 from *Rps. capsulata* St. Louis, *Rps. capsulata* Ala *pho*$^+$, *Rps. viridis* and *R. rubrum*.

The cytochromes used were all more than 90% pure, using the data and criteria in ref. 10, and the *Rps. spheroides* cytochrome c_2 was more than 98% pure.

Disc electrophoresis on acrylamide gels (6.66%) of the cytochrome c_2 from *Rps. spheroides* indicated that several isoenzymes of this cytochrome were present. When the major band on the gel was eluted and concentrated, it was found that the photo-oxidation of this electrophoretically homogeneous cytochrome was identical to the photo-oxidation of the mixture of isozymes before electrophoresis.

The mammalian cytochrome *c* was reduced in solution either by a trace of $NaBH_4$, and subsequent gassing with oxygen-free nitrogen, or by the addition of sodium ascorbate and the subsequent desalting of the cytochrome on Sephadex G-25. The two methods gave reduced cytochrome *c* preparations which were indistinguishable.

The bacterial cytochromes c_2 were kept fully reduced during their isolation by addition of small amounts of sodium ascorbate between purification steps. All cytochromes were stored in the reduced form at −20 °C under N_2.

Determination of the isoelectric point of the reaction centres

The pI values of the cytochromes c_2 are given in ref. 10. The pI of the reaction centres was determined by isoelectric focusing (110 ml column) using carrier ampholine pH range 3–10 (LKB). 1 mg of reaction centre protein was applied, and the column was run in the dark at 4 °C, 250 V and 4 mA until constant voltage and current were attained. The focused reaction centres (which were still photo-active) were collected and the pH measured with a micro-electrode. This method estimated a pI of 6.1 for reaction centres prepared from *Rps. spheroides* R_{26}.

Spectrophotometric methods

Absorbance changes of the reaction centre bacteriochlorophyll (P870) were monitored at 603 nm (P605) in a rapidly responding single beam spectrophotometer and recorded on a Tektronix storage oscilloscope (Type 564) as described by Jackson and Crofts [11]. Cytochrome oxidation was followed with a two photomultiplier double beam spectrophotometer of 10 μs minimum response time using the wavelength pair 550–540 nm. Spectra of the flash-induced changes showed that the wavelengths chosen were optimal for measuring the photochemistry.

Actinic flashes were provided either by 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 200 μs). Under the conditions used in these experiments, both gave a saturating pulse of light which caused a single turnover of the photochemical reaction centres. The xenon flash was used routinely, and the figures refer to experiments using this source.

Preliminary results indicated that the photo-oxidation of reduced cytochromes *c* was independent of the concentration of LDAO present, and that this detergent could be replaced by 0.1% Triton X-100. However, in the absence of cytochrome, the decay of the reaction centre change was very dependent on the concentration of detergent. In 0.008% LDAO the half-time of decay of P605 oxidation could be up

to 1.5 s while in 2% LDAO this was reduced to 140 ms. In these experiments, unless otherwise stated, the reaction mixture routinely used was 2% LDAO in 10 mM Tris at pH 7.5.

Reaction centre preparations contain approx. 1.5 moles of ubiquinone per mole reaction centre, as assayed by the method of Griffiths et al. [12]. In order to allow repeated flash excitation of a single sample, the size of this secondary electron acceptor pool was routinely increased by the addition of 200 μM 1,4-naphthaquinone (B.D.H.).

Poly-L-lysine (average mol. wt 165 000), and Poly-L-glutamic acid (average mol. wt 102 000) were obtained from Sigma. LDAO was a generous gift from A.B.M. Biochemicals, Woodley, Stockport, Cheshire. All other chemicals were Analar grade, or of the highest purity available.

The progress of the reaction was routinely replotted onto a log scale to test for pseudo first-order kinetics, and half-times obtained from these log plots.

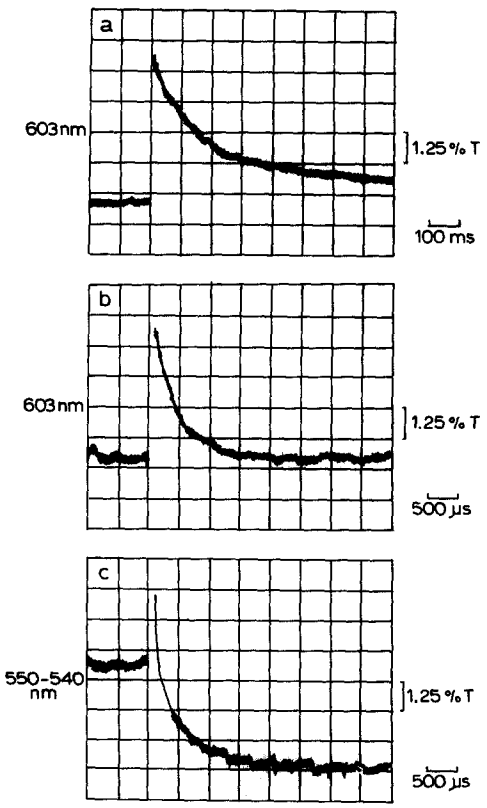


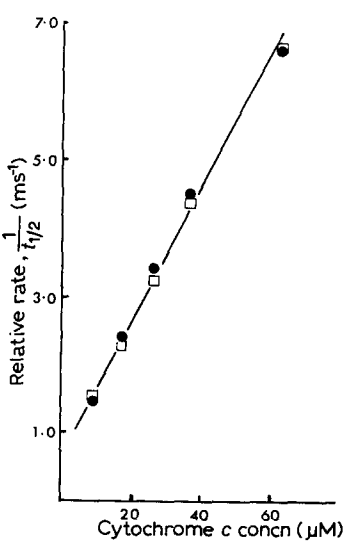
Fig. 1. Kinetics of the absorbance change transients at 603 and 550–540 nm in the absence and presence of reduced cytochrome *c*. (a) The reaction mixture contained 2.5 ml 10 mM Tris–HCl, pH 7.5, 2% LDAO, 200 μM 1,4-naphthaquinone and 0.8 μM reaction centres. The reaction was monitored at 603 nm. (b) as (a), but with the addition of 33 μM reduced cytochrome *c*. (c) as (b), but the reaction was monitored at 550–540 nm. In the absence of reduced cytochrome *c*, there was no net absorbance change at this wavelength pair.

RESULTS

On flash activation of the reaction centre preparation in the absence of cytochrome *c*, the photo-reactive bacteriochlorophyll (P870), measured as the decrease in absorbance seen at 603 nm (P605, ref. 13) was rapidly oxidised (Fig. 1a). Subsequently the oxidised P605 was re-reduced by the return of the electron from the primary acceptor [3]. The half-time for this reaction was 140 ms at pH 7.5 in the presence of 2% LDAO. When reduced mammalian cytochrome *c* was present in the reaction mixture the rate of P605 re-reduction was greatly stimulated (Fig. 1b). The rate of this re-reduction ($t_{\frac{1}{2}} \simeq 300\ \mu s$ under the conditions shown in the figure) exactly equalled the rate of cytochrome *c* oxidation (Fig. 1c).

The rate of P605 re-reduction and cytochrome *c* oxidation depended on the concentration of both cytochrome *c* (Fig. 2) and the reaction centres (Fig. 3); at constant reaction centre concentration the rate of reaction was directly proportional to the concentration of added reduced cytochrome *c*, and when the concentration of cytochrome *c* was kept constant, the initial rate of reaction was directly proportional to the concentration of reaction centres. The extent of the reaction also increased linearly with increasing reaction centre concentration (Fig. 4).

It is clear from these results that the kinetics of the reaction between reduced mammalian cytochrome *c* and the oxidised reaction centres are strictly second order. The second-order rate constant was $3.82 \pm 1 \times 10^8\ s^{-1} \cdot mole^{-1}$ at pH 7.5 in 10 mM Tris–Cl^- buffer. Reaction centres prepared using sodium dodecylsulphate [9] behaved similarly (Fig. 5), obeying second-order kinetics. This contrasts with the re-



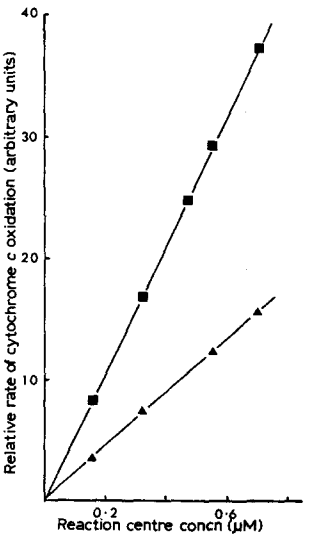


Fig. 2. Rate of re-reduction of P605 and oxidation of cytochrome *c* at different concentrations of reduced cytochrome *c*. Conditions as for Fig. 1, but reduced cytochrome *c* as indicated. ●, rate of re-reduction of P605; □, rate of oxidation of reduced cytochrome *c*.

Fig. 3. Rate of re-reduction of P605 at different concentrations of reaction centres. Conditions as for Fig. 1, but with reaction centres as indicated. ■, with 20 μM reduced cytochrome *c*; ▲, with 10 μM reduced cytochrome *c*.

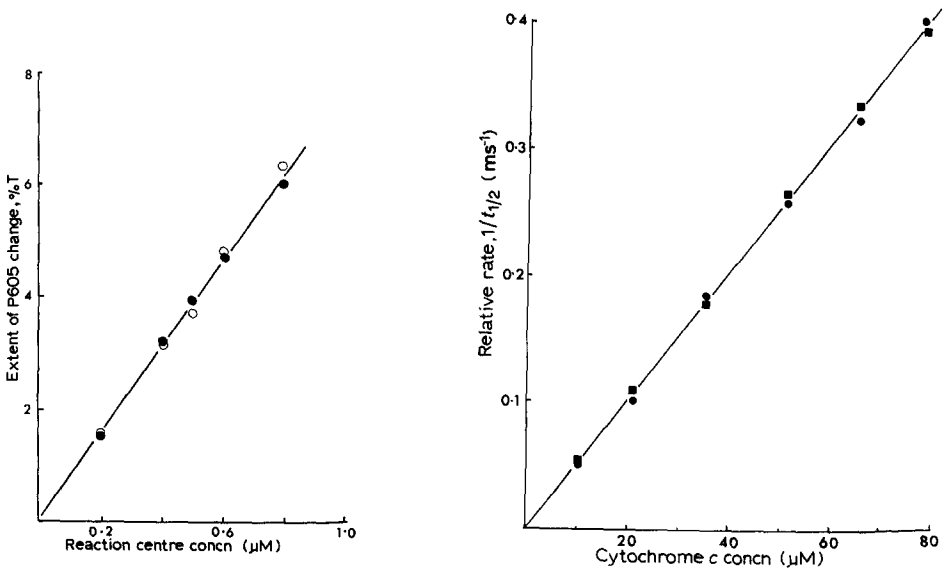


Fig. 4. Extent of P605 change at different concentrations of reaction centres. Conditions as for Fig. 3. ●, with 20 μM reduced cytochrome *c*; ○, with 10 μM reduced cytochrome *c*.

Fig. 5. Rate of re-reduction of P605, and oxidation of cytochrome *c* in sodium dodecylsulphate reaction centres at different concentrations of reduced cytochrome *c*. The reaction mixture contained 2.5 ml of 50 mM Tris–HCl, pH 8.0, 0.2 M NaCl, 2 μM 1,4-naphthaquinone and reduced cytochrome *c* as indicated. ●, rate of re-reduction of P605; ■, rate of oxidation of cytochrome *c*.

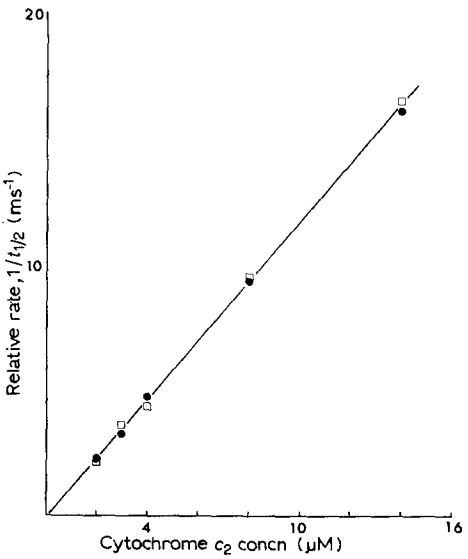


Fig. 6. Rate of re-reduction of P605, and oxidation of cytochrome c_2 at different concentrations of cytochrome c_2. The reaction mixture contained 2.5 ml 10 mM Tris–HCl, pH 7.5, 2 % LDAO, 200 μM 1,4-naphthaquinone, 0.8 μM reaction centres, and reduced cytochrome c_2 from *Rps. spheroides* as indicated. ●, rate of P605 re-reduction; □, rate of cytochrome c_2 oxidation.

sults of Ke et al. [2] who found that the reaction between cytochrome *c* and reaction centres prepared using Triton X-100 was very rapid ($t_{\frac{1}{2}} \simeq 25\ \mu s$) and showed zero-order kinetics. Possible reasons for this discrepancy are discussed below.

The reaction centres prepared with LDAO would also oxidise added reduced *Rps. spheroides* cytochrome c_2. The rate of this reaction, at constant reaction centre concentration was directly proportional to the concentration of reduced cytochrome c_2 (Fig. 6). The reaction between cytochrome c_2 and reaction centre was more rapid than that with cytochrome *c* and had a second-order rate constant of $8.2 \pm 1 \times 10^8$ $s^{-1} \cdot mole^{-1}$ at pH 7.5 in 10 mM Tris–Cl^- buffer. Reaction centres from *Rps. spheroides* would also photo-oxidise cytochromes c_2 isolated from *Rps. viridis*, *Rps. capsulata* St. Louis, *Rps. capsulata* Ala *pho*$^+$, and *R. rubrum*.

Effect of ionic strength

The rate of reaction between either reduced cytochrome *c* (Fig. 7) or c_2 (Fig.

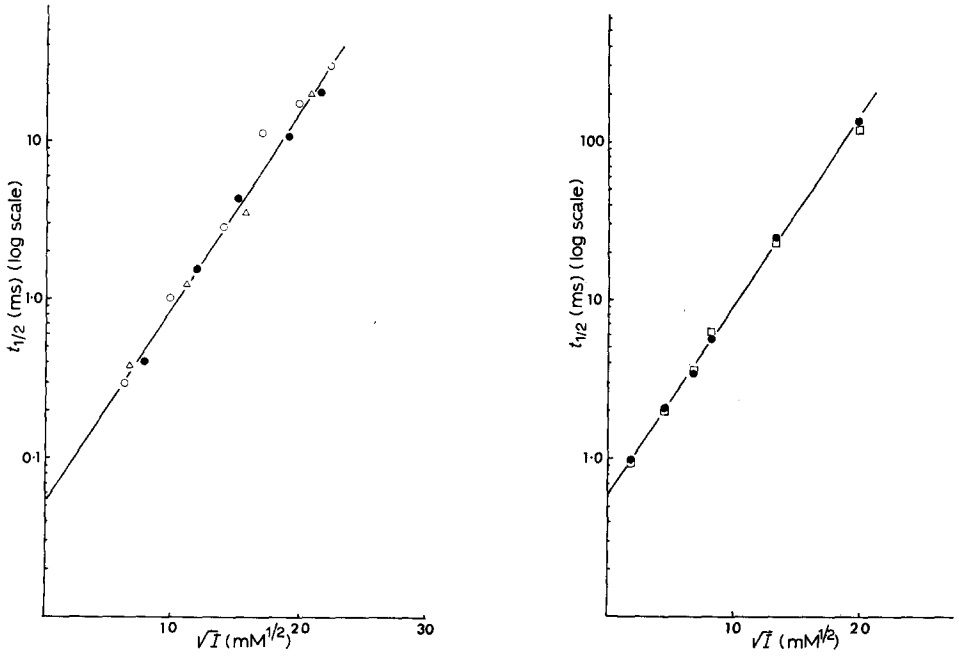


Fig. 7. The effect of ionic strength on the re-reduction of P605 by reduced cytochrome *c* (Bronsted plot). The reaction mixture contained 2.5 ml 10 mM Tris–HCl, pH 7.5, 2 % LDAO, 200 μM 1,4-naphthaquinone, 0.8 μM reaction centres and 33 μM reduced cytochrome *c*. The ionic strength was increased by addition of small volumes of concentrated salt solution. *I* was calculated as in ref. 14. $I = \Sigma\, \frac{1}{2}\, m_i Z_i^2$ where m_i = millimolarity of the ion i, Z_i = the number of charges on the ion i. In this case we have assumed total ionisation of the salt added, and neglected the contribution for the Tris–HCl buffer, which was assumed to be constant throughout the experiment. △, KCl; ○, NaCl; ●, Na_2SO_4.

Fig. 8. The effect of ionic strength on the re-reduction of P605, and the oxidation of reduced cytochrome c_2 (Bronsted plot). The reaction mixture was identical to that of Fig. 7, except that cytochrome *c* was replaced by 1.8 μM reduced cytochrome c_2 from *Rps. spheroides*, and the ionic strength increased by additions of KCl. *I* was calculated as before. ●, rate of re-reduction of P605; □, rate of oxidation of cytochrome c_2.

8) and the photo-oxidised reaction centres decreased with increasing ionic strength. The rate of reaction decreased until the rate of recombination of oxidised reaction centre and reduced primary electron acceptor could occur in preference to cytochrome oxidation. At higher ionic strengths the extent of the coupled cytochrome reaction also declined. However, at all ionic strengths tested the kinetics of the reaction remained second order. Bronsted's [14] theory of ionic reactions predicts that when the logarithm of the rate of the reaction is plotted against the square root of the ionic strength, a straight line graph should be obtained. This prediction was obeyed for the photo-oxidation of both types of cytochrome (Figs 7 and 8). The effect of ionic strength appeared to be independent of the ions involved; the same effect was seen when the ionic strength was varied with NaCl, KCl or Na_2SO_4.

Effect of pH

As described by Ke et al. [2], the rate of the reaction between reduced cytochrome *c* and the oxidised reaction centres depends on pH (Fig. 9). Between pH 6.5 and 10.0 the rate of cytochrome oxidation (or P605 re-reduction) was essentially constant. Below pH 6.5 the rate decreased until at pH 4.0 there was no longer any reaction. Above pH 10.0 the rate decreased until at values of pH higher than pH 12.0 there was again no reaction. The effect of pH was studied at several different ionic strengths, in each case the profile of the pH dependency was similar, but the curves were displaced vertically with increasing ionic strength (Fig. 9). These results may be contrasted with the results of Ke et al. (cf. Fig. 7 of ref. 2). It should be noted that

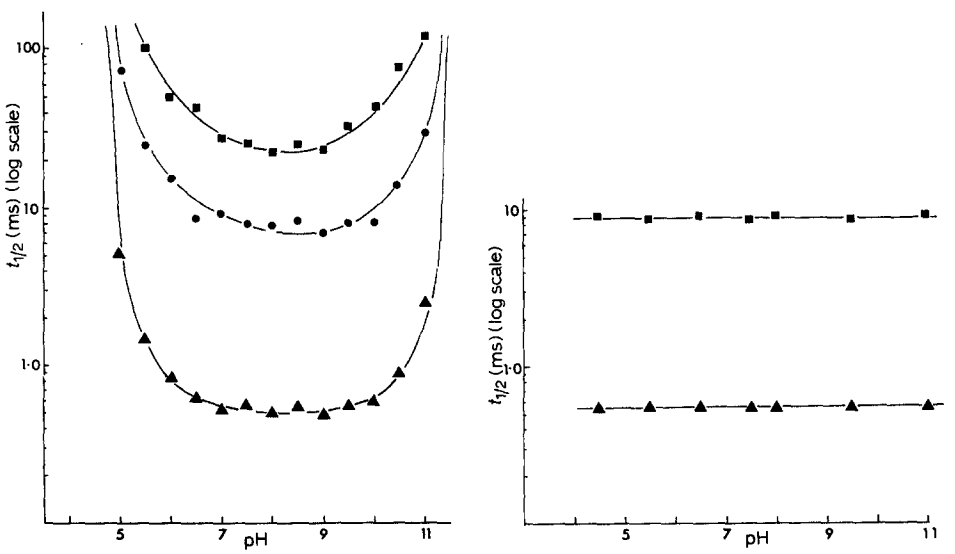


Fig. 9. The effect of pH on the re-reduction of P605 by reduced cytochrome *c*. The reaction mixture contained 2.5 ml 10 mM buffer, pH as indicated, 2 % LDAO, 200 μM 1,4-naphthaquinone, 0.8 μM reaction centres, and 33 μM cytochrome *c*. The buffers used were: pH 4–5.5, sodium succinate; 6.0–6.5, 2-(*N*-morpholino)-ethane sulphonic acid (MES); 7.0–8.5, *N*-tris-(hydroxymethyl)-methyl glycine (Tricine); 8.5–11, glycine. ▲, no additions; ●, plus 200 mM KCl; ■, plus 400 mM KCl.

Fig. 10. The effect of pH on the re-reduction of P605 by reduced cytochrome c_2. The experimental conditions were as for Fig. 9, except that the cytochrome *c* was replaced by 1.8 μM cytochrome c_2. ▲, no additions; ■, plus 200 mM KCl.

the rate of P605 re-reduction (or cytochrome *c* oxidation) was sensitive to ionic strength at all values of pH where a reaction occurred.

In contrast to the reaction with mammalian cytochrome *c* the reaction with cytochrome c_2 was independent of pH between pH 4.0 and 10.0 and the effect of ionic strength on the rate of reaction was similar at all values of pH (Fig. 10).

Effect of polyions

As with the interaction between cytochrome *c* and cytochrome oxidase [15], the photo-oxidation of cytochrome *c* was strongly inhibited by low concentrations of poly-L-lysine (ref. 2, and Fig. 11a). The reaction was also strongly inhibited by low concentrations of poly-L-glutamic acid (Fig. 12a). Interestingly, low concentratrions of poly-L-lysine stimulated the rate of reaction between reduced cytochrome c_2 and reaction centres (Fig. 11b), although higher concentrations of the polycation were again inhibitory. Poly-L-glutamic acid also inhibited the photo-oxidation of cytochrome c_2, but only at a much higher concentration than was required with mammalian cytochrome *c* (Fig. 12b).

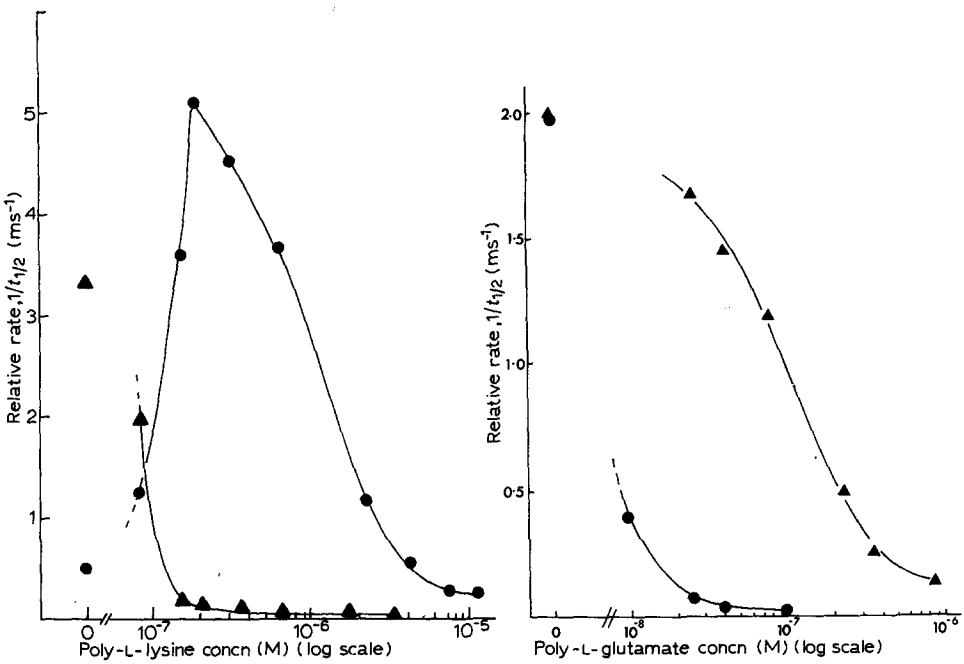


Fig. 11. The effect of poly-L-lysine on the re-reduction of P605. (a) ▲, with cytochrome *c*: The experimental conditions were as for Fig. 7, with the addition of poly-L-lysine as indicated. (b) ●, with cytochrome c_2: The conditions were as for Fig. 11a, except that the cytochrome *c* was replaced by 1 μM cytochrome c_2.

Fig. 12. The effect of poly-L-glutamate on the re-reduction of P605. (a) ●, with cytochrome *c*: The experimental conditions were as for Fig. 11a, except that the cytochrome *c* was 20 μM. Poly-L-glutamate was added as indicated. (b) ▲, with cytochrome c_2: The reaction mixture was as of Fig. 12a, except that the cytochrome *c* was replaced by 2 μM cytochrome c_2.

Reaction of cytochromes with reaction centres from Rps. capsulata

Results essentially similar to those reported above were obtained using reaction centres prepared from the blue green mutant (Ala *pho*$^+$) of *Rps. capsulata* [7], and the same range of mammalian and bacterial cytochromes *c*.

DISCUSSION

The extinction coefficient of P605 in Rps. spheroides and Rps. capsulata

The indentical kinetics of P605 re-reduction and cytochrome *c* oxidation under all conditions allow an equivalence of extinction coefficients of the two reacting species to be calculated. Combining the data for reaction centres prepared from *Rps. spheroides* R_{26} with LDAO and *Rps. spheroides* 2.4.1. with sodium dodecylsulphate gave an average of the ratio P605; cytochrome *c* of 1.30 ($\pm$ 0.05) : 1. Recent measurements of the millimolar extinction coefficient for the absorbance difference between reduced and oxidised forms of equine cytochrome *c* measured at 551 nm minus 540 nm in a 1-cm light path ($E_{\text{mM}\,551-540\,\text{nm}}^{\text{red-ox}}$) vary from 19.6 to 21.1 [16–20]. Using a value of 20.4($\pm$0.7) [20], the millimolar extinction coefficient for reduced minus oxidised reaction centre P605 change ($E_{\text{mM}\,603\,\text{nm}}^{\text{red-ox}}$) in *Rps. spheroides* is 26.5 ($\pm$1.2).

Results obtained under identical conditions on a slower timescale double beam spectrophotometer [11] indicate the ratio P605 : P865 to be 1 : 4.4 ($\pm$0.2). The $E_{\text{mM}\,865\,\text{nm}}^{\text{red-ox}}$ thus becomes 116 ($\pm$8), in close agreement with the data of Straley et al. [20].

From similar experiments with reaction centres prepared from *Rps. capsulata* Ala *pho*$^+$ [7] the millimolar extinction coefficient $E_{\text{mM}\,599\,\text{nm}}^{\text{red-ox}}$ was determined as 48$\pm$4.

The kinetics of cytochrome photo-oxidation

The reaction of both reduced cytochrome *c* and c_2 with oxidised reaction centres prepared with LDAO or sodium dodecylsulphate from *Rps. spheroides* obeyed second-order kinetics. This suggests that, in contrast to the reaction in reaction centres prepared using Triton [2], photo-oxidation of the cytochrome is the result of bimolecular collisions, and that neither LDAO nor sodium dodecylsulphate reaction centres bind significant amounts of cytochrome at the reaction site.

The effect of pH on the photo-oxidation of the cytochromes

The effect of pH on the rate of the reaction between oxidised reaction centres and reduced mammalian cytochrome *c* is marked, and can best be explained if it is assumed that the reaction involves an ionic interaction between two oppositely charged species [2]. Hence, above pH 10, where cytochrome *c* (pI 10.1 [2]) assumes the same charge as the reaction centres, and below pH 5.5, where the reaction centres (pI 6.1) assume the same net charge as the cytochrome *c*, the rate of reaction is strongly inhibited. However, cytochrome c_2 (pI 5.5 [10]) and the reaction centres (pI 6.1) have a similar overall charge at all values of pH, and it is therefore not surprising that the reaction between oxidised reaction centres and reduced bacterial cytochrome c_2 is independent of pH. Using the fluorescent pH probe, 9-amino-acridine [21], it has been shown that bacterial chromatophores may generate a transmembrane pH gradient large enough to bring the internal pH of the chromatophore

as low as pH 4.5 (Prince, R. C., unpublished). The independence of pH of the photo-oxidation of cytochrome c_2 may be advantageous under these conditions, in preventing changes in pH from inhibiting electron flow between cytochrome c_2 and the membrane-bound reaction centre.

The effect of ionic strength upon the rate of cytochrome photo-oxidation

The rates of ionic interactions usually depend on the ionic strength of the solution in which they occur. Bronsted [14] has predicted that the logarithm of the reaction rate in an ionic solution should be proportional to the square root of the ionic strength. Moreover, for small ions in solution, three special cases can be deduced: (1) If the product of the net charges of the reacting species is negative, the rate of reaction should decrease with ionic strength. (2) If the product of the net charges of the reacting species is positive, then the rate of reaction should increase with ionic strength. (3) If one of the reactants is uncharged, then the product of the net charges is zero, and the rate of reaction is independent of ionic strength.

The rate of photo-oxidation of both cytochromes decreases with increasing ionic strength. For the reaction of the positively charged cytochrome *c* with the negatively charged reaction centres, the second special case holds. However, theory would predict that the rate of reaction between the negatively charged cytochrome c_2 and the negatively charged reaction centres should increase with increasing ionic strength, whereas the reverse was found. A possible explanation for this paradox might be that the binding site for the cytochrome c_2 has a local charge of opposite sign to the net charge of the molecule at the pH of the experiment, as indicated by the isoelectric point. However, since the Bronsted theory was derived for small ideal ions, its application to the case of large molecules may be less precise.

The effect of poly-ions on the photo-oxidation of the cytochromes

As described by Ke et al. [2] low concentrations of poly-L-lysine strongly inhibit the rate of cytochrome *c* photo-oxidation. In contrast, low concentrations (approx. 10^{-6} M) stimulate the rate of cytochrome c_2 photo-oxidation. These effects of poly-L-lysine may best be considered if it is assumed that poly-L-lysine can have two separate effects: (1) Poly-L-lysine binds to the negatively charged reactant, and reverses or neutralises the net charge of that species. When binding is favourable, this effect should be seen at low concentrations of poly-L-lysine. (2) Poly-L-lysine will increase the ionic strength of the solution. This effect will predominate at higher concentrations, but because of the poly-ionic nature of the molecule the effect will be complex.

The rate of photo-oxidation of cytochrome *c* was very sensitive to poly-L-lysine as would be predicted, since both the above effects would tend to act additively to inhibit the rate of reaction. On the other hand, in the case of the reaction with cytochrome c_2, the binding of poly-L-lysine would result in a change from a reaction between ions of the same charge to one between ions of opposite charge, and this would be expected to increase the frequency of successful collisions between the reactants. This could explain why low concentrations of poly-L-lysine stimulate the rate of this reaction, especially since the concentrations at which stimulation occurred were in the range where the mole ratio of poly-L-lysine to reaction centres varied from 0.3 to 1.0, suggesting that a stoichiometric binding was involved. At

higher concentrations the reaction was inhibited, presumably because of the increased ionic strength effects.

Poly-L-glutamate inhibited the rate of photo-oxidation of both cytochromes, although the reaction with cytochrome *c* was sensitive to much lower concentrations than that with cytochrome c_2. However, it is not immediately obvious why the two reactions should show a difference in sensitivity.

Physiological significance

Dutton and Jackson [22] have described the anomalous behaviour of the P605 change in chromatophores of *Rps. spheroides*. In contrast to the change in Chromatium chromatophores [23], the rate of decay of the P605 change following activation with a saturating single turnover laser pulse is not identical to the rate of cytochrome c_2 photo-oxidation. In both the LDAO and the sodium dodecylsulphate reaction centres used in the present study, this anomaly is not seen, and the rate of the P605 decay exactly equals the rate of photo-oxidation of either cytochrome *c* or c_2 under all the conditions tested. This illustrates again [24, 25] that there are subtle differences between reaction centre particles in solution, and reaction centres bound to the photosynthetic membrane. These differences should be considered when results obtained with reaction centres are used as models for the true physiological reaction.

The last question which must be considered is whether the reaction between solubilised reaction centres and cytochrome c_2 is the same as that which occurs physiologically. During the preparation of chromatophores from whole cells, some cytochrome c_2 remains associated with the membrane fraction, and is able to undergo rapid photo-oxidation. It is probably this cytochrome that remains associated with the Triton (up to 1 cytochrome/P870 [5] but see also refs 6 and 2), and sodium dodecylsulphate (1 or 0.25 cytochrome/P870 [7]) reaction centres. In the case of the sodium dodecylsulphate reaction centres, the bound cytochrome c_2 appears to be displaced from the "active site" of the reaction centre, since its photo-oxidation following a single flash is slow, varying between 5 and 10 ms in our preparations. In the case of Triton reaction centres, there is no indication that the cytochrome c_2 (when this was present (see ref. 5)) was able to undergo rapid photo-oxidation. In neither preparation does there appear to be a binding site specifically occupied by cytochrome c_2 which modifies the activity of the latter favourably. The discrepancy in order of the reaction of cytochrome *c* and reaction centres, between the preparations used in this work, and those used by Ke et al. [2], throw some doubt on the physiological significance of the cytochrome *c*-binding site(s) of the Triton reaction centre preparations. It is not yet clear whether the cytochrome c_2 extracted from cells is the same species as that which remains with the chromatophores; the two forms have different midpoint potentials (Jones, O. T. G. and Prince, R. C., unpublished) but this may reflect a difference due to membrane binding. Furthermore when the isolated c_2 is electrophoretically purified, it is possible to separate several isozymes. We have performed experiments both with the mixture of isozymes, and with the electrophoretically homogeneous major species of cytochrome c_2, and found no differences in reactivity, but the possibility remains that a minor cytochrome c_2 component may be the physiologically important species.

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